Potential Tumor-Directed T cell Activation and Tumor Inhibition Induced by a 4-1BB x 5T4 ADAPTIR™ Bispecific Antibody

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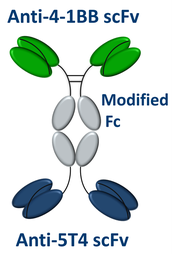
Introduction

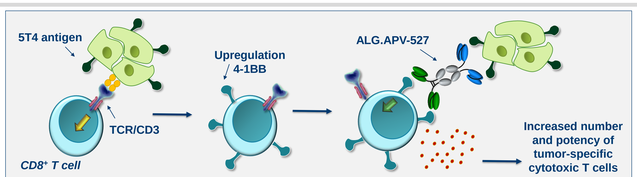
- 4-1BB (CD137) is an activation-induced costimulatory immune receptor expressed on tumor-infiltrating T cells and NK cells
- Stimulation of 4-1BB leads to enhanced proliferation, increased survival, intensified cytolytic activity, and induced IFN-γ production of T and NK cells
- 4-1BB-targeting immunotherapies have shown promising anti-tumor effects clinically however, a monospecific 4-1BB agonist induced dose-limiting hepatic toxicities
- 5T4 is a tumor-associated antigen expressed in a variety of malignancies, including NSCLC, head and neck, mesothelioma, renal, pancreas, bladder, breast, colorectal, gastric, ovarian and cervical cancers

About ALG.APV-527

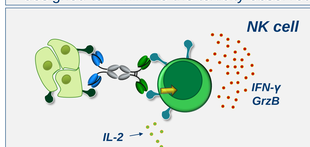
- ALG.APV-527 is an ADAPTIR™ bispecific therapeutic containing two sets of scFv binding domains targeting 5T4 and 4-1BB which are linked to an effector-null Ig Fc domain, providing an antibody-like *in vivo* half-life
- The scFvs originate from the Alligator Gold® human scFv library (Alligator Bioscience)
- Each scFv has been optimized for use in the bispecific ADAPTIR™ format (Aptevo Therapeutics)
- ALG.APV-527 features target-driven T cell stimulation, optimized stability, good manufacturing properties with potential for better risk-benefit in humans than other monospecific 4-1BB antibodies
- ALG.APV-527 is cross-reactive to 4-1BB and 5T4 from cynomolgus monkey. It enhances stimulation of CD3-activated human and cynomolgus T cells *in vitro*



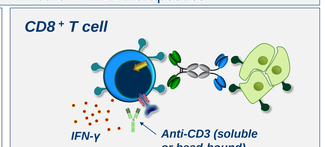
ALG.APV-527 Mode of Action



ALG.APV-527 directs the stimulation of CD8⁺ T and NK cells by 5T4⁺ tumors and is designed to minimize the toxicity observed with other 4-1BB therapeutics

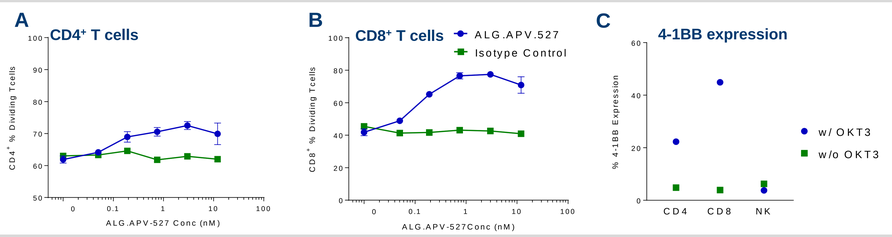


IL-2 upregulates 4-1BB expression on NK cells. Titration of ALG.APV-527 in the presence of 5T4-expressing tumor cells enhances secretion of cytolytic molecules such as IFN-γ and granzyme B (GrzB) and promotes proliferation.



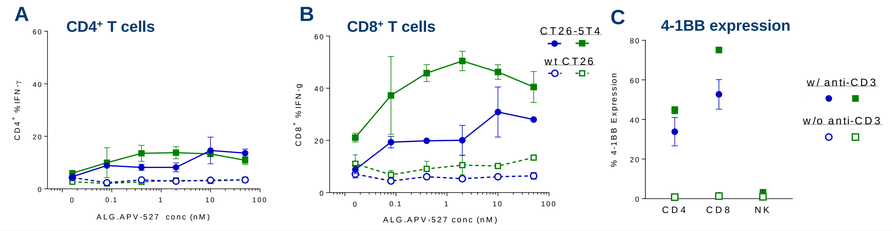
Stimulation of T cells with anti-CD3 induces the upregulation of 4-1BB. Addition of ALG.APV-527 and 5T4⁺ tumors augments primarily CD8⁺ T cells' proliferation and secretion of IFN-γ.

