

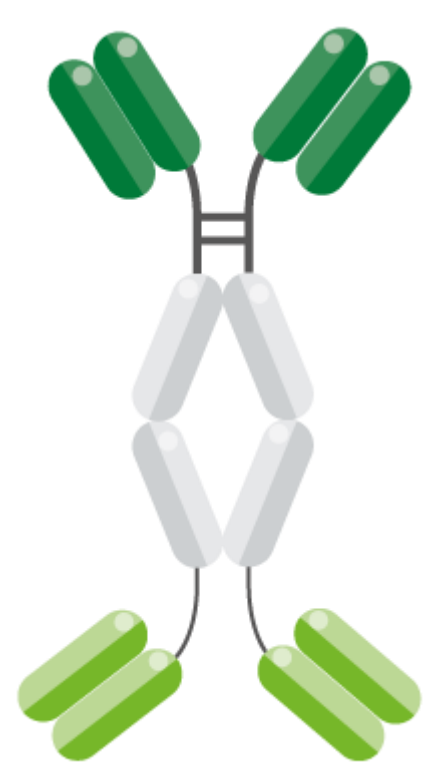
# Tumor Antigen-dependent T Cell Activation And Tumor Localization Induced By A Novel 4-1BB X 5T4 ADAPTIR™ Bispecific Antibody

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## About ALG.APV-527

ALG.APV-527 is a human 4-1BB x 5T4 targeting bispecific antibody in the ADAPTIR format

Anti-4-1BB scFv



Anti-5T4 scFv

- ALG.APV-527 contains two different binding domains targeting 4-1BB and 5T4, generated from the ALLIGATOR-GOLD® human scFv library (Alligator Bioscience)
- Each scFv has then been optimized and developed for use in the bispecific ADAPTIR format (Aptevo Therapeutics)
- The binding domains are linked to a silent immunoglobulin Fc domain providing an antibody-like half-life
- ALG.APV-527 is a bispecific tumor-directed 4-1BB x 5T4 antibody featuring target driven T cell activation, optimized stability and good manufacturing properties with potential for an improved risk-benefit compared with monospecific 4-1BB antibodies

## Summary and Conclusion

Summary, ALG.APV-527:

- Induces a potent tumor directed immune activation in both a 4-1BB reporter assay and a PBMC assay, with minimal activity in the absence of the tumor antigen 5T4
- Activates primary T cells, in the presence of tumor cells with a wide range of 5T4 receptor densities
- Effectively localizes to 5T4 positive tumors *in vivo*
- Inhibits tumor growth in a humanized xenograft tumor model

