Validation data for Anti-TROP2-hIgG1

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Version 23J09-NJ

Anti-TROP2-hIgG1 has been specifically developed to generate antibody-drug conjugates (ADCs). The antibody specificity for TROP2 has been validated using flow cytometry and ELISA **(Figure 1)**. The biological activity of ADCs combining Anti-TROP2-hIgG1 and STG-982, a STING agonist, or TL7-887, a TLR7 agonist, has been validated using cellular assays. In a co-culture of TROP2$^+$ tumor cells (BxPC-3) and human peripheral blood monocytes (PBMCs), Anti-TROP2/STG-982 and Anti-TROP2/TL7-887 induce a significantly higher production of CXCL10 and IL-6, respectively, than unconjugated agonists or negative control ADCs **(Figures 2A and 3A)**. Of note, in absence of tumor cells, PBMCs remain either unresponsive to all stimulations **(Figure 2B)**, or respond to higher doses of Anti-β-gal/TL7-887 control ADC **(Figure 3B)**, which could be explained by cellular uptake through endocytosis/pinocytosis.

Validation of Anti-TROP2-hIgG1 by flow cytometry and ELISA

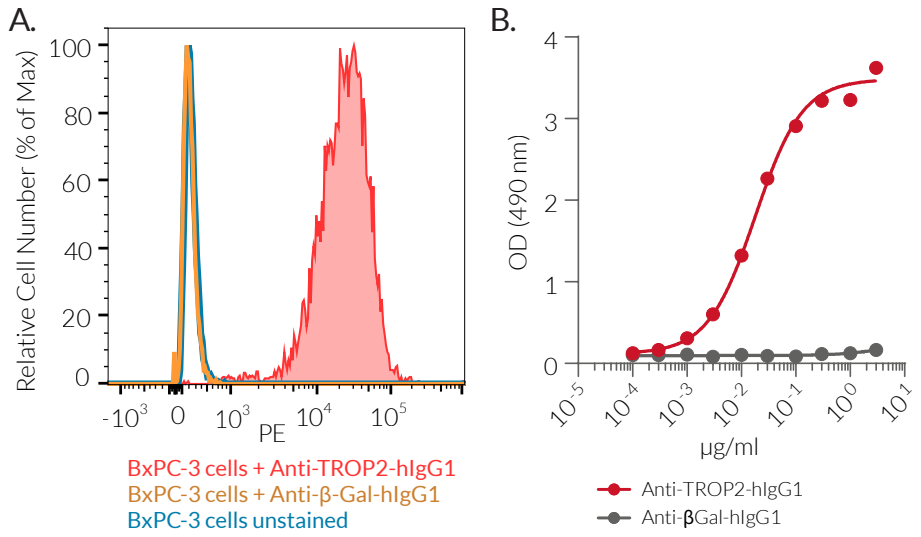

Figure 1: Anti-TROP2-hIgG1 mAb binding to cell-surface TROP2 or plate-bound TROP2.
(A) ~5 x 10^5 BxPC-3 tumor cells were incubated with 1 µg of Anti-TROP2-hIgG1 mAb or an isotype control for 45 min at 4°C. Cells were then washed and incubated with 250 ng of goat anti-human κ light chain antibody coupled to PE for 1h at 4°C. Cell surface staining was analyzed by flow cytometry.
(B) TROP2-Fc fusion protein (1 µg/ml) was coated on ELISA plates overnight. A 3-fold serial dilution of Anti-TROP2-hIgG1 (red curve) or of Anti-β-Gal-hIgG1 control mAb (grey curve) was realized for the capture step. An HRP-labelled anti-human κ light chain antibody (1/1000 dilution) and the HRP substrate OPD (o-phenylenediamine dihydrochloride) were used for the detection step. Absorbance was read at 490 nm.

