INB03: a new immune checkpoint inhibitor that reprograms macrophage polarization, boosts ADCP and reverts T-cell exhaustion markers

Sofia Bruni[1], Maria Florencia Mercogliano[1], Roxana Schillaci[1]

[1]Instituto de Biología y Medicina Experimental. Fundación IBYME. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

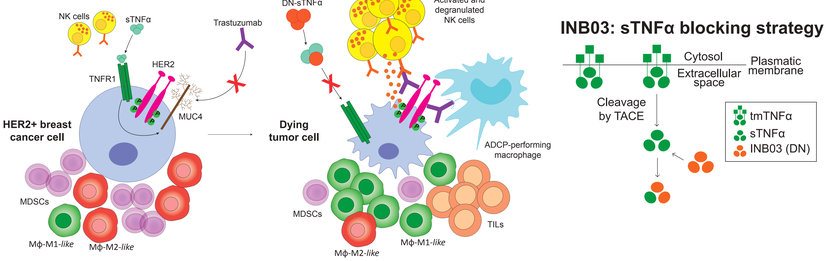
sofibruni@hotmail.com

Poster ID #1365

INTRODUCTION & BACKGROUND

HER2+ breast cancer (BC) affects 10-15% of BC patients. They receive trastuzumab (Tz) as a first line treatment. However, Tz resistance is an important clinical issue and few actionable targets are available.

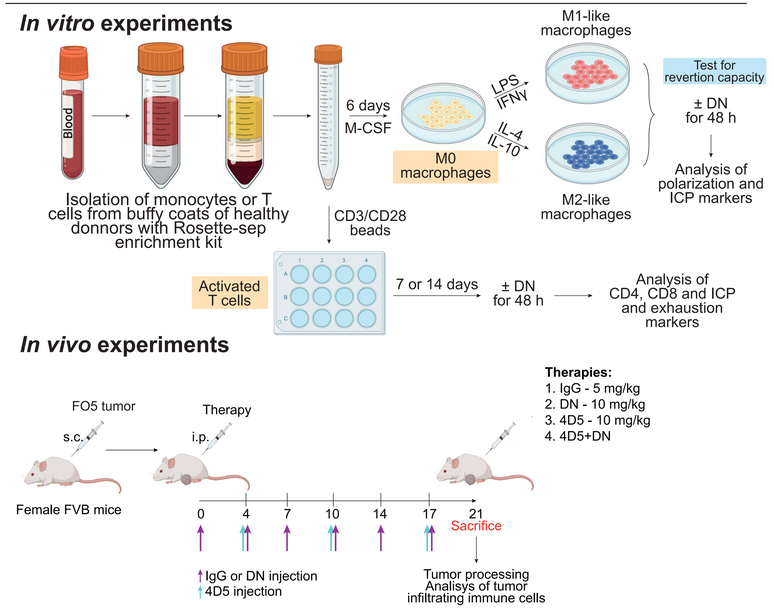
Previously we have demonstrated that soluble TNFα (sTNFα) blockade with INB03 (DN), sensitizes trastuzumab-resistant HER2+ breast cancer, triggering an effective antitumor immune response that relies on the collaboration between M1-*like* macrophages and NK cells [1].



OBJECTIVE

Because immune checkpoint molecules (ICP) and exhaustion of T cells foster tumor immune escape, ***we addressed whether DN could modulates macrophage polarization, T cell exhaustion and ICP expression in both populations to boost an antitumor immune response***

