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ALPN-202, a Conditional CD28 Costimulator and Dual Checkpoint Inhibitor, Utilizes Multiple Mechanisms to Elicit Potent Anti-Tumor Immunity Superior to Checkpoint Blockade

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Abstract

INTRODUCTION: Although immune checkpoint inhibitors (CPI) targeting the CTLA-4 and PD-1/PD-L1 pathways have demonstrated significant clinical activity in many cancers, many patients fail to respond, or they develop acquired resistance due at least in part to insufficient anti-tumor T cell activation and/or exhaustion. Based on this hypothesis, CPIs have been combined with T cell costimulation and shown to enhance and sustain anti-tumor responses preclinically. ALPN-202 is a variant CD80 vIgD™-Fc fusion protein that mediates PD-L1-dependent CD28 costimulation and inhibits the PD-L1 and CTLA-4 checkpoints (Figure 1). Studies were conducted to characterize the unique mechanism of action of this molecule and further elucidate CD80/PD-L1 biology.

EXPERIMENTAL PROCEDURES: X-ray crystallography and cell-based reporter assays were used to demonstrate the simultaneous binding of PD-L1 and CD28 to the single CD80 IgV domain of ALPN-202. PD-L1-dependent CD28 costimulation was evaluated using primary human T cells stimulated by antigen presenting cells (APC) with or without surface PD-L1. ALPN-202 anti-tumor activity was mechanistically evaluated in vivo using a human PD-L1-transduced MC38 tumor model, by combining ALPN-202 treatment with anti PD-L1, anti CTLA-4, or anti CD28 blocking antibodies. Anti-tumor activity was evaluated by serial tumor volume measurements, and intratumoral immune responses were assessed by flow cytometry and/or RNA-Seq analyses.

DATA SUMMARY: The CD80 vIgD:PD-L1 crystal structure was elucidated at a resolution of 3.15 Å, revealing a non-overlapping binding interface distinct from the previously published CD28:CD80 interaction (Figure 2). Using cell-based reporter and cytokine release assays, monomeric and dimeric CD80 vIgD domains elicited conditional CD28 costimulation which was blocked when combined with anti PD-L1 or anti-CD28 antibodies. In vivo, ALPN-202 demonstrated activity superior to PD-L1 blockade alone, while coadministration with either anti CD28 or anti PD-L1 prevented ALPN-202-mediated conditional CD28 costimulation resulting in reduced anti-tumor activity and RNA signatures consistent with lower T cell infiltration and activation (Figures 3 and 4)..

CONCLUSIONS: The CD80 IgV domain utilizes separate, non-competing epitopes to bind CD28 and PD-L1. Exploitation of this attribute via directed evolution-based discovery has yielded ALPN-202, a variant CD80 vIgD-Fc fusion protein capable of simultaneously engaging PD-L1 on tumor cells and CD28 or CTLA-4 on T cells, conferring the unique abilities to block both the PD-L1 and CTLA-4 checkpoints as well as eliciting conditional CD28 costimulation only in the presence of PD-L1. The significantly superior activity of ALPN-202 over CPI-only therapies in vitro and in vivo suggests that conditional CD28 costimulation, together with checkpoint inhibition, may result in meaningfully improved clinical anti-tumor responses.

Results

Figure 1: ALPN-202 is comprised of a variant CD80 IgV domain (vIgD™)fused to an effectorless IgG Fc

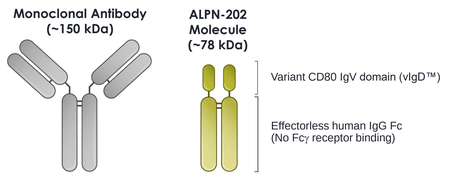
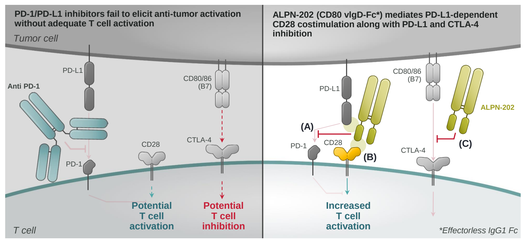
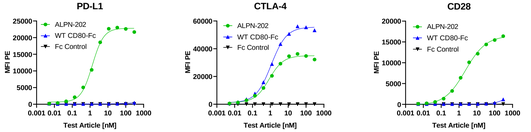


Figure 2: ALPN-202 was engineered for three mechanisms of action: PD-L1 and CTLA-4 antagonism combined with conditional CD28 agonism



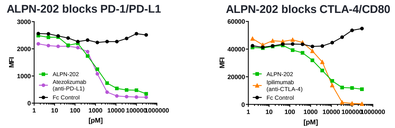
(A) ALPN-202 binds PD-L1 expressed on tumor, blocking PD-L1/PD-1 interactions. (B) Localized, tethered ALPN-202 is able to provide a trans CD28 signal to T cells making contact with the tumor (PD-L1-dependent CD28 costimulation). (C) Additionally, ALPN-202 binds CTLA-4 expressed on T cells, decreasing competition for CD28 signaling, lowering CD3/CD28 signaling thresholds, and promoting TCR repertoire expansion in the periphery.

