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Engineering Nanotechnologies to Improve Immune Responses to Personalized Cancer Vaccines

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Background

Efficient cancer vaccines readily induce an MHC-I-restricted cytotoxic T lymphocyte (CTL) response by delivering tumor neo-antigens to the cytosol of dendritic cells (DCs). Nucleic acid adjuvants have been shown to further enhance CTL responses by triggering intracellular signaling pathways in DCs that are associated with activation, maturation and migration to the lymph nodes where priming of CTLs takes place. Studies show that DCs are present in high numbers in LNs relative to peripheral tissues such as the skin, suggesting that delivery of antigen and adjuvant to the LN might enhance vaccine efficiency[1]. Studies also show that to effectively prime antigen specific CTLs, both the antigen and adjuvant need to be co-delivered to the same DC[2].

Our optimized NP vaccine enables neo-antigen peptides and adjuvants to be loaded on one delivery vehicle and co-delivered to DCs. Since particle size plays a major role in the efficiency and route by which these vaccines reach LNs, we strategically optimized the NPs to be ~30 nm in size with a slight cationic surface charge to improve accumulation in LNs from the injection site. Thereby, allowing the NP vaccine to reach the majority of DCs in the LN. Upon uptake by LN-resident DCs, the pH-responsive NP will respond to the decreased pH within endosomal compartments and disassemble allowing cargo to be released into the cytosol (Fig.1). Cytosolically delivered neo-peptides are subsequently loaded on MHC-I molecules and presented to CTLs. Thus, the optimized NP vaccine can potentially improve targeting of LNs, activation of LN-resident DCs, and enhanced induction of antigen-specific CTLs.

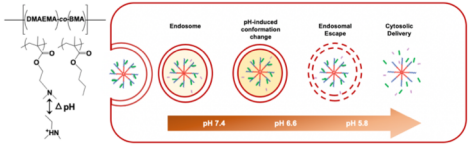

Figure 1: *Proposed mechanism of NP uptake, endosomal disruption, and escape into the cytosol.*

Modified peptides for delivery on nanoparticle vaccine

Name	Sequence	Length	Presentation	Amino Acid Spacer
OVA257-264 decalysine peptide	C(K10)QLESIINFEKL	22-mer	Class I	QLE
Degradable linker peptide	C(K10)SLVRYLLLSIINFEKL	27-mer	Class I	SLVRYLLL

Nanoparticle Vaccine Formulation

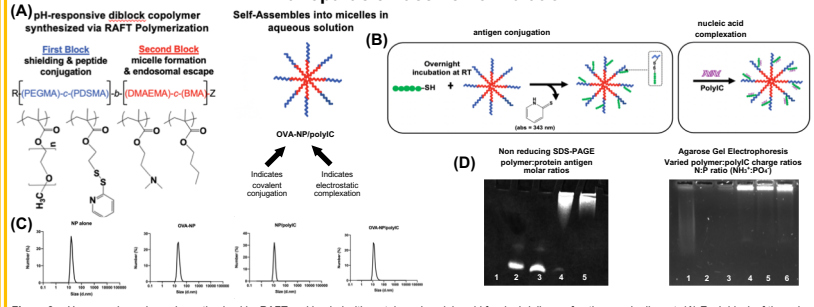

Figure 3: *pH-responsive polymer is synthesized by RAFT and loaded with protein and nucleic acid for dual-delivery of antigen and adjuvant. (A) Each block of the polymer corresponds to a specific function. (B) Schematic of thiolated proteins being conjugated to the block that forms the micelle corona and nucleic acid being electrostatically complexed to the core. (C)Dynamic light scattering (DLS) shows formation of ~40 nm diameter NP; loading with OVA protein and polyIC increases the diameter to ~120 nm. (D) AF647-labeled OVA were run on a non-reducing SDS-PAGE gel; (OVA alone) (Lane 2), (OVA + NP mix]) (Lane 3), 10:1 (OVA-NP) (Lane 4), 10:1 (OVA-NP/polyIC 8:1) (Lane 5).* Gel electrophoresis and GelRed staining were used to confirm adjuvant complexation. Lane (1) PolyIC alone; (2) NP alone; (3) 10:1 OVA-NP; (4) NP/polyIC 8:1; (5) NP/polyIC 10:1; (6) 10:1 OVA-NP/polyIC (8:1).

Results

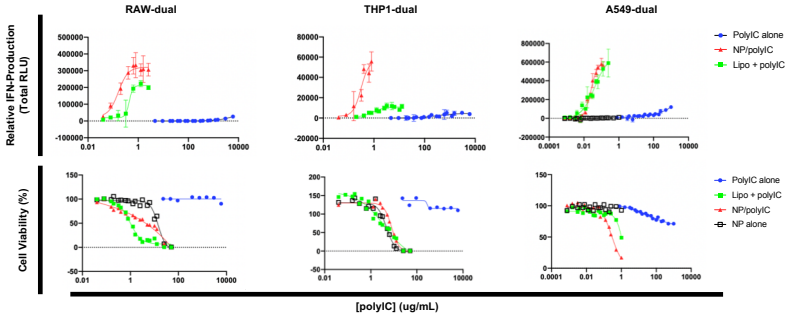

Figure 4: Representative dose response curves in RAW-dual (murine macrophages), THP1-dual (human monocytes), and A549-dual (human lung epithelial cells) reporter cells using polyIC complexed to the core of the NP at a 8:1 N:P ratio (n=2)

Conclusions

- RAFT-synthesized pH-responsive polymer self-assembles to form micelles that can be loaded with OVA protein antigen and nucleic acid adjuvant
- NP/PolyIC formulations enhances the activity of polyIC *in vitro*

Future Work

- Examine MHC-I presentation of OVA257-264 (SIINFEKL) protein antigens engulfed by dendritic cells
- Examine Expression of activation and co-stimulatory markers on primary dendritic cells
- Evaluate the capacity of NP carriers to enhance the cellular uptake of peptide antigen and polyIC
- Assess kinetics uptake of NP vaccine in DCs after subcutaneous injection
- Expression of activation and co-stimulatory markers on dendritic cells:
- Assess magnitude and functionality of CD8+ T cell response:
- Assess the combinatory effects of the NP vaccine combined with anti-PD1

References & Acknowledgements

1. Andorko, J. I., Gammon, J. M., Tostanoski, L. H., Zeng, Q. & Jewell, C. M. Targeted Programming of the Lymph Node Environment Causes Evolution of Local and Systemic Immunity. Cell. Mol. Bioeng. 9, 418–432 (2016).
2. Zhao, H et al. Amplified cancer immunotherapy of a surface-engineered antigenic microparticle vaccine by synergistically modulating tumor microenvironment. ACS Nano 13, 12556-12566 (2019).

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VANDERBILT UNIVERSITY Discovery Grant Program
Alex's Lemonade Stand FOUNDATION FOR CHILDHOOD CANCER
American Cancer Society IRG-58-009-56
CDMRP BC150791
STAND UP TO CANCER