Stimulating Macrophage-Dependent Anti-Tumor Immunity with siRNA-Loaded, Mannosylated Nanoparticles in Ovarian Cancer

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Background

Tumor-associated macrophages (TAMs) are primarily M2-like and promote tumor progression and immunosuppression

- Repolarizing TAMs to an M1, pro-inflammatory phenotype can stimulate anti-tumor immunity
- By targeting the inhibitor of Nuclear Factor-κB alpha (IkBα) with small interfering RNA (siRNA), TAMs can be repolarized to develop anti-tumor immunity
- TAMs overexpress CD206 which can be targeted by decorating nanoparticles with mannose

Hypothesis

Delivery of IkBα siRNA using mannose-decorated polymeric nanoparticles will activate the canonical NF-κB pathway in TAMs to support anti-tumor immunity

MnPEGDB Polymers Complex with siRNA to Form MnNPs that Target and Repolarize M2 BMDMs

- Mannose-Poly(ethylene glycol)-(DMAEMA-co-BMA) (MnPEGDB) forms polymeric complexes with small oligonucleotides (Cy5-dsDNA, scrambled siRNA, IkBα siRNA)
- DLS and zeta revealed size ~140 nm and zeta ~1 mV
- FTIR confirmed mannose conjugation (decrease in azide peak at 2100 cm⁻¹)
- IkBα-MnNPs induce phenotypic shift towards M1 macrophages by activating canonical NF-κB in BMDMs from NGL-reporter mice

In Vivo IP Delivery of MnNPs Targets TAMs in the Ascites and Tumor in TBR5 Ovarian Tumor Models

- Female FVB mice injected IP with TBR5 ovarian tumor cells were treated with Cy5-MnNPs twice per week for 2 weeks (4 total treatments)
- Flow analysis of solid tumor, ascites, and spleen revealed specific uptake of MnNPs in the macrophages and monocytes in the solid tumor and ascites, but not the other immune cells
- Negligible delivery was observed in any immune cell population in the spleen

- Organ comparison of macrophages and monocytes revealed no off-target delivery outside of tumors and ascites
- MnNPs were almost exclusively taken up by CD45+ immune cells, with no delivery to tumor cells

MnNP Treatment with IkBα siRNA Suppresses Tumor Development in Multiple Models of Ovarian Cancer

<table>
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<tr>
<th>ID8-C57BL/6 (Late-Stage Model)</th>
<th>TBR5-FVB (Aggressive Model)</th>
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<tr>
<td>Female C57BI/6 injected with ID8 cells and treated 3x at late-stage</td>
<td>Female FVB injected with TBR5 cells and treated 2x/week to model an aggressive tumor</td>
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<td>Trend in reduction of ascites volume but no change in tumor size</td>
<td>Both MnNP treatments significantly reduced ascites accumulation and tumor growth, only IkBα-MnNP significantly decreased tumor cell populations</td>
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<td>TNF-α and CCL3 RNA expression was increased in the ascites, indicating increase in inflammation</td>
<td>IkBα-MnNPs also significantly decreased the percent of M2-like TAMs, indicating repolarization of macrophages</td>
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Conclusions and Future Directions

- MnNPs form nanoscale micelles that deliver IkBα siRNA to macrophages and alter their phenotype
- In vivo delivery via IP injection revealed specific uptake into macrophages in the solid tumor and ascites with negligible off-target delivery to the spleen
- Treatment with IkBα-MnNPs decreased ascites buildup and tumor burden and altered TAM phenotype
- Preliminary IF studies suggested an increase in infiltrating CD8 T cells, necessary for future combination therapies
- Future Directions:
  - Utilize combination therapies with immune checkpoint blockades to increase therapeutic effects
  - Evaluate potential for MnNP treatments to limit progression of breast cancer metastases using two models:
    - Intubation for direct delivery into lungs with breast metastases generated via orthotopic tumor implants
    - Intravenous delivery to treat pre-existing bone metastases

References


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