# Activation of the CD137 Pathway in T cells by a CD137 x 5T4 bispecific ADAPTIR™ Molecule Requires Co-engagement of CD137 and 5T4

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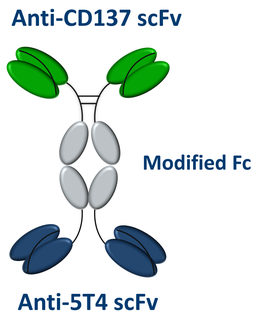
## Introduction

- CD137 (4-1BB) is a key costimulatory immune receptor (member of the TNFR-superfamily) that is highly expressed on tumor infiltrating effector T cells and NK cells.
- Stimulation of CD137 leads to enhanced proliferation, increased survival, intensified cytolytic activity of T cells and induced IFN-γ production.
- CD137 monoclonal antibody therapies have shown promising anti-tumor effects in the clinic, but systemic immune stimulation have induced dose-limiting hepatic toxicities.
- ALG.APV-527, a novel anti-CD137 x anti-5T4 bispecific antibody, was developed based on the ADAPTIR™ technology with binding domains from the Alligator-Gold® human scFv library and optimized for binding, stability and function.
- ALG.APV-527 directs the activation of T cells to 5T4-expressing tumors, thereby minimizing the toxicity observed with other CD137 therapeutics.

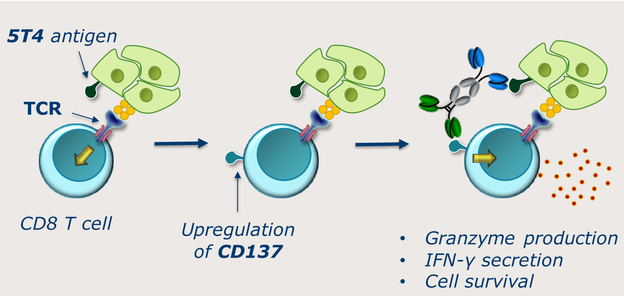
## ALG.APV-527

- ADAPTIR molecules are bispecific antibody-like therapeutics containing two sets of binding domains linked to an immunoglobulin Fc domain to extend the *in vivo* half-life.

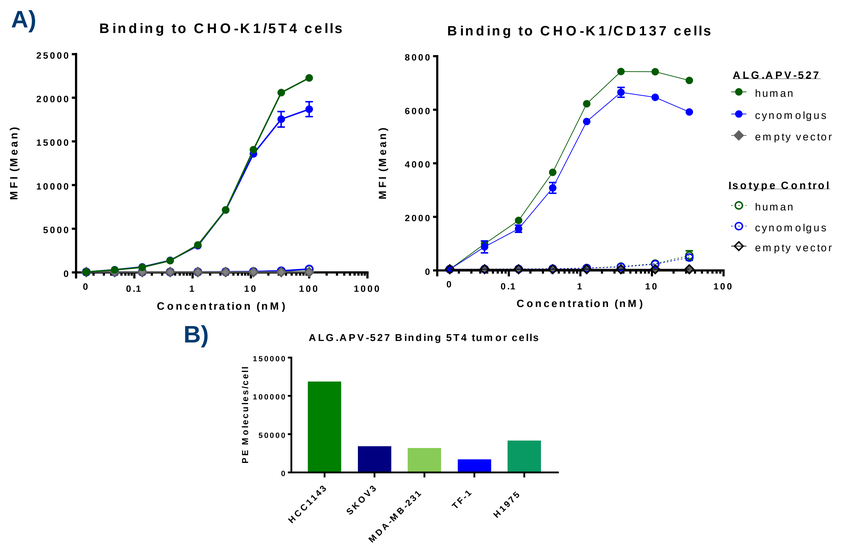



- The anti-CD137 x anti-5T4 ADAPTIR molecule binds both CD137 and 5T4 to enhance the immune response of CD137-expressing T cells.
- To limit interactions with other components of the immune system that could lead to non-specific T cell activation, the Fc region has been engineered to minimize complement fixation and interaction with Fcγ receptors.

