



Screening, Optimization and Characterization of a Novel 4-1BB x 5T4 ADAPTIR™ Bispecific Antibody

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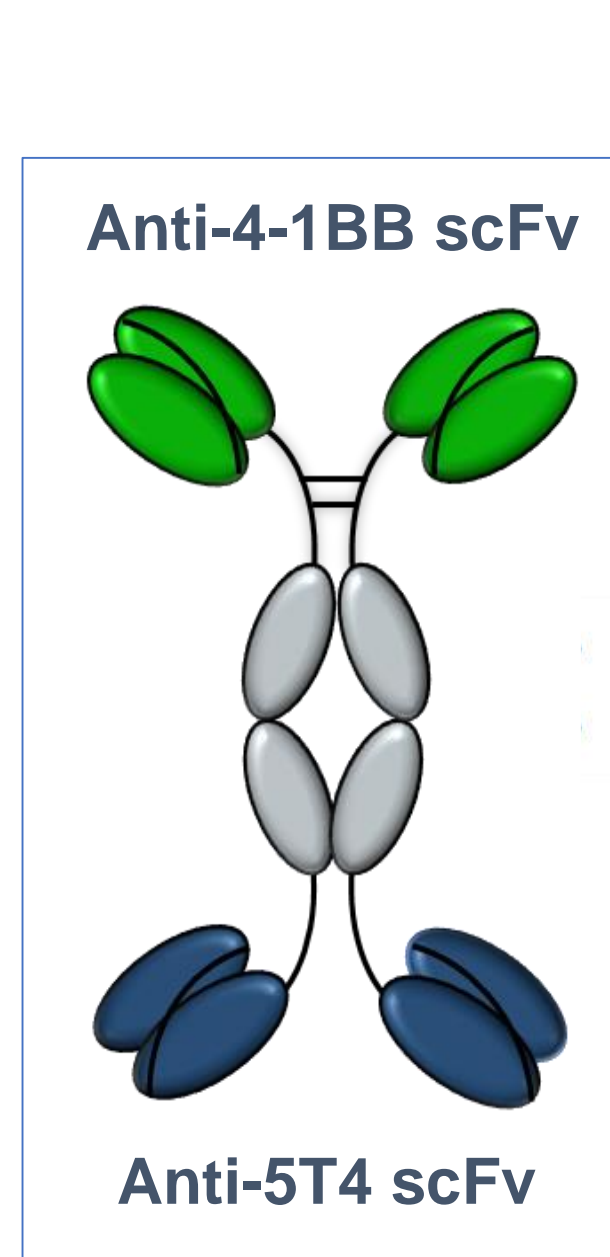
Introduction

A novel 4-1BB (CD137) x 5T4 targeting bispecific antibody, ALG.APV-527, was designed to induce potent tumor specific CD8 T-cell activation, while minimizing unwanted systemic toxicities associated with other 4-1BB targeting therapies. ALG.APV-527 contains binding domains specific to both the co-stimulatory receptor 4-1BB and to 5T4, a tumor associated antigen expressed on multiple solid tumors. ALG.APV-527 only activates 4-1BB when 5T4 is engaged on tumor cells. This feature localizes the immune stimulatory effect of ALG.APV-527 to the tumor microenvironment where both 4-1BB and 5T4 are highly expressed. Therefore, ALG.APV-527 has the potential to be an effective anti-cancer therapeutic agent with an improved safety profile.

5T4 Tumor Associated Antigen

5T4 is a well-defined tumor-associated antigen expressed in a high proportion of patients in a variety of malignancies, including non-small cell lung, renal, pancreas, prostate, breast, colorectal, gastric, ovarian and cervix cancers as well as in acute lymphocytic leukemia. 5T4 has also been shown to be expressed in tumor-initiating cells (Castro et al., 2012; Damelin et al., 2011; Elkord et al., 2009; Southall et al., 1990).

ADAPTIR Molecule Targeting 5T4 and 4-1BB



ADAPTIR Format

ADAPTIR bispecific therapeutics contain two sets of binding domains linked to an immunoglobulin Fc domain which enables an antibody-like half-life. The anti-4-1BB x anti-5T4 ADAPTIR molecules bind both 4-1BB and 5T4 to stimulate activated T-cells to kill 5T4 expressing tumor cells, which leads to more tumor specific cytotoxic T-cells. To avoid interactions with other components of the immune system that could lead to non-specific T-cell activation, the Fc region was engineered to minimize complement fixation and interaction with Fcγ receptors.

