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Pre-clinical Safety and Efficacy of a Tumor-Directed 4-1BB x 5T4 ADAPTIR™ Bispecific Antibody

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Introduction

The ability to induce potent anti-tumor activity by stimulating 4-1BB on tumor specific T cells makes 4-1BB-targeting immunotherapeutic strategies very attractive. However, clinical development of a strongly agonistic monospecific 4-1BB antibody has been hampered by dose-limiting hepatic toxicities. To improve safety of 4-1BB targeting therapies we have developed a 4-1BB x 5T4 bispecific antibody designed to direct tumor-specific T cell responses to the tumor by stimulating 4-1BB only when co-engaged with 5T4. 5T4 is a tumor associated antigen expressed on multiple solid tumors. This feature localizes the immune stimulatory effects of ALG.APV-527 to the tumor microenvironment where both 4-1BB and 5T4 are highly expressed. Here we present pre-clinical data that highlights the mechanism of action and safety profile of ALG.APV-527, supporting its advancement into the clinic.

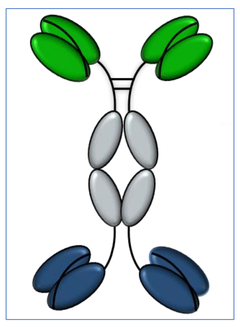
ALG.APV-527 was built using the ADAPTIR™ bispecific platform with binding domains from the ALLIGATOR-GOLD® human scFv library. Its 5T4-dependent agonistic function was assessed using primary CD8⁺ T cells in the presence of 5T4. Secretion of IFN-γ was measured at 72 hrs using ELISA, and proliferation was measured by flow cytometry on Cell Trace™-labeled cells. The murine B16 tumor line transfected with human 5T4 was used to demonstrate drug biodistribution *in vivo*. The pre-clinical safety profile of ALG.APV-527 was evaluated in a dose-range toxicology study, with both single and repeated doses, in non-human primates (NHP). The study design included all the standard repeated dose toxicity parameters and, in addition, pharmacokinetics, immunogenicity, and pharmacodynamic end-points.

5T4 (trophoblast glycoprotein, TPBG)

- Oncofetal tumor associated antigen
- Expressed in trophoblasts
- Overexpressed in numerous solid tumors: NSCLC, bladder, breast, cervical, ovarian, pancreatic, renal, gastric, colorectal, head and neck and mesothelioma
- Limited normal tissue expression
- Involved in cell motility, migration and metastasis
- Expressed on tumor initiating cells
- Has been associated with clinical outcome

About ALG.APV-527 ADAPTIR Molecule

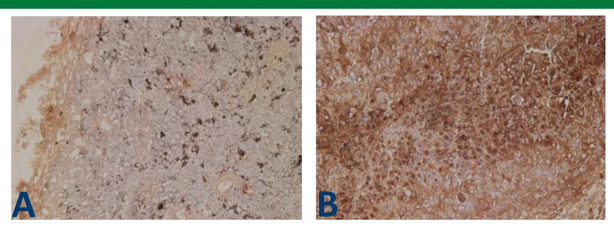
Anti-4-1bb



Anti-5T4

- A tumor-directed 4-1BB x 5T4 targeting antibody in the ADAPTIR™ bispecific platform
- Contains two sets of binding domains, originating from the Alligator Gold® human scFv library, that has been optimized for use in Aptevo´s ADAPTIR™ bispecific platform
- Mutated IgG Fc domain, to reduce FcγR mediated effector functions, providing an extended antibody-like serum half-life of ~ 5-9 days in pre-clinical studies
- Features target-driven T cell activation, optimized stability and good manufacturing properties
- Designed to improve risk-benefit, overcoming efficacy and safety issues of other monospecific 4-1BB agonists

ALG.APV-527 Localized to 5T4+ Tumors

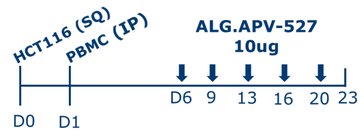


Antigen-dependent localization of ALG.APV-527 was evaluated in a B16 melanoma tumor model. Each mouse received one 5T4 negative and one 5T4 positive tumor at each side of the hind flank/back. The tumor cell lines, growing in log phase, were injected subcutaneously (1x10^5 cells in 100 µL) on Day 0. Intraperitoneal construct treatments (100 µg) were given on day 13 and 15 and mice were sacrificed on day 16 (24 hours after the final administration).