Figure 3: ALPN-202 has elevated PD-L1 and CD28 affinity relative to wild-type CD80



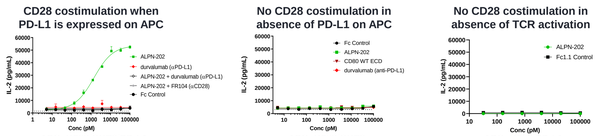
CHO cells stably expressing PD-L1 or CTLA-4, or Jurkat cells endogenously expressing CD28, were used to measure ALPN-202 binding affinity by flow cytometry. ALPN-202 displayed higher affinity for PD-L1 and CD28 than wild-type CD80-Fc.

Figure 4: ALPN-202 antagonizes PD-L1 and CTLA-4



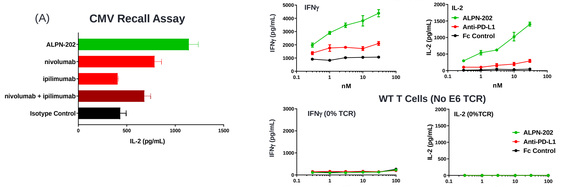
CHO cells stably expressing human PD-L1 (A) or CTLA-4 (B) were plated with titrated ALPN-202 or antibody controls. Cells were washed before adding AF647-conjugated WT PD-1 or CD80-Fc respectively. Binding of conjugated proteins was measured by flow cytometry.

Figure 5: CD28 costimulation requires TCR signal and expression of PD-L1 on the APC



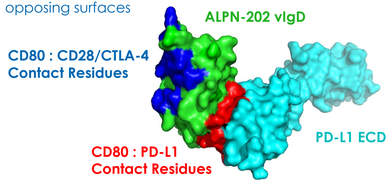
K562 artificial APC (aAPC) stably expressing transmembrane anti-human CD3 and/or human PD-L1 were plated in the presence of primary human T cells and a titration of ALPN-202 or controls. Culture supernatants were harvested at 24 hours and human IL-2 measured by ELISA.

Figure 6: ALPN-202 activity *in vitro* is more potent than dual checkpoint inhibition and requires TCR stimulation



(A) PBMC were stimulated with CMV lysate in the presence of ALPN-202, anti PD-1(nivolumab), anti CTLA-4(ipilimumab), or the combination and IL-2 production measured at 48hr. (B) E6 TCR-transgenic T cells were cultured with the HPV+ tumor line SCC-152. ALPN-202 activity was more potent than anti PD-L1 alone (durvalumab) and dependent on TCR stimulation.

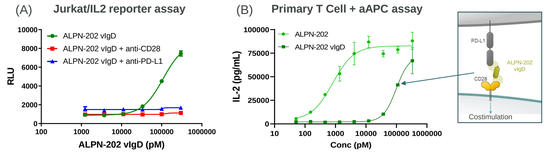
Figure 7: Crystal structure reveals ALPN-202 vIgD binds PD-L1 and CD28/CTLA-4 on opposing surfaces



Crystal structure of a single ALPN-202 variable domain bound to wild type PD-L1 (3.15 Å resolution).

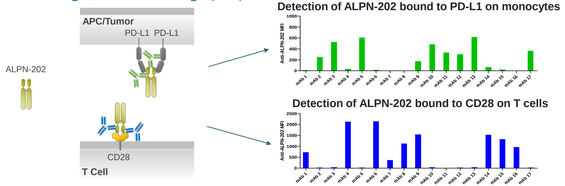
ALPN-202 vIgD : PD-L1 contact residues (red) and known CD80 : CD28/CTLA-4 contact residues (blue).

Figure 8: A single ALPN-202 vIgD domain induces PD-L1-dependent CD28 agonism



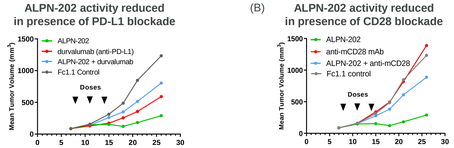
K562 artificial APC (aAPC) expressing anti-human CD3 (OKT3) and WT PD-L1 were plated in the presence of Jurkat/IL2 reporter cells (A) or primary human T cells (B) and incubated with a titration of ALPN-202 alone or with anti CD28 or anti PD-L1 control antibodies. IL-2 reporter activity after 5 hours (RLU) or IL-2 production at 24 hours was measured.

