Preclinical safety and efficacy of a tumor-directed T cell activating 4-1BB x 5T4 ADAPTIR™ bispecific antibody

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Introduction to 4-1BB and 5T4

4-1BB (CD137)

- Activation-induced co-stimulatory immune receptor expressed on tumor-infiltrating T cells and NK cells
- Stimulation of 4-1BB on T cells and NK cells leads to activation, proliferation, enhanced survival, increased cytokine production and increased cytolytic activity
- 4-1BB-antibody therapies in the clinic have shown promising anti-tumor effects, but with dose-limiting hepatic toxicities

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

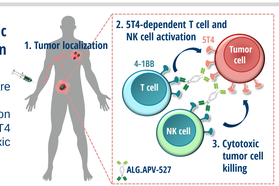
ALG.APV-527

- A tumor-directed 4-1BBx5T4 targeting bispecific antibody in the ADAPTIR™ format
- Contains two sets of binding domains, origin Alligator Gold® human scFv library, that has been optimized for use in Aptevo´s ADAPTIR™ bispecific format
- Silent 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 4-1BB agonists

ALG.APV-527 Mode of Action

ALG.APV-527 is designed to eliminate systemic toxicity by directing and limiting immune activation to the tumor

1. ALG.APV-527 localizes to tumors where both targets are highly expressed
2. ALG.APV-527 binds to 5T4 on tumor cells and 4-1BB on tumor infiltrating T cells and NK cells. Engaging of 5T4 induces multimerization of 4-1BB which activates cytotoxic CD8 T cells and NK cells
3. Cytotoxic CD8 T and NK cells lyse tumor cells



Summary and conclusions

> **ALG.APV-527**
- Augments CD8 T cell proliferation and IFN-γ production but only in the presence of 5T4 positive cells
- Induces NK cell proliferation and enhances the NK cell cytotoxic profile
- Inhibits tumor growth in a human xenograft colon carcinoma model

> 5T4 is expressed in a wide range of human tumor indications, but not in any vital organs such as heart or liver

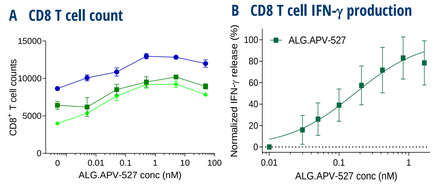
> Due to a similar target expression pattern and similar functional activity of ALG.APV-527 in human and cynomolgus systems, cynomolgus is a relevant species for the preclinical safety evaluation of ALG.APV-527

> ALG.APV-527 was well tolerated in cynomolgus with no clinical symptoms or indications of adverse reactions
- No major changes in liver enzyme levels, cytokine levels or immune cell populations were observed
- Serum half-life of ALG.ALG-527 administered intravenously in cynomolgus monkeys was ~ 5-7 days

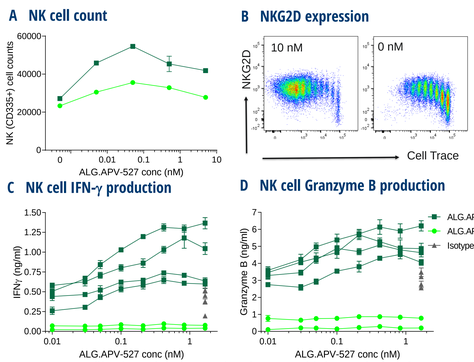
In conclusion, ALG.APV-527 has a favorable pre-clinical safety profile with no indications of systemic activation or liver toxicity

ALG.APV-527: tumor-directed T cell activator with favorable pre-clinical safety profile for treating 5T4-positive tumors with unmet medical need

5T4-dependent T cell and NK cell proliferation and activation



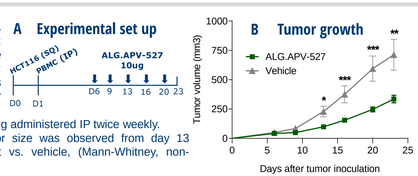
ALG.APV-527 augments CD8 T cell proliferation and IFN-γ production. (**A**) Primary PBMC treated with α-CD3 and ALG.APV-527 in the presence of 5T4-CHO cells. CD8 T cell count in 3 individual donors at 96h (flow cytometry). (**B**) Primary CD8 T cells treated with α-CD3 coated beads and ALG.APV-527 in the presence of HCT116 tumor cells expressing 5T4. IFN-γ was measured in the supernatant at 72h. Normalized IFN-γ levels (n=12) and nonlinear curve fit log (agonist) vs. normalized response variable slope was plotted using GraphPad Prism.



