ALPN-202, a Combined PD-L1/CTLA-4 Antagonist and PD-L1-Dependent CD28 T cell Costimulator, Elicits Potent Intratumoral T cell Immunity Superior to and Differentiated from PD-L1 Inhibitor Monotherapy

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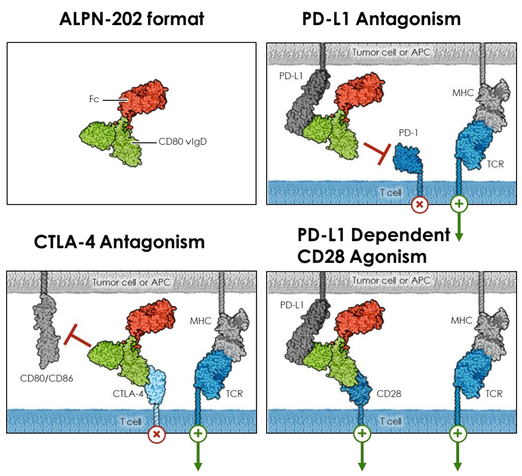
Abstract

Background: ALPN-202 is a variant CD80 vIgD™-Fc fusion protein blocking the PD-L1 and CTLA-4 checkpoints while providing PD-L1-dependent T cell activation via CD28. This strategy delivers potent T cell costimulation, which is currently missing from checkpoint inhibitor only regimens, and may be critical for the generation of clinical anti-tumor responses, seeking to broadly improve cancer outcomes. ALPN-202 has previously demonstrated preclinical anti-tumor activity superior to PD-L1 inhibition, but the specific mechanism(s) of superiority remain unreported.

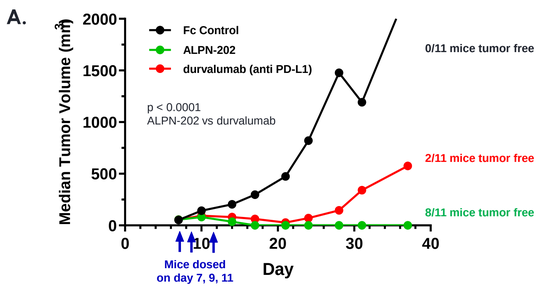
Methods: In an hPD-L1-transduced MC38 tumor model treated with ALPN-202 or durvalumab, an approved PD-L1 inhibitor, anti-tumor responses were evaluated by serial tumor volume measurements, and intratumoral immune responses were assessed by RNA-Seq, flow cytometry, and immunoSEQ TCR repertoire analysis (Adaptive Bio).

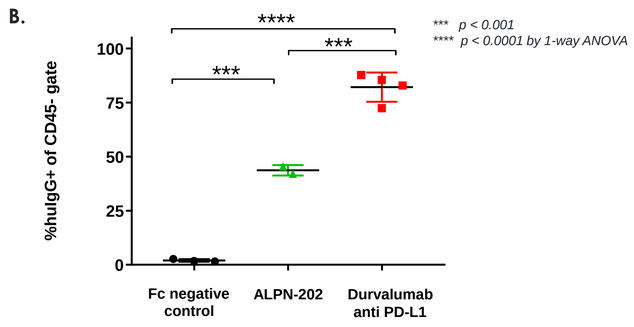
Figure 1: ALPN-202 is Engineered for Three Mechanisms of Action Including PD-L1 and CTLA-4 Antagonism Combined with CD28 Agonism



ALPN-202 is a CD80 vIgD-Fc consisting of the N-terminal IgV domain of CD80 fused to an effectorless human IgG1 Fc. The three proposed mechanisms of action of ALPN-202 include PD-L1/PD-1 antagonism, CTLA-4/CD80/86 antagonism, and PD-L1 dependent CD28 agonism.

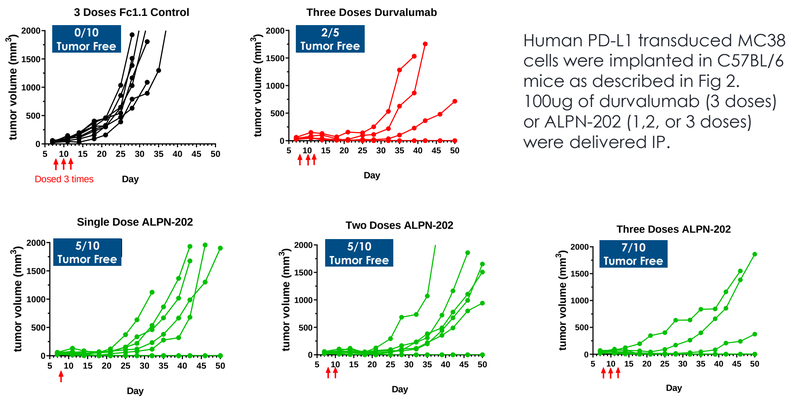
Figure 2: ALPN-202 Induces Superior Anti-Tumor Immunity *In Vivo* Compared to PD-L1 Inhibitor Despite Lower Tumor Exposure





