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ALPN-202, a Conditional CD28 Costimulator and Dual Checkpoint Inhibitor, Enhances the Activity of Multiple Standard of Care Modalities

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Abstract

INTRODUCTION: Checkpoint inhibitors targeting the PD-1 axis have transformed cancer treatment. However, objective response rates remain low, suggesting that novel therapeutics and/or combination treatments are needed. At the same time, non-immuno-oncology therapeutic approaches such as chemotherapy remain standard of care for many malignancies. ALPN-202 is a variant CD80 vIgD™-Fc fusion that mediates PD-L1-dependent CD28 costimulation and inhibits the PD-L1 and CTLA-4 checkpoints. This novel mechanism of action provides potent single agent immunomodulatory activity in mouse tumor models, and thus has the potential to complement other therapeutic modalities, such as checkpoint inhibitors or chemotherapies.

EXPERIMENTAL PROCEDURES: Mice were implanted subcutaneously with human (hu) PD-L1-transduced MC38 colon carcinoma and B16-F10 melanoma cell lines. Once measurable tumors were established, mice were treated with anti-mouse checkpoint (i.e. PD-1 or CTLA-4) blocking monoclonal antibodies (mAbs) or oxaliplatin, a platinum-based chemotherapeutic agent, alone or in combination with ALPN-202, to evaluate compatibility of the novel ALPN-202 protein with existing cancer therapies. Anti-tumor responses were evaluated by serial tumor volume measurements and RNA-Seq analysis of tumors isolated from treated mice.

DATA SUMMARY: Anti PD-1, anti CTLA-4, or oxaliplatin alone were only modestly effective as monotherapy in huPD-L1+ MC38 tumor-bearing mice, while ALPN-202 has potent anti-tumor activity in this model. When the checkpoint inhibitors or chemotherapy were administered in combination with ALPN-202, significantly greater reductions in tumor growth over time were observed than with any of these agents alone. Furthermore, ALPN-202 was extremely effective (92% tumor growth inhibition) in improving the anti-tumor activity of anti PD-1 mAb in mice bearing huPD-L1+ B16-F10 tumors, a tumor that is known to be poorly immunogenic and treatment-recalcitrant. RNA-Seq analysis of tumors from the MC38 studies was performed to explore in-depth the mechanisms, including enhancement of T cell effector transcript expression, that play a role in the ability of ALPN-202 to provide anti-tumor immunity and to enhance the activity of checkpoint inhibitors and the chemotherapeutic oxaliplatin.

CONCLUSIONS: ALPN-202 demonstrates potent anti-tumor efficacy as monotherapy and significantly improves the anti-tumor activity of other only modestly effective treatment modalities, such as checkpoint-only blockade mAbs and chemotherapy. ALPN-202 has the potential to be significantly effective as a monotherapy, and its compatibility with checkpoint inhibitors and chemotherapeutics suggest versatility in its potential to improve outcomes in the frontline setting alone and/or in combination with standard of care of multiple cancer types. A first-in-human clinical study with ALPN-202 is in preparation.

Results

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

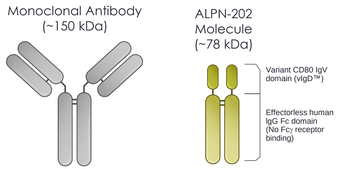
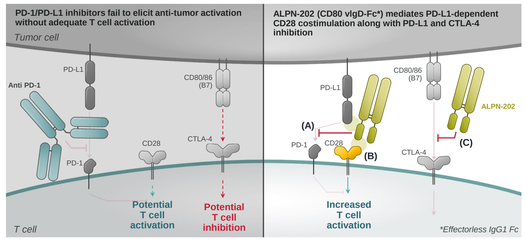
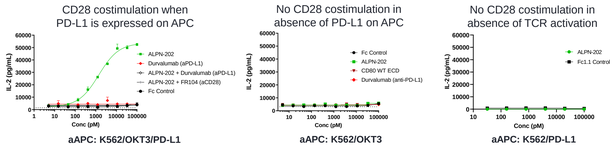


Figure 2: ALPN-202 was engineered for three mechanisms of action: PD-L1 and CTLA-4 antagonism combined with conditional CD28 agonism