Construct Design and Optimization

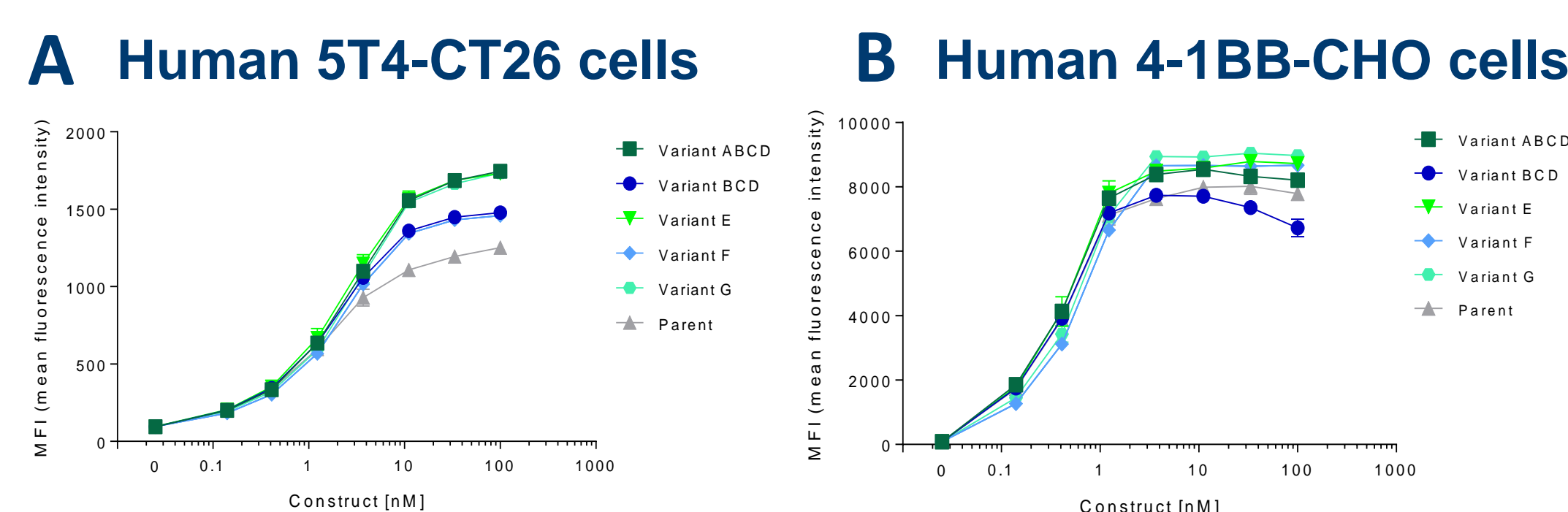
The binding domains of ALG.APV-527 were isolated from the ALLIGATOR-GOLD® human scFv library (Alligator Bioscience AB). Each scFv was then optimized and developed for use in the bispecific ADAPTIR format (Aptevo Therapeutics Inc.). Using phage display, variants for each binding domain were isolated with improved activity or biophysical properties, then combined to select for a bispecific with synergistic benefits. The merger of these elements generated a highly functional, tumor-targeting bispecific molecule featuring enhanced potency binding affinity and improved manufacturability.

Manufacturability Optimization of ALG.APV-527

Construct	Modified Domain	High Salt Solubility, % Protein Loss	Shear Stress, % Protein Loss	Tm (°C) 4-1BB scFv	Tm (°C) 5T4 scFv
Parent	N/A	89	64	56.1	70.5
Variant A	5T4	67	80	57	70.5
Variant B	4-1BB	55	32	60.4	71.0
Variant C	5T4	24	76	56.9	73.0
Variant D	4-1BB	56	71	58.9	70.8
Variant BCD	Both	19	25	61.7	72.3
Variant ABCD	Both	29	27	61.7	72.3

Several mutations, alone and in combination, were discovered to provide additive benefit to the biophysical stability of the ADAPTIR bispecific constructs. Increases in solubility, shear stability and thermostability were achieved.

