Development of an *Ex Vivo* Assay for STING Agonist-Mediated Conventional Type 1 Dendritic Cell Activation

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Objectives:
Stimulator of interferon gene (STING) agonists, such as cyclic dinucleotides (CDNs), have potential to drive antitumor immunity in cancers, including head and neck squamous cell carcinoma (HNSCC). We previously established that peptide-based hydrogel delivery systems improve the efficacy of CDN monotherapy in HNSCC preclinical models. However, the immune mechanism leading to this improved survival is not entirely known. We hypothesize that CDN-mediated conventional type 1 dendritic cell (cDC1) activation may lead to tumor regression in our HNSCC preclinical models. To test this, an ex vivo assay was developed to investigate cDC1 activating CDN treatments.

Methodology:
Bone-marrow-derived cells (BMDCs) were isolated from 8-12 week old C57BL/6 mice via the femur and tibia under sterile conditions. Then we differentiated BMDCs using 20 µg/L of GM-CSF. After a 10-day culture, differentiated cells were treated with 1µM, 5µM, and 50µM CDN overnight, and flow cytometry analysis was conducted to evaluate subpopulations and activation.

Results:
Although about 35% of CD45+CD3- were DCs, less than 1% of the total DC population were cDC1. While these cells were CD86+, marking of activated DCs, we are unable to confirm the activation of cDC1 cells due to low differentiation yields.

Conclusions:
We developed an ex vivo assay to confirm DC activation following CDN treatment. Further optimization is required to increase cDC1 differentiation yield to study the role of activated cDC1s in our treatment models. A 17-day culture period with specific growth factors and future assays, including sorting and flow analysis, are required to increase the yield of cDC1s and confirm activation. Collectively, these studies delineate the STING agonist CDN-mediated cDC1 activation mechanism leading to tumor regression in preclinical HNSCC models of oral cancer.

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