Case Study for ADCC and CDC Assays

I. Antibody-dependent cell-mediated cytotoxicity (ADCC) Assay

- Subject antibody: Trastuzumab (Herceptin®);
- Target cell lines: Human breast cancer cell line SK-BR-3 (ATCC HTB-30) and MDA-MB-453 (ATCC HTB-131);
- Effector cells: human peripheral blood lymphocytes (PBL) purified from the blood of the healthy donors;
- Assay readout: LDH release, or Calcein release.

1. ADCC assay at different E/T ratios

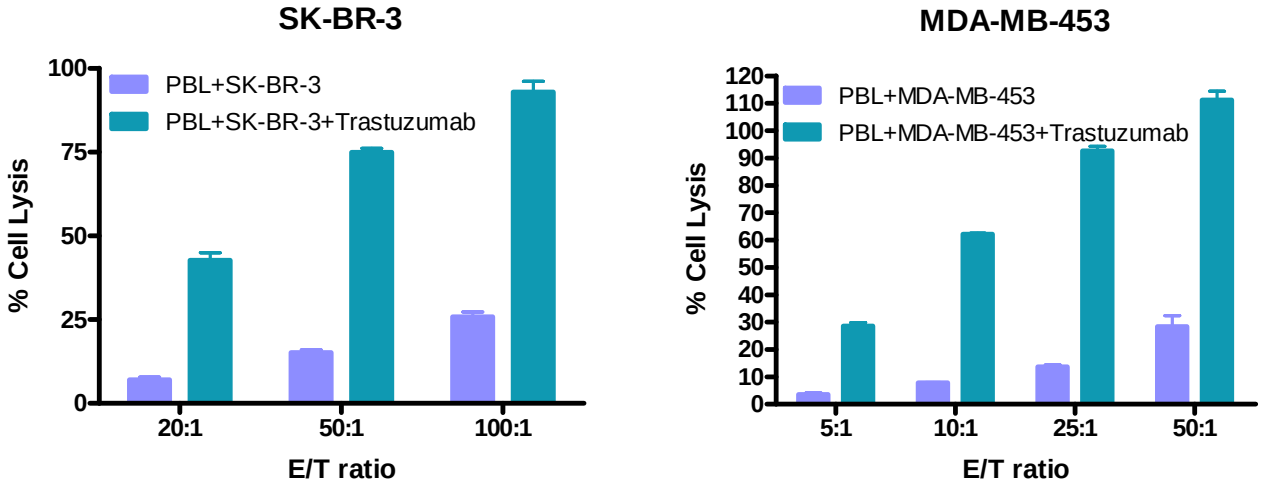

Figure 1. Trastuzumab induced human PBL mediated ADCC to SK-BR-3 cells or MDA-MB-453 cells. 10,000 SK-BR-3 cells or 20,000 MDA-MB-453 cells were incubated with the effector cells at the indicated ratios (freshly prepared human PBL) with or without the presence of 20 μg/ml Trastuzumab for 4 hours at 37 °C/5%CO_2. LDH release to the cell supernatants was analyzed with LDH kit. Absorbance was detected at 490 nm (formazan absorbance) and 650 nm (nonspecific absorbance) by FlexStation® 3.

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E/T	SK-BR-3	MDA-MB-453
5:1	-	25.02 ± 1.27
10:1	-	54.31 ± 0.47
20:1	35.64 ± 2.23	-
25:1	-	78.94 ± 1.63
50:1	59.71 ± 1.26	82.75 ± 3.23
100:1	67.10 ± 3.19	-

Table 1. Percentage of Trastuzumab induced human PBL mediated ADCC to SK-BR-3 cells or MDA-MB-453 cells. (Average ± s.e)