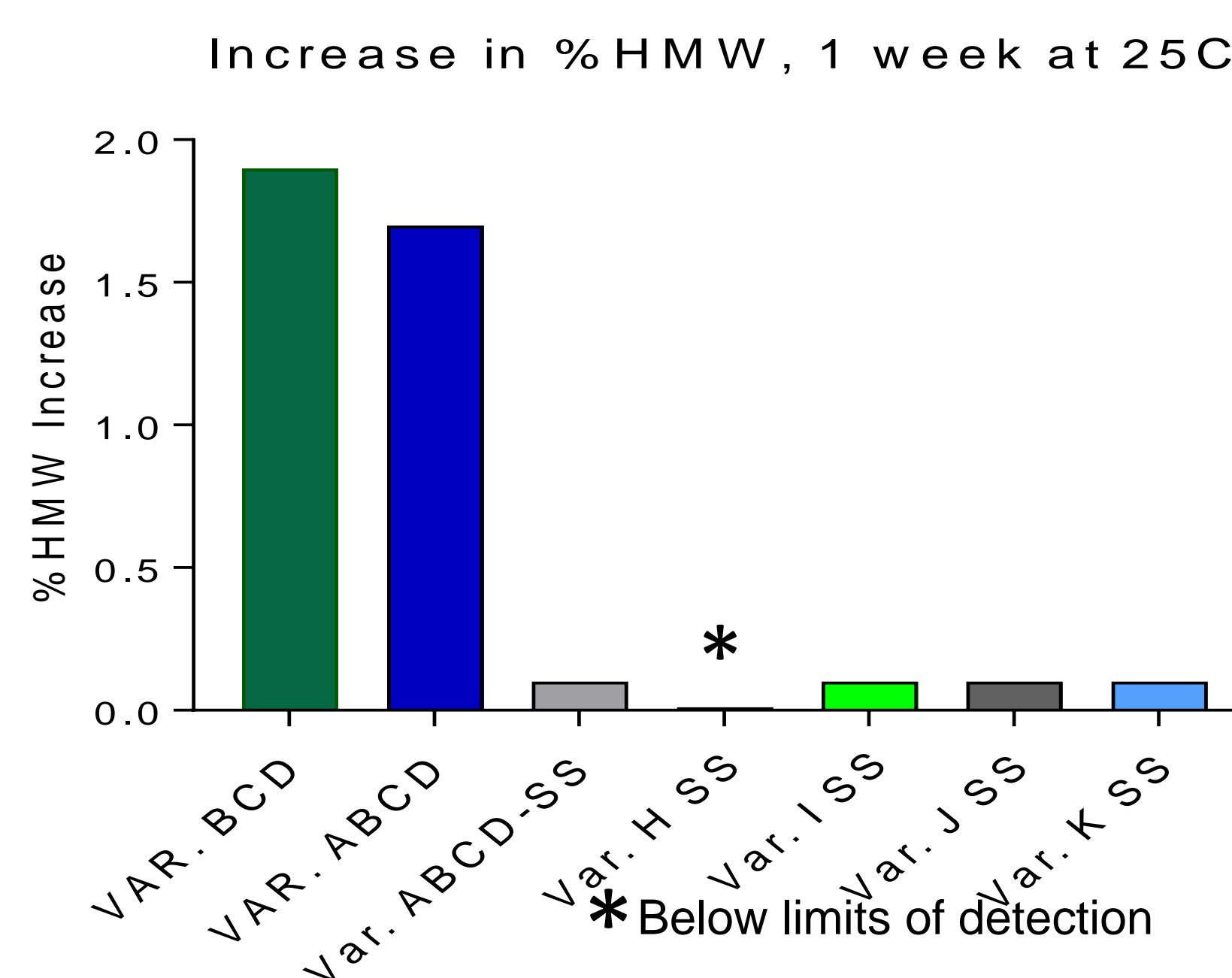
Binding to Human 5T4 and 4-1BB Expressing Cells



A Several variants were evaluated for binding to CT26 cells expressing human 5T4. Several constructs had similar EC50 values, but improved max levels of saturation.

