Tumor-activated PD1-directed IL-2 increased antigen specific T cells in tumors and demonstrated anti-tumor activity in mice

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Introduction

PD-1/PD-L1 therapies have shown significant activity across a range of tumor types, but only 10-30% of patients achieve durable responses. With the goal of improving the response to PD-1/PD-L1 therapies, we have leveraged our proprietary Xilio Advanced Cytokine Therapies (X-ACT) platform to develop PD1/IL2-ACT, a multifunctional PD1 blocker enhanced with a tumor-activated, engineered IL-2 agonist. PD1/IL2-ACT is designed to block the activity of IL-2 by a protein domain that prevents IL-2R binding until activated in the tumor microenvironment (TME) by matrix metalloproteinases (MMPs). Tumor-selective activation of IL-2 enabled dosing of PD1/IL2-ACT at sufficient exposure levels to block PD1/PD-L1 signaling as well as mitigating systemic IL-2 driven toxicity. PD1/IL2-ACT is designed to enable in cis engagement of IL-2 receptors on antigen experienced PD-1$^+$ CD8$^+$ T cells in the TME, inducing effector T cell functions while preventing regulatory T cell (T_{reg}) mediated immune suppression.

PD1/IL2-ACT is a PD1/PD-L1 blocker with a tumor activated engineered IL-2 agonist

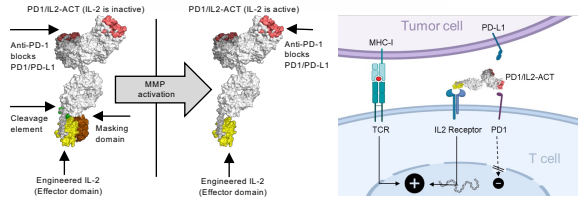

Figure 1: The anti-PD1 portion of PD1/IL2-ACT blocks the PD1/PD-L1 interaction. The masking domain keeps IL-2 pharmacologically inactive in non-tumor tissue. Upon proteolytic cleavage by proteases that are enriched in the TME, the IL-2 moiety can induce effector T cell function in the tumor. The cis-targeting of PD1 and IL2R enables effective delivery of IL-2 to PD1+ immune cells.

In vitro demonstration of efficient masking of IL-2, activation by MMP, and PD-1 blockade

PD1/IL2-ACT exhibited pharmacologically active IL-2 upon proteolytic activation and constitutive PD-1 blockade in reporter gene assays

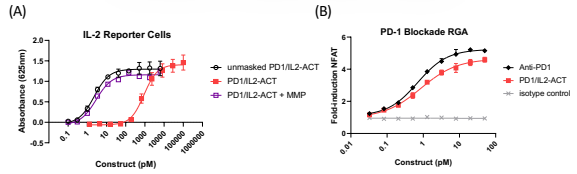

Figure 2: (A) IL-2 activity was measured with an IL-2 reporter gene cell line, which is engineered to express a colorimetric reporter upon IL-2-mediated STAT5 activation. Cells incubated with PD1/IL2-ACT demonstrated reduced reporter activity compared to cells treated with unmasked control molecule. Upon activation by MMP, PD1/IL2-ACT demonstrated parental (unmasked) level potency. (B) PD-1 blockade activity was measured with a two-cell PD-1/PD-L1 blockade bioassay, which is engineered for NFAT-induced luciferase activity upon blockade of PD-1/PD-L1 interaction. Cells incubated with PD1/IL2-ACT demonstrated activity comparable to anti-PD1 benchmark.

PD1/IL2-ACT can be activated in vitro, PD1 targeting enhanced potency of IL-2

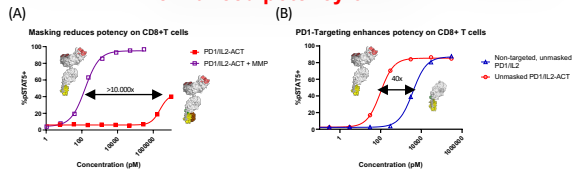

Figure 3: (A) Primary human peripheral blood mononuclear cells (PBMCs) were preactivated to upregulate PD1 expression. Preactivated PBMCs were incubated with varying doses of PD1/IL2-ACT and MMP-activated PD1/IL2-ACT for 12 minutes followed by evaluation for STAT5 phosphorylation. PD1/IL2-ACT exhibited minimal activity on hPBMCs while MMP-activated PD1/IL2-ACT exhibited potent signaling on CD8+ T cells. (B) Cells were incubated with varying doses of test articles including unmasked PD1/IL2-ACT and evaluated for STAT5 phosphorylation by flow cytometry. PD1-targeting enhanced the potency of unmasked PD1/IL2-ACT in comparison to a non-targeted version of this molecule.