Figure 9: Antibodies can differentiate ALPN-202 bound to CD28 or PD-L1, corroborating different binding epitopes



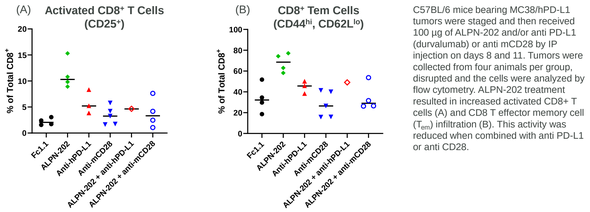
A panel of 17 mouse anti ALPN-202 antibodies were screened for detection of ALPN-202 bound to CD28 on T cells or PD-L1 on activated monocytes. Two distinct groups of antibodies were identified suggesting ALPN-202 binds CD28 on one epitope and PD-L1 on a separate, non-competing epitope.

Figure 10: Selective blockade of ALPN-202 targets *in vivo* confirms PD-L1-dependent CD28 agonism is a major contributor to anti-tumor activity



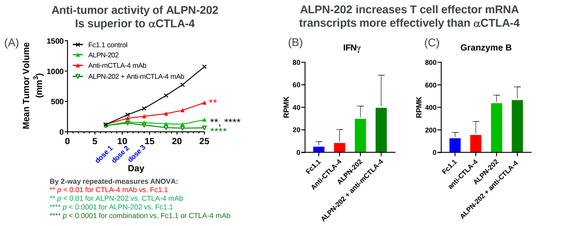
C57BL/6 mice were subcutaneously implanted with human PD-L1 transduced MC38 cells on day 0 and staged on day 7. On days 8, 11, and 14, mice received 100µg of ALPN-202 and/or anti PD-L1 (A) or anti mCD28 (B) antibody by IP injection. Tumor volumes were measured through day 27.

Figure 11: ALPN-202-mediated increase in CD8⁺ T_{em} cell infiltration requires engagement of both CD28 and PD-L1



C57BL/6 mice bearing MC38/hPD-L1 tumors were staged and then received 100 µg of ALPN-202 and/or anti PD-L1 (durvalumab) or anti mCD28 by IP injection on days 8 and 11. Tumors were collected from four animals per group, disrupted and the cells were analyzed by flow cytometry. ALPN-202 treatment resulted in increased activated CD8+ T cells (A) and CD8 T effector memory cell (T_{em}) infiltration (B). This activity was reduced when combined with anti PD-L1 or anti CD28.

Figure 12: ALPN-202 is a potent mono-therapeutic and significantly improves the anti-tumor activity of anti CTLA-4 mAb when given in combination



By 2-way repeated-measures ANOVA:
** $p < 0.01$ for CTLA-4 mAb vs. Fc1.1
** $p < 0.01$ for ALPN-202 vs. CTLA-4 mAb
**** $p < 0.0001$ for ALPN-202 vs. Fc1.1
**** $p \leq 0.0001$ for combination vs. Fc1.1 or CTLA-4 mAb

C57BL/6 mice were implanted s.c. with MC38/hPD-L1 cells on day 0 and staged on day 7. On days 8, 11, and 14, mice received 100 µg of ALPN-202 and/or anti-mouse CTLA-4 antibody (mIgG2b) by IP injection. (A) Tumor volumes were measured through day 33. (B, C) 72 hours after second dose, a subset of tumors (n=5/group) was collected, RNA isolated and RNAseq performed. Data as reads per kilobase of transcript, per million mapped reads (RPKM) (mean + SEM).

Visit Poster #467 (K. Lewis, et al): **"**ALPN-202, a Conditional CD28 Costimulator and Dual Checkpoint Inhibitor, Enhances the Activity of Multiple Standard of Care Modalities**"**

Summary and Conclusions

- ALPN-202 is a conditional CD28 agonist and a dual PD-L1 and CTLA-4 antagonist
- Blockade of CD28 or PD-L1 inhibits ALPN-202-mediated CD28 costimulation
- ALPN-202 binds PD-L1 and CD28/CTLA-4 at distinct non-overlapping epitopes – enabling its unique functionality
- RNAseq and TIL analyses reveal ALPN-202 treatment results in improved T cell infiltration and pro-inflammatory cytokine production relative to monotherapy CPI
- This potential novel mechanism of action may represent a new, best-in-class immunotherapy for cancer patients
- A first-in-human clinical study with ALPN-202 is in preparation