2. Dose-response study of Trastuzumab induced PBL mediated ADCC

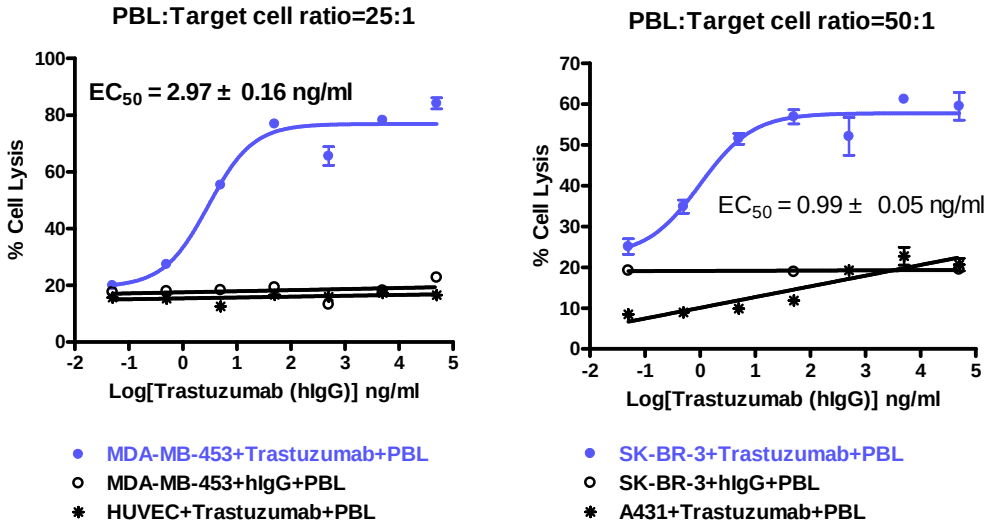

Figure 2. Dose-dependent study of Trastuzumab triggered PBL mediated target cell lysis.

The effector cells, human PBL, were incubated with HER2 overexpressing human breast carcinoma cell line MDA-MB-453 or SK-BR-3 at indicated E/T ratio in a 4 h assay. HER2 negative Human Umbilical Vein Endothelial Cells (HUVEC) and human epithelial carcinoma cell line A431 were used as the negative controls for MDA-MB-453 and SK-BR-3, respectively. Human whole IgG was used as an isotype control. LDH release to the cell supernatants was analyzed with LDH kit. Absorbance was detected at 490 nm (formazan absorbance) and 650 nm (nonspecific absorbance) by FlexStation® 3.

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II. Complement-dependent cytotoxicity (CDC) Assay

- Subject antibody: Rituximab (Rituxan®);
- Target cell lines: Daudi (ATCC CCL-213™) (peripheral blood Burkitt's lymphoma);
- Complement source: human sera from the healthy donors;
- Assay readout: LDH release, or CellTiter-Glo® Luminescent Cell Viability Assay.

1. Cell morphology

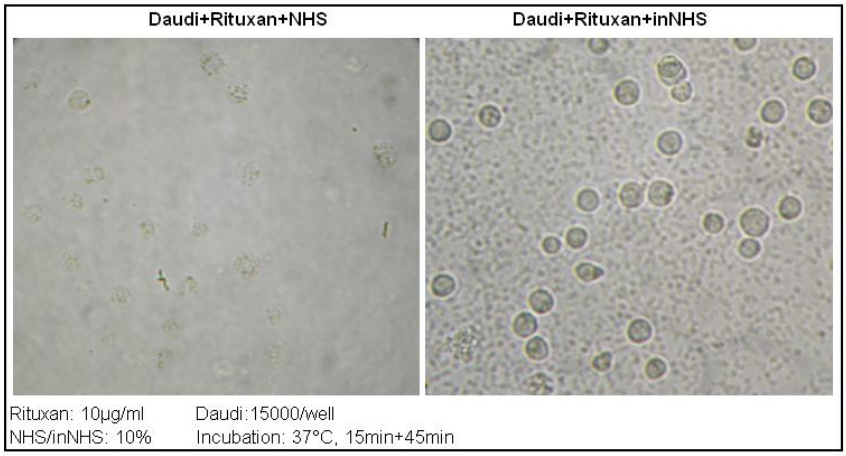

Figure 3. Cell morphology of Rituximab triggered CDC effect on Daudi Cells

(NHS, normal human serum; inNHS, heat inactivated normal human serum)