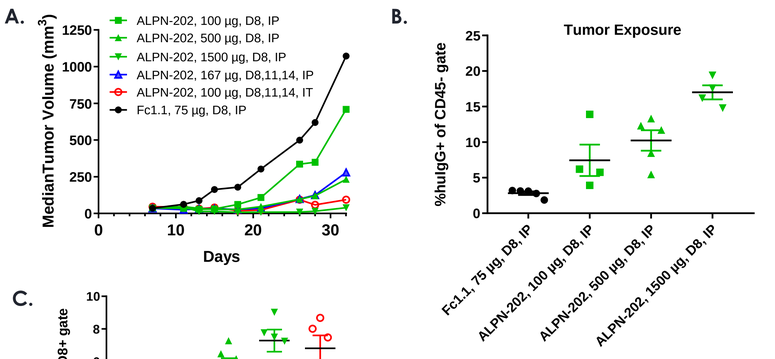
C57BL/6 mice were subcutaneously implanted with 1.5 million human PD-L1 transduced MC38 cells on day 0, with 11 mice per group staged with mean 50mm³ tumor volume on day 7. On days 7, 9, and 11, mice received 100ug of test articles by IP injection. Tumor volumes were measured through day 37 (A). Tumor exposure (B) of human Fc containing therapeutics was determined by staining isolated tumor cells with anti-human IgG secondary.

Figure 3: ALPN-202 Demonstrates Potent, Single Dose Efficacy Comparable to Repeat Dosing of PD-L1 Inhibitor



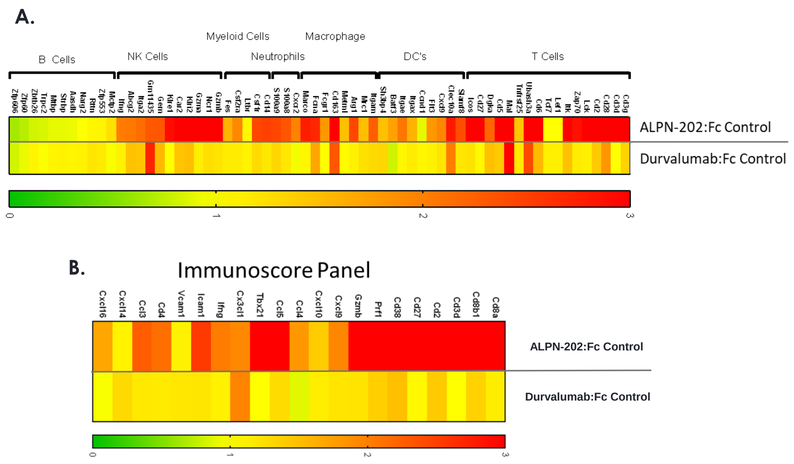
Human PD-L1 transduced MC38 cells were implanted in C57BL/6 mice as described in Fig 2. 100ug of durvalumab (3 doses) or ALPN-202 (1,2, or 3 doses) were delivered IP.

Figure 4: Systemic and Intratumoral Delivery of ALPN-202 Result in Potent Anti-Tumor Immunity in a Dose Dependent Manner



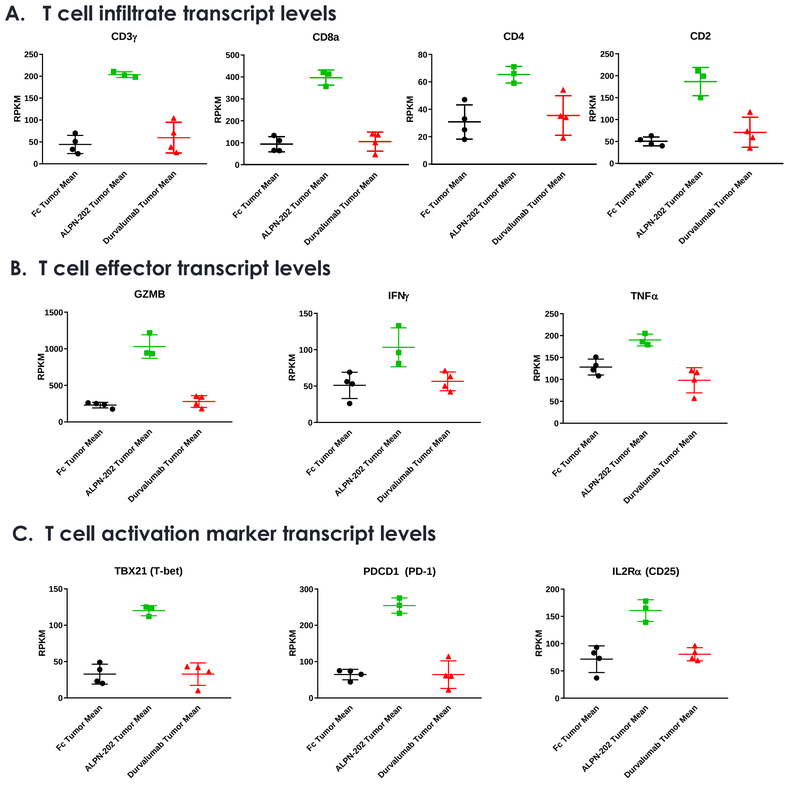
Human PD-L1 transduced MC38 cells were implanted as described in Fig 2. Single, escalating doses of ALPN-202 were administered IP, as well as three 100ug ALPN-202 doses delivered intratumorally. Tumor volumes were measured over time (A). Tumor therapeutic exposure (B) was determined as described in Fig 2. Antigen specific CD8 responses in tumor were measured (C) ex vivo by staining digested tumors with a mouse MHC Class I tetramer containing a retroviral p15E peptide epitope present in infected MC38 cells.

