

# Chitosan/ $\gamma$ -PGA nanoparticles-based immunotherapy as adjuvant to radiotherapy in breast cancer

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Radiotherapy (RT) is an essential treatment modality for several types of cancer. Despite its therapeutic potential, RT is frequently insufficient to overcome the immunosuppressive nature of the tumor microenvironment, failing to control tumor metastases. Innovative immunomodulatory strategies, like immunostimulatory biomaterials could be used to boost the immunogenic effects of RT. We have reported that chitosan (Ch)/poly( $\gamma$ -glutamic acid) ( $\gamma$ -PGA) nanoparticles (NPs) modulate immature/immunosuppressive APCs, namely macrophages and dendritic cells, towards an immunostimulatory profile, impairing their ability to induce cancer cell invasion [1]. Furthermore, Ch/ $\gamma$ -PGA NPs have also been shown to be potential carriers for immunomodulatory drugs, such as interferon (IFN)- $\gamma$  [2].

Herein, we addressed the synergistic potential of immunostimulatory chitosan/poly( $\gamma$ -glutamic acid) nanoparticles (Ch/ $\gamma$ -PGA NPs) [1] combined with RT to induce antitumor immunity in the 4T1 breast tumor mouse model. Therefore, animals were divided in 4 groups: non-treated, treated with NPs, with RT or with NPs+RT. Briefly, 4T1 cells were injected orthotopically in BALB/cByJ. After 7 days, animals from RT and RT+NPs groups were locally irradiated with hypofractionated 10Gy. Then, animals from NPs and RT+NPs groups were subcutaneously injected with NPs. Tumor burden, lung metastasis and immune cell profile were explored.

Non-treated animals had progressive primary tumor growth and developed splenomegaly and lung metastases. While RT decreased primary tumor burden, Ch/ $\gamma$ -PGA NPs-treatment decreased systemic immunosuppression and lung metastases. The combination therapy (RT+NPs) synergistically impaired 4T1 tumor progression, which was associated with a significant primary tumor growth and splenomegaly reduction, a decrease in the percentage of splenic immunosuppressive myeloid cells and an increase in antitumoral CD4<sup>+</sup>IFN- $\gamma$ <sup>+</sup> population. Notably, animals from the combination therapy presented less and smaller lung metastatic foci and lower levels of the systemic pro-tumoral cytokines, and of the CCL4 chemokine, in comparison to non-treated animals. Overall, these results evidenced that Ch/ $\gamma$ -PGA NPs potentiate and synergize with RT, headlining their promising role as adjuvant anticancer strategies [3].

## References:

[1] F. Castro, M. L. Pinto, A. M. Silva, C. L. Pereira, G. Q. Teixeira, M. Gomez-Lazaro, S. G. Santos, M. A. Barbosa, R. M. Gonçalves, M. J. Oliveira. *Acta Biomaterialia*, 63 (2017) 96-109

[2] F. Castro, M. L. Pinto, R. Almeida, F. Pereira, A. M. Silva, C. L. Pereira, G. Q. Teixeira, S. G. Santos, M. A. Barbosa, R. M. Gonçalves, M. J. Oliveira. *Biomaterials Science*, 7(8) (2019) 3386-3403

[3] F. Castro, M. L. Pinto, C. L. Pereira, K. Serre, M. A. Barbosa, K. Vermaelen, F. Gartner, R. M. Gonçalves, O de Wever, M. J. Oliveira. *Biomaterials* (2020).