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Stimulation of antibody dependent cellular cytotoxicity (ADCC) upon failure of cellular tumor suppression of breast cancer cells by Trastuzumab

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INTRODUCTION

Therapy of Her2-positive breast cancer patients using Trastuzumab can prolong the recurrence free and overall survival in both metastatic and the (neo-)adjuvant setting. However, about 40-60 % of all patients acquire cellular resistance during therapy. The therapeutic efficiency of Trastuzumab treatment is not only due to inhibition of tumor cell growth e. g., by inhibiting Her2 signaling but moreover can cause tumor cell eradication by triggering the immune system. Hence, aim of this study was to evaluate the interaction of NK- (effector) and tumor- (target) cells initiated by Trastuzumab and to evaluate strategies to enhance NK-cell mediated tumor cell defense in Trastuzumab sensitive and insensitive tumor cell lines.

METHODS

In order to generate cellular resistance the Her2-positive Trastuzumab-sensitive breast cancer cell line BT474 was continuously exposed to 10 µg/ml Trastuzumab for a period of four months. The proliferative capacity was repeatedly analyzed by flow cytometry. Simultaneously binding efficiency of Trastuzumab was evaluated. The immunologically mediated cytotoxicity was investigated in cocultures of NK- and Trastuzumab sensitive vs. resistant BT474 cells. Effector and target cells were analyzed by multiparametric phenoytping (NK cell activation e.g., Perforin-, IFNγ release) and Annexin-Dapi assays (tumor cell killing). In addition the kinetics of Trastuzumab treatment efficiency in terms of target cell number and viability was monitored by using the xCELLigence technique.

RESULTS

The originally highly sensitive BT474 cells acquired resistance to Trastuzumab after prolonged treatment (four months). The proliferative fraction was 25.3% ± 2.4% in sensitive wildtype (WT) cells vs. 27.7 ± 0.27% in resistant cells ***(Figure 1)***. Resistant BT474 cells maintained the original Her2 expression level and binding capacity of Trastuzumab was unaltered.

$$\frac{\text{Trastuzumab – binding}}{\text{Her2 – expression}}$$ WT: 0.496 ± 0.06 vs. Res: 0.54 ± 0.07

Despite of cellular resistance BT474 remained susceptible to immunological anti-tumor effects: NK-cell mediated antibody-dependent cellular cytotoxicity (ADCC) was 164% ± 3.0% in WT-BT474 and 176% ± 13,6% in resistant cells after 72h of coculture ***(Figure 2)***. xCelligence kinetics revealed a gradually increasing tumor cell killing in coculture over the course of 96 hrs ***(Figure 3)***. Tumor cell killing is due to an antibody mediated colocalisation of NK- and tumor cells that triggers Perforin release ***(Figure 4)***. ADCC in resistant BT474 cells can be moderately enhanced by IL-15 stimulation [10 ng/ml] of NK cells and to a greater extent by an increased effector target cell ratio ***(Figure 5)***.

CONCLUSION

Multiparametric phenotyping by flow cytometry enables the phenotypic and functional characterization of effector (NK) and target (tumor) cells derived from cocultures. In this experimental setup Trastuzumab triggers ADCC both in Trastuzumab sensitive and resistant cells. ADCC efficiency can be enhanced by IL-15 stimulation of NK cells, a promising approach that can potentially be extended to other antibody therapy regimens.

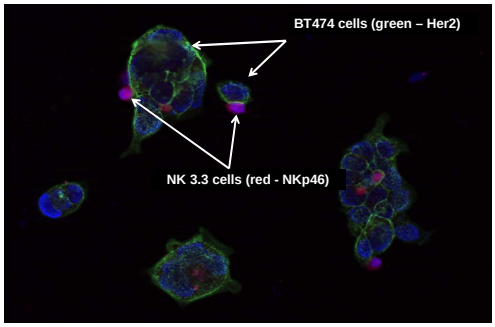

Figure 4

Antibody mediated colocalisation of NK- and tumor cells analyzed by immuno-fluorescence microscopy.

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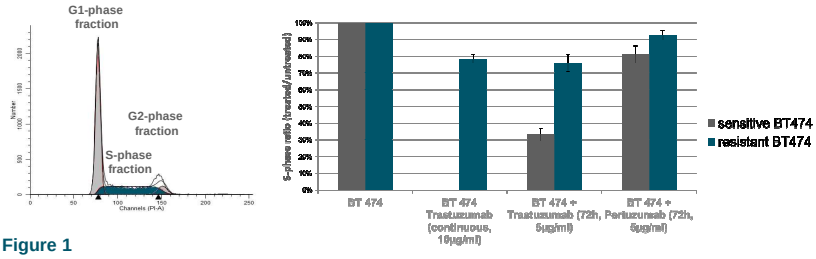

Figure 1

Generation of a cellular Trastuzumab resistant Her2-positive breast cancer cell line BT474. Flow cytometry analysis of the proliferative capacity.

