Enhancing the Effectiveness of Chemotherapy in Osteosarcoma by Targeting Tumour-Associated Macrophages (TAMs) Through STING Activation

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Introduction

Osteosarcoma is an aggressive bone cancer, primarily affecting children. Despite advances in chemotherapy, the 5-year survival rate for resistant metastatic forms remains below 20%. Tumour-associated macrophages (TAMs) can constitute up to 50% of the osteosarcoma tumour microenvironment, and therefore they are an enticing target for immunotherapies. Recently, cGAS/STING signalling has emerged as a valuable immunotherapeutic target due to its role in immunomodulation and driving anti-tumour. Herein, bioinformatic analysis revealed a loss of STING in osteosarcoma patient samples and an enrichment of M2-like TAMs. Using an indirect co-culture model, we found the STING agonist CL656 to induce immune of remodelling TAMs towards a pro-inflammatory M1-like phenotype. We found that CL656 stimulation of M1-like TAMs synergised with doxorubicin to enhance osteosarcoma cell elimination. Lastly, utilising poly(ß-amino ester) (pBAE) nanoparticles, we achieved significant cellular responses with a 40-fold reduction in STING agonist concentration versus free drug.

Materials and methods

We utilised an indirect co-culture system, wherein the conditioned media of SaOS-2 osteosarcoma cells (± doxorubicin IC_{50} dose **(1.7µM)** for 24 hours) was applied to THP-1 monocytes subpolarised to either an M0-like **[+PMA]**, M1-like **[+LPS and IFNγ]** , or M2-like **[+IL-4 and IL-13]** phenotype.

Macrophages were treated with **10µM** of the STING agonist CL656 for 6 hours prior to the administration of osteosarcoma cell conditioned media. Lastly, to assess the impact of nanoparticle delivery on STING stimulation, we synthesised both electrostatic encapsulated **(pBAE/CL656)** and covalently conjugated **(pBAE/STG-968)** nanoparticle delivery platforms using two different STING agonists.

Outcomes were assessed via ELISAs (IL-6, IL-10), Flow cytometry (CD86), Western blot analysis (*STING, IRF3*, *pIRF3*), Immunofluorescent imaging (*STING*), RT-qPCR analysis (*cGAS, STING, IRF3*) and WST-1 assay (cell survival).

Results

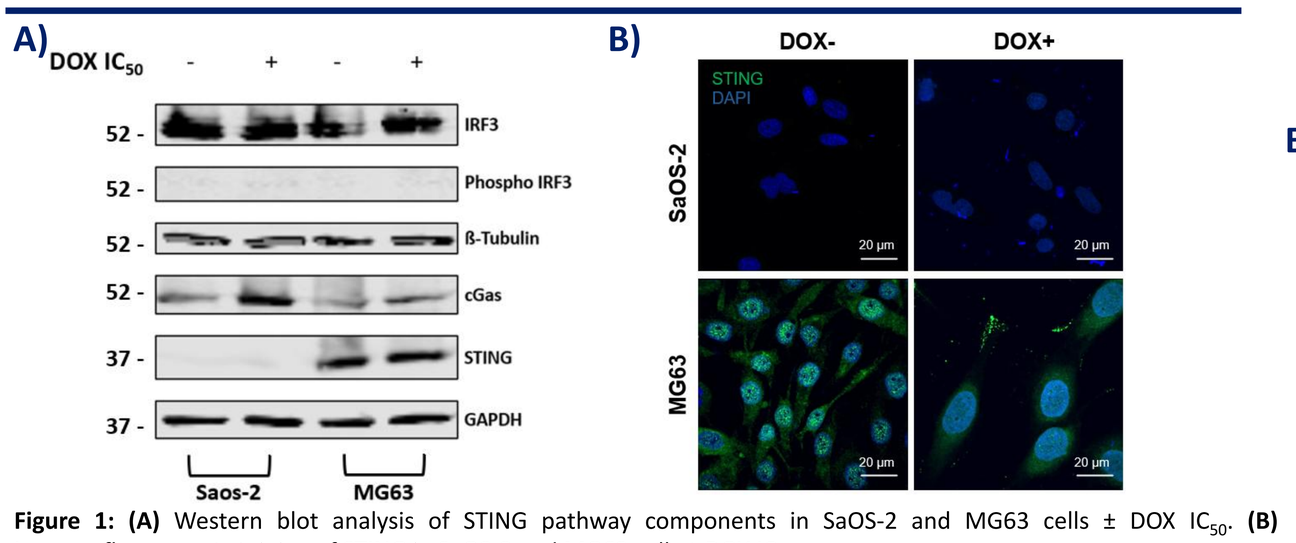


Figure 1: (A) Western blot analysis of STING pathway components in SaOS-2 and MG63 cells ± DOX IC_{50}. **(B)** Immunofluorescent staining of STING in SaOS-2 and MG63 cells ± DOX IC_{50}.