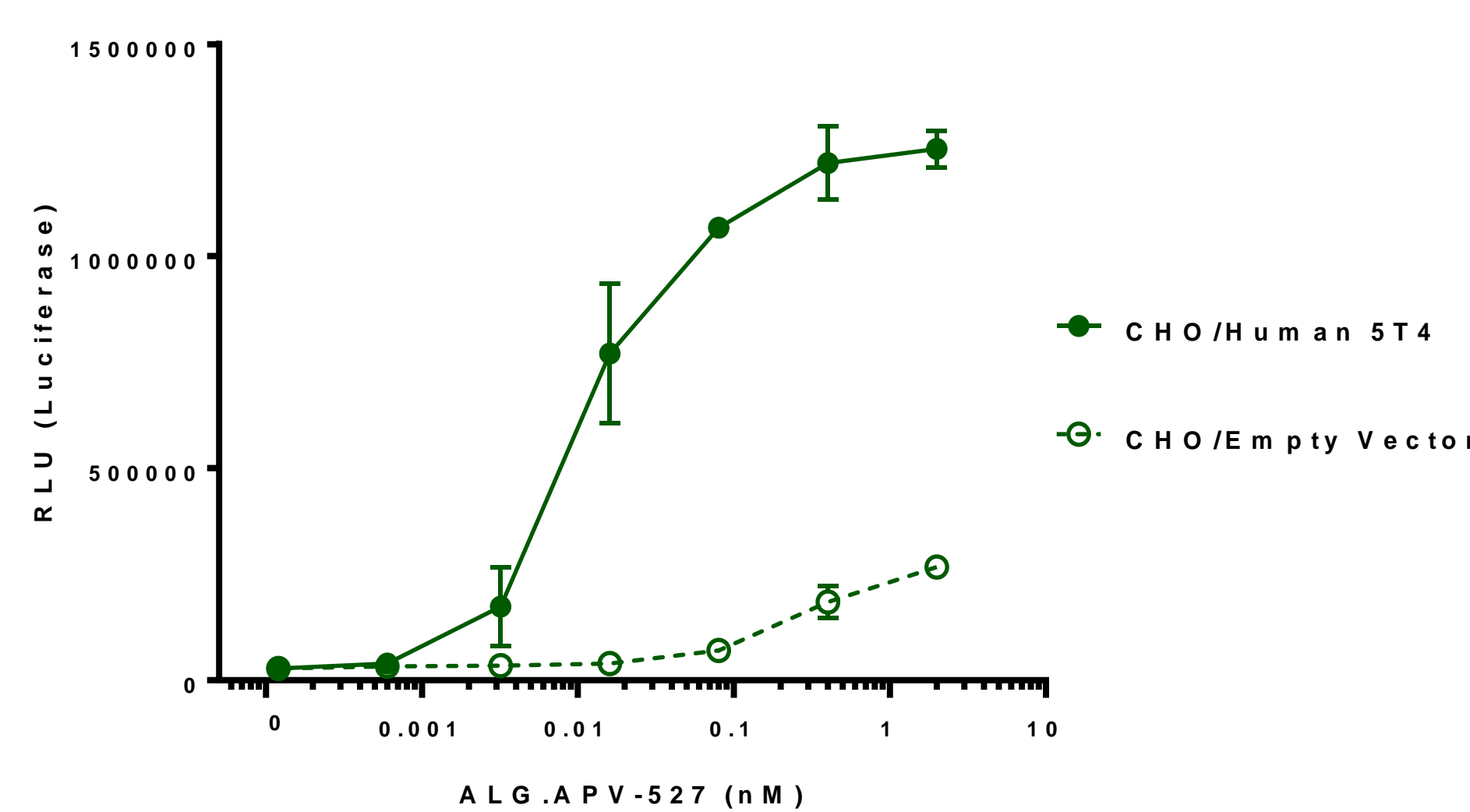
B Variants were also evaluated for binding to CHO cells expressing human 4-1BB. The constructs had similar binding properties as the unoptimized parent sequence.

