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ALPN-202 Combines Checkpoint Inhibition with Conditional T Cell Costimulation to Overcome T Cell Suppression by M2c Macrophages and Improve the Durability of Engineered T Cell Anti-Tumor Responses

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Abstract (#10640)

INTRODUCTION: Despite significant clinical benefit of checkpoint inhibitors (CPI) in some settings, unfortunately the majority of patients still fail to respond and/or develop resistance. This is likely due to a complex set of factors including, but not limited to, the presence of immunosuppressive myeloid cells and/or T cell exhaustion due to chronic TCR activation in the absence of sufficient costimulation. ALPN-202, a variant CD80 vIgD™-Fc fusion protein that mediates PD-L1-dependent CD28 costimulation and blocks PD-L1 and CTLA-4, was designed to overcome several of these suppressive mechanisms. The objective of these studies was to measure effects of ALPN-202 on the suppression of T cell activation by M2c macrophages and to determine *in vivo* effects of ALPN-202 on T cell exhaustion in an adoptively transferred human TCR transgenic tumor model.

METHODS: M2c macrophages differentiated from primary monocytes with M-CSF and IL-10 were cocultured for 72 hrs with autologous T cells, anti-CD3, and a titration of ALPN-202, anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibodies. Cytokine concentrations in the culture media were measured at 24 and 72 hrs, and T cells and macrophages were characterized at 72 hrs by flow cytometry. To measure its anti-tumor activity and effect on T cell exhaustion *in vivo*, ALPN-202 was evaluated in a humanized model using anti-HPV E6 TCR-transduced human T cells transferred into immunodeficient NSG mice bearing HPV$^+$ SCC152 squamous cell tumors stably expressing PD-L1. Tumor volume was measured twice weekly and on day 38 tumors were harvested, digested, and intratumoral T cells characterized for expression of exhaustion markers.

RESULTS: In the *in vitro* coculture assay, ALPN-202 increased T cell proliferation and production of IL-2, IFNγ, TNFα, GM-CSF, IL-6, and IL-21 significantly more potently than CPI alone. Additionally, the M2c macrophages in the presence of ALPN-202 displayed a dose-dependent elevation of MHC II, CD80, and CD86, indicative of a more pro-inflammatory, M1-like phenotype. In the SCC152 tumor model, ALPN-202 induced a more robust anti-tumor response than CPI.

CONCLUSION: As a dual checkpoint inhibitor and conditional CD28-costimulator, ALPN-202 induced robust and selective T cell costimulation *in vitro* which overcame M2c macrophage-mediated suppression more potently than CPI alone. Intriguingly, not only were T cells vigorously activated, but M2c macrophages transitioned to a more M1-like proinflammatory phenotype. In a humanized tumor model, ALPN-202 treatment resulted in potent anti-tumor activity. The data suggest that by combining CD28 costimulation with CPI, ALPN-202 may provide a more robust and persistent anti-tumor T cell response compared to CPI alone.

Figure 1: ALPN-202 is composed of a variant CD80 IgV domain (vIgD™) fused to an effectorless IgG1 Fc

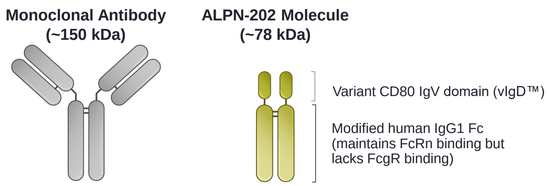
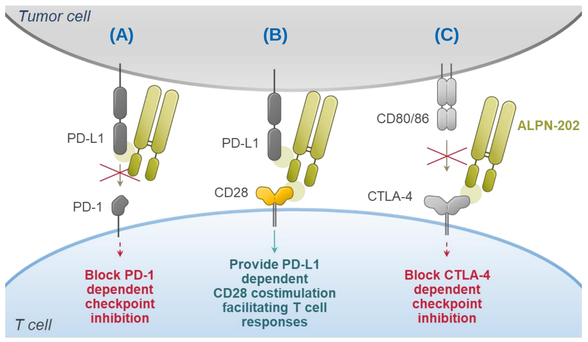
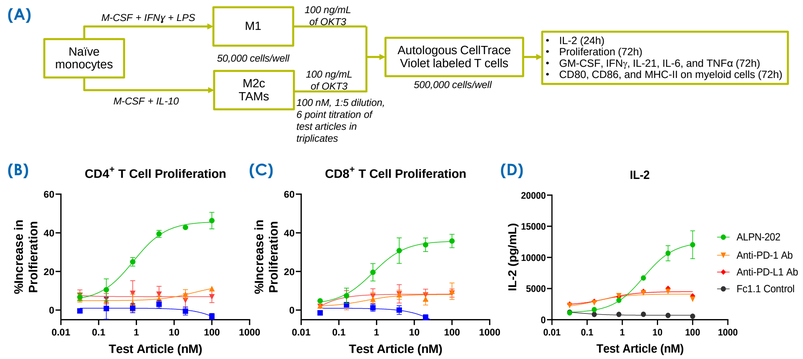