Figure 5: ALPN-202 Induces a More Robust Tumor Inflammatory Gene Signature than PD-L1 Inhibitors



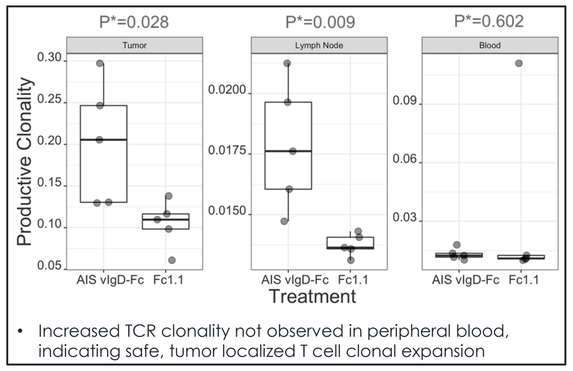
MC38 tumor cells were transduced with human PD-L1 and implanted as described in Fig 2. Three days post first dose (day 11), four mice treated with Fc Control, ALPN-202, or durvalumab were euthanized for tumor harvest. RNA was extracted from tumor and RNA-Seq was performed. Presented heat maps illustrate the mean expression level fold change of individual transcript for ALPN-202 and durvalumab treated animals compared to Fc Control treated animals.

Figure 6: ALPN-202 Induces Increased T Cell Tumor Infiltration, T Cell Effector Function, and T Cell Activation in Tumor Compared to PD-L1 Inhibitors

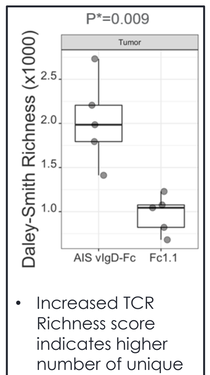


From the same RNA-Seq data set described in Fig 5, individual transcript levels were graphed representing (A) T cell infiltrate levels (B) T cell effector function and (C) T cell activation status

Figure 7: TCR Repertoire Analysis Reveals Increased TCR Clonality and Richness in Tumors Treated with ALPN-202



- Increased TCR clonality not observed in peripheral blood, indicating safe, tumor localized T cell clonal expansion



- Increased TCR Richness score indicates higher number of unique TCR clones also in the tumor of ALPN-202 treated mice

MC38 tumor cells were transduced with human PD-L1 and implanted as described in Fig 2. Three days post third dose (day 15), five mice treated with Fc Control or ALPN-202 were euthanized for tumor harvest. Genomic DNA was extracted from tumor and ImmunoSEQ analysis was performed at Adaptive Biosciences.

Summary and Conclusions

- ALPN-202 was successfully engineered to bind human PD-L1 with increased affinity compared to wild-type CD80, creating a first-in-class therapeutic with PD-L1 and CTLA-4 antagonism coupled with CD28 agonism
- ALPN-202 promotes dose-dependent anti-tumor immunity via systemic or intratumoral delivery, including potent single dose activity, superior to repeat dosing with the approved single checkpoint inhibitor and anti-PD-L1 antagonist durvalumab
- ALPN-202 induces a significantly greater tumor inflammation signature compared to durvalumab, as well as significantly increases in T cell clonality and richness, which may translate into improved anti-tumor immunity
- Our data support that ALPN-202 has the potential to afford superior monotherapy efficacy over single checkpoint antagonists
- ALPN-202 is in preclinical development for treating advanced malignancies and clinical trials are expected to begin in 2H-2019