Nanoparticle delivery of STING immunotherapy enhances anti-tumour immunity in metastatic Osteosarcoma

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Introduction

Osteosarcoma remains a therapeutic challenge due to an aggressive malignant phenotype and poor response to conventional therapies. Recent advances in cancer immunotherapy have highlighted the cGAS-STING pathway as a promising target to reprogram the tumour microenvironment (TME). However, systemic delivery of STING agonists have been limited by tumour specificity. Our research aims to develop a novel nanoparticle delivery method of a STING agonist to selectively target and activate the STING pathway in osteosarcoma to reprogram anti-tumour immunity.

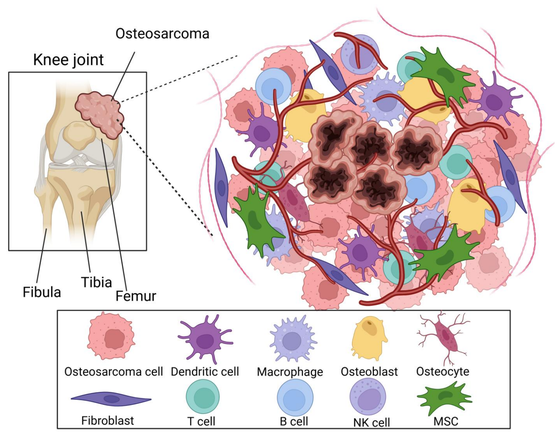


Figure 1: Osteosarcoma is cancer of the bone that most commonly forms on long bones near joints and is most common in children and young adults.

Materials and methods

Macrophages were isolated from BALB/c mouse femurs and characterized by flow cytometry. STING nanoparticles were formulated and characterized by dynamic light scattering (DLS).

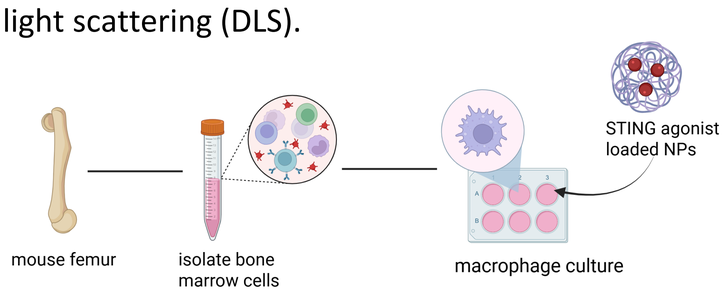


Figure 2: Bone marrow derived macrophages (BMDMs) were isolated from BALB/c mouse femurs, cultured and treated with STING agonist and NPs in 2D.

We generated and characterized a 3D metastatic Osteosarcoma spheroid model to mimic the Osteosarcoma TME.

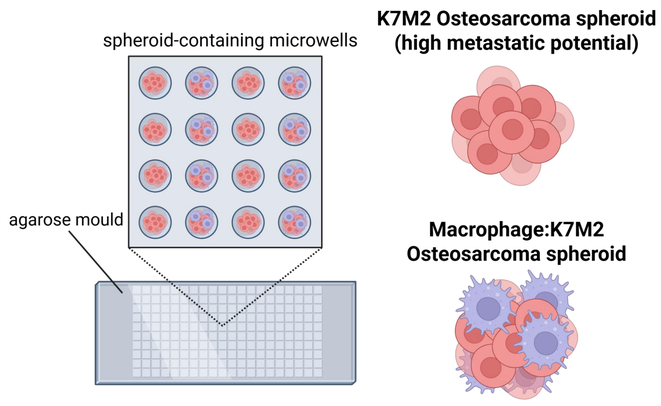


Figure 3: K7M2 murine Osteosarcoma spheroids with and without bone marrow derived macrophages (BMDMs) were generated via centrifugation in agarose microwells in 24 well cell culture plates and incubated for 96 hours for spheroid formation.

The effects of the addition of a STING targeting immunotherapy on immune cell STING activation and cancer cell cytotoxicity in 3D Osteosarcoma spheroids was assessed by flow cytometry, WST-1 cell proliferation, mouse IFN-beta and IL-6 ELISA and STING mRNA expression via PCR.