METHODS



RESULTS

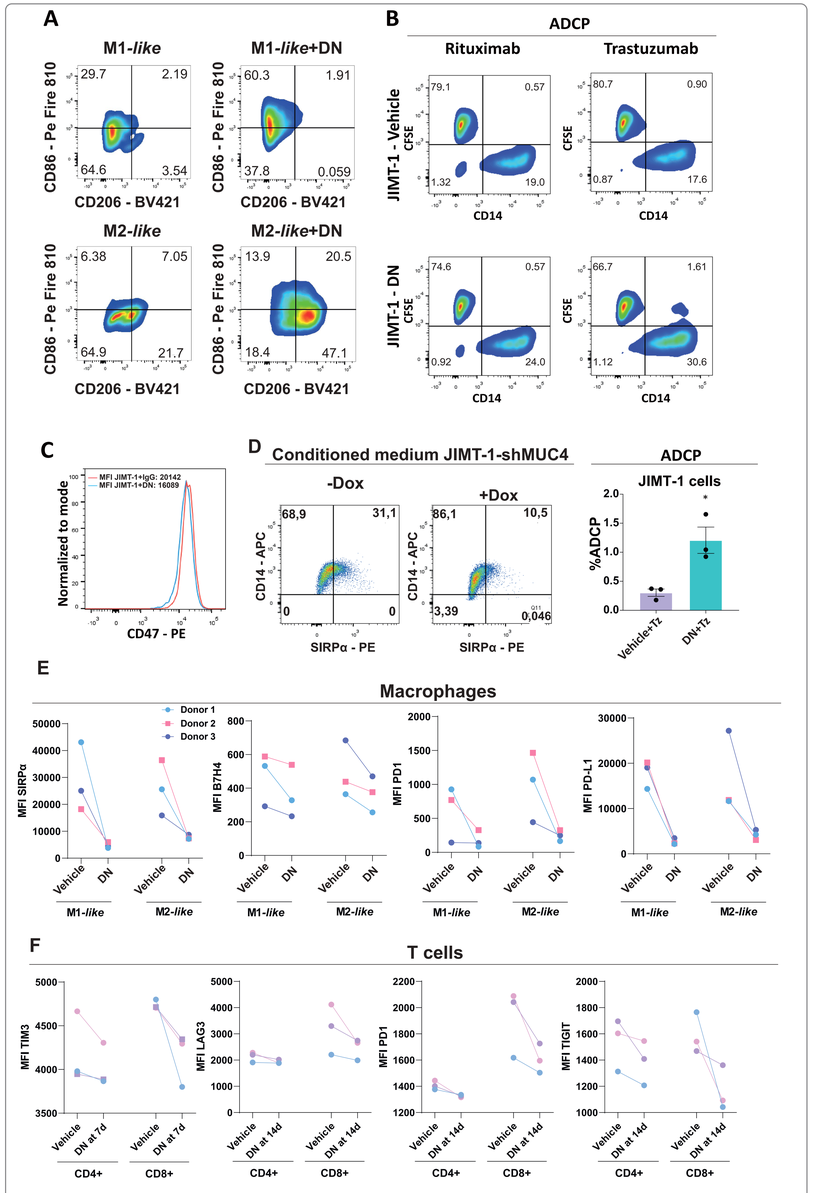


Figure 1. sTNFα blockade modulates human macrophage polarization and enhances ADCP and reduces human T cell ICP and exhaustion markers. **A)** sTNF blockade with INB03 (DN) enhances M1-*like* phenotype in already-polarized M1-*like* macrophages and reverts M2-*like* polarization into M1-*like*. **B)** Treatment of human HER2+ breast cancer cell line (JIMT-1) with DN for 48h enhances trastuzumab-mediated ADCP and **C)** decreases CD47 expression. **D)** Co-culture of conditioned media from JIMT-1 cells with silenced MUC4 expression (JIMT-1-shMUC4 cells) decreased the expression of SIRPα on human macrophages. Addition of DN to human M1-*like* and M2-*like* macrophages **(E)** and activated T cells **(F)** decreases the expression of ICP molecules and exhaustion markers. *p<0.05.

