

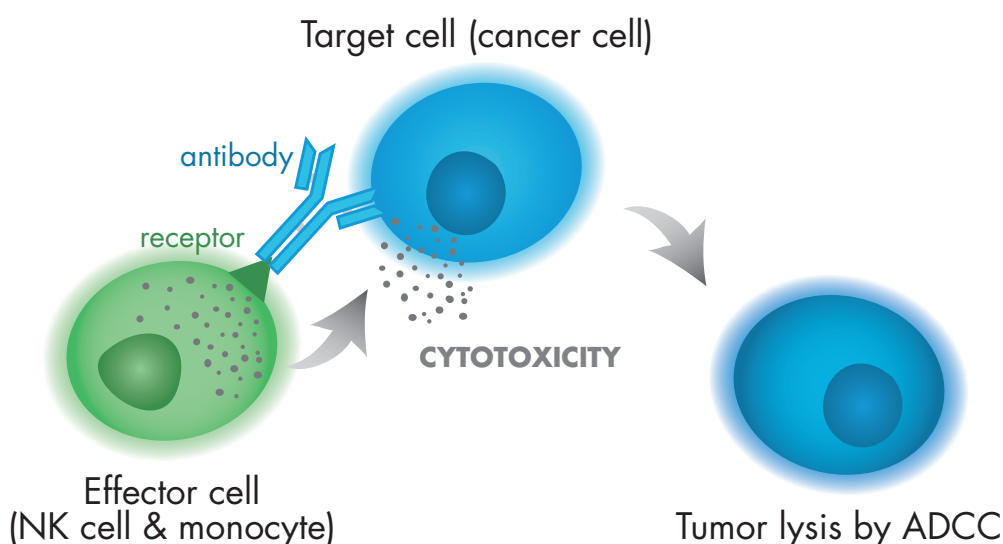
Antibody-Dependent Cell-mediated Cytotoxicity

Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) is a major mechanism by which therapeutic antibodies directed against cell surface targets on cancer cells exert their clinical effect.

ADCC is a cell-mediated innate immunity mechanism whereby an effector cell of the immune system (natural killer (NK), monocyte, macrophage, eosinophil) actively lyses a target cell (cancer cell) that has been recognized by specific antibodies (opsonization).

At first an antibody, such as an IgG, binds to a cancer cell. The Fc portion of this antibody is recognized by the Fc receptor of an NK (or PBMC) cell. Then, the NK cell releases cytokines such as interferon (IFN), and cytotoxic granules containing perforin and granzymes. These cytotoxic granules enter the target cell and trigger apoptosis leading to cell death.

In cancer therapy, antibodies can be raised against specific antigens present on the surface of tumors. As an example, approximately 30% of breast cancer cells display abnormally high levels of the constitutively active receptor, HER2, on their surface. Such breast cancer is known as a HER2 positive breast cancer and HER2 can be used as a target antigen in this case.



Experimental approach

The assay has been conducted with the following parameters:

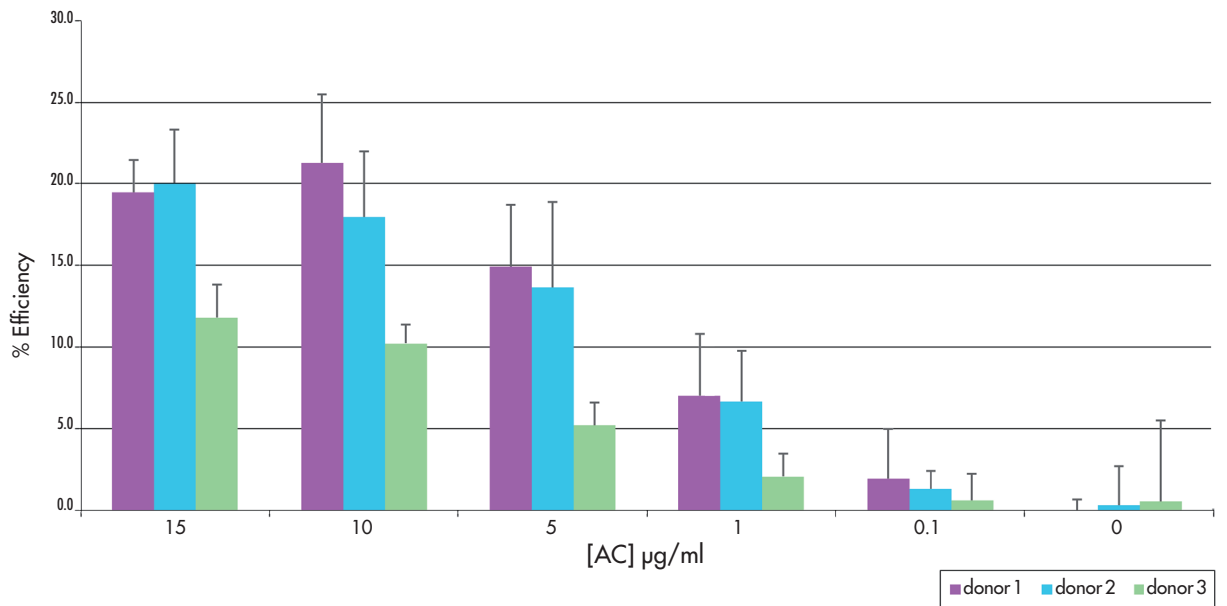
- PBMCs from 3 donors were used as effector cells.
- The SKBR3 breast cancer cell line was used as the target cell.
- Target cell: Effector cell ratio was 1: 50.
- Mouse monoclonal antibody against human HER2 was tested at 5 concentrations.

The assay was performed in 96-well format. Each data point was tested in triplicate and a reading was taken at 4 hours. ADCC activity was measured by LDH (lactate

dehydrogenase) release. Experimental release was measured in the presence of the PBMCs, SKBR3s, and antibody. Spontaneous release was measured in the presence of the PBMCs and SKBR3s, without the antibody. Maximal release was measured in the presence of the SKBR3s and the detergent Triton X-100. The percentage of cytotoxicity was measured using the following formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental lysis} - \text{Spontaneous lysis})}{(\text{Maximal lysis} - \text{Spontaneous lysis})} \times 100$$

Results



The graph represents the percentage of cytotoxicity induced by PBMC from 3 donors on HER2 mAb-coated Her2-positive cell line SKBR3.

Cerep possesses a customizable ADCC assay platform that facilitates the selection of antibody drug candidates. We provide ADCC assay services to determine antibody ADCC activity against specified target cell lines.

Our ADCC assay uses freshly-isolated effectors cells (PBMC) and accurately detects cell lysis based on LDH-release. Assay specifications are customizable: a variety of common target cell lines are available in-house, or we can use your unique cell line.

The assay service includes optimization of the target cell: Effector cell ratio and measurement of ADCC activity at a series of antibody concentrations, in triplicate. Tests with PBMC cells from multiple donors can also be requested.