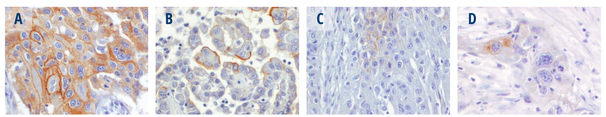
ALG.APV-527 promotes proliferation, enhances expression of NKD2G and induce production of IFN-γ and granzyme B. (**A-B**) Primary NK cells were stimulated with IL-2 and ALG.APV-527 in the presence of 5T4-CHO-K1 cells (**A**) Number of CD335+ NK cells were assessed in 2 healthy donors on day 6 by flow cytometry (**B**) Representative expression of NKG2D on treated NK cells (**C-D**) IL-2 pre-stimulated NK cells were incubated with ALG.APV-527 in co-culture with HCT116 tumor cells expressing 5T4. Secretion of (**C**) IFN-γ and (**D**) Granzyme B was measured in the supernatant at 72h (ELISA).

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

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 10ug 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).



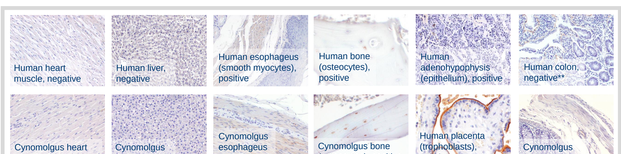
5T4 detection in TMAs of different tumor indications



A Squamous cell lung carcinoma (1-3+, >75%)
B Malignant epithelial mesothelioma (1-3+, >75%)
C Larynx squamous cell carcinoma (H&N) (1-2+, <25%)
D Pancreatic ductal adenocarcinoma (1-2+, <25%)

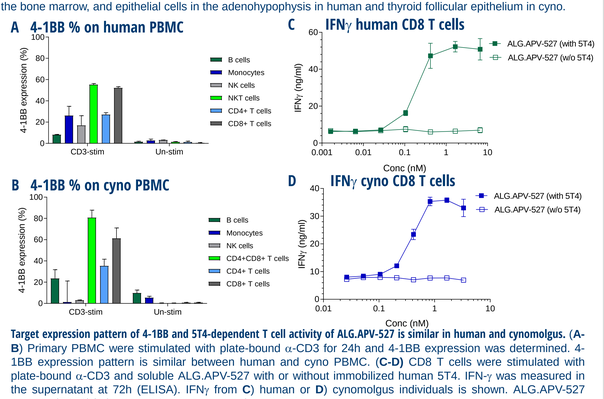
5T4 expression is detected in human tumors with variable incidence, frequency and intensity. Formalin-fixed paraffin-embedded tissue micro-arrays (TMAs) were acquired from US Biomax Inc, and stained for 5T4 detection (clone MAB4975, R&D Systems). (**A**) 5T4 expression was detected in tumors from NSCLC, Head and Neck, mesothelioma, pancreatic, bladder, renal and ovarian cancer with variable incidence (percentage of positive samples/indication), frequency (percentages of 5T4 positive cells of total cells, 0-100%) and intensity in staining (0-3+), as exemplified in images above.

Cynomolgus is a relevant toxicology species for ALG.APV-527



*cytoplasmic 5T4 staining in smooth myocytes **cytoplasmic 5T4 staining of spindle cells in lamina propria

Target expression pattern of 5T4 in normal tissue is low and is similar in human and cynomolgus. Formalin-fixed paraffin-embedded TMAs were stained for 5T4 detection. No 5T4 was detected in any major organs such as cardiovascular, respiratory or hepatic systems. Human and cynomolgus had a similar 5T4 expression pattern with occasional membranous staining of 5T4 detected in specific cell populations (smooth myocytes of the esophagus, osteocytes in the bone marrow, and epithelial cells in the adenohypophysis in human and thyroid follicular epithelium in cyno.