In vivo anti-tumor activity and demonstration of tumor-selective pharmacokinetics (PK), pharmacodynamics (PD) in mouse model

PD1/IL2-ACT demonstrated anti-tumor activity in anti-PD1 insensitive setting at well-tolerated exposures

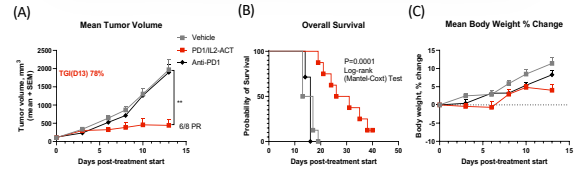

Figure 4: PD1/IL2-ACT demonstrated anti-tumor activity in an anti-PD1 insensitive MC38 syngeneic tumor model. C57BL/6-B-hPD1 mice were implanted subcutaneously with MC38 tumor cells and received two intravenous injection of 8mg/kg PD1/IL2-ACT (N=8), an equimolar concentration of anti-PD1 (N=7), or vehicle (N=8). Tumor and body weight measurements were taken two or three times a week. Data represent mean ± SEM (Standard error of the mean). (A) Tumor volume was assessed by a two-way ANOVA with Bonferroni's multiple comparisons test compared to vehicle treated animals.** P <0.0019 compared to vehicle-treated animals on Day 13. (B) Kaplan-Meier survival curves; animals were sacrificed when the ethical burden limit was reached (2000 mm^3);(Log-rank (Mantel-Cox) test, ****P < 0.0001). (C) Body weight data are displayed until Day 13 when 100% of the vehicle-treated group were alive (N=8). PR: Partial Regression

PD1/IL2-ACT was effectively masked in the periphery of MC38 murine tumor model

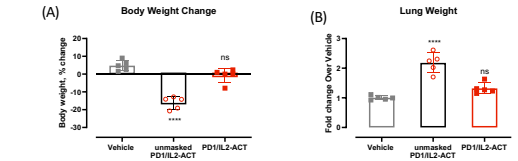

Figure 5: Evaluation of peripheral activity of PD1/IL2-ACT in MC38 syngeneic tumor model. (A) PD1/IL2-ACT (8mg/kg) resulted in no body weight change. Unmasked PD1/IL2-ACT (0.75mg/kg) resulted in significant body weight change. (B) PD1/IL2-ACT resulted in no pulmonary edema. Unmasked PD1/IL2-ACT resulted in significant pulmonary edema. Statistical comparisons by a one-way ANOVA with Bonferroni's post-hoc pairwise comparison test compared to vehicle.**** P <0.0001 compared to vehicle-treated animals on Day 6.

PD1/IL2-ACT in vivo activity is associated with activation in MC38 murine tumor model

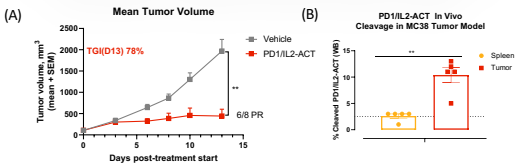

Figure 6: Tumor-associated protease-dependent activation of PD1/IL2-ACT in vivo. (A) C57BL/6-B-hPD1 mice were implanted subcutaneously with MC38 tumor cells and received two intravenous injection of PD1/IL2-ACT (8mg/kg). Data represent mean ± SEM. Tumor measurements were assessed by a two-way ANOVA with Bonferroni's multiple comparisons test compared to vehicle treated animals.** P <0.0047 compared to vehicle-treated animals on Day 13. TGI: tumor growth inhibition. PR: Partial regression. (B) Measurement of % of cleaved PD1/IL2-ACT in the tumor and spleen, in the MC38 syngeneic tumor model. MC38 mice were dosed with 8mg/kg PD1/IL2-ACT. On Day 6, mice were sacrificed, Spleen and the tumor were collected. Spleen and tumor tissues were lysed in RIPA buffer. PD1/IL2-ACT in tissue lysate was immunoprecipitated using anti-human IgG and the percentage of cleaved PD1/IL2-ACT was determined by WB (LLOQ 2.5%). Statistical comparisons were assessed by an unpaired t test versus tumor, where ***P < 0.0021

PD1/IL2-ACT increased memory and antigen specific CD8 T cells in MC38 murine tumor but not in the periphery