ALG.APV-527 augments CD8⁺ T cell proliferation



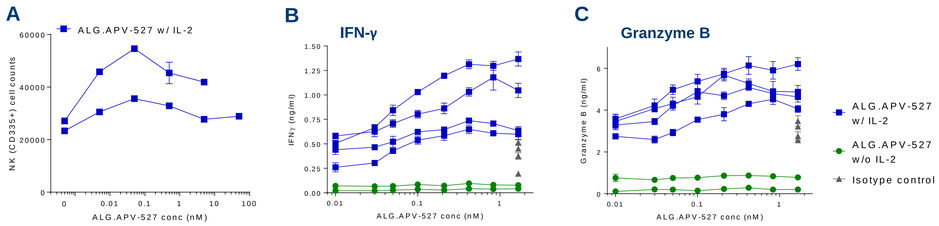
ALG.APV-527 augments T cell proliferation. Primary PBMC were stimulated with anti-CD3 Ab in solution and serial dilutions of ALG.APV-527 in the presence of human 5T4-expressing CT26 cells. Representation of the percentage of proliferating (**A**) CD4⁺ or (**B**) CD8⁺ T cells or were calculated on day 5 via flow cytometry. One representative donor from 4 donors. (**C**) 4-1BB expression on T or NK cells after stimulation +/- anti-CD3 (OKT3).

ALG.APV-527 enhances IFN-γ production in the presence of 5T4+ cells



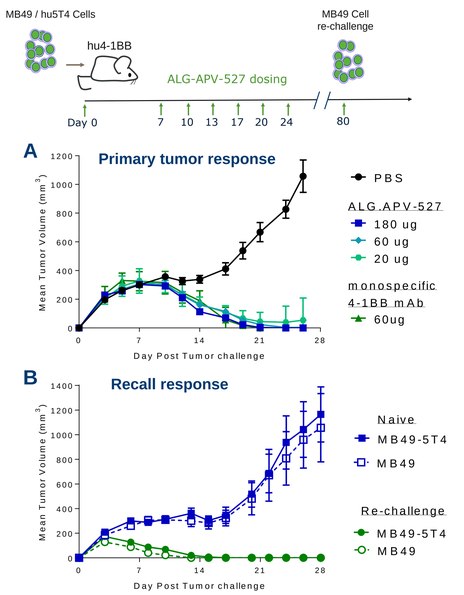
ALG.APV-527 enhances IFN-γ production from activated T cells. Primary PBMC were stimulated with anti-CD3 Ab and dilutions of ALG.APV-527 in the presence of wildtype CT26 or human 5T4-expressing CT26 cells. Representation of the percentage of (**A**) CD4⁺ or (**B**) CD8⁺ T cells producing IFN-γ were analyzed at 48 hours via flow cytometry following treatment with Brefeldin A. Two representatives from 4 donors. (**C**) 4-1BB expression on T or NK cells after stimulation +/- anti-CD3 (OKT3).

ALG.APV-527 augments NK cells



ALG.APV-527 enhances NK cells' effector functions. (**A**) IL-2 stimulated primary NK cells in the presence of 5T4-expressing CHO-K1 cells and ALG.APV-527. CD335⁺ NK cells were assessed in 2 donors on day 6. (**B-C**) IL-2 pre-stimulated primary NK cells co-cultured with 5T4-expressing HCT116 cells and ALG.APV-527. IFN-γ and Granzyme B was measured in the supernatant at 72h using ELISA. 4 representatives from 12 donors.