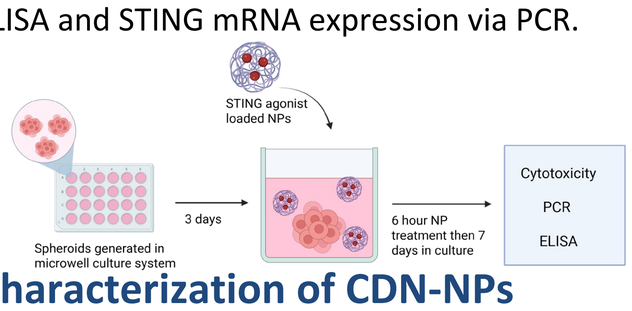


Figure 4: Spheroids were treated with STING agonist loaded pBAE nanoparticles for 6 hours and analysed for STING activation via IFN-Beta production, immune cell activation by flow cytometry and spheroid diameters were measured.

Characterization of CDN-NPs

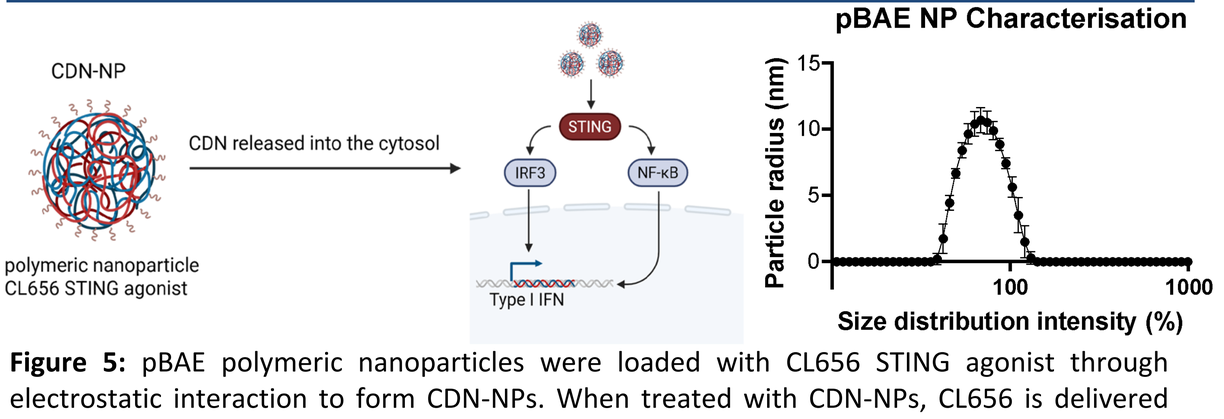


Figure 5: pBAE polymeric nanoparticles were loaded with CL656 STING agonist through electrostatic interaction to form CDN-NPs. When treated with CDN-NPs, CL656 is delivered which stimulates the cGAS-STING pathway causing a type I interferon response. CDN-NPs were characterised by DLS for their size and zeta-potential and were shown to have ~70nm particle size.

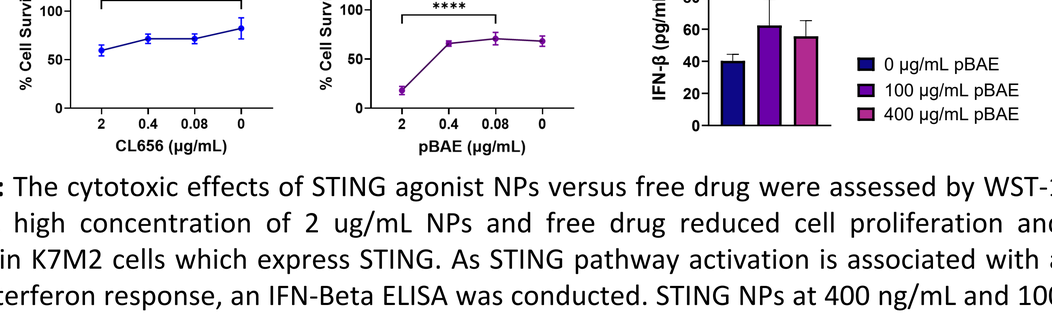


Figure 6: The cytotoxic effects of STING agonist NPs versus free drug were assessed by WST-1 assay. A high concentration of 2 ug/mL NPs and free drug reduced cell proliferation and viability in K7M2 cells which express STING. As STING pathway activation is associated with a type I interferon response, an IFN-Beta ELISA was conducted. STING NPs at 400 ng/mL and 100 ng/mL increase IFN-Beta production in K7M2 cells.