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 24h and 4-1BB expression was determined. 4-1BB expression pattern is similar between human and cyno 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 (**C**) human or (**D**) cynomolgus individuals is shown. ALG.APV-527 potency (EC50) in human: 0.2 nM vs. cynomolgus 0.4 nM.

ALG.APV-527 has a favorable safety profile with no indications of systemic activation or liver toxicity in a dose-range finding pilot toxicology study

Dose-range finding pilot toxicology study design

The safety of ALG.APV-527 was evaluated in a dose-range finding pilot toxicology study performed in cynomolgus. 3 repeated-dose groups and one single-dose treated group were included in the study (see table). ALG.APV-527 was administered intravenously in a total volume of 2.5 mL/kg into the tail vein for >1h. 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.

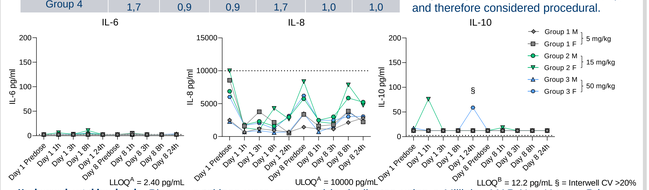
Treatment group	Administration Route	Dose (mg/kg)	Dose Days	Administered Volume (mL/kg)	Group Size
Group 1	IV, infusion	5	1, 8, 15, 22	2.5	1 male + 1 female
Group 2		15			
Group 3		50			
Group 4		15	1		3 males

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 were included and showed no elevation, 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 decreased on both dosing occasions but recovered.
- 22 immune cell populations were measured on Day 2, 8, 15 and 30 with no indication of drug-induced changes

Fold change of liver enzymes	AST (U/L)			ALT (U/L)		
Treatment group	Day 2	Day 15	Day 30	Day 2	Day 15	Day 30
Group 1	1,8	0,9	0,9	1,3	1,0	1,1
Group 2	4,4	0,9	0,9	2,5	0,9	0,9
Group 3	2,7	1,1	1,0	1,6	0,9	1,0
Group 4	1,7	0,9	0,9	1,7	1,0	1,0

Unchanged liver enzyme levels. 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 in fold change seen on day 2 is transient and is not seen day 15 or 30 despite repeated administration weekly, and therefore considered procedural.

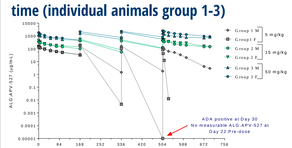


LLOQ[A] = 2.40 pg/mL ULOQ[A] = 10000 pg/mL LLOQ[B] = 12.2 pg/mL § = Interwell CV >20%

Unchanged cytokine levels. Plasma cytokines were measured in duplicates using a Milliplex MAP Non-Human Primate Cytokine Magnetic Bead Panel 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. All groups displayed recoveries in IL-8. After the second dose, all groups showed a decline in IL-8 levels.

Kinetic profile of ALG.APV-527 in cynomolgus monkeys

Serum concentrations of ALG.APV-527 over time (individual animals group 1-3)



Serum concentrations of ALG.APV-527 over time (individual animals in repeated dose groups 1-3). ALG.APV-527 was detected after the 4th (final) dose until study termination (196 hours post dose) in all repeated dose groups. There was exposure during the entire duration of the study in all dose groups. The serum half-life was in the range of 5-7 days. There is a slight tendency towards accumulated serum concentration in the dosed groups. One animal in Group 1 (F) developed ADA (confirmed) leading to rapid decline after fourth dose. Development of ADA in preclinical species is not considered translatable to the clinical setting (ICH S6, 2011). In the remaining animals, there was antibody-like clearance and volume parameters, with exposure (Cmax values and AUC) remaining high following four doses.