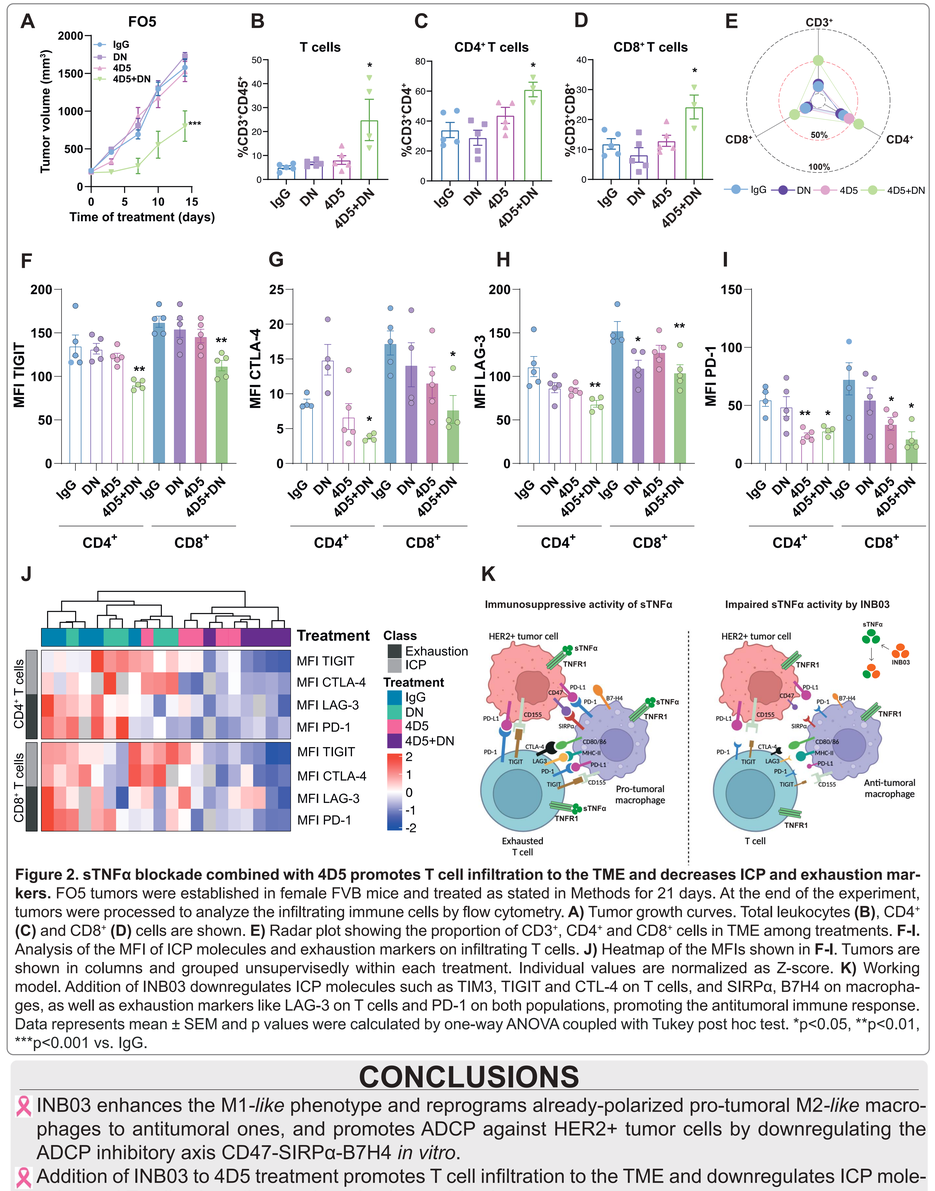


Figure 2. sTNFα blockade combined with 4D5 promotes T cell infiltration to the TME and decreases ICP and exhaustion markers. FO5 tumors were established in female FVB mice and treated as stated in Methods for 21 days. At the end of the experiment, tumors were processed to analyze the infiltrating immune cells by flow cytometry. **A)** Tumor growth curves. Total leukocytes **(B)**, CD4⁺ **(C)** and CD8⁺ **(D)** cells are shown. **E)** Radar plot showing the proportion of CD3⁺, CD4⁺ and CD8⁺ cells in TME among treatments. **F-I.** Analysis of the MFI of ICP molecules and exhaustion markers on infiltrating T cells. **J)** Heatmap of the MFIs shown in **F-I**. Tumors are shown in columns and grouped unsupervisedly within each treatment. Individual values are normalized as Z-score. **K)** Working model. Addition of INB03 downregulates ICP molecules such as TIM3, TIGIT and CTL-4 on T cells, and SIRPα, B7H4 on macrophages, as well as exhaustion markers like LAG-3 on T cells and PD-1 on both populations, promoting the antitumoral immune response. Data represents mean ± SEM and p values were calculated by one-way ANOVA coupled with Tukey post hoc test. *p<0.05, **p<0.01, ***p<0.001 vs. IgG.

CONCLUSIONS

- INB03 enhances the M1-*like* phenotype and reprograms already-polarized pro-tumoral M2-*like* macrophages to antitumoral ones, and promotes ADCP against HER2+ tumor cells by downregulating the ADCP inhibitory axis CD47-SIRPα-B7H4 *in vitro*.
- Addition of INB03 to 4D5 treatment promotes T cell infiltration to the TME and downregulates ICP molecules and T cell exhaustion markers *in vitro* and *in vivo*.
- We speculate that these effects could avoid tumor immune evasion to anti-HER2 targeted therapies by reinvigorating the immune infiltrate.

FINANCIAL SUPPORT AND REFERENCES

References:

1. Bruni et al. J Immunother Cancer 2023