2. Optimization of serum concentration

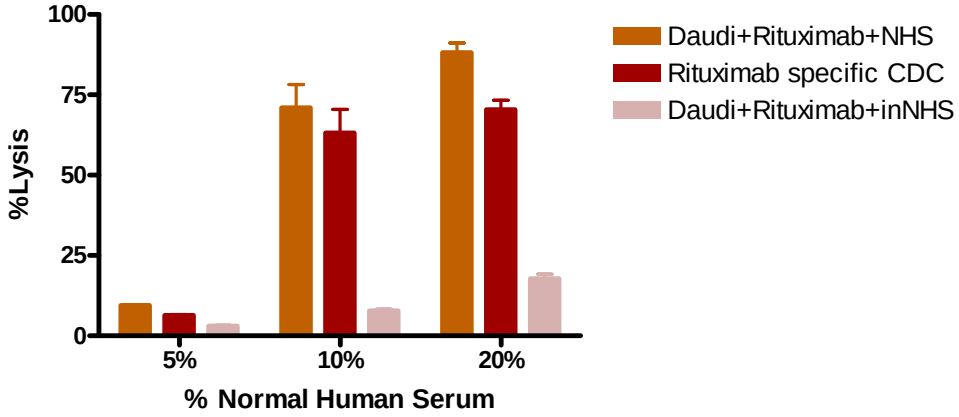

Figure 4. Optimization of human serum concentration for Rituximab based CDC assay

(Rituximab specific CDC was obtained by subtracting "Daudi+ Rituximab+ inNHS" from "Daudi+ Rituximab+ NHS")

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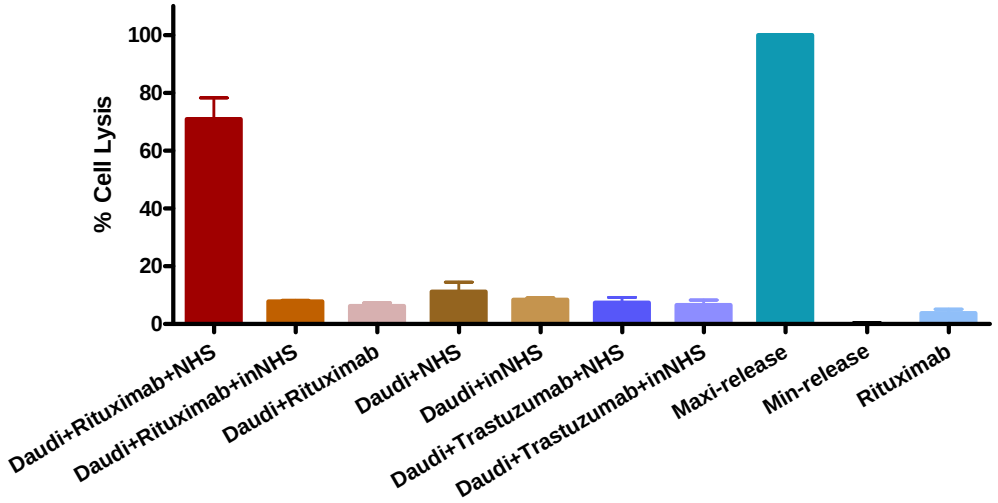

Figure 5. Rituximab induced CDC lysis to Daudi cells with 10% normal human serum. 15,000 Daudi cells were pre-incubated with Rituximab (10 µg/ml) at RT for 15 min followed by addition of 10% NHS, inNHS or other controls as indicated in the figure for another 45 min. Maxi-release was obtained by disrupting the cells with 0.2% Triton. Min-release was obtained by spontaneous LDH release from the un-treated target cells. Cell lysis was analyzed with LDH method.