STING NPs increase macrophage activation

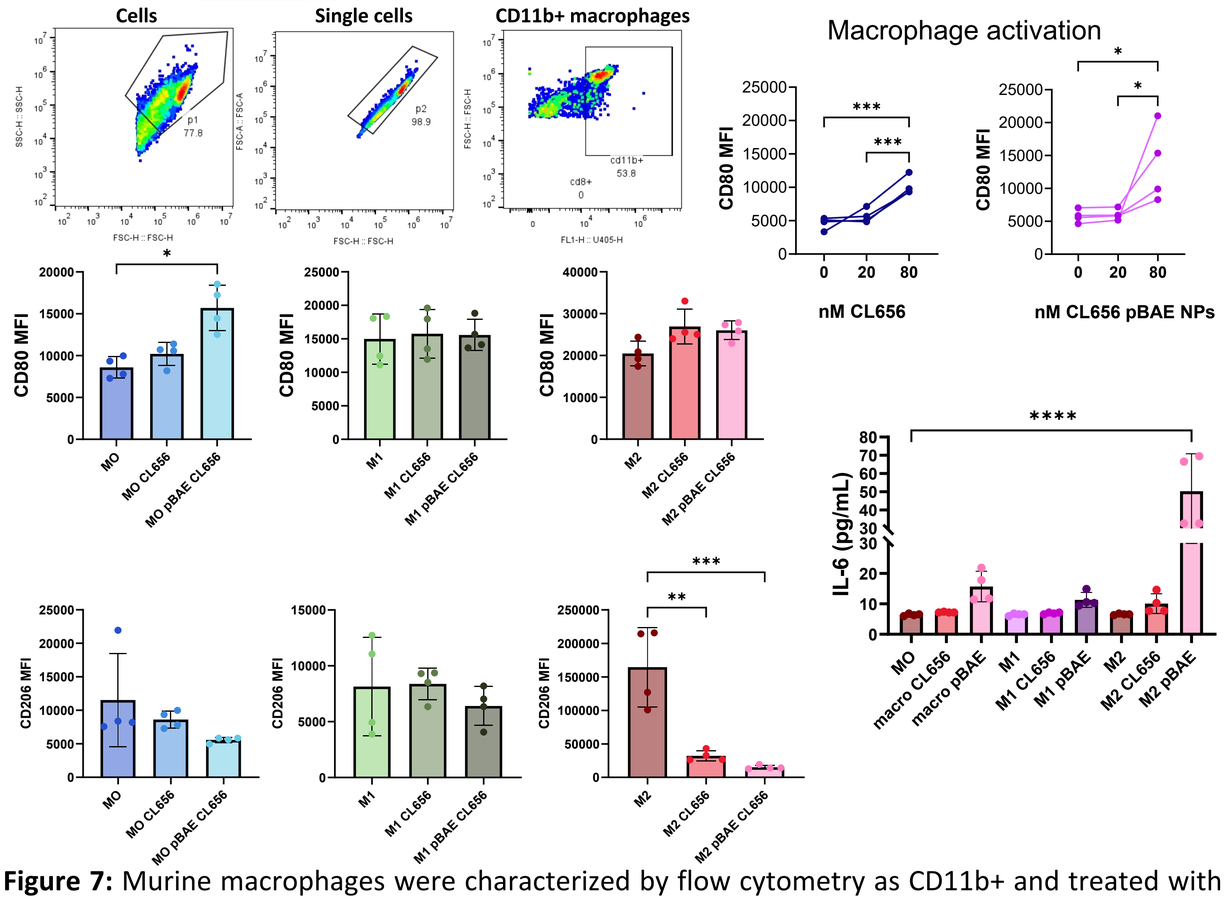


Figure 7: Murine macrophages were characterized by flow cytometry as CD11b+ and treated with free STING agonist and STING loaded NPs. Macrophage activation and inhibition was assessed by CD80 and CD206 expression. Macrophage inflammatory cytokine secretion was also assessed by ELISA for IL-6 secretion. One-way ANOVA analysis, n=4, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Generation of murine Osteosarcoma spheroids