Stabilizing Disulfide Reduces Aggregate Formation During Storage

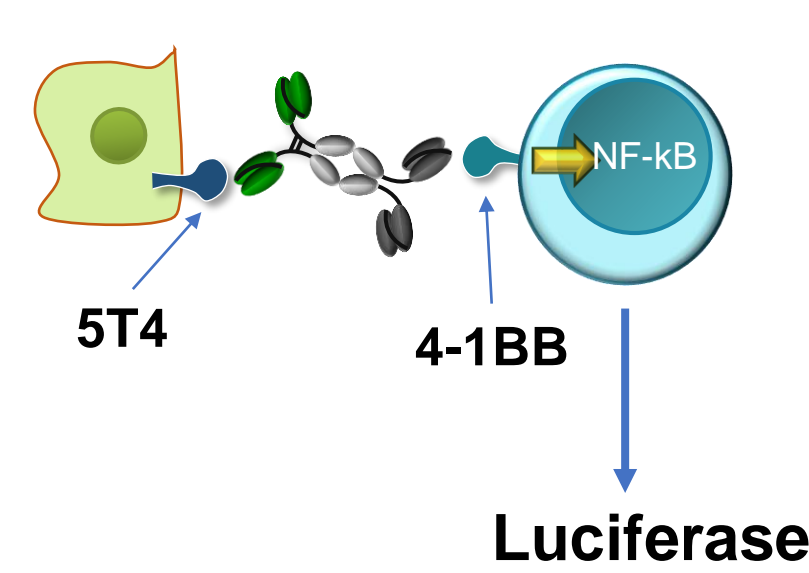


Additional cysteine residues were incorporated into the VH and VL of the c-terminal scFv to form a disulfide bond. A significant reduction in aggregate formation during storage was observed with constructs containing the disulfide (denoted by SS).

