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DELIVERING TLR AGONISTS IN PROTEIN NANOPARTICLE CANCER VACCINES REVEALS PLASMACYTOID DENDRITIC CELLS (pDCs) SUPPORT OF MYELOID DENDRITIC CELLS (mDCs)

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Background Cancer vaccine immunotherapy facilitates the immune system's recognition of tumor-associated antigens (TAAs), and the biomolecular design of these vaccines using nanoparticles is one important approach to elicit strong anti-tumor responses. The majority of cancer vaccines focus on generating a robust CD8+ T cell-mediated anti-tumor immune response. TAA presentation is conventionally attributed to myeloid DCs (mDCs). Plasmacytoid DCs (pDCs) are less capable of antigen presentation but produce high levels of cytokines. Their role in eliciting anti-tumor immune responses is less understood and is often overlooked.¹ Our lab has formulated efficacious cancer vaccines for C57BL/6 mouse tumor models using the hollow E2 caged protein as a platform, which is composed of 60 identical monomers to yield nanoparticles with diameters of ~30 nm.²⁻⁴ We previously hypothesized that such efficacy could be in part attributed to the exceptional ability to activate mDCs by E2 nanoparticles encapsulating TLR9 agonist CpG1826.⁵ However, we did not evaluate whether the formulations could additionally activate pDCs or the mechanistic role of pDCs in immune responses to cancer vaccine.

Methods The E2 protein nanoparticle platform was formulated to deliver encapsulated TLR9 or TLR7 agonist to determine their activity over free agonists in activating both mDCs and pDCs. CpG1826, the clinically-used CpG1018, or a 20-mer ssRNA sequence derived from HIV-1 was conjugated to the inner surface of the E2 nanoparticle. Murine mDCs and pDCs were differentiated *in vitro* and evaluated for viability and activation markers 24 hours post-nanoparticle exposure. To evaluate antigen presentation by DCs, SIINFEKL peptide antigen was conjugated to nanoparticles encapsulating CpG1826, and secreted factors from vaccine-stimulated pDCs or control were transferred to mDCs to evaluate pDC aid of mDC antigen display.

Results Although mDCs were only activated by nanoparticle-encapsulated TLR9 agonists, pDCs were activated by all the individually tested constructs, and CpG1826 was shown to induce robust cytokine production in pDCs. Transfer of culture supernatant from pDCs that were exposed to the antigen and nucleic acid conjugated E2 nanoparticle enhanced mDC display of the antigen, particularly when also delivered in nanoparticles. This effect was not observed with the non-conjugated individual components.

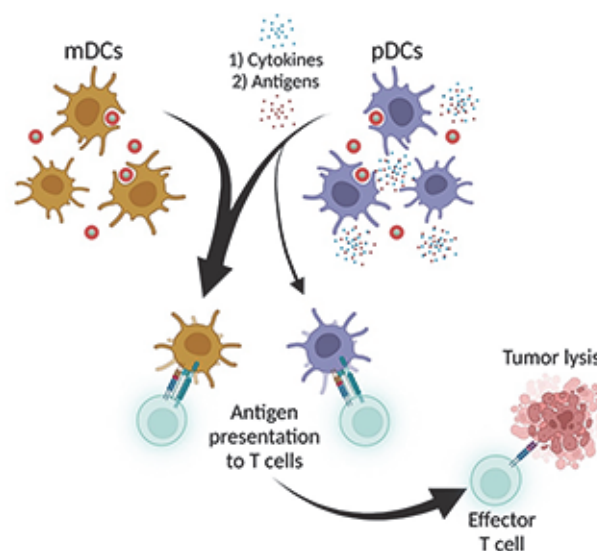
Conclusions These results reveal that pDCs can enhance mDC function (figure 1), suggesting that engaging both pDCs and mDCs will be critical in designing optimally effective cancer vaccines, and demonstrate the advantage of using nanoparticle-based vaccine delivery.⁶

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REFERENCES

1. Gulubova M. Myeloid and Plasmacytoid Dendritic Cells and Cancer - New Insights. *Open Access Maced J Med Sci*. 2019;**7**(19):3324–3340.
2. Molino NM, *et al*. Viral-mimicking protein nanoparticle vaccine for eliciting anti-tumor responses. *Biomaterials*. 2016;**86**:83–91.
3. Neek M, *et al*. An Antigen-Delivery Protein Nanoparticle Combined with Anti-PD-1 Checkpoint Inhibitor Has Curative Efficacy in an Aggressive Melanoma Model. *Advanced Therapeutics*. 2020;**3**(12):2000122.
4. Neek M, *et al*. Co-delivery of human cancer-testis antigens with adjuvant in protein nanoparticles induces higher cell-mediated immune responses. *Biomaterials*. 2018;**156**:194–203.
5. Molino NM, *et al*. Biomimetic protein nanoparticles facilitate enhanced dendritic cell activation and cross-presentation. *ACS Nano*. 2013;**7**(11):9743–52.
6. Butkovich N, *et al*. Nanoparticle vaccines can be designed to induce pDC support of mDCs for increased antigen display. *Biomater Sci*. 2023;**11**(2):596–610.

Ethics Approval All animal studies were carried out in accordance with protocols approved by the Institute for Animal Care and Use Committee (IACUC) at the University of California, Irvine, which is fully accredited by AAALAC.



Abstract 1120 Figure 1 Response of mDCs and pDCs to nanoparticle cancer vaccines. After uptake and processing of a nanoparticle vaccine, mDCs (and to a lesser extent pDCs) can directly activate T cells, which may subsequently recognize and eliminate tumor cells. Secreted factors including cytokines and antigens from pDCs may aid mDCs in this process. Represented are mDCs (orange cells), pDCs (purple cells), nanoparticle vaccine (red circles), antigens (red dots), cytokines (blue dots), T cells (turquoise cells), and lysed tumor cells (pink).

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