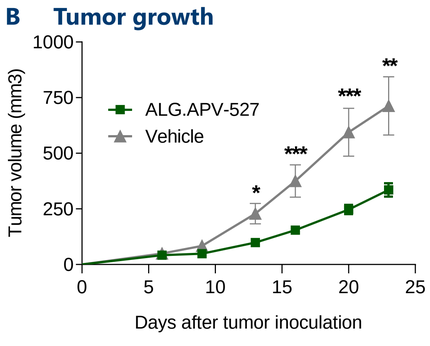
IHC of 5T4-negative (**A**) and 5T4-positive (**B**) tumor stained with an antibody detecting human IgG

ALG.APV-527 inhibits tumor growth in human xenograft model

A Experimental set up



B Tumor growth

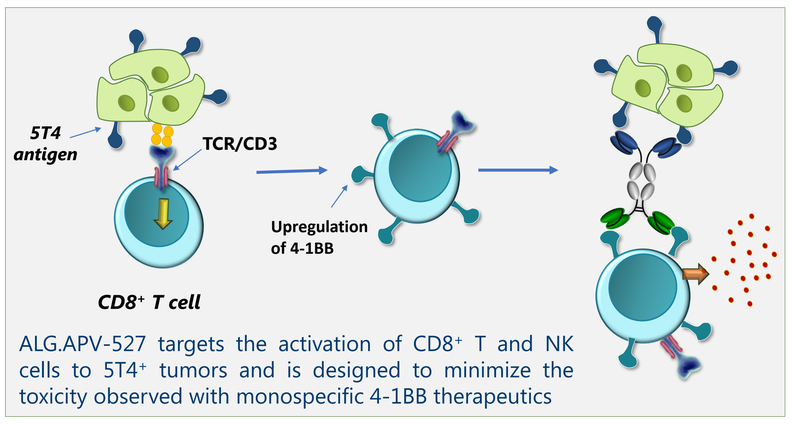


ALG.APV-527 inhibits tumor growth.

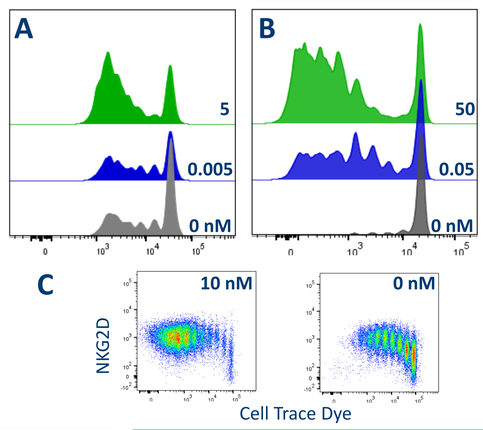
A) Experimental set up. Day 0: HCT116 cells injected SQ into the flank of SCID-beige mice. Day 1: Human PBMC's from 4 donors injected IP. 5 mice/group/donor total 20 mice/ treatment. Day 6-20. Treatments of ALG.APV-527 at 10µg administered IP twice weekly.

B) Significant decreases in tumor size was observed from day 13 following ALG.APV-527 treatment vs. vehicle, (Mann-Whitney, non-parametric 2-tailed *t* test).

