Macrophage-Targeted Polymer-Drug Conjugates for STING Pathway Activation to Improve Cancer Immunotherapy

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Background

The Cancer Immunity Cycle (CIC)

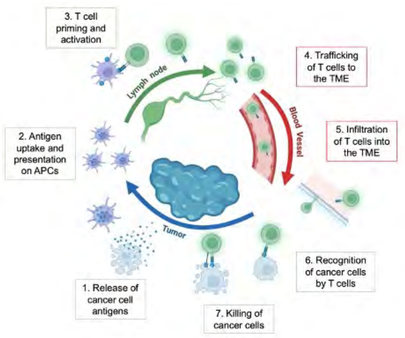


Figure 1: Cancer remains the second leading cause of death nationwide. One promising approach to combat cancer is by modulating the cancer immunity cycle, which outlines the series of steps the immune system undergoes to recognize and eliminate cancer cells. The CIC serves as a framework for developing novel immunotherapies that harness and enhance the immune response to target and eliminate cancer more effectively.

STING Signaling to Promote the CIC

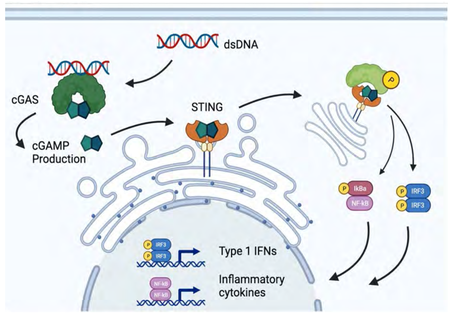
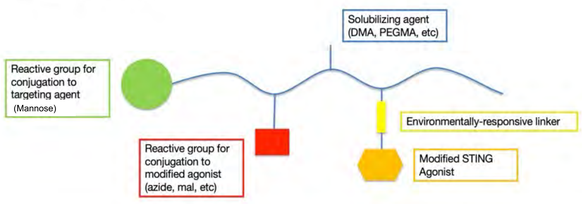


Figure 2: The Stimulator of Interferon Genes (STING) pathway is a cellular signaling pathway that detects the presence of cytosolic DNA, including DNA released by cancer cells. Activation of STING triggers the production of immune-stimulating molecules and enhances various aspects of the cancer immunity cycle. Due to the crucial role of STING in antitumor immunity, STING pathway agonists are being developed as next-generation cancer immunotherapeutics to activate this pathway and enhance the anti-tumor immune response.

Polymer Delivery Technologies



Advantages of Drug Conjugation to a Polymer Carrier:

- Drug solubilization
- Improved pharmacokinetics
- Tumor targeting
- Reduced systemic toxicity
- Environmentally-responsive drug release

Polymeric Carrier Design

DMA is a water-soluble and biocompatible monomer that will solubilize the STING prodrug

AzEMA allows for chemical conjugation to the DBCO reactive handle on the STING prodrug

Mannose is the biological moiety that targets tumor associated macrophages (TAMs)

Deprotected mannose is readily recognized by mannose receptors present on TAMs

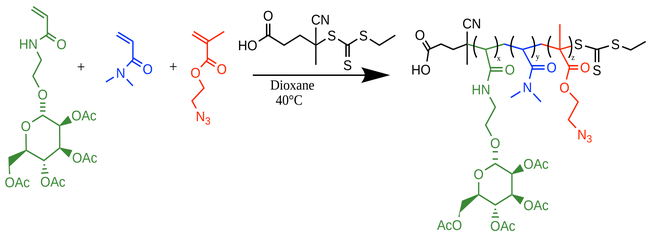


Figure 3: Synthesis of 100kDa poly(N,N-dimethylacrylamide-co-Azide ethylmethacrylate-co-Mannose) terpolymers via Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization

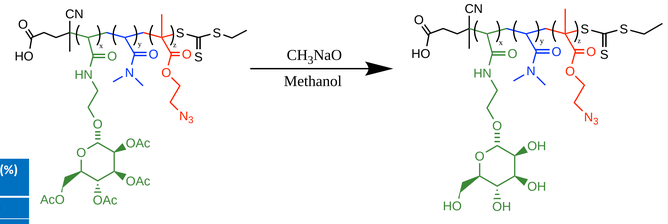


Figure 4: Mannose deprotection in sodium methoxide for 100kDa poly(N,N-dimethylacrylamide-co-Azide ethylmethacrylate-co-Mannose) terpolymers to increase cellular uptake

Mannose (%)	AzEMA (%)	DMA (%)
0	10	90
5	10	85
15	10	75
30	10	60

In Vitro Macrophage Polarization and Uptake

Macrophage Polarization

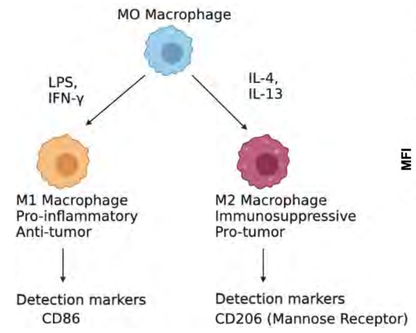


Figure 5: M0 macrophage polarization to M1 and M2 phenotypes using stimuli factors

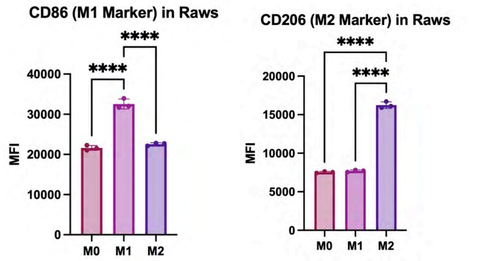


Figure 6: RAWs exhibit M1 polarization, characterized by CD86 detection marker expression and M2 polarization, marked by CD206 detection

