Immuno-conjugates and chimeric molecules

851 POTENT TUMOR-DIRECTED T CELL ACTIVATION AND IN VIVO TUMOR INHIBITION INDUCED BY A 4–1BB X ST4 ADAPTIR™ BISPECIFIC ANTIBODY

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Background 4-1BB (CD137) is an activation-induced co-stimulatory receptor that regulates immune responses of activated CD8+ T cells and NK cells, by enhancing proliferation, survival, cytolytic activity and IFN-γ production. Its ability to induce potent anti-tumor CD8+ and NK cell activity makes 4-1BB an attractive target for designing novel therapeutics for immuno-oncology. However, clinical development of a monospecific 4-1BB agonistic antibody has been hampered by dose-limiting hepatic toxicities. To minimize systemic immune toxicities and enhance activity at the tumor site, we have developed a novel 4-1BB x ST4 bispecific antibody that stimulates 4-1BB function only when co-engaged with ST4, a tumor-associated antigen. The combined preclinical dataset presented here provides an overview of the mechanism of action and the efficacy and safety profile of ALG.APV-527, supporting its advancement into the clinic.

Methods ALG. APV-527 was built based the ADAPTIR™ platform with binding domains to 4-1BB and ST4 generated using the ALLIDGATOR-GOLD® human scFv library. ALG.APV-527 was tested using primary cells in the presence or absence of cells expressing ST4. Cell Trace-labelled PBMC sub-optimally stimulated with anti-CD3, to induce 4-1BB expression, cells were gated using flow cytometry. T cell cytotoxicity was assessed by quantifying cell death in CD8+ T cell/tumor cell co-cultures, and images were obtained using a cell live imaging system (Cytation 5). For tumor inhibition studies, human 4-1BB knock-in mice were injected subcutaneously with MB49 cells transfected with human ST4. Cured mice were subsequently used in a toxicity study and liver pathology was evaluated.

Results In vitro, ALG.APV-527 enhances primary CD8+ T cell and NK cell function and proliferation in the presence of ST4-expressing cells. Using imaging, ALG.APV-527 in combination with a bispecific T cell engager caused increased cell death in T cell/tumor cell co-cultures. ALG.APV-527 inhibited growth of established tumors at doses as low as 2 μg/mouse in a syngeneic bladder cancer model. Following recovery, mice exhibited a memory response when rechallenged with tumor. In a high dose safety study in human 4-1BB knock-in mice, ALG.APV-527 did not cause significant systemic immune activation, whereas urelumab analogue treated mice induced dermatitis, elevated serum cytokines, CD8+ T-cell liver infiltration and systemic T-cell proliferation.

Conclusions ALG. APV-527 induces potent CD8+ T cell and NK cell co-stimulation and T-cell cytotoxicity and has potent in vivo anti-tumor activity, without inducing systemic toxicity. Based on preclinical data, ALG.APV-527 is a promising anti-cancer therapeutic for the treatment of a variety of ST4-expressing solid tumors.

Ethics Approval All studies were reviewed and approved by the Internal Animal Care and Use Committee (IACUC) of Aptevo Therapeutics.