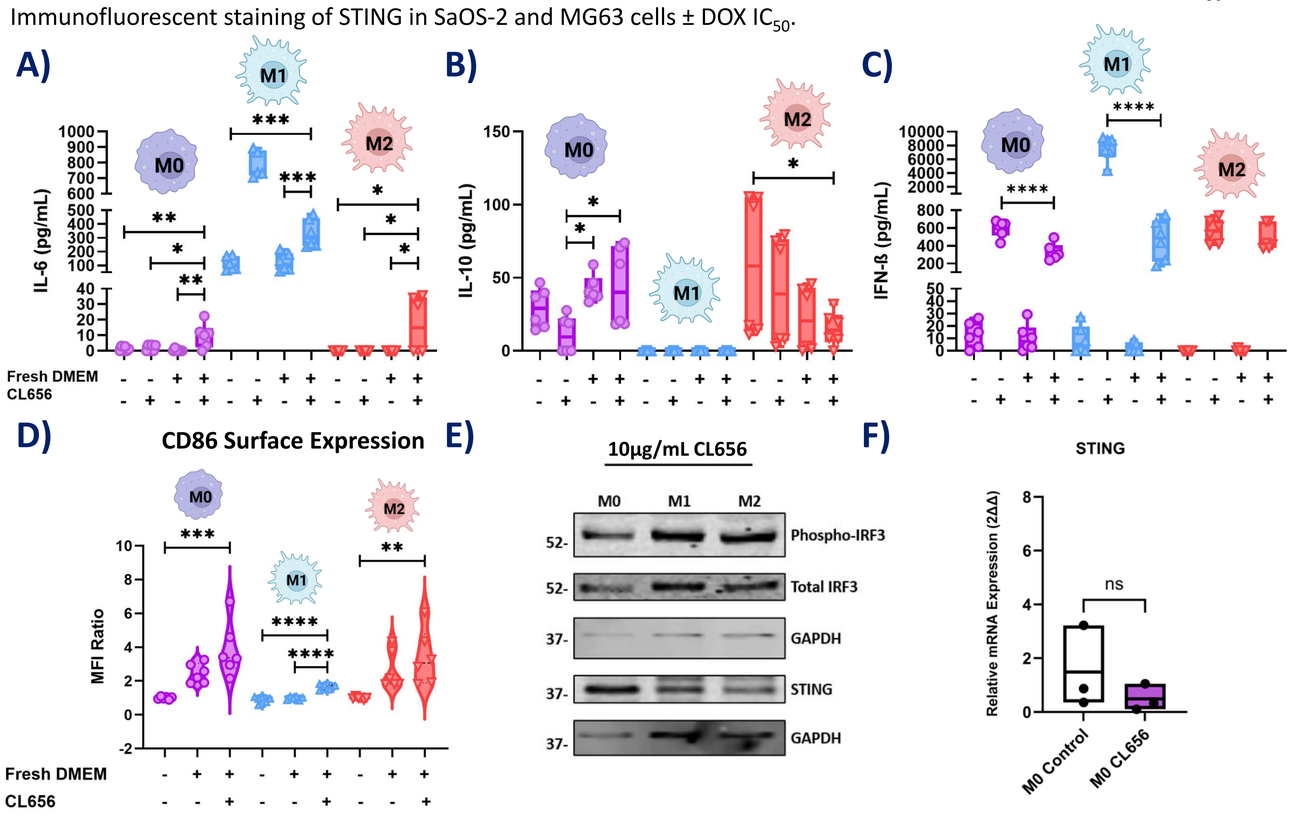


Figure 2: (A-C) ELISA analysis of A) IL-6, B) IL-10, and C) IFN-ß expression by M0-, M1-, and M2-like macrophages ± CL656 stimulation for 6h prior to the administration of SaOS-2 CM. **(D)** Flow cytometry analysis of CD86 expression on M0-, M1-, and M2-like macrophages ± CL656 6h prior to administration of SaOS-2 CM. **(E)** Western blot analysis of STING, IRF3, and phospho-IRF3 protein levels in M0-, M1-, and M2-like following CL6566h. **(F)** RT-qPCR analysis of STING mRNA expression in M0-like macrophages following CL656 6h. All data is represented as mean ± SD, n = 6. Statistical differences were conducted on each macrophage phenotype and assessed using a one-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

