Validation data for Raji-h4-1BB Cells

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Version 21A12-ED

Raji-h4-1BB cells were developed from the Raji cell line to overexpress the human 4-1BB gene. Raji-h4-1BB cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human 4-1BB monoclonal antibodies (mAbs). Human 4-1BB expression by Raji-h4-1BB cells has been verified by flow-cytometry **(Figure 1)**, and induction of ADCC has been validated using a collection of anti-human 4-1BB 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. Antibodies displaying lower EC_{50} have higher ADCC potency.

Validation of 4-1BB expression by flow cytometry

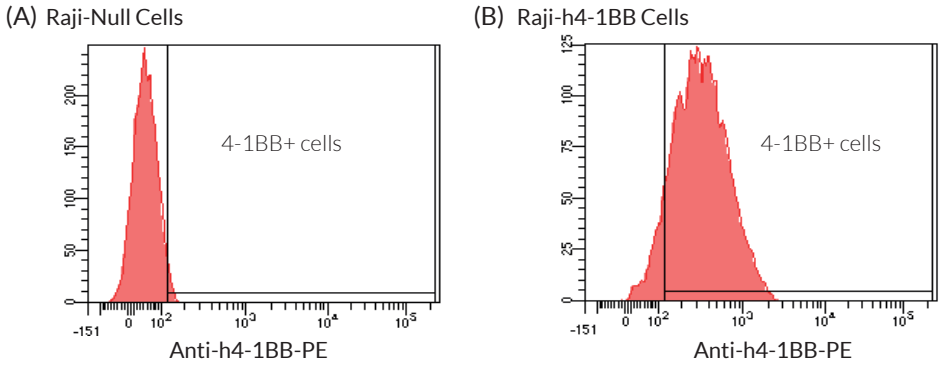

Figure 1: Validation of the expression of human 4-1BB by Raji-h4-1BB cells. Raji-Null (A) and Raji-h4-1BB (B) cells were incubated with a PE-conjugated Anti-h4-1BB mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human 4-1BB antibody isotypes and Raji-h4-1BB target cells

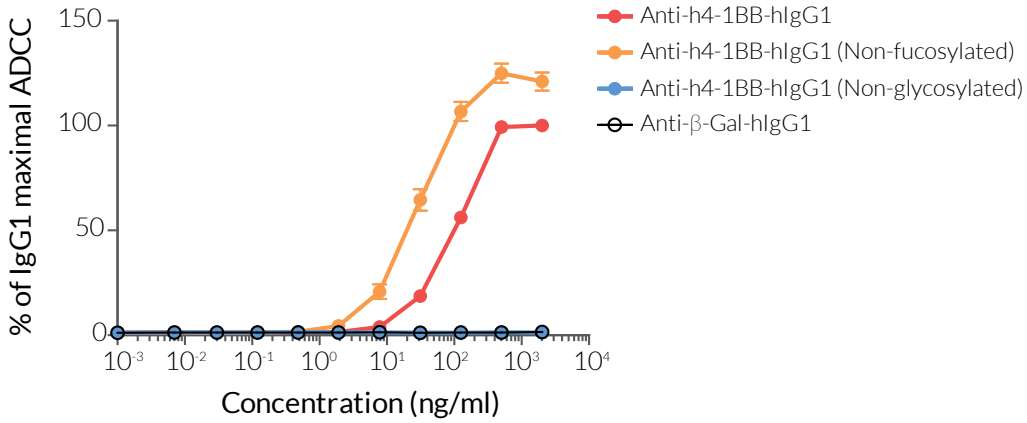

Figure 2: Comparison of ADCC potency for native and engineered anti-human 4-1BB antibody isotypes. Raji-h4-1BB cells were incubated with gradient concentrations of Anti-h4-1BB or Anti-β-galactosidase (β-Gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells 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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