Validation data for Raji-HER2 Cells

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Version 21B17-ED

Raji-HER2 cells were developed from the Raji cell line to overexpress the human HER2 gene. Raji-HER2 cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-HER2 monoclonal antibodies (mAbs). HER2 expression by Raji-HER2 cells has been verified by flow-cytometry **(Figure 1)**, and induction of ADCC has been validated using InvivoGen's collection of anti-HER2 antibody isotypes and Jurkat-Lucia™ NFAT-CD16 reporter cells **(Figure 2)**. The level of ADCC induction is measured by an NFAT-dependent Lucia luciferase reporter protein.

Validation of HER2 expression by flow cytometry

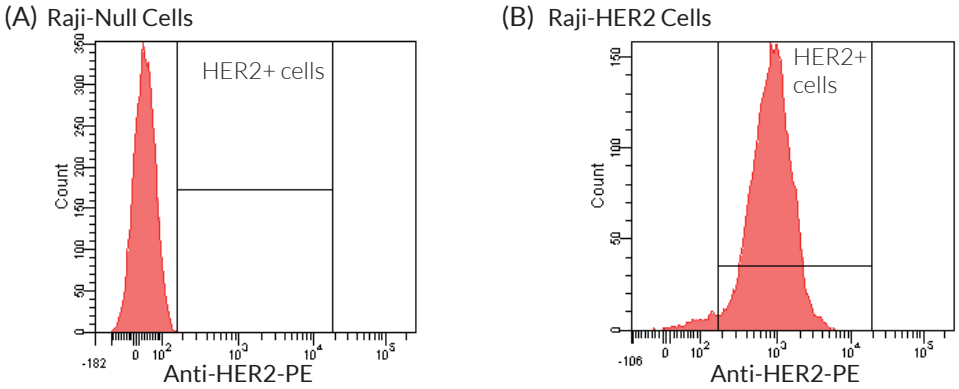

Figure 1: Validation of the expression of HER2 by Raji-HER2 cells. Raji-Null (A) and Raji-HER2 (B) cells were incubated with a PE-conjugated Anti-HER2 mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-HER2 antibody isotypes and Raji-HER2 target cells

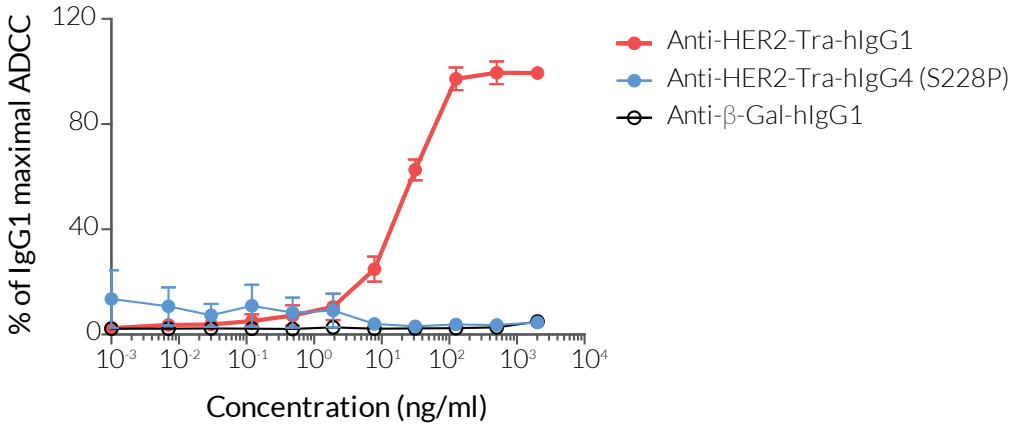

Figure 2: Comparison of ADCC potency for native and engineered anti-HER2 antibody isotypes. Raji-HER2 cells were incubated with gradient concentrations of Anti-HER2-Tra mAbs, which feature the Trastuzumab variable region, or an Anti-β-galactosidase (β-Gal) mAb for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells for 6 hours. NFAT activation, reflecting the induced ADCC response, was assessed by determining Lucia luciferase activity in the supernatant using QUANTI-Luc™. Percentages of the maximal response normalized to the IgG1 isotype are shown.

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