

# Microenvironment Developed In Breast Cancer Spheroids Induced Resistance To Trastuzumab And Modulates Cancer Stem Cells And HER2 Expression.

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**INTRODUCTION.** HER2 is overexpressed in 20% invasive breast tumors and correlates with low free disease survival. Trastuzumab (Tz), monoclonal antibody anti HER2, is used to treat HER2+ tumors; however, more than half of them are resistant or acquire resistance during treatment. Spheroids represent a 3D cell growth model that mimics *in vivo* avascular tumors. We have previously demonstrated that cells cultured as spheroids are more resistant to Tz than monolayers. Cancer stem cells (CSC), associated with chemotherapy resistance, are targeted by Tz.

**OBJETIVE.** Analyze the resistance acquired in 3D and the impact of the CSC developed in spheroids.

**MATERIALS AND METHODS.** BT474 and MCF7 human mammary adenocarcinoma cell lines were cultured as spheroids using the hanging drop method. Flow cytometry and viability assays were performed.

**RESULTS.** We analyzed the presence of CD44 and CD24 molecular markers in BT474 (HER2+) spheroids treated with Tz, as CD44+CD24<sup>low</sup> phenotype is associated with breast cancer stems cells. 15 days of Tz treatment induced Tz resistance and increased CD44+CD24<sup>low</sup> subpopulation by 1.5 fold compared to controls (p<0.05). Moreover, we found 2 HER2 subpopulations, HER2<sup>high</sup> and HER2<sup>low</sup> (37% vs 63%, respectively p<0.05). Interestingly, after 15 days Tz treatment, HER2<sup>high</sup> subpopulation increased to 50%.

In MCF7 cells, without HER2 amplification and unresponsive to Tz, we observed HER2<sup>low</sup> expression in 82% of the cells that was reduced to 61% in Tz treated spheroids (p<0.05). Even though Tz did not changed spheroids size, we detected a 30% reduction in the CD44+CD24<sup>low</sup> CSC subpopulation after treatment.

**CONCLUSION.** The hostile microenvironment developed in 3D has a key role in the development of resistance to Tz. These conditions could favor an increase of CSCs, no longer responsive to Tz despite HER2 expression. Furthermore, MCF7 results suggest that HER2<sup>low</sup> expression increased in 3D developed CSCs could become a target for Tz treatment.

**KEYWORDS:** Trastuzumab, 3D cultures, cancer stem cells