ALG.APV-527 Only Stimulates 4-1BB in the Presence of 5T4+ cells

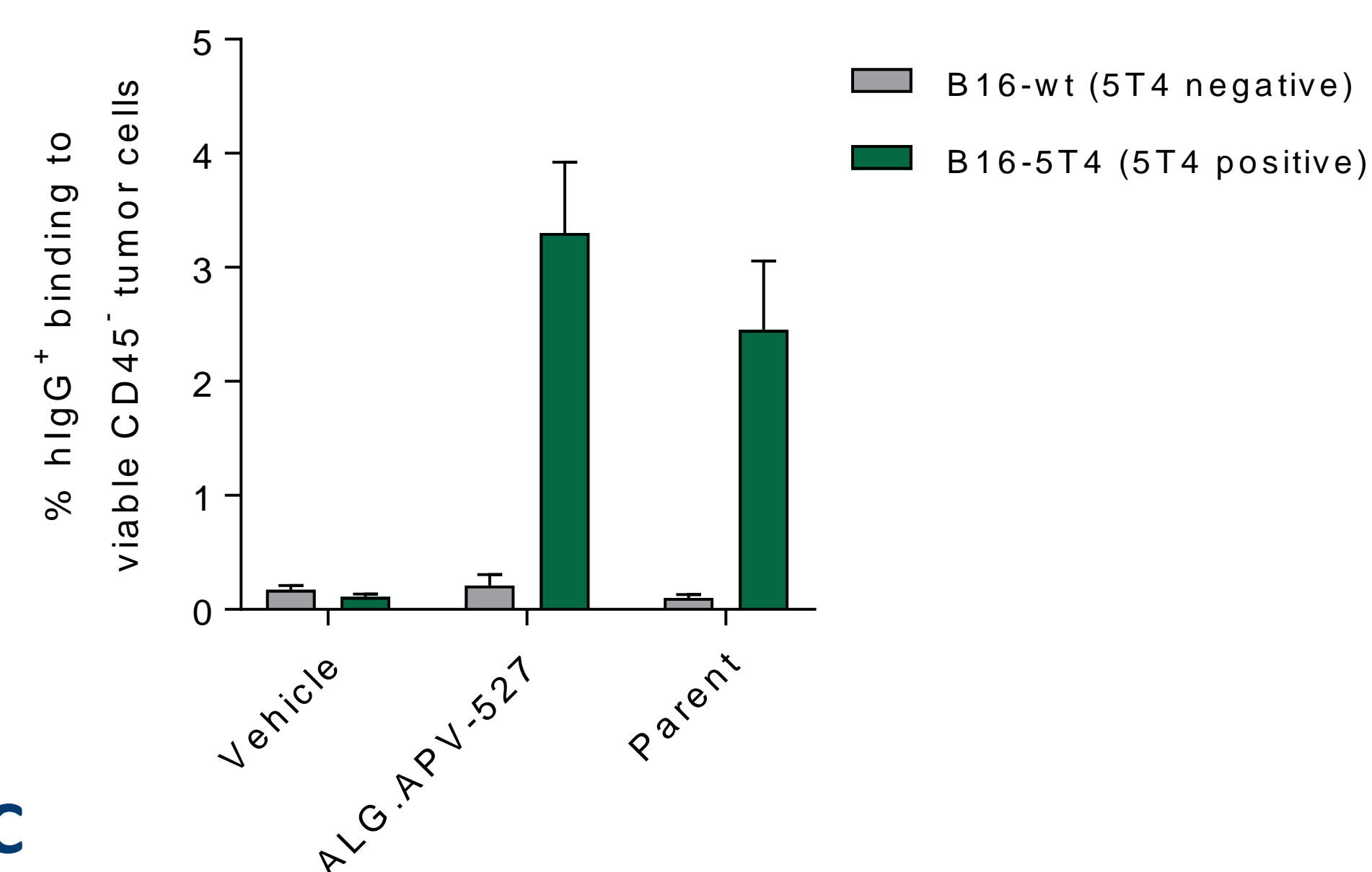
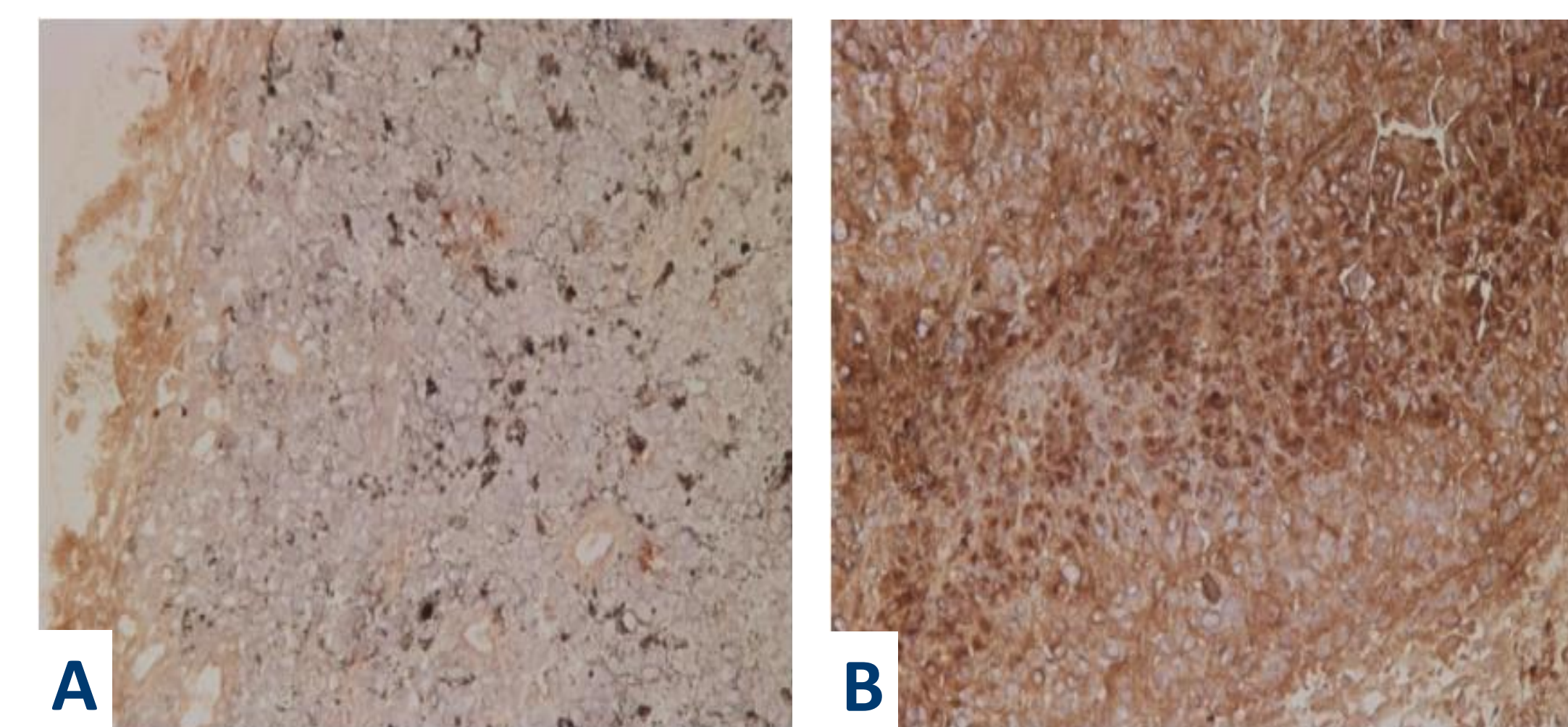


