Poster 433: Receptor-antibody clustering induced endocytosis of HER2 has therapeutic potential in breast cancer

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BACKGROUND: HER2 is an oncogenic receptor, promoting tumour development and disease progression in 15-25% of breast cancers. HER2 overexpressing tumours are more aggressive and associated with poorer prognosis, thus the receptor is a priority target for anticancer therapy. HER2 is notably 'endocytosis resistant", frustrating targeting approaches where efficacy is contingent on lysosomal delivery e.g. antibody-drug conjugates (ADC) such as trastuzumab-emtansine. We extend our recently published research showing that receptor-antibody clustering induced endocytosis (RACIE) can be strategically used to promote lysosomal delivery and degradation of HER2 in breast cancer cells (Moody et al. 2015; Ogris and Sami 2015).

MATERIALS AND METHODS: HER2-RACIE was examined in two breast cancer cell lines overexpressing this receptor: BT474 and SKBR3 cells. Clustering was achieved by sequential application of biotinylated, fluorescently-labelled trastuzumab and streptavidin. Trastuzumab was clinically sourced and labelled in-house. Following RACIE, downstream analyses were performed: Western blotting to study changes in HER2 levels, activation and downstream signalling and to measure the levels of other HER family members, EGFR and HER3. Live cell confocal microscopy was conducted to monitor endocytosis.

RESULTS AND DISCUSSION: Examination of HER2 levels at time points up to 48 hr demonstrated that after an initial reduction, HER2 levels then recover, potentially allowing for retargeting. Investigation of HER2 activation and downstream signalling revealed HER2-RACIE specific activation of the MEK/ERK pathway. The levels of HER2 dimerization partners were examined and while EGFR levels were unaffected, HER3 was concomitantly downregulated with HER2. Highly biotinylated trastuzumab (6.0 biotins per antibody) was compared with low biotin (1.7 per antibody) for RACIE. The data showed that HER2 and HER3 downregulation was only induced by the highly biotinylated trastuzumab while ERK activation was stimulated by both antibodies.

CONCLUSIONS: Our data suggest that RACIE, induced by antibodies of sufficient valency, represents an effective approach for enhanced delivery of ADCs in HER2+ breast cancer. This strategy may also have the potential extend to other receptor targets in a wide range of cancers.