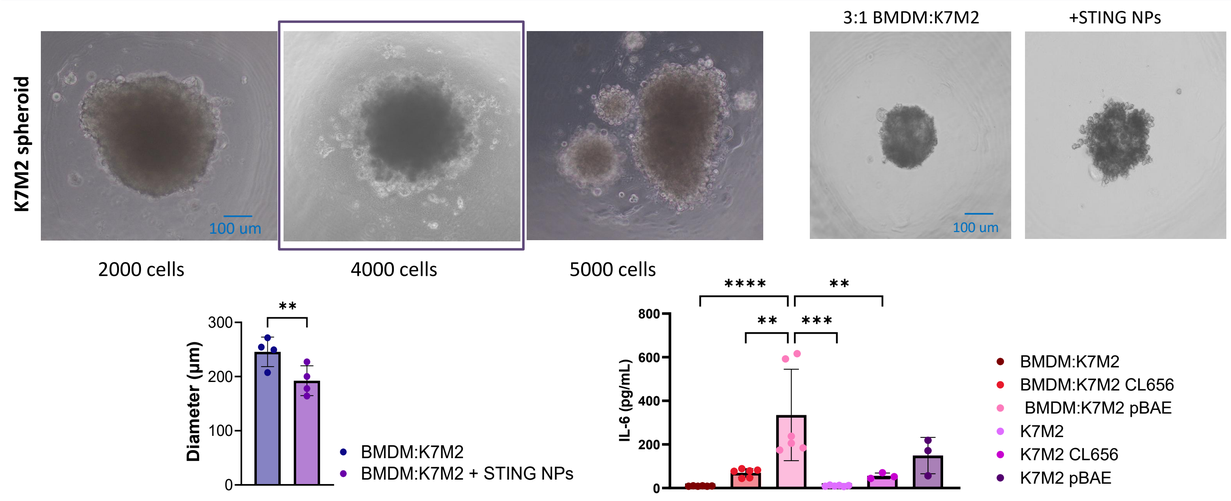


Figure 8: K7M2 metastatic murine osteosarcoma spheroids were formed at three different cell densities and analysed via microscopy for the optimal cell density for spheroid formation. 4000 cells per spheroid was taken forward for the formation of macrophage cancer cell spheroids (BMDM:K7M2) at a 3:1 ratio. Treatment with STING NPs reduced tumour spheroid size and increased inflammatory IL-6 secretion in 3D osteosarcoma spheroids. One-way ANOVA analysis, n=4, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

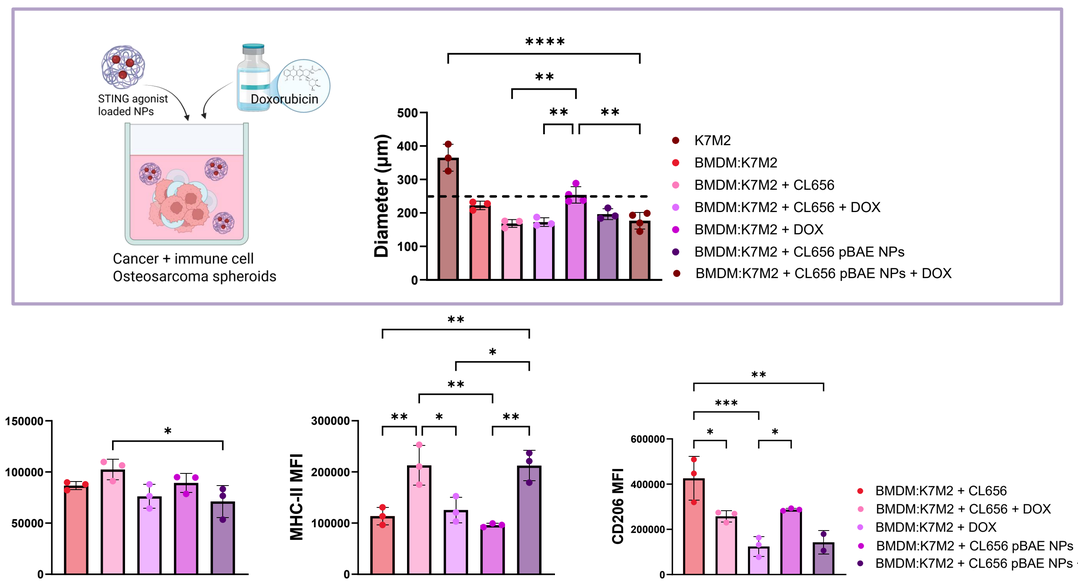


Figure 9: Delivery of a STING agonist immunotherapy in combination with doxorubicin chemotherapy (DOX) reduces osteosarcoma spheroid size. STING in combination with doxorubicin also increases macrophage CD80 activation, MHC-II pro-inflammatory marker expression and significantly reduces CD206 pro-tumorigenic marker expression. One-way ANOVA analysis, n=4, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Discussion

CL656 STING agonist nanoparticles CDN-NPs were generated and characterized to be ~70 nm. Comparing CDN-NPs to free drug we observed increased cytotoxicity of cancer cells with STING agonist delivery in NPs versus free drug, and the stimulation of IFN-Beta production in cancer cells. STING NPs also reduced tumour spheroid size and increased the effectiveness of doxorubicin chemotherapy in a 3D model of metastatic murine osteosarcoma. Similarly, STING NPs increased M0 macrophage CD80 activation and repolarized M2 macrophages back to an M1 phenotype with a reduction in M2 CD206 expression and an increase in IL-6 inflammatory cytokine secretion.

Acknowledgements

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