Figure 2: ALPN-202 was engineered for three mechanisms of action: PD-L1 and CTLA-4 antagonism, and conditional CD28 agonism



(**A**) ALPN-202 binds PD-L1 and blocks PD-1 interaction. (**B**) Localized, PD-L1 bound ALPN-202 is able to provide a trans CD28 signal to T cells that are in contact with the tumor (PD-L1-dependent CD28 costimulation). (**C**) 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.

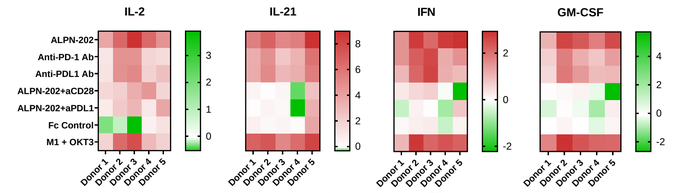
Figure 3: ALPN-202 helps T cells overcome M2c macrophage mediated suppression *in vitro* more potently than CPI alone



(**A**) Schematic of primary M2c + T cell co-culture assay. *In vitro* derived M2c macrophages were co-cultured with T cells and submaximal anti-CD3 (clone OKT3). The effects of ALPN-202 or checkpoint blockade on cytokine production, T cell proliferation, and changes in the surface phenotype of myeloid cells were characterized. (**B-C**) CD4+ and CD8+ T cell proliferation was characterized by CellTraceViolet™ dilution and graphed as percent increase relative to Fc control. ALPN-202 treatment resulted in a dose-dependent increase in proliferation compared to anti-PD-1 (nivolumab) or anti-PD-L1 (durvalumab). (**D**) Similarly, IL-2 concentration in the supernatant was measured at 24hrs revealing a dose-dependent increase following ALPN-202 treatment. ALPN-202 increased IL-2 production more potently than either PD-1 or PD-L1 blockade alone. Data are representative of results from 5 donors tested.

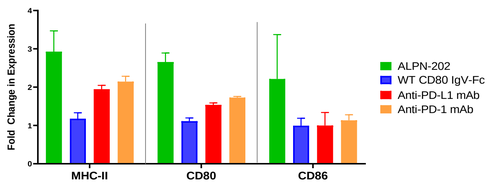
Figure 4: ALPN-202 activity in M2c *in vitro* co-culture system requires PD-L1 and CD28 and can drive a shift from M2c→M1 phenotype

(A) ALPN-202 activity induces production of multiple proinflammatory cytokines and is dependent on both PD-L1 and CD28 binding



To understand the breadth of response in this co-culture system, analysis of 72 hrs culture supernatants revealed elevated production of several proinflammatory cytokines: IL-2, IL-21, IFNγ, and GM-CSF. Fold change in concentration of cytokines with respect to untreated control (M2c+OKT3+T cells) indicates ALPN-202 activity was superior to checkpoint blockade alone and was dependent on PD-L1 and CD28 binding (decreased cytokine production when ALPN-202 was combined with anti-CD28 or anti-PD-L1). Heat map of the fold change in cytokine levels for all treatments. Red indicates an increase and green a decrease relative to control. Data shown from all 5 donors tested.

