Validation data for Anti-HER2-hIgG1

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Version 23J09-NJ

Anti-HER2-hIgG1 has been specifically developed to generate antibody-drug conjugates (ADCs). The antibody specificity for HER2 has been validated using cell surface staining and analysis by flow cytometry **(Figure 1)**. The biological activity of ADCs combining Anti-HER2-hIgG1 and STG-982, a STING agonist, or TL7-887, a TLR7 agonist, has been validated using cellular assays. In a co-culture of HER2^{+} tumor cells (SK-BR-3) and human peripheral blood monocytes (PBMCs), Anti-HER2/STG-982 and Anti-HER2/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-HER2/TL7-887 **(Figure 3B)**. This latter observation could be explained by cellular uptake through endocytosis after binding to Fc-receptor and/or to HER2 which is expressed at low levels by some PBMC subpopulations[1].

1. You F. et al., 2008. Low-level expression of HER2 and CK19 in normal peripheral blood mononuclear cells: relevance for detection of circulating tumor cells. J Hematol Oncol. 1(2): DOI: 10.1186/1756-8722-1-2.

Validation of Anti-HER2-hIgG1 by flow cytometry

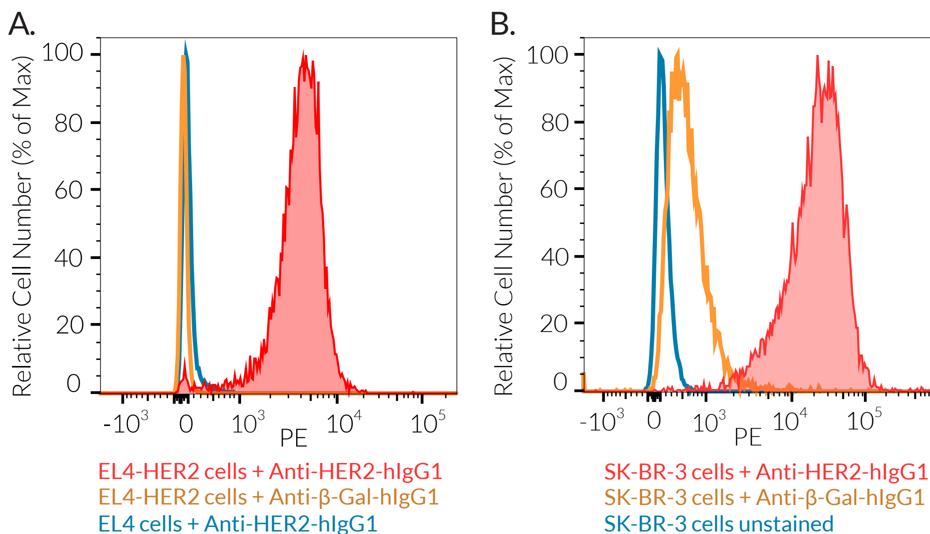

Figure 1: Cell surface staining of HER2 using Anti-HER2-hIgG1 mAb.
~5 x 10^5 EL4-HER2 **(A)** or ~3 x 10^5 SK-BR-3 **(B)** cells were incubated with 500 ng **(A)** or 1 µg **(B)** of Anti-HER2-hIgG1 mAb or an isotype control for 30 min to 1h at 4°C. Cells were then washed and incubated with 250 ng of goat anti-human κ light chain antibody coupled to PE for 45 min to 1h at 4°C. Cell surface staining was analyzed by flow cytometry.

Biological activity of Anti-HER2/STG-982 in co-cultures

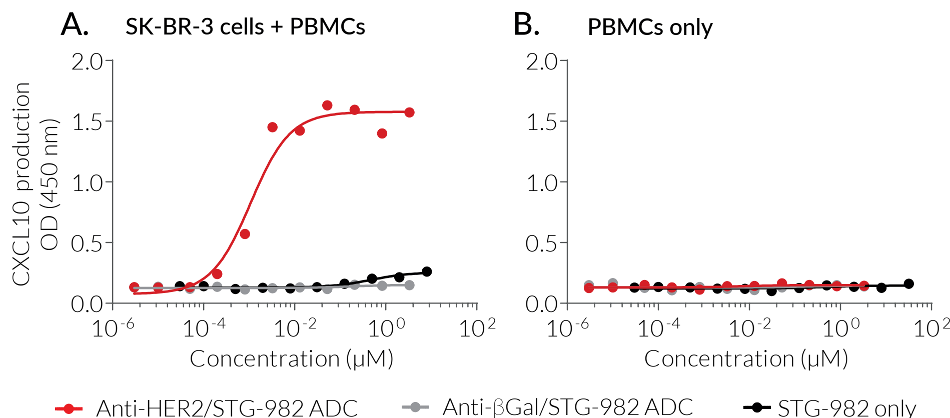

Figure 2: Dose-response of human PBMCs co-cultured with SK-BR-3 tumor cells and Anti-HER2/STG-982 ADC.
1.5 x 10^5 human PBMCs and 5 x10^4 SK-BR-3 tumor cells **(A)** or 1.5 x 10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-HER2/STG-982 ADC (DAR ~5), Anti-β-Gal/STG-982 ADC (DAR ~5), or STG-982 only. After overnight incubation, the STING-mediated response was assessed by measuring the production of CXCL10 in PBMC and SK-BR-3 co-culture supernatants, using an ELISA. The optical density (OD) at 450 nm is shown.

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Biological activity of Anti-HER2/TL7-887 in co-cultures

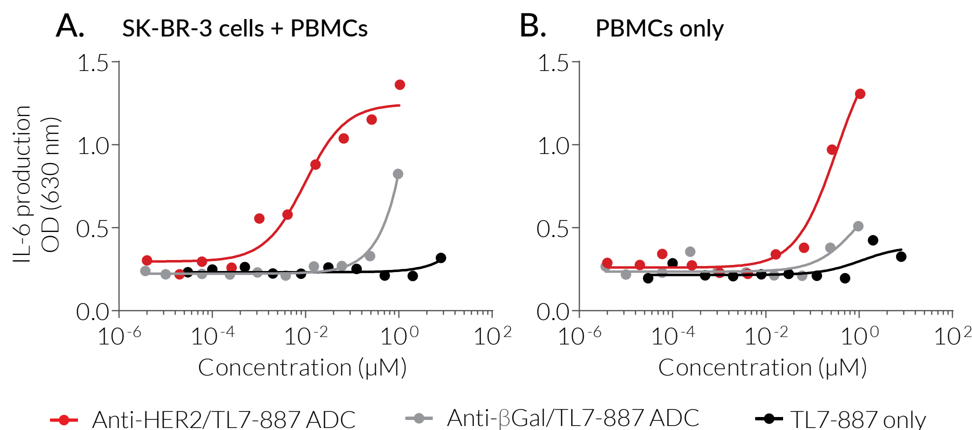

Figure 3: Dose-response of human PBMCs co-cultured with SK-BR-3 tumor cells and Anti-HER2/TL7-887 ADC.
1.5 x 10^5 human PBMCs and 5 x 10^4 SK-BR-3 tumor cells **(A)** or 1.5 x 10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-HER2/TL7-887 ADC (DAR ~6), Anti-β-Gal/TL7-887 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 SK-BR-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.