## ALG.APV-527 Mechanism of Action



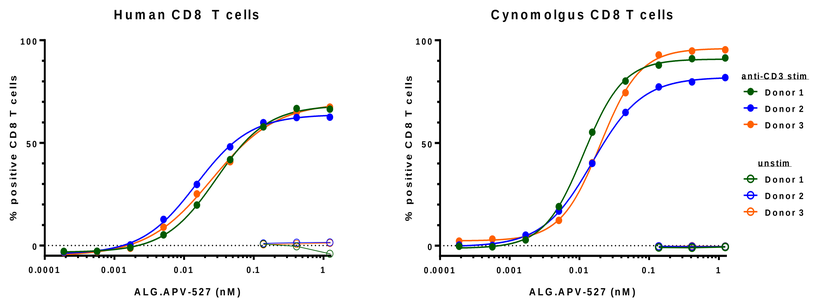

## Figure 1. ALG.APV-527 Binds to 5T4⁺ and CD137⁺ Cells




(A) Binding of ALG.APV-527 to 5T4 and CD137 was assessed by flow cytometry on CHO-K1 cells stably expressing full length human and cynomolgus 5T4 or CD137. Isotype control ADAPTIR and empty vector transfected CHO-K1 cells were used. An anti-human IgG secondary antibody was used to detect binding. .

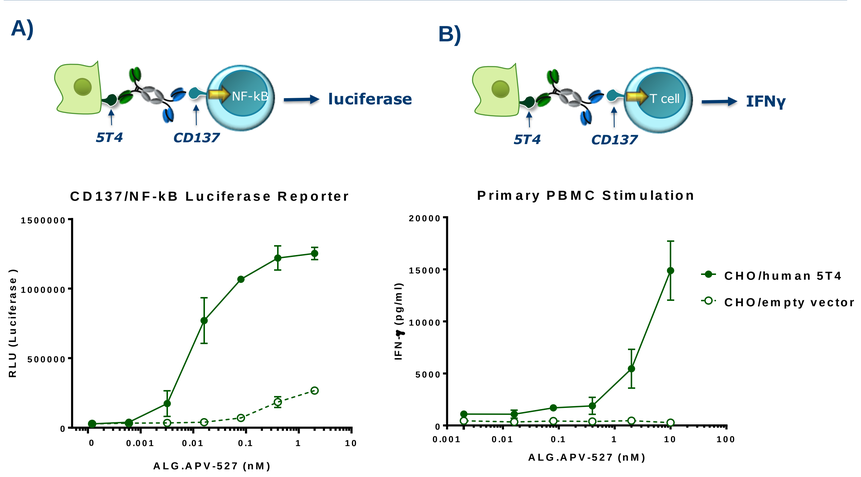
(B) ALG.APV-527 binding at 100nM was tested on 5T4-expressing tumor lines, HCC1143, SKOV3, MDA-MD-231, H1975 and TF-1 cells and assessed by flow cytometry. PE molecule numbers were determined using Quantibrite beads.

## Figure 2. ALG.APV-527 Binds Human & NHP Activated T cells




Human and cynomolgus PBMC were stimulated or not for 48h with anti-CD3. Biotinylated ALG.APV-527 was serially diluted and incubated for 30 minutes, followed by streptavidin-APC and Ab to CD3 and CD8. Cells were gated on CD3⁺CD8⁺ cells and analysed for ALG.APV-527 binding using flow cytometry.

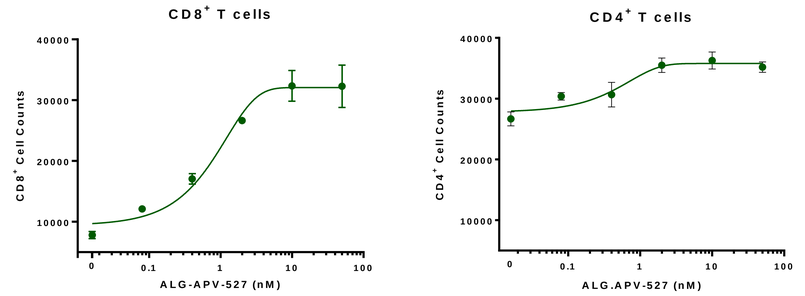
## Figure 3. ALG.APV-527 Only Stimulates CD137 in the Presence of 5T4




A) CD137 (NF-kB/luciferase) reporter cells were stimulated with serial dilutions of ALG.APV-527 in the presence of 5T4 or empty vector transfected CHO-K1 cells for 5 hr. ALG.APV-527 induces CD137 activation only when 5T4-expressing cells are present- indicating the requirement of tumor antigen dependency.