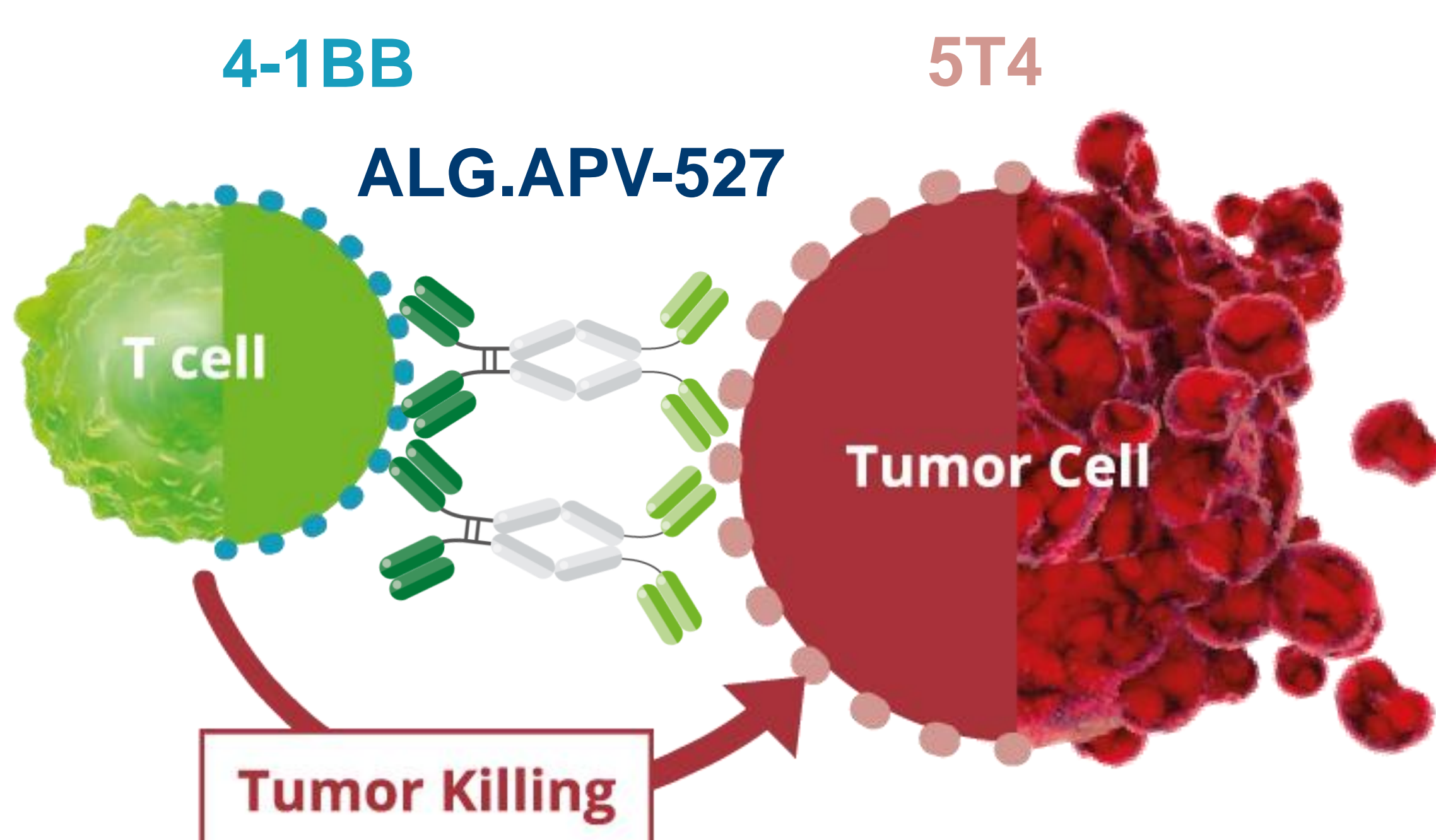
Conclusion

ALG.APV-527 has the potential to be a unique anti-cancer therapeutic agent with an improved 4-1BB safety profile for the treatment of a variety of 5T4-expressing solid tumors with a high unmet medical need

