Preclinical anti-tumor immune-mediated synergy of selenium nanoparticles and a nanovaccine against luminal B breast cancer

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Introduction: Selenium (Se) is an element crucial for human health, with anticancer properties. Although selenium nanoparticles (SeNPs) have shown lower toxicity and higher biocompatibility than the organic or inorganic Se compounds, bare SeNPs are unstable in aqueous solutions and prone to aggregation. [1] Furthermore, SeNPs have been demonstrated to induce apoptosis in cancer cells as well as to stimulate the immune system to target and destroy cancer cells. [2] Therefore, the aim of this work was to access the main immune cell populations responsible for SeNPs immunotherapy against luminal B breast cancer.

Methods:

SeNPs production and stability: SeNPs were produced using sodium selenite, ascorbic acid as reducing agent and bovine serum albumin (BSA) as stabilizing agent, adjusting the pH reaction environment to 1. The stability of BSA-SeNPs was performed in human plasma, cell medium, pH 7.4 and 5.5.

Therapeutic Intervention Study Design of the Combination Treatment of BSA1-SeNPs and the $KRAS_{wt}$ Nanovaccine: A breast cancer orthotopic model was established by inoculating 1×10^6 E0771 cells in the fourth inguinal mammary fat pad of C57BL/6J mice. For intervention therapeutic study evaluating the antitumor efficacy of the combinational treatments of the $KRAS_{wt}$ nanovaccine and the BSA1-SeNPs, once the average volume of tumors reached ≈50–100 mm^3, mice were randomly divided into a control group and 4 treatment groups (n = 6 animals per group), as reported in **Figure 1**. $KRAS_{wt}$ nanovaccine were subcutaneously (s.c.) administered to mice via injection proximal to both left and right sides inguinal lymph nodes (50 µL per side containing 40 µg of MHC II-restricted $KRAS_{wt}$ peptide antigen, 20 µg of CpG-ODN and 40 µg of Poly(I:C)), on days 7 and 14 following tumor inoculation. BSA1-SeNPswere both intratumorally (i.t.) or i.v. administered at 1.25 mg kg^{-1} every 2 days. Tumors and spleens were collected from mice (n = 6 animals per group) after euthanasia and homogenized in a single-cell suspension in cold sterile PBS. Tumor single-cell suspensions were obtained by mechanical disruption and enzymatic digestion. pleens were also mechanically disrupted, and single-cell suspensions were depleted of erythrocytes using ACK lysing buffer for 5 minutes at 37 °C, being further filtered. Cells were seeded in 96-well plates, washed with PBS, and incubated with Ghost Dye Red 780. Afterwards, cells were stained with extracellular and intracellular fluorochrome-labeled anti-mouse antibodies, according to the manufacturer's instructions.

Results: SeNP were spherical, smaller than 50 nm, and with a narrow size distribution and stable in medium, plasma, and at physiologic pH, maintaining their size around 50-60 nm, for a prolonged period. Moreover, the combination of BSA1-SeNPs with a KRAS-loaded PLGA-mannose nanovaccine resulted in a strong reduction of tumor growth in an EO771-breast cancer mouse model. Indeed, the synergistic effect of $KRAS_{wt}$ nanovaccine combined with SeNPs was confirmed by the tumor growth inhibition of 62.2%, compared to 34% and 16.6% for SeNPs (i.t.) and $KRAS_{wt}$ nanovaccine, respectively (**Figure 2**). This synergistic anticancer effect of the combined treatment significantly increased the tumor infiltration of both B, NK, and CD8+ T effector cells. Furthermore, the tumor infiltration of Tregs and PD1-expressing T cells were decreased for the combined treatment with the SeNPs and the nanovaccine. (**Figure 3**).

Conclusion: Stable SeNPs at physiologic pH and plasma were produced. Also, SeNPs presented anticancer properties in EO771-bearing mice, presenting synergy with a $KRAS_{wt}$ nanovaccine. Therefore, this study offers valuable insights for the development of innovative combinatorial approaches using SeNPs to improve the outcomes of cancer immunotherapy.

References

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[2] G. Chen, F. Yang, S. Fan, H. Jin, K. Liao, X. Li, G. Liu, J. Liang, J. Zhang, J. Xu, J. Pi. Front Immunol. 2022; 13: 956181. DOI: 10.3389/fimmu.2022.956181