ALG.APV-527 Mode of Action



ALG.APV-527 targets the activation of CD8⁺ T and NK cells to 5T4⁺ tumors and is designed to minimize the toxicity observed with monospecific 4-1BB therapeutics

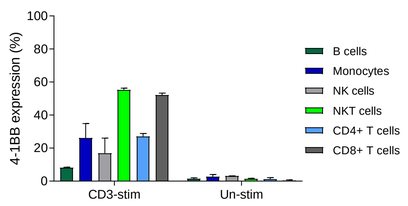


ALG.APV-527 augments CD8⁺T cell proliferation. Primary PBMC were stimulated with α-CD3 Ab in solution and serial dilutions of ALG.APV-527 in the presence of 5T4-expressing CHO-K1 cells. **(A)** Representation of the proliferation of CD8⁺ T cells from a single individual at different concentrations of ALG.APV-527.

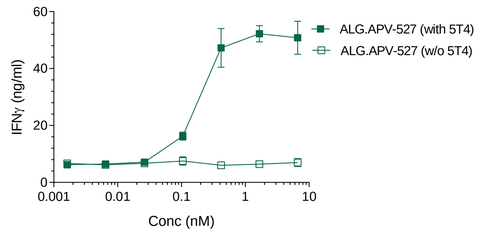
ALG.APV-527 enhances the proliferation and upregulation of NKG2D on NK cells. (B-C) Primary NK cells were stimulated with IL-2 and serial dilutions of ALG.APV-527 in the presence of 5T4-expressing CHO-K1 cells. **(B)** Representative proliferation of NK cells. **(C)** Representative expression of NKG2D on treated NK cells.

Cynomolgus Macaque is a Relevant Toxicology Species

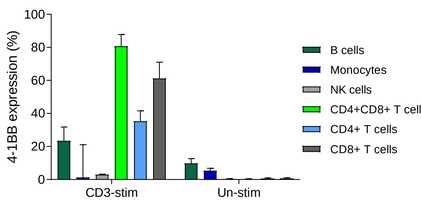
A) 4-1BB % on human PBMC



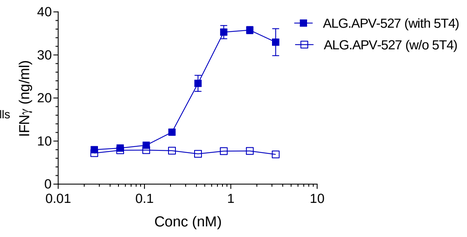
C) IFNγ - human CD8 T cells



B) 4-1BB % on cyno PBMC



D) IFNγ - cyno CD8 T cells



Target expression pattern of 4-1BB and 5T4-dependent T cell activity of ALG.APV-527 is similar in human and cynomolgus.

(**A-B**) Primary PBMC were stimulated with plate-bound α-CD3 for 48 hours, and 4-1BB expression was determined. 4-1BB expression pattern is similar between human (A) and cyno (B) PBMC.

(**C-D**) CD8 T cells were stimulated with plate-bound α-CD3 and soluble ALG.APV-527 with or without immobilized human 5T4. IFN-γ was measured in the supernatant at 72h (ELISA). IFNγ from human (C) or cynomolgus (D) individuals is shown. ALG.APV-527 potency (EC50) in human: 0.2 nM vs. cynomolgus 0.4 nM in the INFγ assay.

Non Human Primate (NHP) Study Design and Key Findings

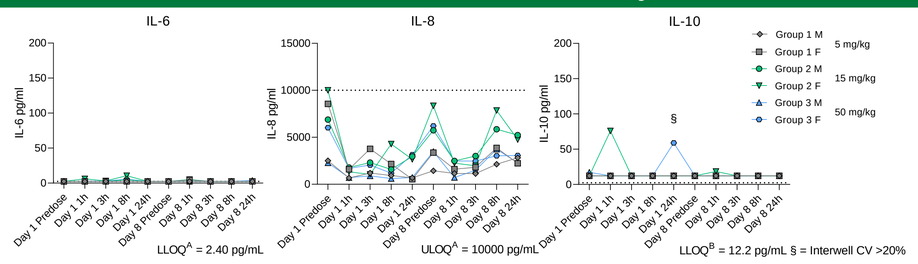
Group	Dose	Dose Days	Group Size
Group 1	5 mg/kg	1, 8, 15, 22	1 m + 1 f
Group 2	15 mg/kg	1, 8, 15, 22	1 m + 1 f
Group 3	50 mg/kg	1, 8, 15, 22	1 m + 1 f
Group 4	15 mg/kg	1	3 m