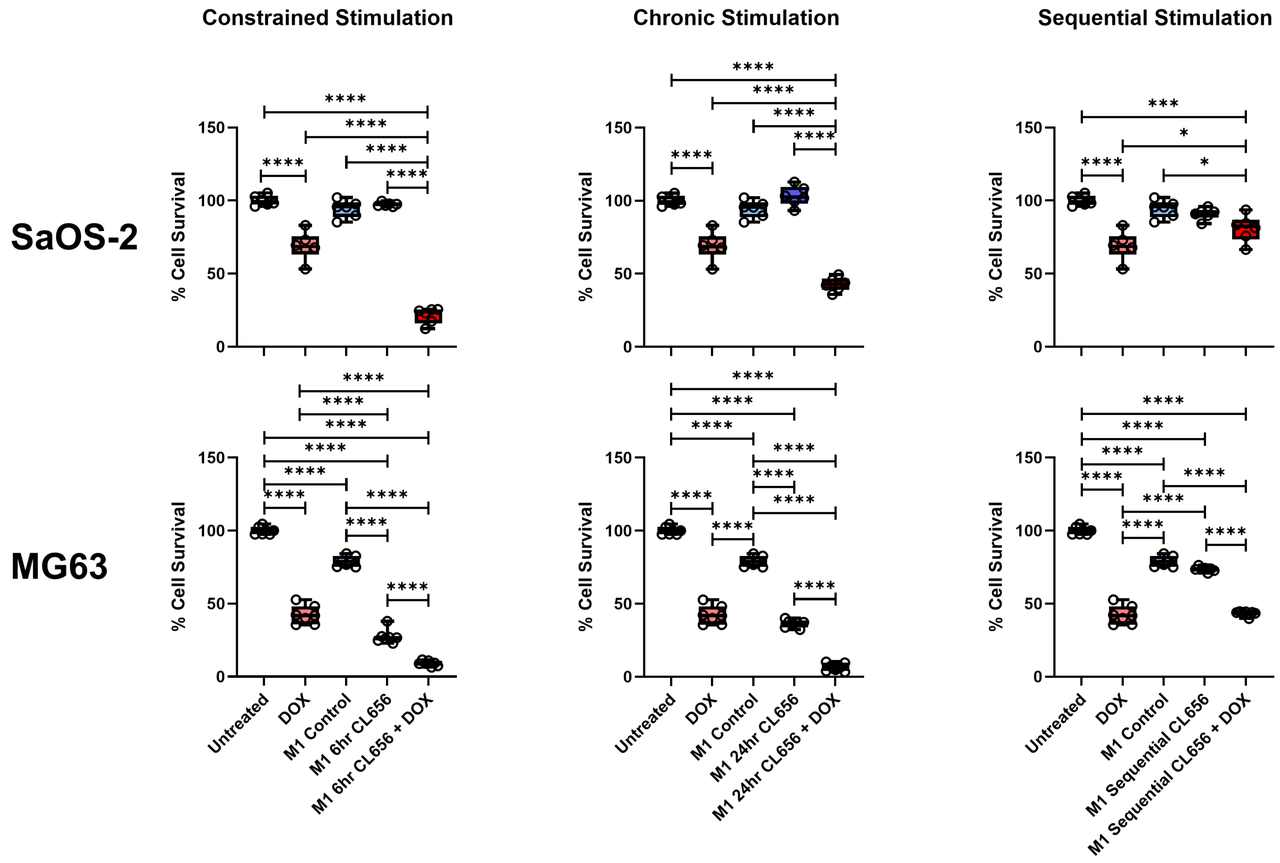


Figure 3: Percentage cell survival SaOS-2 and MG63 cells after 24-h treatment with DOX, M1-like macrophage CM, or M1-like macrophage CM combined with STING stimulation (*chronic, constrained*, or *sequential*), or a combination of both (chemo-immunotherapy). Data are presented as mean ± SD (n = 6). Statistical analysis was performed for each macrophage phenotype using one-way ANOVA (*$p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$).