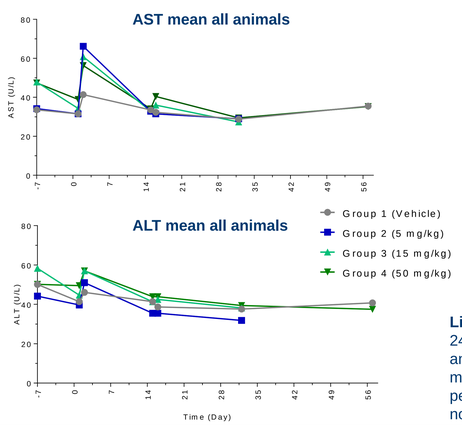
ALG.APV-527 induces rejection of established tumors and promotes anti-tumor memory response



ALG.APV-527 induces the generation of memory cells. (**A**) Day 0, MB49 cells expressing human 5T4 were injected SQ into 4-1BB knock-in mice. Starting on day 7, treatments of ALG.APV-527 or 4-1BB mAb control (w/ wildtype Fc) were administered IP twice weekly until day 24, (8 mice/ treatment). (**B**) Surviving mice that had cleared their primary tumor were re-challenged with MB49 tumor cells on day 80. Naïve mice were used as controls. No bispecific therapy was given.

ALG.APV-527 has a favorable safety profile in a non-human primate GLP toxicology study

The safety of ALG.APV-527 was evaluated in a GLP toxicology study performed in cynomolgus monkeys. 4 repeated-dose groups were included in the study, one as vehicle control. ALG.APV-527 was administered by intravenous infusion (over 1hr) into the tail vein. Samples were collected throughout the study for clinical pathology, PK, ADA, and immunophenotyping by flow cytometry. Samples were also collected at necropsy for histology and histopathology.



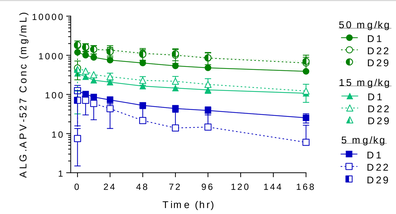
Dose (mg/kg)	Dose Days	Group Size	
		Main (Day 32)	Recovery (Day 60)
0	1, 8, 15, 22, 29	3 male + 3 female	2 male + 2 female
5		3 male + 3 female	n/a
15		3 male + 3 female	n/a
50		3 male + 3 female	2 male + 2 female

Findings from the GLP toxicology study

- No adverse events were observed in any of the animals during or after dose administration.
- Clinical pathology evaluations included all standard hematology, coagulation and clinical chemistry parameters, with no indication of adverse reactions.
- 22 immune cell populations were measured on Day 2, 15, 32 and end of recovery with no indication of ALG.APV-527-induced changes

Liver enzyme levels (mean) were not impacted by ALG.APV-527. Samples were collected pre and 24 hours post relative to dosing on Day 1 and 15. Additional samples were collected on Day 29 for all animals and on Day 59 for recovery animals. Liver enzymes AST and ALT compared to pre-treatment measurements. No general elevation in liver enzymes were observed during treatment. The slight peak seen in the mean AST on day 2 was due to individuals with higher spikes. The changes were not considered dose dependent for ALG.APV-527.

ALG.APV-527 has an antibody-like half life in non-human primates (NHP)



Serum half-life ranged from 5.1 to 9.5 days

Dose (mg/kg)	Day 1		Day 22		Day 29	
	Cmax_D	AUC_D	Cmax_D	AUC_D	Cmax_D	AUC_D
5	25	1633	25	632	14	NA
15	23	1744	32	2378	29	NA
50	24	1930	38	3347	37	7436

GLP toxicokinetic data for ALG.APV-527. ALG.APV-527 was detected after the 5th (final) dose until study termination (168 hours post dose) in all repeated dose groups. There was exposure during the entire duration of the study in all dose groups. From non-compartmental analysis, the serum half-life was in the range of 5.1 and 9.5 days. Exposure by Cmax values remained high following all doses. Cmax_D = Mean Cmax/Dose (kg*ug/mL/mg); AUC_D = Mean AUC/Dose (hr*kg*ug/mL/mg).

Summary and Conclusions

> ***ALG.APV-527***:

- Augments CD8⁺ T cell proliferation and IFN- γ production in the presence of 5T4⁺ expressing cells
- Enhances the cytotoxic profile of NK cells via production of IFN-γ and Granzyme B
- Inhibits growth of a bladder cancer expressing human 5T4 in a human 4-1BB knock-in murine model
- Displays antibody-like half-life in NHP and is well tolerated with repeated dosing

> The anti-4-1BB x anti-5T4 targeting ADAPTIR molecule, ALG.APV-527, has the potential to be a unique anti-cancer therapeutic agent with an improved safety profile for the treatment of numerous 5T4-expressing solid tumors with an unmet medical need

> ALG.APV-527 has a favorable non-clinical safety profile with no indications of systemic activation or liver toxicity in NHP