ALG.APV-527 is expected to begin Phase 1 clinical development in 2019

## ALG.APV-527 Mode of action

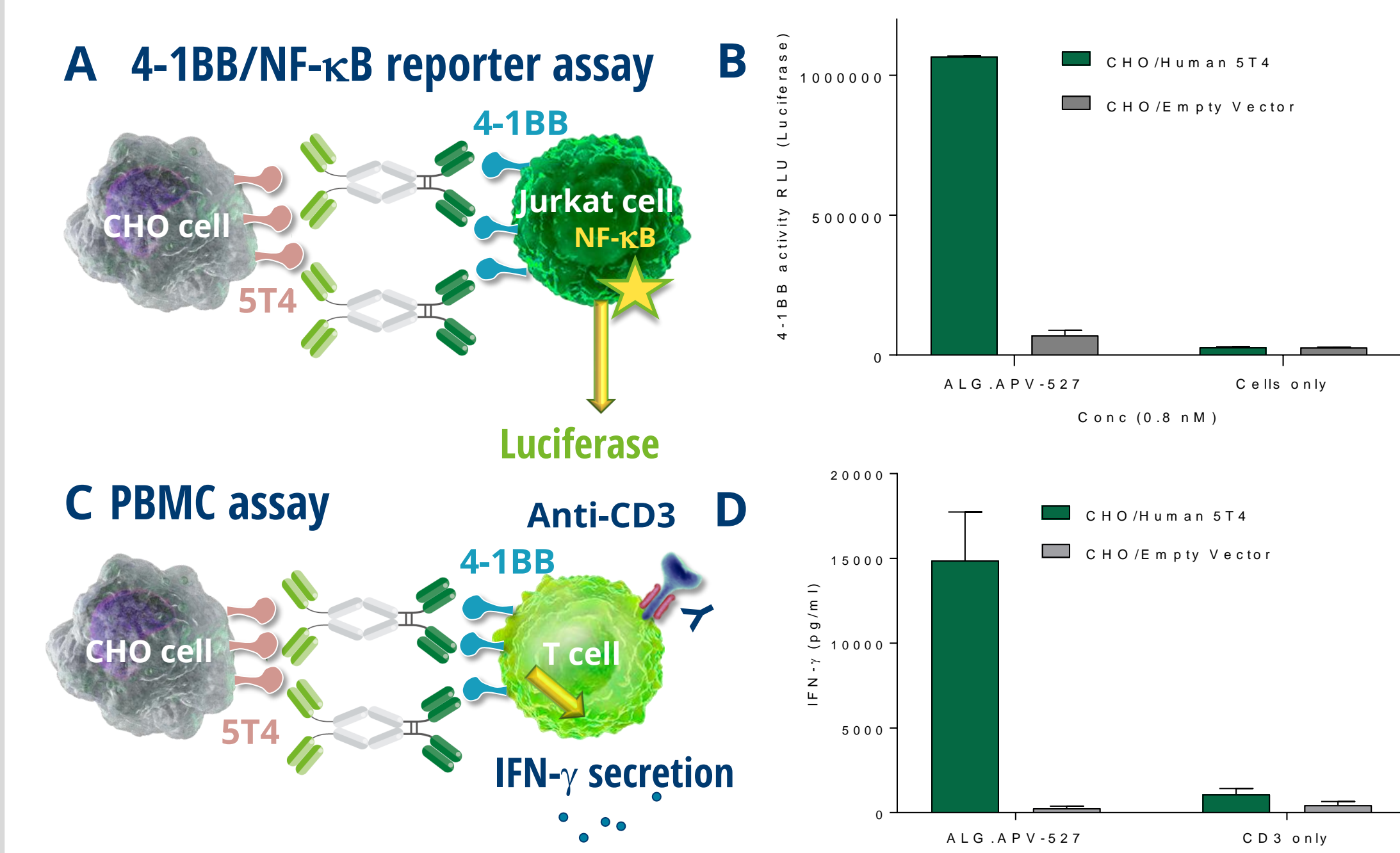
ALG.APV-527 is designed to induce potent tumor specific CD8 T cell activation in the tumor, while minimizing unwanted systemic toxicities



- 4-1BB is highly expressed on tumor infiltrating T cells
- 5T4 is highly expressed on tumor cells
- ALG.APV-527 binds and stimulates 4-1BB on T cells, but only when 5T4 is engaged on tumor cells