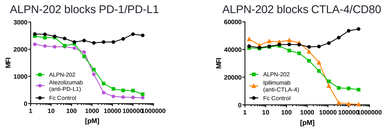
(A) ALPN-202 binds PD-L1 expressed on tumor, blocking PD-L1/PD-1 interactions. (B) Localized, tethered ALPN-202 is able to provide a trans CD28 signal to T cells making contact with the tumor (PD-L1-dependent CD28 costimulation). **(C)** Additionally, ALPN-202 binds CTLA-4 expressed on T cells, decreasing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

Figure 3: CD28 costimulation requires TCR signal and expression of PD-L1 on the APC



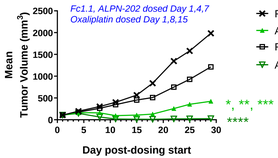
K562 artificial APC (aAPC) stably expressing transmembrane anti-human CD3 and/or human PD-L1 were plated in the presence of primary human T cells and a titration of ALPN-202 or controls. Culture supernatants were harvested at 24 hours and human IL-2 measured by ELISA.

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



CHO cells stably expressing human PD-L1 or CTLA-4 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: ALPN-202 is a potent mono-therapeutic and significantly improves the anti-tumor activity of the chemotherapeutic oxaliplatin when given in combination

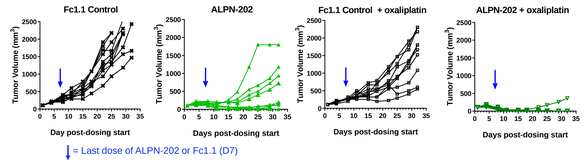


Test Article	Mean % TGI (D25)*
Fc1.1 control	0
ALPN-202	77.5
Fc1.1 + Oxaliplatin	41.2
ALPN-202 + Oxaliplatin	**98.6**

*Last day when 100% of mice still alive on study

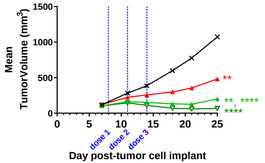
ALPN-202 group differences by repeated-measures 2-way ANOVA:
* $p < 0.05$ vs. ALPN-202+oxaliplatin combo group
** $p < 0.01$ vs. Fc1.1+oxaliplatin combo group
*** $p < 0.001$ vs. Fc1.1 control group
**** p < 0.0001 vs. Fc1.1 or Fc1.1+oxaliplatin combo group

%Tumor Growth Inhibition (TGI) = [(mean Fc1.1 tumor vol. – mean treated tumor vol.)/mean Fc1.1 tumor vol.] x 100)



C57BL/6 mice were implanted with s.c. 1.5 x 10^6 huPD-L1 transduced MC38 cells on day -11. On Day 1, mice were placed into treatment groups (mean tumor volume = 108 mm^3; 10 mice/group). Mice received 100 ug ALPN-202 or 75 ug Fc control by IP injection; oxaliplatin was dosed by IP injections at 5 mg/kg.

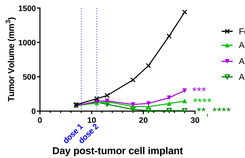
Figure 6: ALPN-202 is a potent mono-therapeutic and significantly improves the anti-tumor activity of anti CTLA-4 mAb when given in combination



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
**** $p < 0.0001$ for combination vs. Fc1.1 or CTLA-4 mAb

C57BL/6 mice were implanted s.c. with 1.5 x 10^6 huPD-L1 transduced MC38 cells on day 0. On Day 7, mice were placed into treatment groups (mean tumor volume = 113 mm^3; 9 mice/group). Mice received 100 ug of ALPN-202 or anti-mCTLA-4 mIgG2b mAb, or 75 ug of Fc1 control by IP injection, with means shown up to the day when at least 75% of mice in each group were still alive on study (Day 25).