3. Dose-response study of Rituximab induced CDC lysis to Daudi Cells

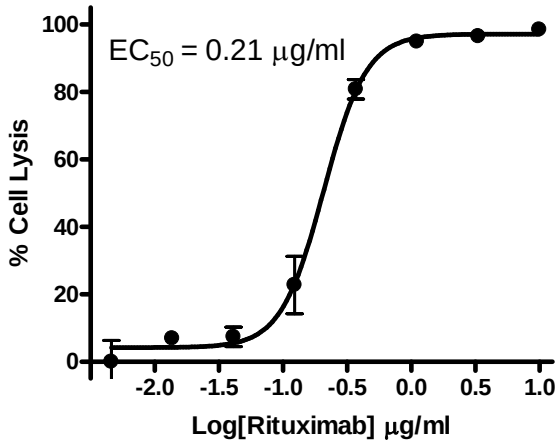

Figure 6. Dose-response study of Rituximab induced CDC lysis to Daudi Cells. 15,000 Daudi cells were pre-incubated with indicated concentration of Rituximab at RT for 15 min followed by addition of 10% NHS and incubated for another 45 min. Cell viability was analyzed with CellTiter-Glo® Luminescent Cell Viability Assay Kit.

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III. References

(1) Paul Carter, *et al*. (1992) Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl. Acad. Sci.* USA 89:4285-4289;

(2) Christoph Uherek, *et al*. (2002) Retargeting of natural killer-cell cytolytic activity to ErbB2-expressing cancer cells results in efficient and selective tumor cell destruction. *Blood.* 100: 1265-1273

(3) Helene Gazzano-Santoro *et al*. (1997) A non-radioactive complement-dependent cytotoxicity assay for anti-CD20 monoclonal antibody. *Journal of Immunological methods*.202:163-171.

(4) Frank J. Beurskens, *et al*. (2008) Complement activation impacts B-cell depletion by both type I and type II CD20 monoclonal antibodies. *Blood*. 112: 4354-4355.

(5) Bohua Li *et al*. (2009) Characterization of a rituximab variant with potent antitumor activity against rituximab-resistant B-cell lymphoma. *Blood.* 114: 5007-5015.

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GenScript ADCC assay services

- **Provided by client:**
  - Antibody sample (400 μl, 1 mg/ml is needed)
  - Target cell line(s) (or purchased by GenScript)
    Note: Some 250 tumor cell lines are available at GenScript for free as ADCC target cells
  - Antigen expression level on the target cell line (optional)
- **Service details:**
  - Effector cell preparation: human peripheral blood lymphocytes (PBL) purified from the blood of the healthy donors (4 donors are recommended);
  - Detection of antigen expression level of target cell(s) on FACS (optional, if not provided by the client);
  - Effector cell CD16 polymorphism genotyping (optional)
  - ADCC assay at 3 different E/T ratios (50:1, 25:1, and 10:1 by default or defined by the client) with a fixed antibody concentration (20 μg/ml by default or defined by the client);
  - ADCC assay at 8 concentrations in triplicate (50, 5, 0.5, 0.05, 0.005, 0.005, 0.0005, and 0 μg/ml by default or defined by the client);
  - Readout: LDH release, or Calcein release;
  - Timeline: 3 weeks;
  - Deliverables: raw and analyzed data, dose-response curves.

GenScript CDC assay services

- **Provided by client:**
  - Antibody sample (300 μl, 1 mg/ml is needed)
  - Target cell line(s) (or purchased by GenScript)
    Note: Some 250 tumor cell lines are available at GenScript for free as CDC target cells
  - Antigen expression level on the target cell line (optional)
- **Service details:**
  - Normal human serum preparation;
  - Detection of antigen expression level of target cell(s) on FACS (optional, if not provided by the client);
  - CDC assay at 8 concentrations in triplicate (50, 5, 0.5, 0.05, 0.005, 0.005, 0.0005, and 0 μg/ml by default or defined by the client);
  - Readout: Calcein release, or LDH release;
  - Timeline: 2 weeks;
  - Deliverables: raw and analyzed data, dose-response curves.

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