## 4-1BB activity is 5T4 dependent

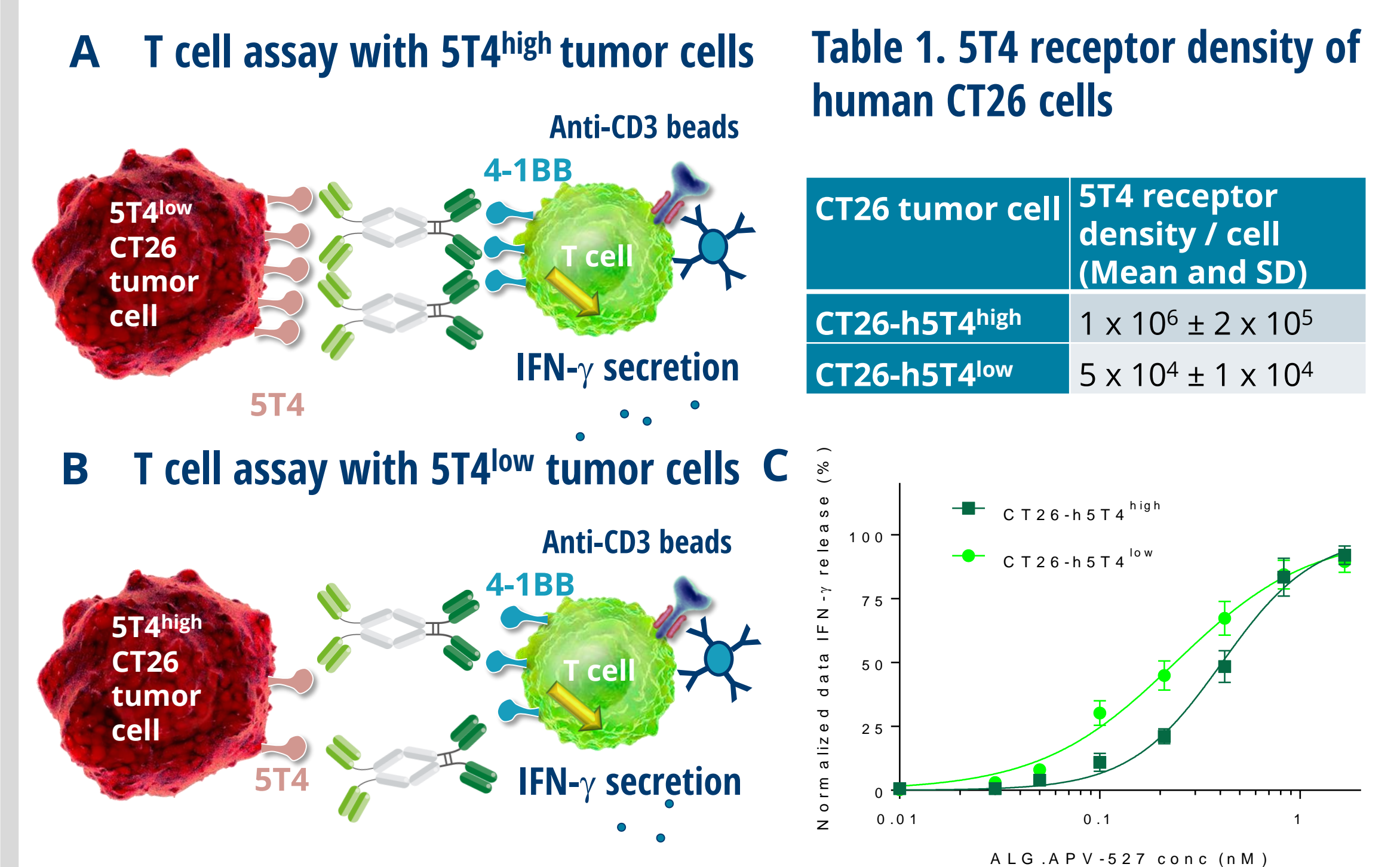
ALG.APV-527 demonstrates a potent 5T4-dependent activity in both a 4-1BB NF-κB reporter assay and a PBMC assay



**Figure 1. 5T4-dependent activation of 4-1BB in a reporter and a PBMC assay**  
A Experimental setup of a 4-1BB reporter assay using NFκB/ 4-1BB/ Jurkat cells and CHO-K1 cells expressing either human 5T4 or empty vector. The RLU (luminescence) was used to measure 4-1BB pathway activation.  
B ALG.APV-527 activates 4-1BB in the reporter assay in the presence of 5T4.  
C Experimental setup of a PBMC assay using PBMC, anti-CD3 and CHO-K1 cells expressing 5T4 or empty vector. Human PBMC were co-cultured in plates with CHO cells, anti-CD3 mAb in solution and ALG.APV-527 at 10 nM. PBMC activation was assessed via IFN-γ production by ELISA.  
D In the presence of 5T4, ALG.APV-527 enhances the production of IFN-γ. In the absence of 5T4, IFN-γ was not detected above background. Graph show mean ± SD of a representative donor.

