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

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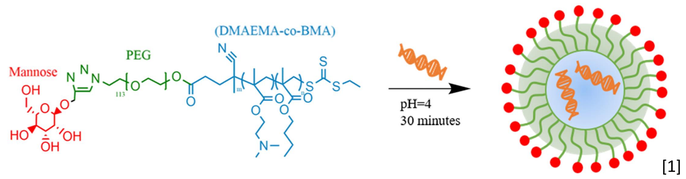
Background

- 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 (IκBα) 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

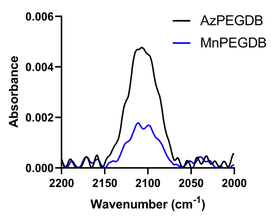
Delivery of IκBα siRNA using mannose-decorated polymeric nanoparticles will activate the canonical NF-κB pathway in TAMs to support anti-tumor immunity

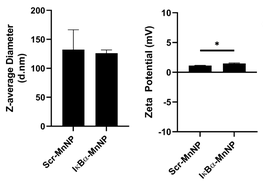
MnPEGDB Polymers Complex with siRNA to Form MnNPs that Target and Repolarize M2 BMDMs



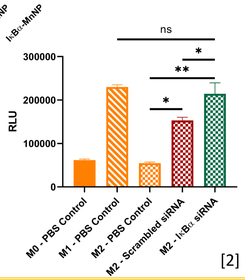
[1]

- Mannose-Poly(ethylene glycol)-(DMAEMA-co-BMA) (MnPEGDB) forms polymeric complexes with small oligonucleotides (Cy5-dsDNA, scrambled siRNA, IκBα siRNA)





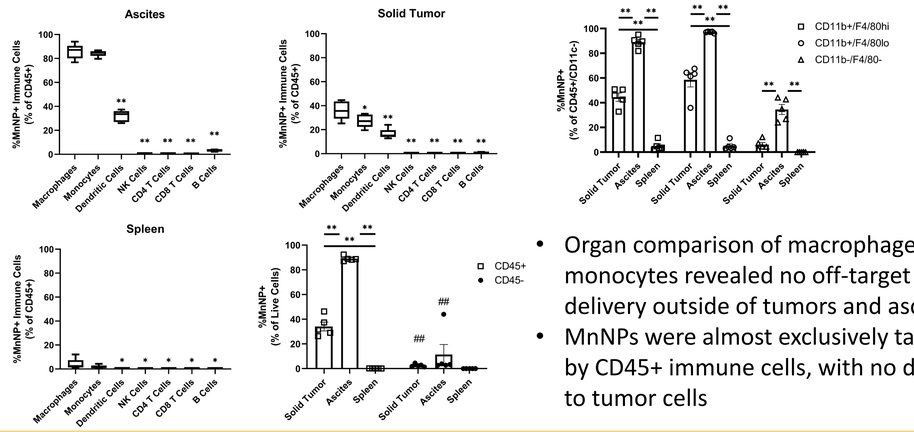
- DLS and zeta revealed size ~140 nm and zeta ~1 mV
- FTIR confirmed mannose conjugation (decrease in azide peak at 2100 cm^{-1})
- IκBα-MnNPs induce phenotypic shift towards M1 macrophages by activating canonical NF-κB in BMDMs from NGL-reporter mice



[2]

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

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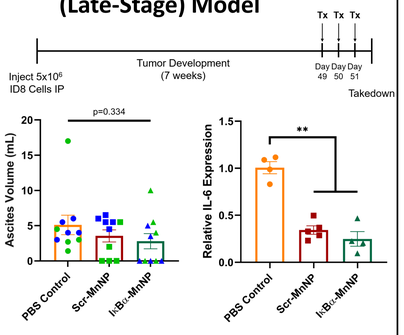
MnNP Treatment with IκBα siRNA Suppresses Tumor Development in Multiple Models of Ovarian Cancer

ID8-C57Bl/6 (Late-Stage) Model



- Female C57Bl/6 injected with ID8 cells and treated 3x at late-stage
- Trend in reduction of ascites volume, but no change in tumor size
- TNF-α and CCL3 RNA expression was increased in the ascites, indicating increase in inflammation

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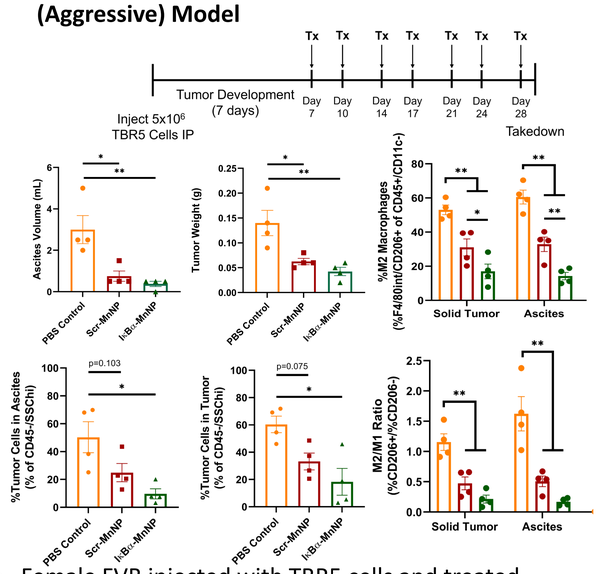
TBR5-FVB (Aggressive) Model

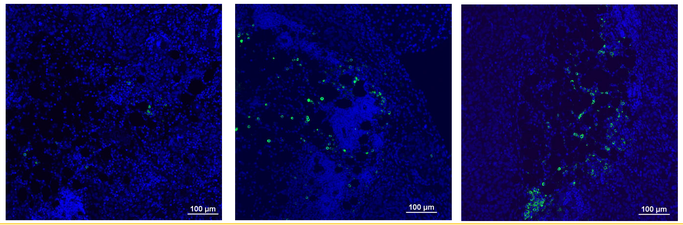


- Female FVB injected with TBR5 cells and treated 2x/week to model an aggressive tumor
- Both MnNP treatments significantly reduced ascites accumulation and tumor growth, only IκBα-MnNP significantly decreased tumor cell populations
- IκBα-MnNPs also significantly decreased the percent of M2-like TAMs, indicating repolarization of macrophages

Treatment with IκBα-MnNPs Increases CD8+ T Cell Infiltration in TBR5 Tumors

- TBR5 tumors treated biweekly with MnNPs were fixed, sectioned, and stained
- IF staining with DAPI (nuclei) and AF-488 (CD8) demonstrated an increase in T cell infiltration in treated tumors

PBS Control **Scr-MnNP** **IκBα-MnNP** [2]



Conclusions and Future Directions

- MnNPs form nanoscale micelles that deliver IκBα 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 IκBα-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

1. Glass EB, et. al. *ACS Omega* 2019.
2. Glass EB, et. al. *BMC Cancer* Submitted.

Acknowledgements

- Todd Giorgio and Fiona Yull Research Groups
- J. and K. Rathmell Labs for flow cytometry assistance
- VINSE for use of FTIR and Zetasizer
- Funding:
  - NIH RO1CA214043

Also supported by a generous gift from Mr. Chris Hill through Anglo-American Charity Ltd.