877 BLOCKING SOLUBLE TNFα SENSITIZES HER2-POSITIVE BREAST CANCER TO TRASTUZUMAB THROUGH MUC4 DOWNREGULATION AND SUBVERTS IMMUNOSUPPRESSION

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Background Trastuzumab resistance is an important clinical issue. Although a plethora of resistance mechanisms have been characterized, few have been shown to be actionable. We have demonstrated that soluble TNFα isoform (sTNFα) upregulates mucin 4 (MUC4) expression, which shields the trastuzumab epitope on HER2, hindering its therapeutic effect *in vitro* and *in vivo*.[1,2] Since the success of trastuzumab treatment relies on immune response, we addressed the role of MUC4 on modulating the tumor immune infiltrate to foster immune evasion in sTNFα-induced trastuzumab-resistant HER2-positive (HER2+) breast cancer.

Methods *De novo* trastuzumab-resistant JIMT-1 and KPL-4 cell lines were engineered to express a doxycycline-inducible MUC4 shRNA (JIMT-1-shMUC4 and KPL-4-shMUC4, respectively). Female nude mice bearing these s.c. tumors (~100 mm^3), were treated i.p with IgG or trastuzumab (5mg/kg), a dominant negative (DN) sTNFα inhibitor (10 mg/kg) or trastuzumab+DN (n=4-6 per group). After 3 weeks of treatment, tumor-infiltrating immune cells were studied by immunofluorescence and flow cytometry. For macrophage and NK cell depletion, clodronate or anti-asialo GM1 was used, respectively. ADCP was studied using parental JIMT-1 cells pre-cultured for 48h with DN (10 μg/ml) or vehicle and then co-cultured with human macrophages for 1.5 h. A cohort of 91 HER2+ breast cancer patients treated with trastuzumab was used to correlate tumor MUC4 expression with tumor-infiltrating lymphocytes (TILs).

Results Upon MUC4 silencing through doxycycline induction, trastuzumab antitumor effect was reinstated (80% or 85% tumor growth inhibition, JIMT-1-shMUC4 or KPL-4-shMUC4, respectively; $p<0.0001$). The addition of DN did not further decrease tumor burden. In the absence of doxycycline, trastuzumab+DN inhibited tumor growth and modified the immunosuppressive tumor milieu, increasing M1-like macrophage polarization ($p<0.01$) and NK cell degranulation ($p<0.01$). In MUC4-silenced tumors, trastuzumab treatment alone mimics this tumor infiltrate. Depletion experiments revealed a cross-talk between macrophages and NK cells necessary for trastuzumab+DN antitumor effect. When MUC4 was silenced, trastuzumab antitumor effect was lost upon macrophage depletion, but it was preserved when NK cells were absent. Furthermore, JIMT-1 cells pre-treated with DN were more susceptible to trastuzumab-dependent cellular phagocytosis ($p<0.05$). Finally, MUC4 expression in HER2+ breast cancer negatively correlated with TILs ($p=0.004$), reflecting "immune desert" tumors.

Conclusions In all, we conclude that sTNFα isoform blockade is able to tackle MUC4 expression and, together with trastuzumab, triggers an effective antitumor immune response that relies on M1-macrophage-NK cell collaboration. These findings provide rationale to pursue sTNFα blockade combined with trastuzumab or trastuzumab drug-conjugates for MUC4+ and HER2+ breast cancer patients to overcome trastuzumab resistance.

REFERENCES

1. Mercogliano MF, De Martino M, Venturutti L, *et al*. TNFalpha-Induced Mucin 4 Expression Elicits Trastuzumab Resistance in HER2-Positive Breast Cancer. *Clin Cancer Res* 2017 23:636–48.
2. Bruni S, De Martino M, Mauro FL, *et al*. Soluble TNFα induced mucin 4 is a mediator of trastuzumab resistance and of an immunosuppressive tumor microenvironment in HER2+ breast cancer. *J. Immunotherapy Cancer 7*, 2019;**283:**O39. doi:10.1186/s40425-019-0764-0

Ethics Approval Patient samples were collected with the patient's informed consent and with Helsinki approval from Hospital Fernández (CEI # 201629) and Henry Moore Institute of Oncology, (Buenos Aires, Argentina) and from Hospital Oncológico Provincial de Córdoba (Cordoba, Argentina). All animal studies were conducted in accordance with the highest standards of animal care as outlined by the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of IBYME.

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