ImmunoTools *multiplex* Award 2014

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The impact of chemotherapy on Trastuzumab-mediated ADCC in breast cancer

In 2013, breast cancer continued to be the most common type of cancer in women worldwide and second leading cause of cancer death. The overexpression of HER2, occurring in approximately a quarter of these patients, predicts for poorer outcome as its amplification is associated with an aggressive anti-apoptotic alteration promoting rapid proliferation. The monoclonal antibody trastuzumab targets the growth factor receptor Her2 and has profoundly improved the course and outcome of disease for women with Her2-overexpressing breast cancer. The clinical activity of Trastuzumab is largely credited to inhibition of intracellular signaling in tumor cells. However, many patients (~50%) whose tumors overexpress Her2 do either *ab-initio* not respond to Trastuzumab or become insensitive during antibody treatment. On the one hand these tumors acquire cellular resistance to the antibody based targeting. On the other hand a profound deficiency of immunological tumor defense (i.e. predominantly NK cell related antibody dependent cellular cytotoxicity = ADCC) has to be assumed.

Natural killer (NK) cells are considered the key players in ADCC. Via their Fcγ (IIIa) receptor (CD16), NK cells recognize the Fc portion of the mAb Trastuzumab bound to its target (Her2) on the tumor cell, resulting in target cell lysis and killing. Accumulating data indicate that ADCC is a major mechanism of trastuzumab's action. Thus, a functioning immune response seems essential for mAb-mediated ADCC.

Today's standard of care in the HER2^{+} status is a combined chemo-immunotherapy: Trastuzumab in combination with chemotherapy, particularly Paclitaxel. Chemotherapeutics, however, exert serious immunosuppressive side effects. Hence, it has been hypothesized that concomitant chemotherapy is likely to compromise NK cell function and may affect ADCC adversely. In contrast, emerging evidence suggests even immunogenic effects of some conventional chemotherapeutics on immune response. Consequently, the influence of combined chemotherapy on specific immune functions needs to be scrutinized.

Therefore, the aim of our study is to assess the impact of concomitant chemotherapy on ADCC mediated by the mAb Trastuzumab. We imitated an adjuvant breast cancer treatment, by combining Herceptin (Trastuzumab) with either Epirubicin or the taxane Paclitaxel. Cord blood-derived mononuclear cells (MNC) containing polyclonal peripheral blood NK cells and the IL-2 dependent NK cell line NK3.3 are co-cultured with BC cell lines differing in their intrinsic Herceptin sensitivity. NK cell activity is assessed by an extensive phenotyping of activation markers (e.g. CD16, CD56, NKp46, NKG2D, CD137) and the apoptosis induction of target cells (quantified by an Annexin-V/Dapi staining).

Conclusively, our project will elucidate the impact of concomitant chemotherapy on NK cell induced ADCC in order to help optimizing the treatment strategy of breast cancer.

These complex flow cytometric analyses require a multiplexed analytical, antibody based cell differentiation and characterization. Thus, the ImmunoTools multiplex array would be of great value to further analyze NK cell activity in this experimental setup.

ImmunoTools *multiplex* AWARD for **Lisa Turinsky**

includes free analysis of samples on several antibody arrays with large range of antibodies against human CDs, human cytokines, and others