The safety of ALG.APV-527 was evaluated in a dose-range finding pilot toxicology study performed in cynomolgus macaques. ALG.APV-527 was administered intravenously in a total volume of 2.5 mL/kg into the tail vein for >1h. Three repeated-dose groups and one single-dose treated group were included in the study (see table). Samples were collected throughout the study for hematology and clinical chemistry, PK, ADA, cytokines and immunophenotyping of 22 immune cell populations analyzed by flow cytometry. Samples were also collected at necropsy for histology and histopathology (m, male; f, female)

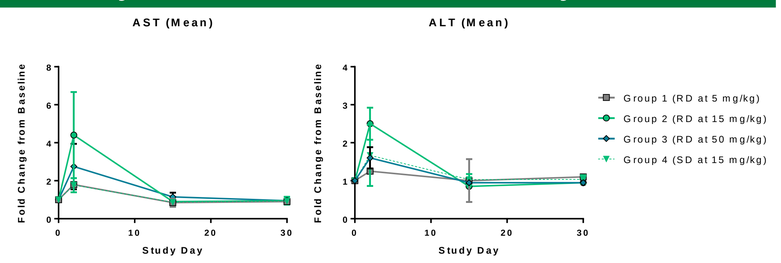
Key Findings from the Pilot Toxicology Study

- No adverse events were observed in any of the animals during or after dose administration
- Clinical pathology evaluations included standard hematology, coagulation and clinical chemistry parameters, with no indication of adverse reactions
- Liver enzymes AST and ALT levels showed minimal elevation at 24 hr that resolved to baseline by Day 15, indicating low risk for liver toxicity
- Cytokines measured after first and second dose were all low or below the limit of quantification (<LLOQ) of the assay except for IL-8 levels which decreased following doses, but recovered by the next dose
- Flow cytometry phenotypic analysis of 22 immune cell populations on Day 2, 8, 15 and 30 with no indication of drug-induced changes

ALG.APV-527 has a Favorable Safety Profile with no Indication of Systemic Activation or Liver Toxicity



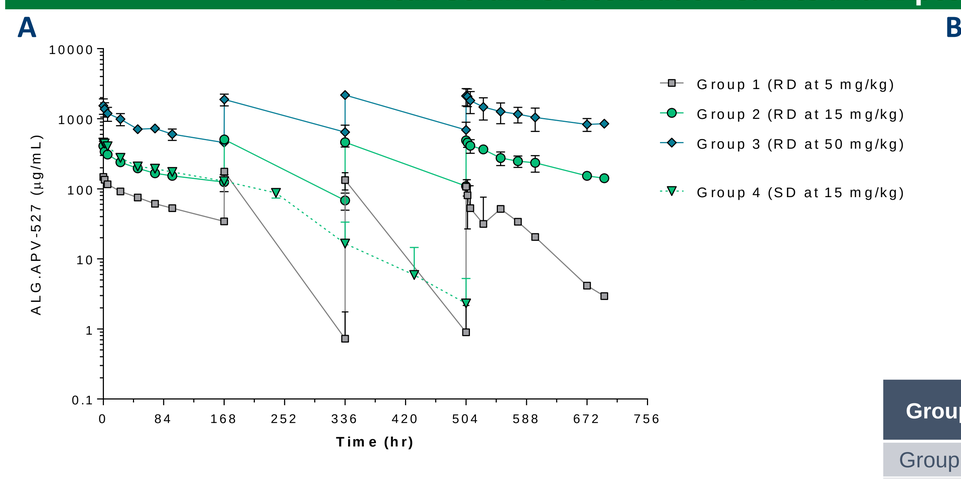
Unchanged cytokine levels. Plasma cytokines were measured using a Milliplex MAP Non-Human Primate Cytokine Kit (Millipore). For IL-1β, IL-2, IL-4, IL-6, IL-10, IFN-γ and TNF-α the concentrations were low or below the assay limit of quantification at all timepoints with no obvious dose effects. For IL-8, concentrations decreased from Day 1 Predose to Day 1 +24h, and after the second dose (Day 8); all groups showed recoveries in IL-8 levels.