Figures

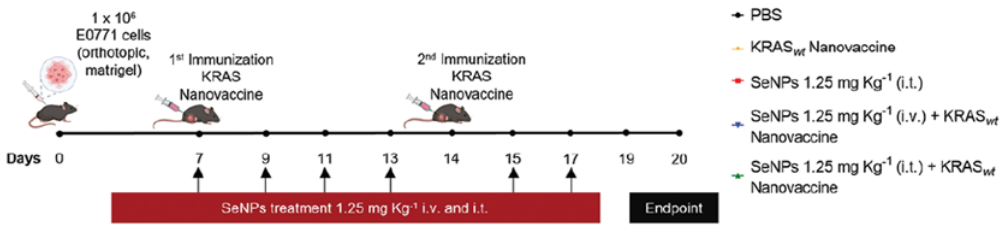

Figure 1. Experimental design of the *in vivo* study. C57BL/6J mice were orthotopically inoculated with 1 × 10^6 EO771 tumor cells and treated with BSA1-SeNPs every other day and $KRAS_{wt}$ nanovaccine on days 7 and 14

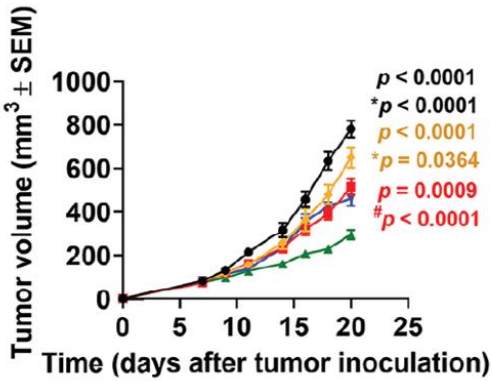

Figure 2. Effect of the individual and combined treatments of SeNPs and $KRAS_{wt}$ on EO771 tumour growth Data are presented as mean ± s.e.m of EO771-bearing mice (N = 2 *in vivo* assays, n = 6 animals per assay for all treatment groups except for SeNPs 1.25 mg Kg^{-1} (i.v.) + $KRAS_{wt}$ Nanovaccine (n = 6)). Statistical significance was analyzed by one-way ANOVA followed by Tukey multiple comparisons post-hoc test and p, p*, and p # values correspond to tumor volume at day 20 after tumor

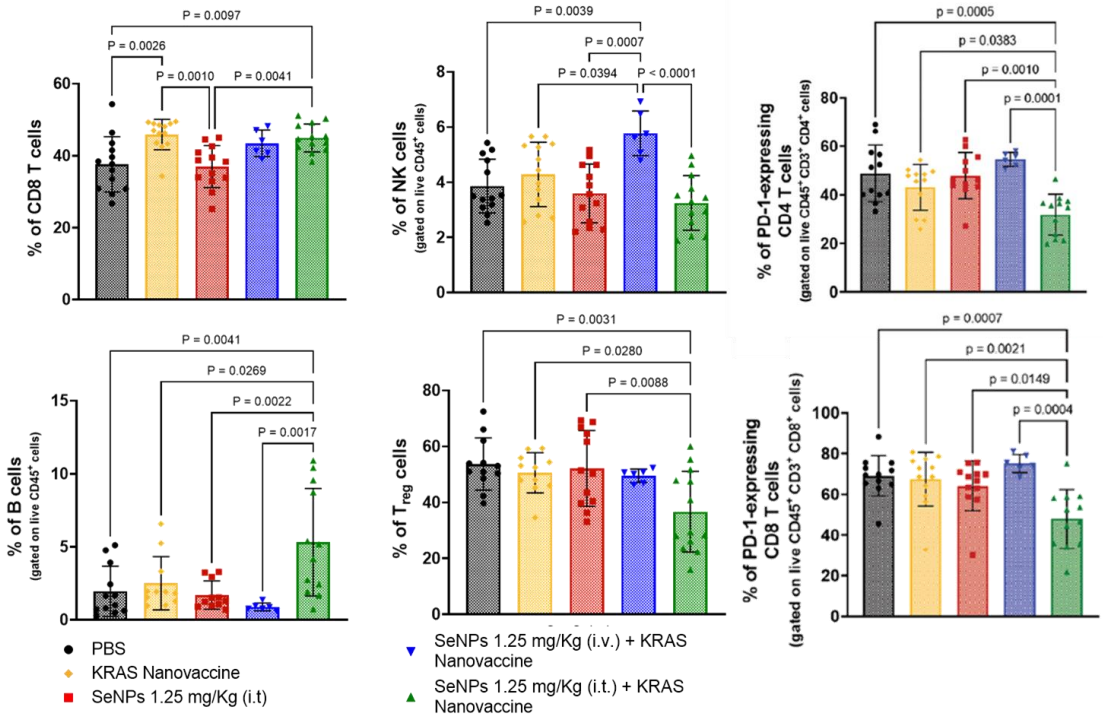

Figure 3. Effect of the individual and combined treatments of SeNPs and KRASwt on the immune cells' population in the tumour microenvironment. Highest infiltration of B and CD8+ T cells, and decreased Treg and PD1-expressing T cell levels for divalent combination of SeNPs intratumorally administered with $KRAS_{wt}$ nanovaccine was observed