Validation data for STG-982

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Version 23J12-NJ

STG-982 is a ready-to-use "pre-linked" conjugatable STING ligand, synthesized from an analog of CL656, a well-known STING agonist. STG-982 efficiently triggers IRF- and NF-κB-mediated cellular responses **(Figure 1)**. STG-982 can be used to generate immunostimulatory antibody-drug conjugates (ADCs) as conjugation to a Anti-TROP2-hIgG1 and subsequent activation of STING 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 induces a significantly higher production of CXCL10 than unconjugated STG-982 or a negative control ADC **(Figure 2)**.

Biological activity of STG-982

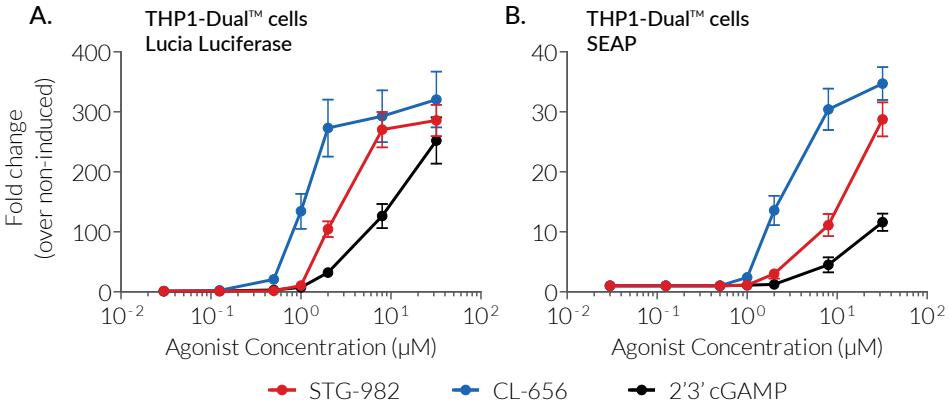

Figure 1: IRF and NF-KB responses induced by STING conjugatable ligand STG-982.
THP1-Dual™ cells were stimulated with increasing concentrations of STG-982, CL-656, or 2'3'-cGAMP. After overnight incubation, the IRF and NF-κB responses were determined by measuring Lucia luciferase and SEAP activity in the supernatant using QUANTI-Luc™ (A), or QUANTI-Blue™ Solution (B), respectively. Data are shown as a fold increase (mean ± SEM) over non-induced cells.

Biological activity of STG-982 conjugated to Anti-HER2-hIgG1

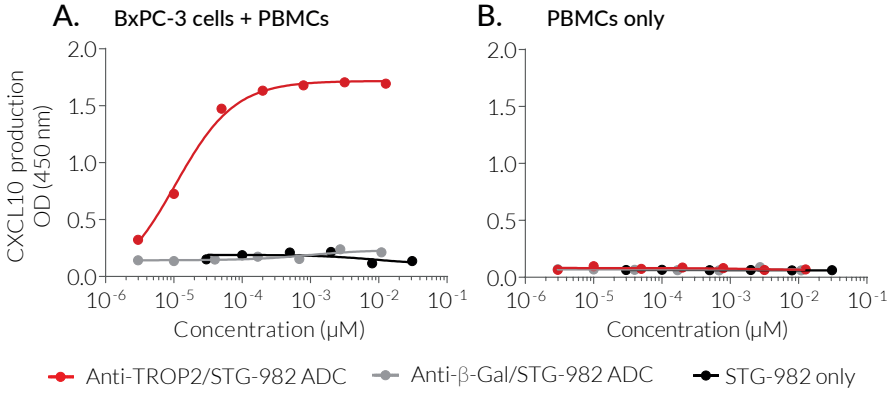

Figure 2: Dose-response of human PBMCs co-cultured with BxPC-3 tumor cells and Anti-TROP2/STG-982 ADC.
1.5 x 10^5 human PBMCs and 5 x 10^4 BxPC-3 tumor cells **(A)** or 1.5 x 10^5 human PBMCs only **(B)** were incubated with increasing concentrations of Anti-TROP2/STG-982 ADC (DAR ~4), Anti-β-Gal/STG-982 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.

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