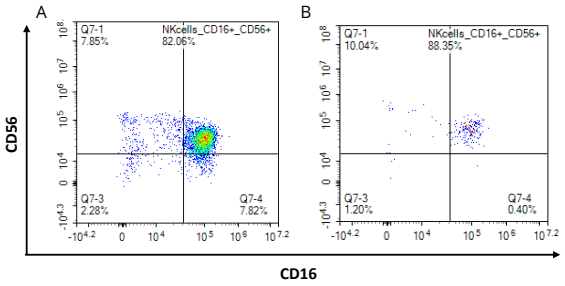
ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY ASSAY

Many cells of the innate immunity work in concert with the adaptive immune system to effectively afford protection. Furthermore, many of these cells, including Natural Killers (NK) and even granulocytes, express CD16 – a low affinity receptor for immunoglobulins, which, upon immunoglobulin binding, triggers immune cell response and destruction of the target – a mechanism referred to as antibody-dependent cell-mediated cytotoxicity (ADCC). This mechanism is the basis of several monoclonal antibody therapies which have been proven powerful and effective in cancer treatment.

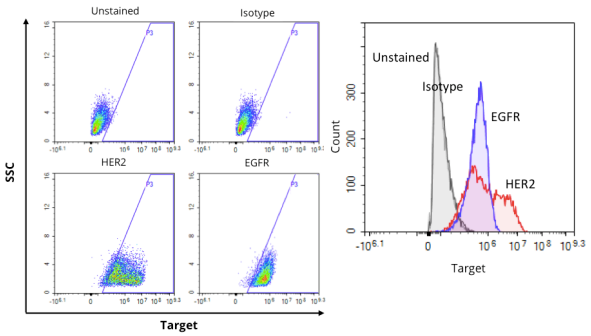
Herceptin (Trastuzumab) and Erbitux (Cetuximab) are therapeutic monoclonal antibodies that bind specifically to the human epidermal growth factor receptors HER2 and EGFR, respectively, overexpressed in many tumor types. In the case study presented here, the ADCC activity of Cetuximab and Trastuzumab was evaluated and highlighted through the promotion of the primary NK-mediated cytotoxicity towards SKOV3 ovarian tumor cells, which express high levels of human HER2 and EGFR, through monitoring of IFNγ release and tumor apoptosis.

While NK cell addition induces a cytotoxic activity as pointed by the increase in the IFNγ levels and apoptosis, tumor cell pre-exposure to anti-HER2 or anti-EGFR antibodies further enhances this NK cell-mediated cytotoxicity and thus underlines the antibody-dependent activity of those antibodies in directing the NK-induced killing of tumor cells.

Primary NK effector cells and tumor target cells



Evaluation of the expression of CD16 and CD56 in primary NK cells by flow cytometry, after NK isolation from PBMCs (A) and following 24h activation (B).

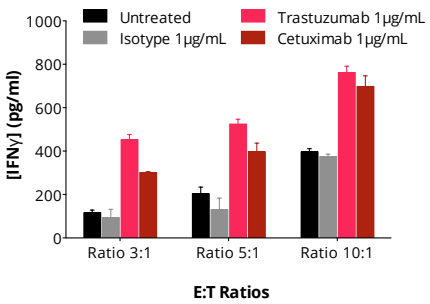


Expression of HER2 and EGFR antigens was assessed in SKOV3 tumor cells by flow cytometry.

Combination of NK cells with anti-HER2 or anti-EGFR antibodies enhances NK-mediated cytotoxicity in SKOV3 ovarian tumor cells, in a NK ratio-dependent manner

SKOV3 tumor cells were cultured under control, Cetuximab-, or Trastuzumab-treated conditions and IL2- activated primary NK cells were added at different E:T ratios. 24h later, supernatants were collected and analyzed for IFN***γ*** release by HTRF as a surrogate of NK-induced cell toxicity.

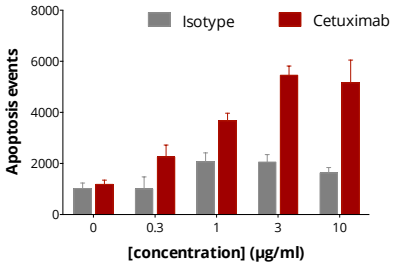
While IFN***γ*** levels are increased in a NK-ratio dependent manner, Cetuximab and Trastuzumab treatments of tumor cells further promote this increase whatever the E:T ratio, thereby showing an ADCC activity.

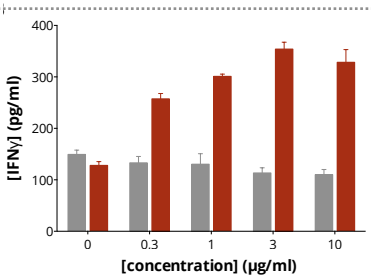


Dose-dependent anti-EGFR antibody (Cetuximab)-dependent cell cytotoxicity in SKOV3 ovarian tumor cells

SKOV3 tumor cells were cultured under control or increasing doses Cetuximab-treated conditions and IL2- activated primary NK cells were added at an appropriate E:T ratio (3:1).

Integration of live cell imaging data at 15h after NK cell addition as AUC of tumor cell apoptosis as a surrogate measure of NK-mediated tumor cytotoxicity.





24h following NK cell addition, supernatants were collected and analyzed for IFN***γ*** release by HTRF.

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