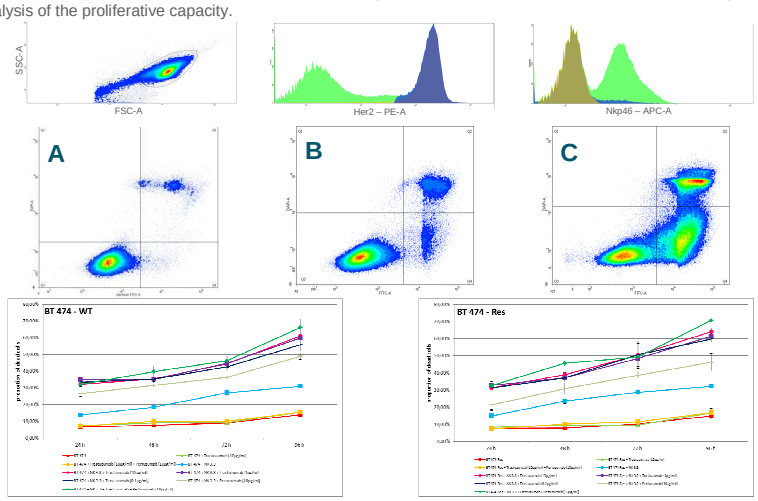

Figure 2

Cocultures of BT474 and NK- cells with/without Trastuzumab in a flow cytometry based assay to analyze immuno-logically mediated cytotoxicity.

Above: Gating strategy of the Annexin-Dapi assays to determine tumor cell killing.
3 Examples: A: BT474 + Trastuzumab / B: BT 474 + NK-cells / C: BT 474 + NK-cells + Trastuzumab

Below: Kinetic presentation of the tumor cell killing for BT474-WT und BT474-Res.

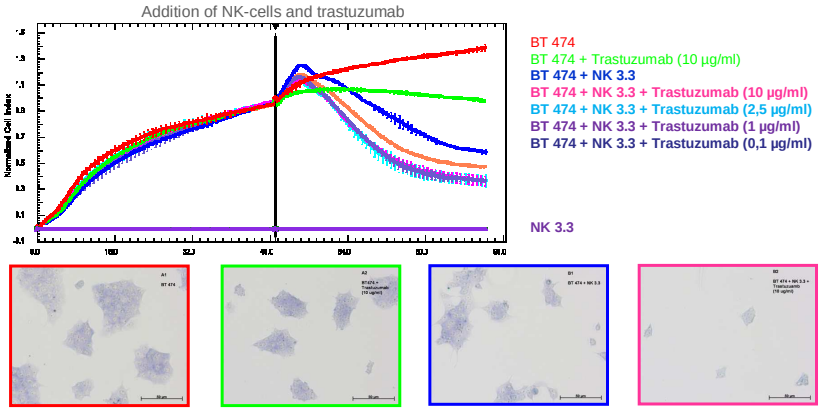

Figure 3

Above: Kinetic study of Trastuzumab treatment efficiency by using the xCELLigence technique.
Below: On the right side: correlating microscopic images of these cocultures.

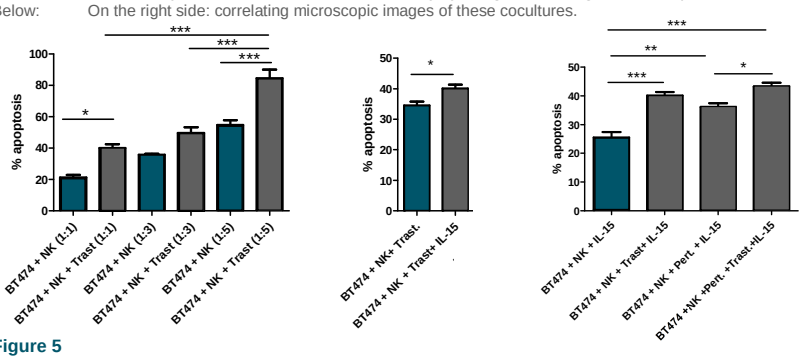

Figure 5

Possibilities to enhance the ADCC effect in a 48 h coculture of BT474 and NK cells.
A: significant ADCC enhancement by an increased effector target cell ratio (* p<0.05 / *** p<0.001)
B: moderate ADCC enhancement by IL-15 stimulation of NK cells (* p<0.05)
C: ADCC effect of IL-15 stimulated NK cells (* p<0.05 / ** p<0.01 / *** p<0.001)