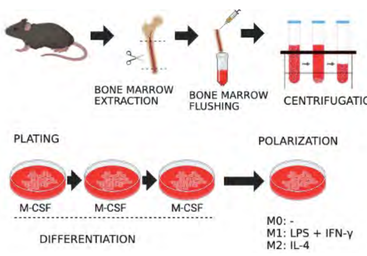


Figure 7: Bone Marrow-Derived Macrophages (BMDMs) Extraction and Polarization

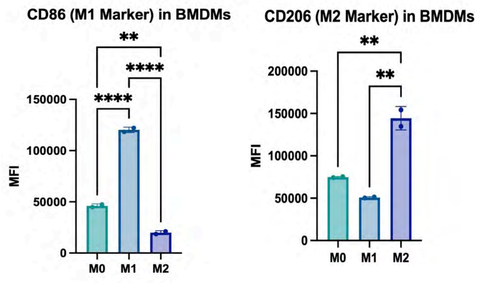


Figure 8: BMDMs exhibit M1 polarization, characterized by CD86 detection marker expression, and M2 polarization, marked by CD206 detection

Polymer Uptake Study

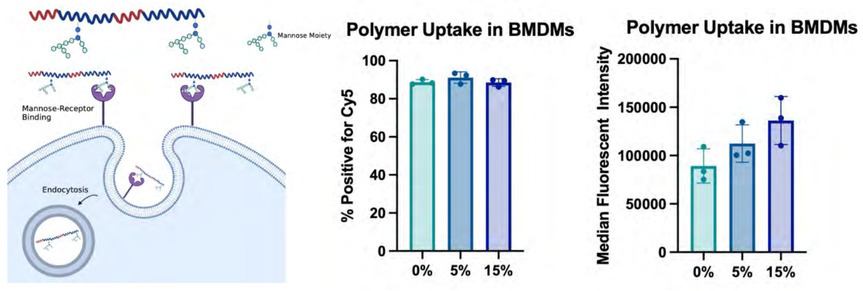


Figure 9: *In vitro* understanding of preferential uptake by M2 BMDMs with Cy5-labeled, mannose-functionalized platforms and non-functionalized platform. Read out: Flow cytometry looking at % Cy5-positive cells.

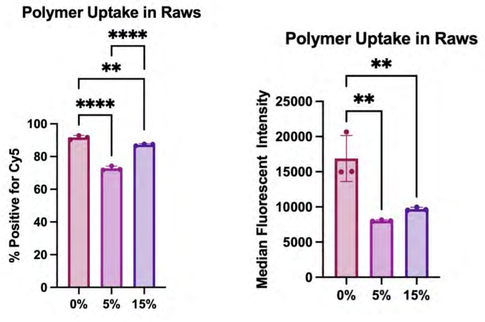


Figure 10: *In vitro* understanding of preferential uptake by M2 RAWs with Cy5-labeled, mannose-functionalized platforms and non-functionalized platform. Read out: Flow cytometry looking at % Cy5-positive cells.

Polymer Biodistribution

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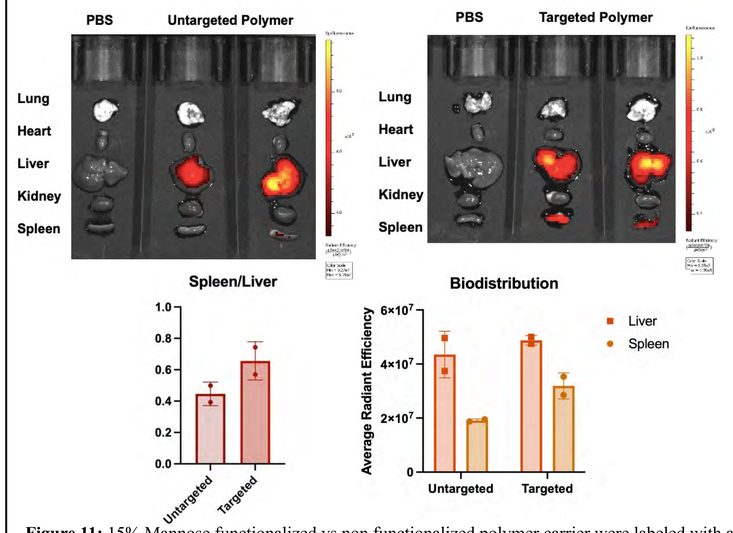

Figure 11: 15% Mannose functionalized vs non functionalized polymer carrier were labeled with a Cy5 dye and systemically injected into mice. At 24 hours, organs were harvested, and organ fluorescence was measured via IVIS. Increased uptake in the spleen can be visualized with the mannose-functionalized carrier.

Conclusions and Future Directions

Conclusions

- We successfully synthesized poly(N,N-dimethylacrylamide-co-Azide ethylmethacrylate-co-Mannose) terpolymers via RAFT Polymerization
- We polarized both BMDMs and RAWS to M1 and M2 phenotypes
- Macrophage uptake may increase with increasing mannose density
- Targeted polymer accumulates in macrophage-rich organs

Future Directions

- Conjugation of STING prodrug to our lead polymer carrier via copper-free click chemistry and characterize via UV-Vis
- Measure STING activation in M2 macrophages via IFN ELISA comparing mannose-functionalized vs non-functionalized platform
- Measure the ability of the mannose platform to repolarize M2 macrophages towards an M1 phenotype
- Biodistribution and therapeutic efficacy of targeted vs non targeted platforms using E0771 murine tumor model

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