Validation data for Raji-hTIGIT Cells

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Raji-hTIGIT cells were developed from the Raji cell line to overexpress the human TIGIT gene. Raji-hTIGIT cells were designed as target cells in InvivoGen's antibody-dependent cellular cytotoxicity (ADCC) assay using clinically-relevant anti-human TIGIT monoclonal antibodies (mAbs). Human TIGIT expression by Raji-hTIGIT cells has been verified by flow-cytometry **(Figure 1)**, and induction of ADCC has been validated using InvivoGen's collection of anti-human TIGIT antibody isotypes and Jurkat-Lucia™ NFAT-CD16 reporter cells **(Figure 2)**. The level of ADCC induction is measured as a bioluminescent signal produced by an NFAT-dependent Lucia luciferase reporter protein. Antibodies displaying lower EC_{50} have higher ADCC potency.

Validation of TIGIT expression

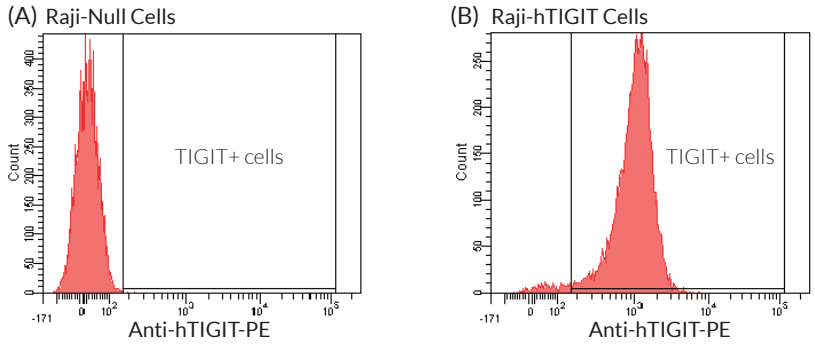

Figure 1: Validation of the expression of human TIGIT by Raji-hTIGIT cells. Raji-Null (A) and Raji-hTIGIT (B) cells were incubated with a PE-conjugated Anti-hTIGIT mAb for 30 minutes. The binding affinity was then measured using flow cytometry.

ADCC assay using various anti-human TIGIT antibody isotypes and Raji-hTIGIT target cells

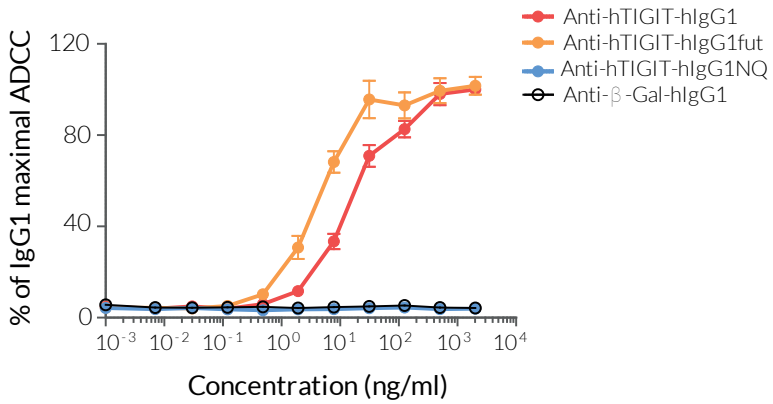

Figure 2: Comparison of ADCC potency for native and engineered anti-human TIGIT antibody isotypes. Raji-hTIGIT cells were incubated with gradient concentrations of Anti-hTIGIT or Anti-β-galactosidase (β-gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with target cells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