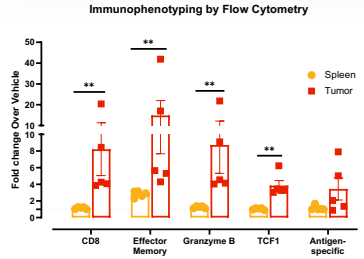

Figure 7: PD1/IL2-ACT demonstrated intra-tumor CD8 T cell expansion. C57BL/6-B-hPD1 mice were implanted subcutaneously with MC38 tumor cells and received two (i.v) injections of PD1/IL2-ACT (8mg/kg) or vehicle control. Immune cells were phenotyped using fluorescence-activated cell sorting (FACS). The percentage of cells for each immune phenotype was calculated as percentage of live CD45+ cells and the ratio of percent cells after PD1/IL2-ACT treatment to vehicle treatment is presented as mean ± SEM. Changes in the ratio of each cell type in spleen and tumor were assessed by Mann Whitney t test. *P < 0.05, **P < 0.005, ***P < 0.0021, ****P < 0.0001. Effector memory (CD44$^+$CD62L$^-$), Antigen-Specific (p15E-Pentamer)

PD1/IL2-ACT showed antibody-like PK in mice and was effectively retained in MC38 tumor

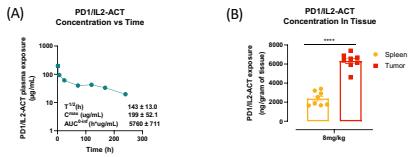

Figure 8: PD1/IL2-ACT exposure in non-tumor bearing and tumor bearing mouse models. (A) Exposure after a single 8mg/kg intravenous (i.v) injection in non-tumor bearing C57BL/6-hFcRn mice. (B) Exposure 72hrs after second intravenous (i.v) injection in MC38 tumor-bearing C57BL/6-B-hPD1 mice. Changes between spleen and tumor were assessed by an unpaired t test. *P < 0.05, **P < 0.005, ***P < 0.0021, ****P < 0.0001.

PD1/IL2-ACT demonstrated significant anti-tumor activity in vivo in a murine bladder tumor model

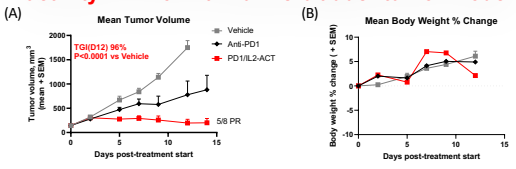

Figure 9: PD1/IL2-ACT demonstrated superior anti-tumor activity in an anti-PD1 sensitive MB49 syngeneic tumor model. C57BL/6-B-hPD1 mice were implanted subcutaneously with MB49 tumor cells and received two intravenous injection of 3mg/kg PD1/IL2-ACT, an equimolar concentration of anti-PD1, or vehicle (N=8). Tumor and body weight measurements were taken two/three times a week. Data represent mean ± SEM (Standard error of the mean). (A) Tumor volume was assessed by a two-way ANOVA with Bonferroni's post-hoc pairwise comparison test compared to vehicle treated animals.** P <0.0001 compared to vehicle-treated animals on Day 12. (B) Body weight data are displayed until Day 12 when 100% of the vehicle-treated group were alive (N=8). PR: Partial regression

PD1/IL2-ACT pharmacokinetics (PK) and safety profile in non-human primates (NHP)

PD1/IL2-ACT demonstrated dose-proportional PK and was well tolerated following a single IV Dose in non-human primates

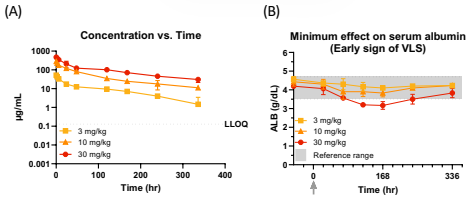

Figure 10: Female cynomolgus monkeys were given a single 30-minute intravenous infusion of PD1/IL2-ACT at 3, 10, and 30 mg/kg and samples were collected for PK and clinical pathology analysis. (A) PK analysis demonstrated dose-proportional exposure and linear elimination across all doses tested. (B) Albumin remained within normal ranges in animals receiving 3 and 10 mg/kg PD1/IL2-ACT, and was transiently decreased in animals receiving 30 mg/kg PD1/IL2-ACT. There were no observed adverse clinical observations, and transaminase levels remained within normal ranges for all animals (data not shown).

Conclusions

The MMP activated, masked, anti-PD1 targeted IL-2 multifunctional, PD1/IL2-ACT, represents a potential novel approach for the treatment of cancer. Preclinical data presented demonstrated:

- PD1/IL2-ACT effectively antagonized PD-1 signaling in vitro
- PD1/IL2-ACT exhibited >10,000 fold masking of IL-2 activity in PBMC assays
- In vivo, PD1/IL2-ACT was activated in the TME and effectively masked in the periphery
- PD1/IL2-ACT exhibited significant anti-tumor activity in both PD1 insensitive and sensitive in vivo models
- PD1/IL2-ACT increased memory and antigen specific CD8 T cells in preclinical tumor models
- PD1/IL2-ACT demonstrated antibody-like PK and was well tolerated in NHPs