## ALG.APV-527 enhances T cell activation

ALG.APV-527 increases CD8 T cell IFN-γ production in the presence of 5T4 high- and low-expressing tumor cells



**Figure 2. Enhanced T cell effector function (IFN-γ production)**  
A-B Experimental setup of a T cell assay using primary CD8 T cells and UV-irradiated CT26 cells transfectants expressing either high or low levels of human 5T4 (Table 1). Primary CD8 T cells were incubated with ALG.APV-527 and anti-CD3 coated on beads at a 1:1 T cell/ beads ratio and T cell activation was measured after 72 h, via IFN-γ production by ELISA.  
C ALG.APV-527 increases CD8 T cell effector function, as measured by IFN-γ production in the presence of tumor cells expressing both high and low levels of 5T4, while in the absence of the 5T4 it is essentially inert (not shown). Graph show normalized data mean ± SEM of 12 donors.

CT26 tumor cell	5T4 receptor density / cell (Mean and SD)
CT26-h5T4 <sup>high</sup>	1 × 10 <sup>6</sup> ± 2 × 10 <sup>5</sup>
CT26-h5T4 <sup>low</sup>	5 × 10 <sup>4</sup> ± 1 × 10 <sup>4</sup>

## Dual targeting of 4-1BB and 5T4

More about 4-1BB (CD137)

4-1BB is an activation-induced costimulatory receptor expressed on antigen-specific activated CD8 T cells and NK cells. Upon 4-1BB stimulation, CD8 T cells undergo enhanced proliferation, increased survival, intensify their cytotoxic activity and enhance IFN $\gamma$  production. The ability to induce a potent and long-lived anti-tumor activity by stimulating 4-1BB on tumor specific T cells makes 4-1BB-targeting immuno-therapeutic strategies very attractive. However, clinical development of a strongly agonistic monospecific 4-1BB antibody has been hampered by dose-limiting hepatic toxicities.

More about 5T4

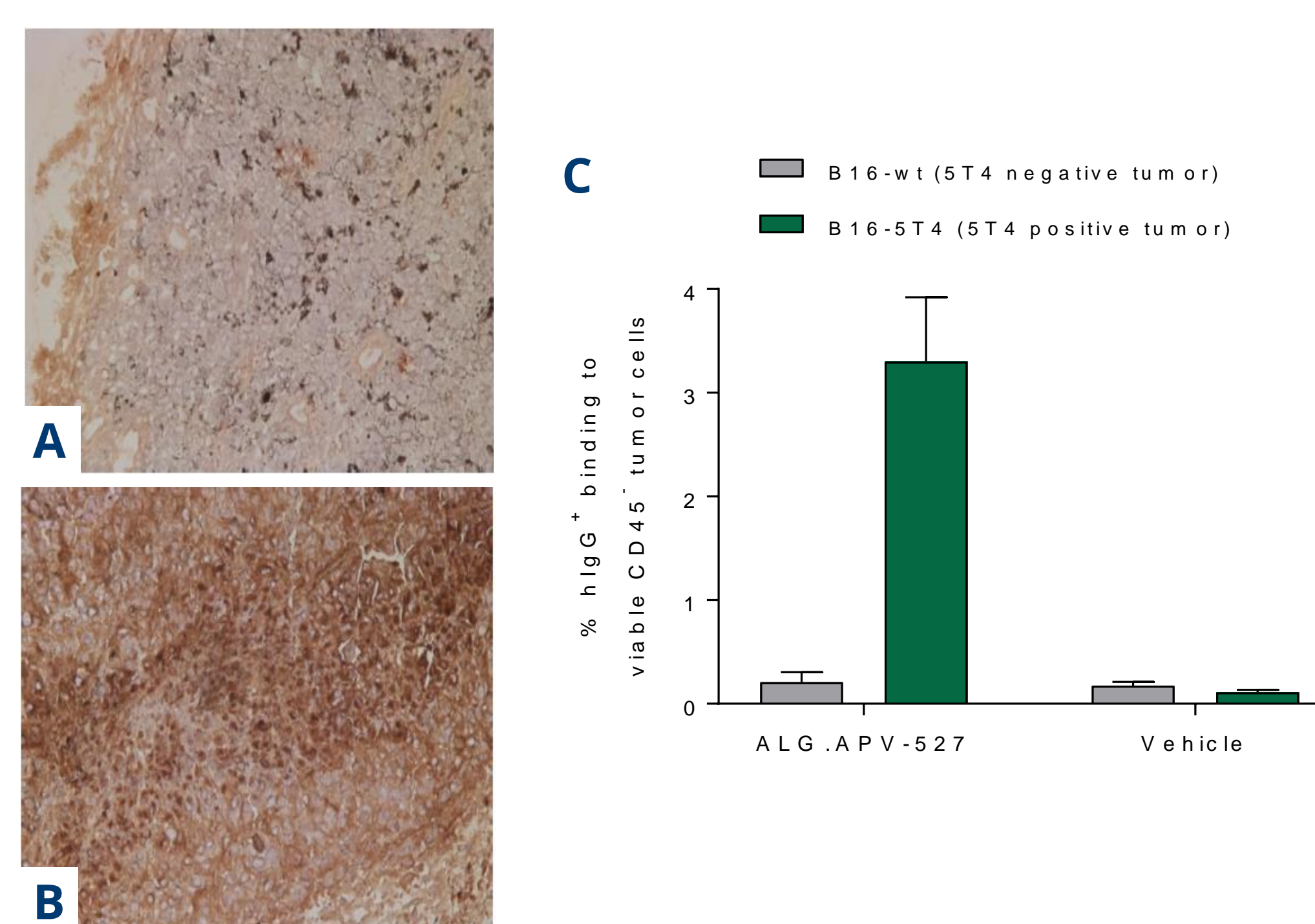
5T4 is a well-defined tumor-associated antigen expressed in a high proportion of patients in a variety of malignancies, including non-small cell lung, renal, pancreas, prostate, breast, colorectal, gastric, ovarian and cervix cancers and in acute lymphocytic leukemia.

