INB03: A NEW IMMUNE CHECKPOINT INHIBITOR THAT REPROGRAMS POLARIZATION AND PROMOTES ADCP IN HUMAN MACROPHAGES

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Background Trastuzumab resistance is an important clinical issue and few actionable targets are available. We have demonstrated that soluble TNF (sTNF) upregulates mucin 4 (MUC4) expression, which shields trastuzumab epitope on HER2 hindering its therapeutic effect, 1 2 and that sTNF blockade with INB03 decreases MUC4 expression which, together with trastuzumab, triggers an effective antitumor immune response that relies on M1-macrophage-NK cell collaboration. Because macrophages have a plastic phenotype and express immune checkpoint molecules (ICP) to foster immune escape, we addressed whether INB03 could modulate macrophage polarization and ICP expression to boost the antitumor immune response.

Methods Human monocytes were isolated from buffy coats and enriched with RosetteSep enrichment cocktail. Macrophages (Mφ) were differentiated for 7 days with M-CSF (10 ng/ml) to M0 (day 0) and then for 72h to the M1 subtype with LPS (50 ng/ml)+IFNg (20 ng/ml), or to the M2 subtype with IL-4 (20 ng/ml)+IL-10 (20 ng/ml) (day 3). INB03 was added (10 μg/ml) for 72h with corresponding cytokines to M0 (day 0) or to already-polarized M1 or M2 (day 3) to test its ability to inhibit or revert polarization, respectively. M1, M2 and ICP markers were analyzed by flow cytometry. M1 were considered CD14+CD86+CD206- and M2 CD14+CD86-CD206+. For functional assays, Mφ antibody-dependent cellular phagocytosis (ADCP) against the human HER2+ breast cancer cells JIMT-1 was performed using IncuCyte live-cell imaging.

Results At day 0, addition of INB03 showed no effect on Mφ polarization fate. However, adding it to already-polarized M1 enhanced polarization towards M1 (CD86+CD206-). M2 treated with INB03 lost CD206 and gained CD86, reverting their polarization commitment towards M1. Mφ exposed to INB03 showed a decreased in B7H4 and PD1 expression, in both unpolarized M0 and M2. This effect was stronger when INB03 was added 72h after polarization to M2 was induced. The expression of PD-L1 and of SIRPα, which inhibits ADCP, diminished when INB03 was added to M1. M2 exhibited a more powerful downregulation of these inhibitory molecules. Finally, Mφ exhibited an increased ADCP against JIMT-1 cells treated with INB03, which showed downregulation of the inhibitory signal CD47.

Conclusions In all, sTNF blockade commits M0 Mφ to the M1 phenotype and reprograms already-polarized pro-tumoral M2 Mφ to antitumoral ones. INB03 can tailor the tumor microenvironment by acting as an ICP inhibitor and by promoting ADCP against tumor cells. HER2+MUC4+ BC patients could benefit from the administration of INB03 to boost targeted-therapy efficacy and overcome macrophage immune escape.

REFERENCES

Ethics Approval Healthy donor’s blood was collected with the donor’s informed consent from Fundación Hematologica Sarmento under IRB approval from Instituto de Biología y Medicina Experimental (IBYME-CONICET) in Buenos Aires, Argentina (CEI # 6217).

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