B) Primary PBMC were stimulated with anti-CD3 Ab in solution and serial dilutions of ALG.APV-527 in the presence of 5T4 or empty vector transfected CHO-K1 cells, and cultured for 72 hr; Supernatants were assessed for IFNγ production. ALG.APV-527 enhanced the production of IFNy in the presence but not absence of 5T4⁺ cells.

## Figure 4. ALG.APV-527 Induces CD8 T cell Proliferation



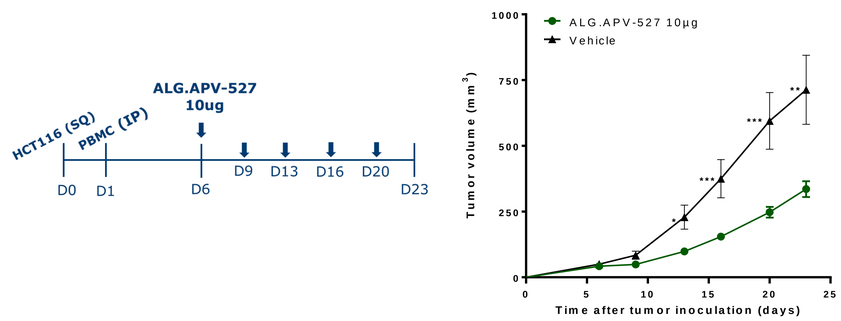

PBMC were stimulated with irradiated CHO-K1/human5T4, anti-CD3 and serial dilutions of ALG.APV-527 for 72 hours. T cell proliferation was assessed as an increase in CD4 and CD8 cell counts by flow cytometry. ALG.APV-527 primarily increased the number of CD8 but not CD4 T cells.

## Figure 5. Antibody-Like Half-Life

| Half-life | Cmax | Serum Clearance | Volume of Distribution | Area under the curve |
|---|---|---|---|---|
| 215 hr | 252 µg/ml | 0.204 mL/hr/kg | 63 mL/kg | 38705 hr x µg/ml |

Female Balb/c mice were injected intravenously (IV) with 200 µg (~10 mg/kg) of ALG.APV-527 in 200 µl volume. Anesthetized mice were exsanguinated via cardiac puncture for serum collection at t = 15 minutes, and 2, 6, 24, 48, 72, 96, 168, 336 and 504 hours after injection. Serum concentrations were determined using ELISA method that detects full length construct. Three mice were used per timepoint; 3 samples were excluded due to apparent ADA.

## Figure 6. ALG.APV-527 inhibits tumor growth of a 5T4⁺ human colon carcinoma *in vivo*




HCT116 colon carcinoma cells were injected SQ delivered into the flank of SCID-beige mice. Fresh human PBMC's from 4 donors were IP injected the day after tumor inoculation. There were 5 mice per group and donor for a total of 20/treatment. Treatments of ALG.APV-527 at 10ug were given twice weekly starting on day 6. Significant decreases in tumor size were observed starting from day 13 to 23 compared to the vehicle group. * P<0.05; ** P<0.1; *** P<0.001; Mann-Whitney, non-parametric 2-tailed *t* test.

## Summary

***In vitro***:

- Binds human and cynomolgus 5T4 and CD137 expressing cells
- Binds activated human and cynomolgus T cells
- CD137 reporter activity only when 5T4⁺ targets are present
- CD8⁺ T cell proliferation and IFN-γ production only in the presence of 5T4⁺ targets

***In vivo***:

- Demonstrated an extended antibody-like serum half-life of 9 days
- Reduction of colon carcinoma HCT116 tumor growth *in vivo*

## Conclusions:

The anti-CD137 x anti-5T4 ADAPTIR molecule ALG.APV-527 is a promising therapeutic for 5T4-expressing solid tumors, that may be able to enhance a patient's cytolytic T cell response. Because it is a targeted agent, it has the potential to avoid the dose-limiting hepatic toxicities seen with anti-CD137 antibody therapies currently in the clinic.