Unchanged liver enzyme levels. Mean fold change of liver enzymes AST and ALT compared to duplicate pre-treatment samples. No long-lasting elevation in liver enzymes were observed during treatment. The slight peak seen on day 2 is transient and is not seen day 15 or 30 despite repeated administration weekly, and is therefore considered procedural and not drug-related.

Mean Serum Concentration Curves and Exposure Over Time for ALG.APV-527

A



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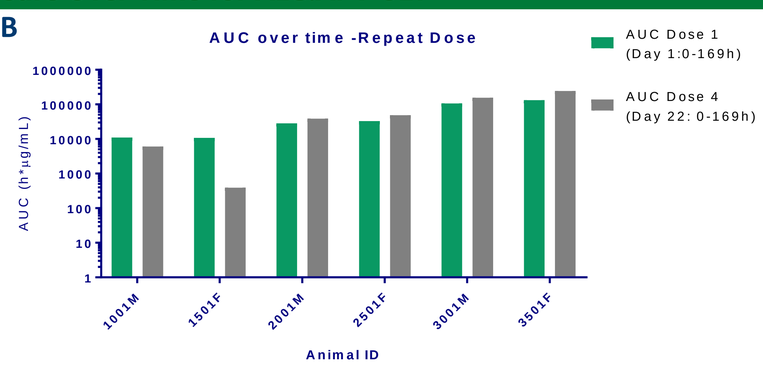

Pharmacokinetic Profile of ALG.APV-527 in NHP

A) ALG.APV-527 was detected after the 4th (final) dose until study termination (196 hours post dose) in all repeated dose groups. There is a slight tendency towards accumulated serum concentration in the dosed groups. Exposure by Cmax values remained high following all four doses.

B) There was exposure during the entire duration of the study in all repeated dose groups. All animals, with the exception of one animal in Group 1, had similar AUC following the 1st and 4th doses.

Group	Dose	Clearance*	Volume of Distribution*	Half-life (T ½)*
Group 1	5 mg/kg (RD)	0.297 mL/h/Kg	48.1 mL/Kg	112 h (4.7 days)
Group 2	15 mg/kg (RD)	0.2365 mL/h/Kg	62.8 mL/Kg	218.5 h (9.1 days)
Group 3	50 mg/kg (RD)	0.2315 mL/h/Kg	48.6 mL/Kg	145.5 h (6.1 days)
Group 4	15 mg/kg (SD)	0.294 mL/h/Kg	38.6 mL/Kg	90.9 h (3.8 days)

RD, repeat dose on Day 1, 8, 15 and 22; SD, single dose on Day 1

*Mean parameters were determined after the first dose for Groups 2 and 3 (n = 2/group); There was antibody-like clearance and volume parameters, and the serum half-life was in the range of ~4 - 9 days following one dose.

Summary and Conclusions

ALG.APV-527 induces a 5T4-dependent increase in IFN-γ production and enhances proliferation of T cells and NK cells *in vitro*. ALG.APV-527 induces potent CD8⁺ T cell co-stimulation and NK cell expression of NKG2D, but only in the presence of the tumor antigen 5T4. Furthermore, ALG.APV-527 localizes to 5T4-expressing tumors *in vivo*. Four repeated doses (administered once weekly) did not cause clinical symptoms in the NHP toxicity study. Clinical pathology, cytokine measurement and immune cell phenotyping showed no indication of systemic immune activation. These results support progression of ALG.APV-527 towards a clinical Phase I study, where the current pharmacokinetic profiling supports dosing every other week.

In conclusion, ALG.APV-527 has a favorable non-clinical safety profile with no indications of systemic activation or liver toxicity