Biological activity of Anti-TROP2/STG-982 in co-cultures

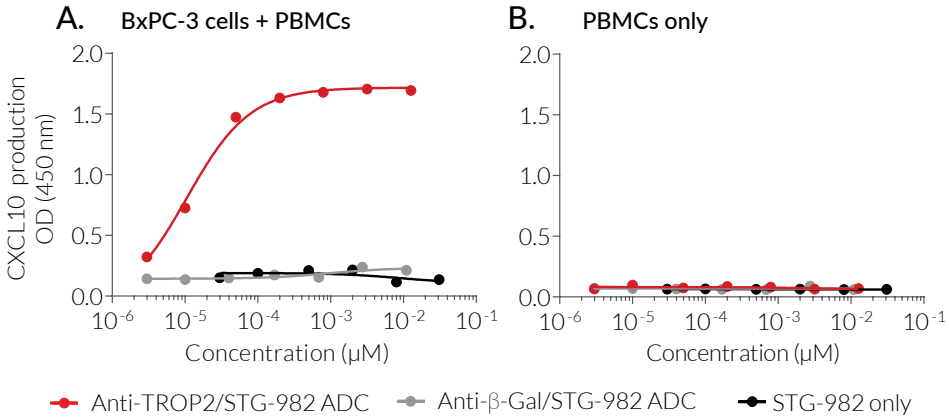

Figure 2: Dose-response of human PBMCs co-cultured with BxPC-3 tumor cells and Anti-TROP2/STG-982 ADC.
1.5 x 10^5 human PBMCs and 5 x 10^4 BxPC-3 tumor cells **(A)** or 1.5 x 10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-TROP2/STG-982 ADC (DAR ~4), Anti-β-Gal/STG-982 ADC (DAR ~4), or STG-982 only. After overnight incubation, the STING-mediated response was assessed by measuring the production of CXCL10 in PBMC and BxPC-3 co-culture supernatants, using an ELISA. The optical density (OD) at 450 nm is shown.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com

Biological activity of Anti-TROP2/TL7-887 in co-cultures

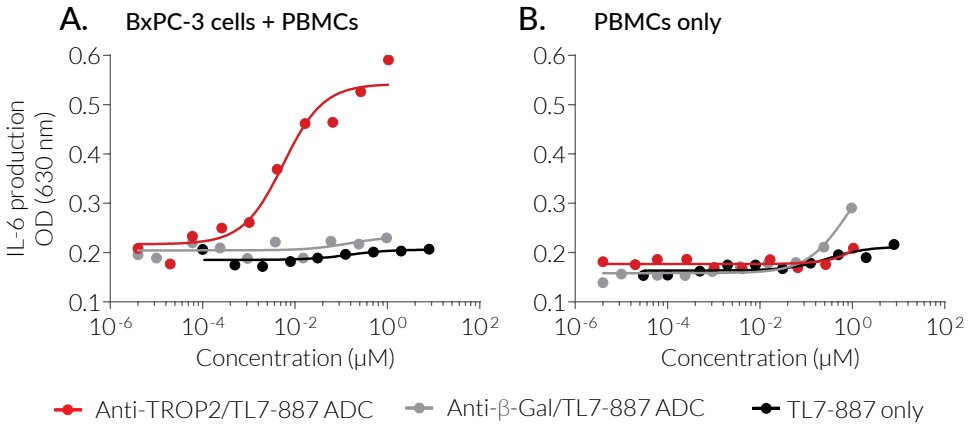

Figure 3: Dose-response of human PBMCs co-cultured with BxPC-3 tumor cells and Anti-TROP2/TL7-887 ADC.
1.5×10^5 human PBMCs and 5×10^4 BxPC-3 tumor cells **(A)** or 1.5×10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-TROP2/TL7-887 ADC (DAR ~6), Anti-β-Gal/TL7-887 ADC (DAR ~6), or TL7-887 only. After overnight incubation, the TLR7-mediated response was determined using HEK-Blue™ IL-6 reporter cells. Briefly, the levels of IL-6 production in PBMC and BxPC-3 co-culture supernatants were assessed by measuring the SEAP activity of HEK-Blue™ IL-6 reporter cells, using QUANTI-Blue™, a SEAP detection reagent. The optical density (OD) at 630 nm is shown.