Why dual targeting of 4-1BB and 5T4?

Dual targeting of ALG.APV-527 directs the immune stimulatory effect of 4-1BB to the 5T4-expressing tumor area, potentially minimizing dose-limiting toxicities. This feature makes ALG.APV-527 an attractive drug candidate for many different tumor indications.

## ALG.APV-527 localizes to the tumor

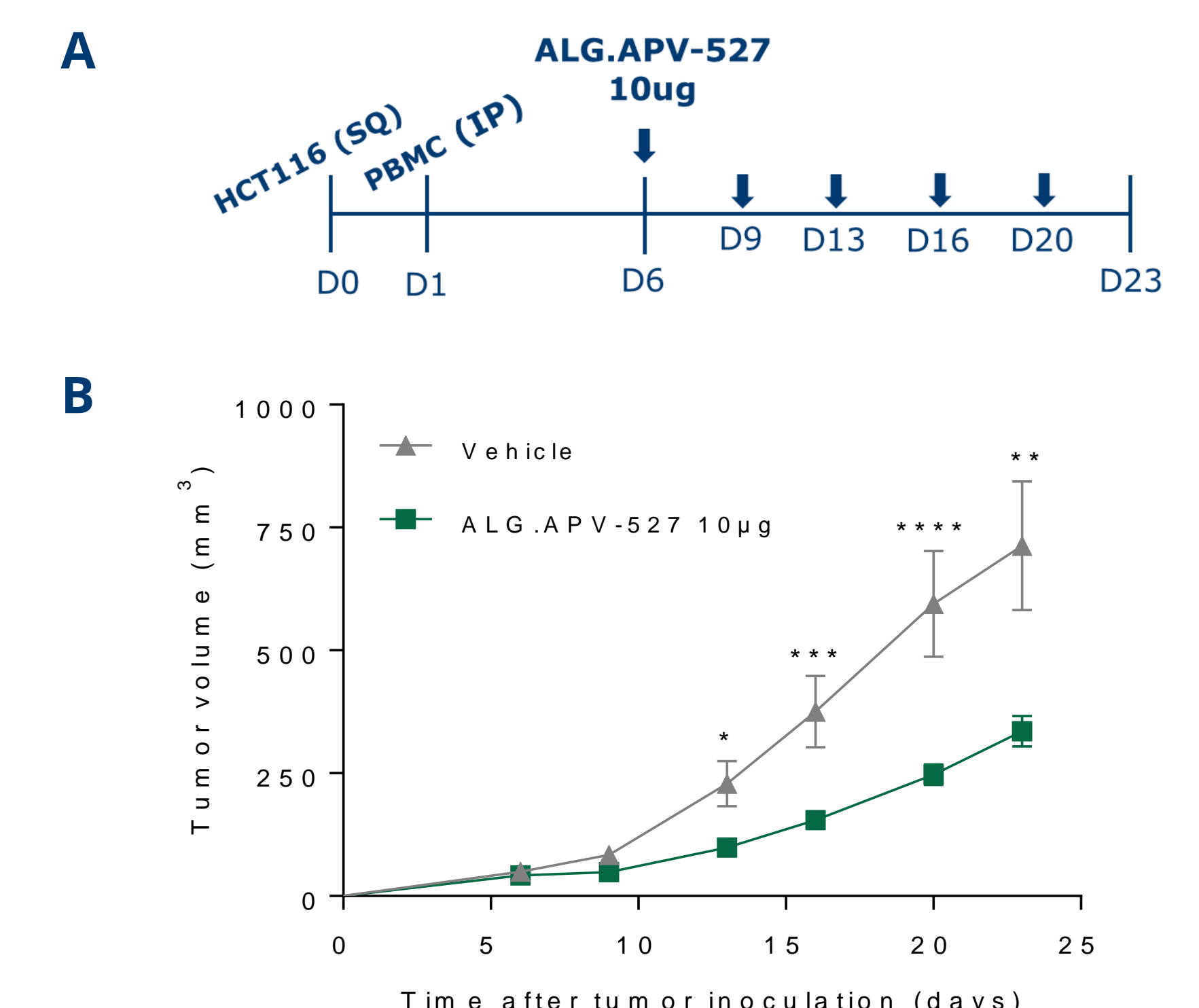
ALG.APV-527 localizes to 5T4-positive tumors in an antigen-dependent manner in a B16 melanoma twin tumor model



**Figure 3. 5T4-dependent localization of ALG.APV-527 in a B16 twin tumor model**  
Day 0: each mouse received one 5T4 negative and one 5T4 positive B16 tumor injected subcutaneously (SQ, 1x10<sup>6</sup> cells in 100  $\mu$ L) at each side of the hind flank/back. Intraperitoneal (IP) treatment of ALG.APV-527 (100  $\mu$ g) was given on day 6 and 13 and mice were sacrificed on day 14 (24 h after the final treatment). Tumors were collected and the levels of human IgG (hlgG) positive cells were assessed either by IHC or flow cytometry using an antibody detecting human IgG.  
A-B IHC of ALG.APV-527 binding to A 5T4-negative tumors or B 5T4-positive tumors  
C Flow cytometry of ALG.APV-527 binding to dissociated 5T4-positive and -negative tumor cells (percentage of hlgG+ cells out of live CD45+ cells)

## ALG.APV-527 inhibits tumor growth

ALG.APV-527 inhibits tumor growth in a human xenograft HCT116 colon carcinoma model



**Figure 4. ALG.APV-527 inhibits tumor growth in a 5T4-positive human xenograft HCT116 colon carcinoma model**  
A Experimental setup: HCT116 colon carcinoma cells with endogenous expression of 5T4 were injected SQ into the flank of SCID-beige mice. Fresh human PBMCs from 4 donors were injected IP the day following tumor inoculation (n=5 mice per treatment group/donor, total of 20 mice/treatment). ALG.APV-527 treatments (10  $\mu$ g) were given twice weekly starting on day 6 and ending on day 20.  
B A statistically significant decrease in tumor size was observed from day 13 to 23 in comparison with the vehicle group, as evaluated by Mann-Whitney, non-parametric 2-tailed t test. Graph shows mean ± SEM.