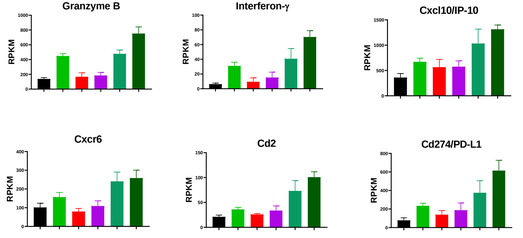
Figure 7: ALPN-202 is a potent mono-therapeutic and significantly improves the anti-tumor activity of anti PD-1 mAb when given in combination



By 2-way repeated-measures ANOVA:
*** $p < 0.001$ for PD-1 mAb vs. Fc1.1
**** $p < 0.01$ for ALPN-202 vs. Fc1.1
** $p < 0.01$ for combination vs. PD-1 mAb
**** $p < 0.0001$ for combination vs. Fc1.1

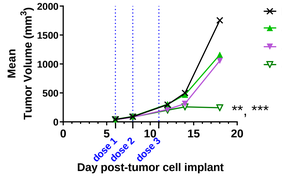
C57BL/6 mice were implanted s.c. with 1.5 x 10^6 huPD-L1 transduced MC38 cells on Day 0. On Day 7, mice were placed into treatment groups (mean tumor volume = 85 mm^3; 10 mice/group). Mice received 100 ug of ALPN-202 or anti-mouse (m) PD-1 mAb, or 75 ug Fc control by IP injection, with means shown up to the day when at least 75% of mice in each group were still alive on study (Day 28).

Figure 8: ALPN-202 enhances the expression of effector T cell transcripts in tumors, improving upon the anti-tumor activity of anti PD-1 and CTLA-4 mAbs



Tumors (4/group) were harvested from huPD-L1+ MC38 tumor-bearing mice 72 hr after a single IP injection of 75 ug Fc1.1, 100 ug ALPN-202, anti-mCTLA-4 mAb, or anti-mPD-1 mAb, or the indicated combination of ALPN-202 + either mCTLA-4 mAb or mPD-1 mAb. RNA was isolated and RNA-Seq performed. RPKM = reads per kilobase of transcript, per million mapped reads (mean + SEM). ALPN-202 significantly increased the expression of each gene over the level for CTLA-4 ($p < 0.05$ or better) or PD-1 mAb alone ($p < 0.01$ or better) by 1-way ANOVA.

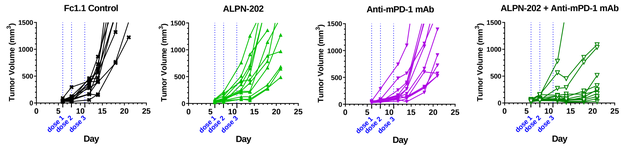
Figure 9: In the poorly immunogenic B16-F10 model, ALPN-202 significantly improves the anti-tumor activity of anti PD-1 mAb when given in combination



ALPN-202+PD-1 mAb combination comparisons by repeated measures 2-way ANOVA::
** $p < 0.01$ vs. PD-1 mAb or ALPN-202 monotherapy groups
*** $p < 0.001$ vs. Fc1.1 control

Test Article	Mean % TGI (D18)*
Fc1.1 control	0
ALPN-202	33.9
Anti-mouse PD-1 mAb	39.8
ALPN-202 + Anti-mouse PD-1 mAb	**86.1**

*Last day when at least 70%/group still alive on study



C57BL/6 mice were implanted s.c. with 0.5 x 10^6 huPD-L1 transduced B16-F10 cells on day 0. On Day 6, mice were placed into treatment groups (mean tumor volume = 45 mm^3; 12 mice/group). Mice received 100 ug of ALPN-202 or mPD-1 mAb, or 75 ug Fc control by IP injection.

Visit Poster #793 (M. Maurer, et al): "**ALPN-202, a Conditional CD28 Costimulator and Dual Checkpoint Inhibitor, Utilizes Multiple Mechanisms to Elicit Potent Anti-Tumor Immunity Superior to Checkpoint Blockade Alone**"

Summary and Conclusions

- ALPN-202 is a conditional CD28 costimulator and dual PD-L1 and CTLA-4 antagonist with demonstrated potent monotherapy anti-tumor efficacy in preclinical tumor models
- In combination, ALPN-202 significantly improves the anti-tumor activity of conventional standard of care chemotherapy or checkpoint-only inhibition, which are only modestly effective
- ALPN-202 augments the expression of intratumoral T effector cell transcripts and, in combination with checkpoint-only blockade, expression is further enhanced
- ALPN-202 may represent a new first-in-class immunotherapy with the potential to improve outcomes as monotherapy and in combination with standard of care in multiple cancer types.
- A first-in-human clinical study with ALPN-202 is in preparation.