Jurkat/NF-κB reporter cells



4-1BB reporter assay set up using NFκB/4-1BB/Jurkat cells and CHO-K1 cells. ALG.APV-527 and a negative control were run with CHO-K1 cells expressing either human 5T4 or empty vector. The RLU (luminescence) was used to measure 4-1BB pathway activation.

Tumor Localization of ALG.APV-527



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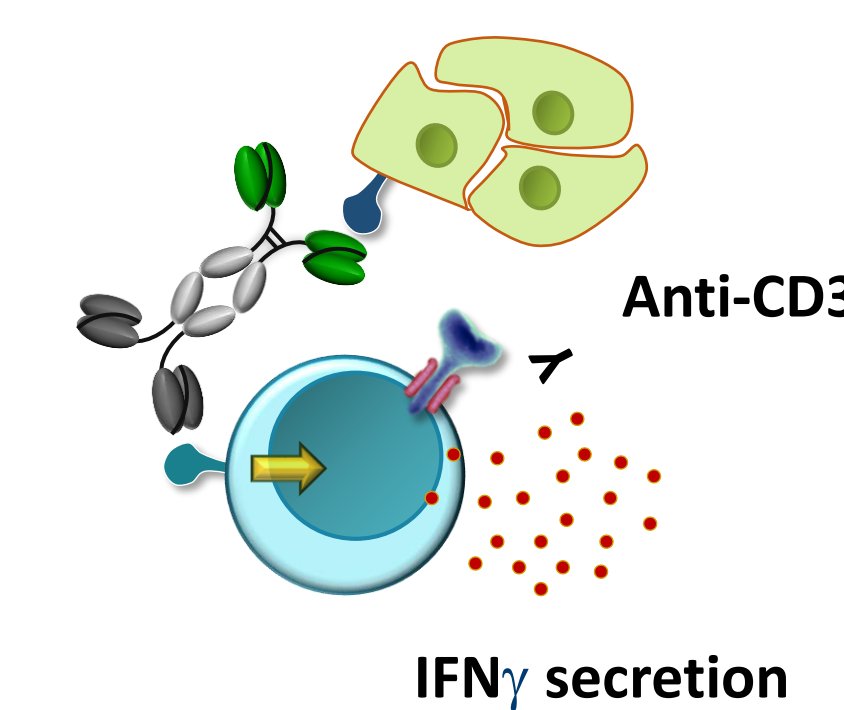
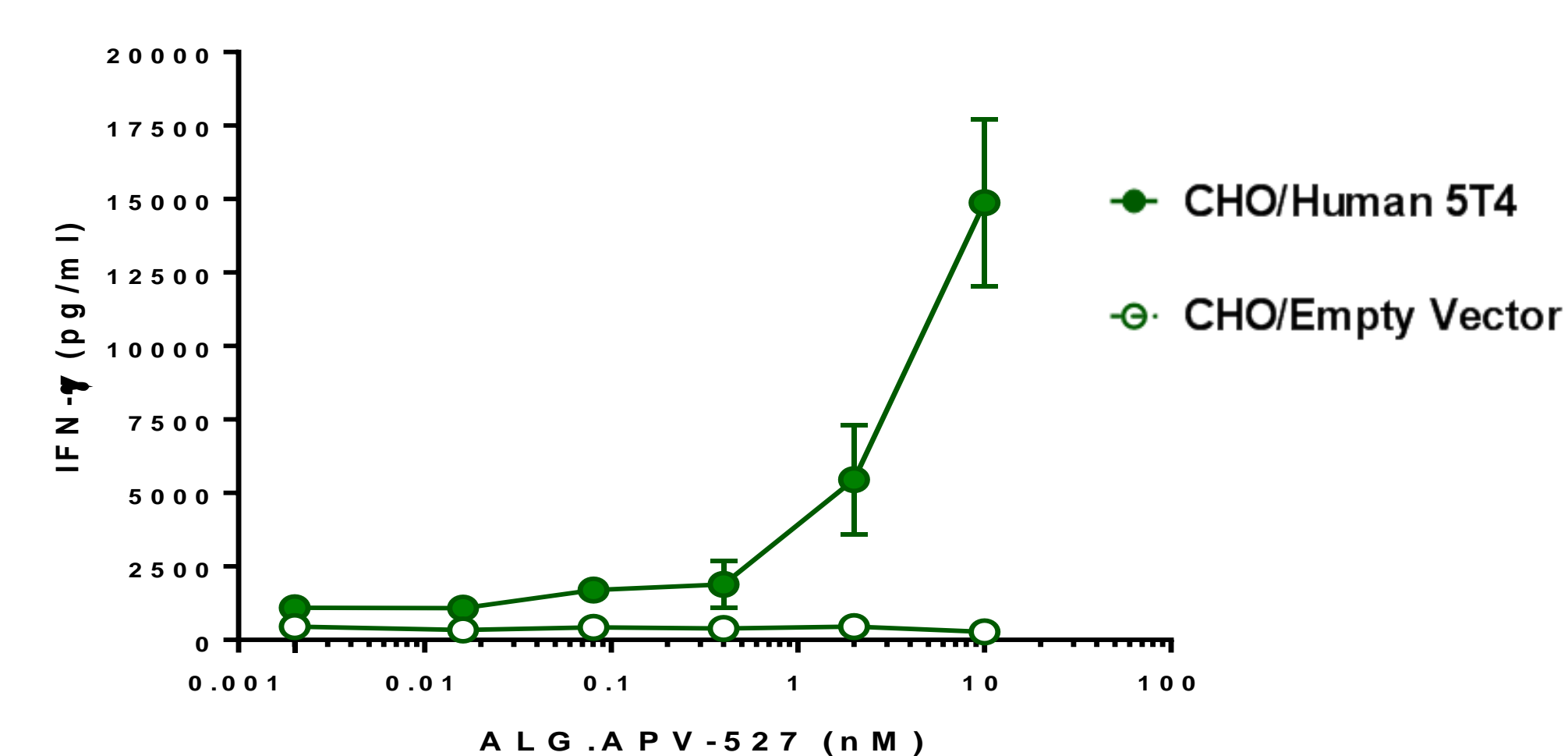
Antigen-dependent localization of ALG.APV-527 was evaluated in a B16 melanoma tumor model. Each mouse received one 5T4 negative and one 5T4 positive tumor at each side of the hind flank/back. The tumor cell lines, growing in log phase, were injected subcutaneously (1×10^5 cells in 100 μ L) on Day 0. Intraperitoneal construct treatments (100 μ g) were given on day 6 and 13 and mice were sacrificed on day 14 (24 h after the final treatment).