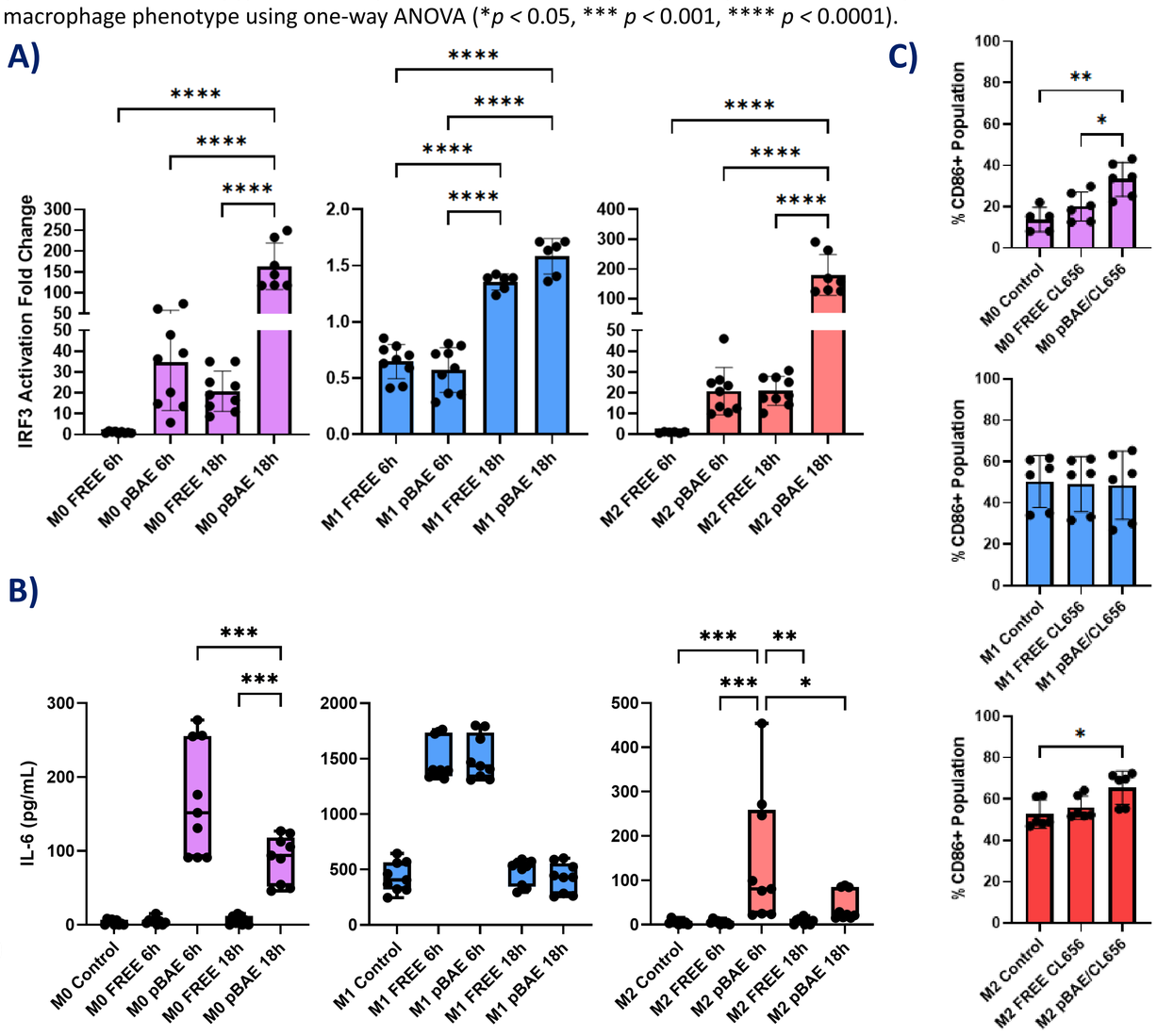


Figure 4: (A) IRF3 activity fold change in M0-, M1-, and M2-like macrophages treated with 250nM of free CL656 or pBAE/CL656. **(B)** ELISA analysis of IL-6 secretion by M0-, M1-, and M2-like macrophages treated with 250nM of free CL656 or pBAE/CL656. **(C)** Flow cytometry percentage of CD86+ M0-, M1-, and M2-like macrophages treated with 250nM of free CL656 or pBAE/CL656 per the overall cellular population. All data re presented as mean ± SD (n = 9). Statistical analysis was performed using a one-way ANOVA (*$p < 0.05$, **$p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Discussion

Our initial analysis of STING pathway component expression in two commonly utilised osteosarcoma cell lines (SaOS-2 and MG63 cells) revealed divergent expression of STING, consistent with previous reports in the literature. Following dose response and time response analysis, we found 6hr STING agonist stimulation to produce the maximal pro-inflammatory response, coinciding with M1-like TAM reprogramming. Critically, this 6hr stimulation did not induce loss of STING expression at the protein or gene level, as is observed with longer chronic STING agonist administration. We found that STING stimulation was able to synergise with the chemotherapeutic drug doxorubicin to drive enhanced osteosarcoma cell elimination.

Lastly, preliminary data indicates that pBAE nanoparticle delivery of STING agonists results in greatly enhanced cellular responses at vastly lower drug concentrations. Together this data provides a basis for the use of STING agonists in combination with classical chemotherapeutic agents as a novel chemoimmunotherapeutic approach for the treatment of osteosarcoma

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