Validation data for Raji-Null Cells

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Raji-Null cells were developed from the human lymphoblast Raji cell line which constitutively expresses high level of CD20 and low level of PD-L1 (programmed cell death ligand 1) surface antigens. Raji-Null cells were designed as target cells in antibody-dependent cellular cytotoxicity (ADCC) assays using clinically-relevant anti-human CD20 monoclonal antibodies (mAbs). Human CD20 and PD-L1 expression by Raji-Null cells has been verified by flow-cytometry, and induction of ADCC has been validated using InvivoGen's combinations of anti-human CD20 antibody isotypes and Jurkat-NFAT Lucia™ CD16 reporter cell line (Figure 1). 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. Raji-Null cells can also be used as negative control target cells in ADCC assays for other molecules of interest such as the immune checkpoints CTLA-4, PD-1, or PD-L1 (Figure 2). Of note, the low level of PD-L1 expression on Raji-Null cells allows ADCC induction, albeit with lower potency than with Raji-hPD-L1 cells overexpressing human PD-L1.

ADCC assay using various anti-human CD20 (Rituximab) antibody isotypes and Raji-Null target cells

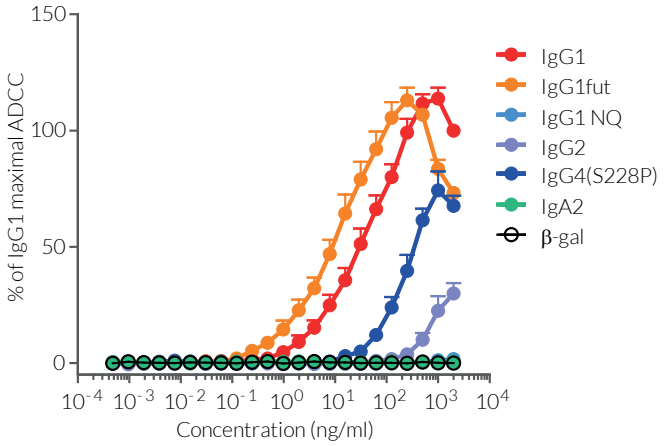

Figure 1. Comparison of ADCC potency for native and engineered anti-human CD20 antibody isotypes: Raji-Null cells were incubated with gradient concentrations of Anti-hCD20 or Anti-β-galactosidase (β-gal) mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with targets cells 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.

ADCC induction on antigen-expressing Raji cells and Raji-Null control target cells

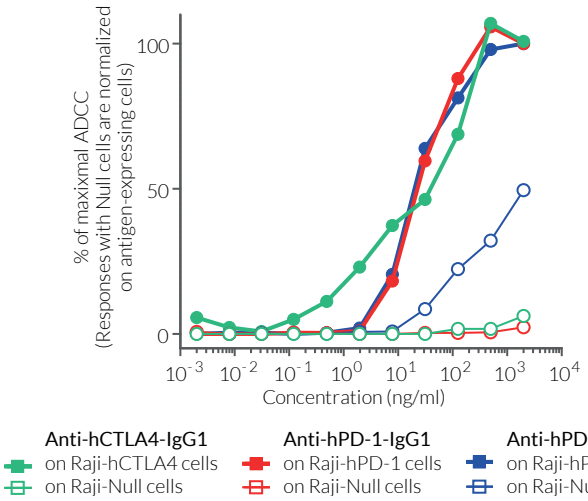

Figure 2. Comparison of ADCC potency of anti-human CTLA-4, PD-1, or PD-L1 IgG1 antibodies on Raji-derived target cells: Raji-hCTLA4, Raji-hPD-1, Raji-hPD-L1 or Raji-Null control cells were incubated with gradient concentrations of anti-hCTLA4-IgG1, anti-hPD-1-IgG1, or anti-hPD-L1-IgG1 mAbs for 1 hour. Jurkat-Lucia™ NFAT-CD16 effector cells were then co-incubated with targets 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 are shown, and responses with Raji-Null cells are normalized on appropriate antigen-expressing cells.

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