(B) ALPN-202 treatment results in upregulation of proinflammatory M1 phenotypic markers: MHC-II, CD80, and CD86

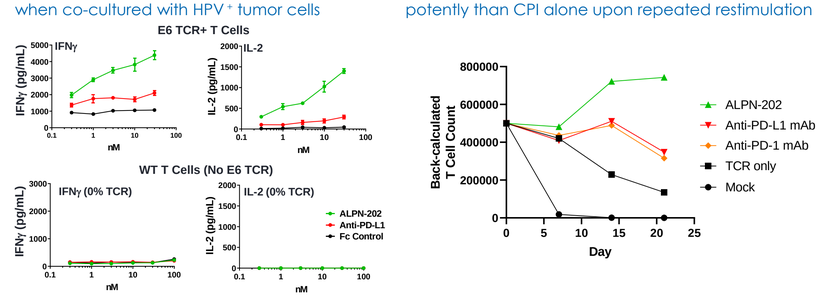


Changes in surface expression of proinflammatory markers on myeloid cells were measured by flow cytometry. Expression of MHC-II, CD80, and CD86 on the myeloid cells increased with ALPN-202 treatment to a greater extent than PD-L1 (durvalumab) or PD-1 (nivolumab) blockade alone suggesting a shift from a suppressive to proinflammatory phenotype. Data shown are representative of results from two donors tested at a 100 nM concentration of each test article.

Figure 5: ALPN-202 drives *in vitro* expansion and promotes survival of HPV-specific E6 TCR transduced T cells

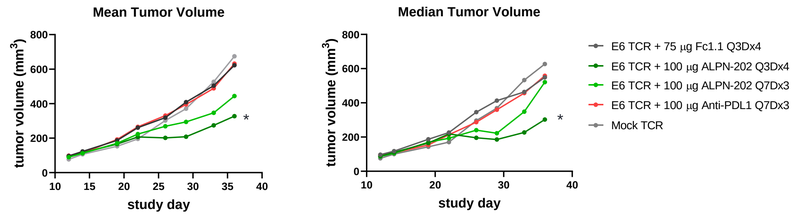
(A) ALPN-202 increases E6 TCR$^+$ T cell activation when co-cultured with HPV$^+$ tumor cells

(B) ALPN-202 promotes survival of E6 TCR$^+$ T cells more potently than CPI alone upon repeated restimulation



(**A**) Primary human T cells were created by transduction with lentivirus encoding the HPV peptide-specific E6 TCR α and β chains. Wild type (WT) or E6 TCR$^+$ T cells were co-cultured with HLA-A2$^+$/HPV$^+$ SCC-152 cells stably expressing human PD-L1 and a titration of ALPN-202, anti-PD-L1 (durvalumab), or Fc control. ALPN-202 treatment increased IFNγ and IL-2 production in a dose dependent manner more potently than PD-L1 blockade alone. (**B**) E6 TCR$^+$ T cells were activated and then restimulated every seven days by co-culturing with SCC-152/PD-L1 cells and 10 nM of test article. Every 7 days, cultures were harvested, counted, and the total number of T cells grown under each condition was back-calculated based on the fold expansion or contraction at each time point.

Figure 6: ALPN-202 increases anti-tumor activity of E6 TCR$^+$ T cells *in vivo* more potently than anti-PD-L1 antibody treatment



SCC-152 cells stably expressing human PD-L1 were implanted subcutaneously into NSG mice on day 0. E6 TCR$^+$ T cells were retro-orbitally implanted on day 15. Treatments started on day 16 with 100 μg doses of ALPN-202 every three days (dark green) or once weekly (light green), 100 μg doses of anti-PD-L1 antibody (durvalumab) administered once weekly (red), and 75 μg doses of isotype control administered every three days to match the ALPN-202 dosing. Tumor volume was measured every 3-4 days throughout the study. n=10 animals per group. Mock TCR group n=5.
* $p < 0.05$ vs. Fc1.1 and anti-PD-L1 groups by 2-way repeated measures ANOVA for treatment effects.

Summary and Conclusions

- ALPN-202 is a dual checkpoint inhibitor and conditional CD28 costimulator that may overcome M2c macrophage-mediated suppression more potently than CPI alone.
- ALPN-202 activity can drive suppressive M2c macrophages toward an anti-tumor M1-like phenotype, and promote T cell expansion and survival during multiple rounds of *in vitro* stimulation.
- In a humanized tumor model, ALPN-202 treatment results in more potent anti-tumor activity than CPI alone.
- These novel data indicate ALPN-202 may overcome aspects of CPI resistance that will translate into significantly improved outcomes in multiple cancers.
- A phase 1 study of ALPN-202 in patients with advanced solid tumors and lymphomas has been initiated (NEON-1, NCT04186637)

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