Panel A: IHC of 5T4-negative tumor stained with an antibody detecting human IgG

Panel B: IHC of 5T4-positive tumor stained with an antibody detecting human IgG

Panel C: FACS analysis of 5T4 positive or negative tumors using an antibody detecting human IgG binding to 5T4 positive tumor cells.

ALG.APV-527 Enhances IFN γ Release After Primary T-Cell Stimulation



T-cell activation was assessed via IFN γ production. Human PBMCs were cultured on plates coated with anti-CD3 mAb, 5T4-Fc, and different concentrations of ALG.APV-527. IFN γ concentration was determined by ELISA. In the presence of 5T4, ALG.APV-527 enhanced the production of IFN γ . In the absence of 5T4, IFN γ was not detected above background.

Summary and Conclusions

- Significant improvement in the solubility and stability of the anti-4-1BB x anti-5T4 ADAPTIR molecule, ALG.APV-527, was achieved through the use of phage display and inclusion of a stabilizing disulfide bond
- ALG.APV-527 was shown to localize to the site of 5T4 positive tumors and only stimulate T cells in the presence of 5T4-expressing cells
- ALG.APV-527 is a promising therapeutic for 5T4-expressing solid tumors that may enhance generation of tumor specific cytolytic T cells without the dose-limiting toxicities observed with another anti-4-1BB monotherapy