A Bifunctional Anti-PD-L1 Antibody/IL-10 Fusion Protein Targeting Exhausted T Cells for Cancer Immunotherapy

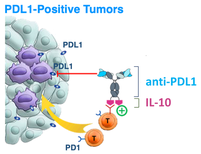
Huey-Wen Hsiao, Chih-Lun Hsiao, Pandelakis A. Koni, and Hung-Kai Chen

Elixiron Immunotherapeutics Inc., Taipei, Taiwan

INTRODUCTION

Immune checkpoint inhibitors are effective only in a fraction of patients with advanced malignancies. Resistance to PD-1 or PD-L1 blockade may be mediated by additional exhaustion pathways. Interleukin-10 (IL-10) has demonstrated utility in aiding CD8 T cell-mediated tumor control in mouse models but phase 2 clinical trials with pegylated IL-10 did not show clear and meaningful benefit. More recent studies revealed that another half-life-extended form of IL-10 (IL10-Fc) can activate terminally-exhausted CD8$^+$ T cells to boost anti-tumor activity.

We designed a novel, bifunctional anti-PDL1/IL10 fusion protein with the goal of delivering IL-10 to PD-L1-high cells while blocking PD-L1, and evaluated its *in vitro* activity as well as *in vivo* anti-tumor efficacy.



Dual Mechanism

- **anti-PDL1** for checkpoint blockade and tumor-targeting
- **IL-10** to revitalize exhausted T cells

METHODS

Anti-PD-L1 antibodies were first developed by screening an in-house human variable domain phage display library for anti-PD-L1 clones. A lead anti-PD-L1 antibody was then C-terminally-fused with human IL-10 polypeptide. Recombinant anti-PDL1/IL10 fusion proteins were generated and produced by expiCHO expression system (Thermo Fisher). *In vitro* PD-L1 blocking activity was evaluated by ELISA, PD-1/PD-L1 signaling bioassay (Eurofins) and mixed lymphocyte reaction (MLR) assays. *In vitro* IL-10 activity was evaluated by ELISA, MC/9 cell proliferation and CD8 T cell activation assays. Mouse syngeneic CT26 colon cancer and EMT6 breast cancer tumor model studies employed the services of Oneness Biotech and Crown Bioscience, respectively. Doses, and schedules of dosing, of antibodies and recombinant proteins (by i.p. injection) are in each Figure.

RESULTS

Anti-PDL1/IL10 Blocks PD-1/PD-L1 Signaling and Increases T Cell Activation

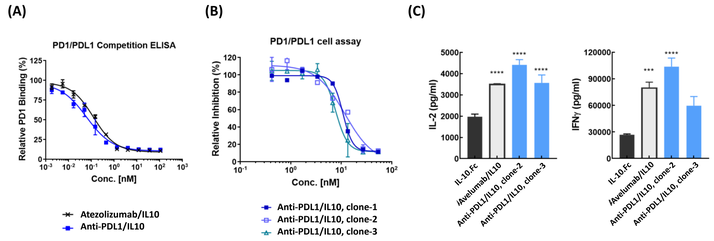


Figure 1. (A) Anti-PDL1/IL10 fusion proteins block the specific binding of PD-L1 to PD-1. Recombinant human PD-L1 was immobilized on microtiter wells, biotin-conjugated human PD1 was added and detected by streptavidin using ELISA. To test the competition activity, serial dilutions of anti-PDL1/IL10 fusion proteins were added. **(B)** anti-PDL1/IL10 fusion proteins block PD-1 activation mediated by a U2OS PD-L1 cell line co-culture. The U2OS PD-L1 cells (Eurofins) were pre-treated with anti-PDL1/IL10 fusion proteins for 1 hour and then stimulated with Jurkat PD-1 cells (Eurofins) for 2 hours. **(C)** anti-PDL1/IL10 fusion proteins enhance T-cell activation in MLR. CD4 T cells were co-cultured with allogeneic mature DC in the presence of anti-PDL1/IL10 fusion proteins. The level of IL-2 and IFNγ in supernatants were measured by ELISA (BioLegend) at day 2 and 5, respectively.

Anti-PDL1/IL10 Induces IFNγ And Granzyme B Production From Activated CD8 T cells

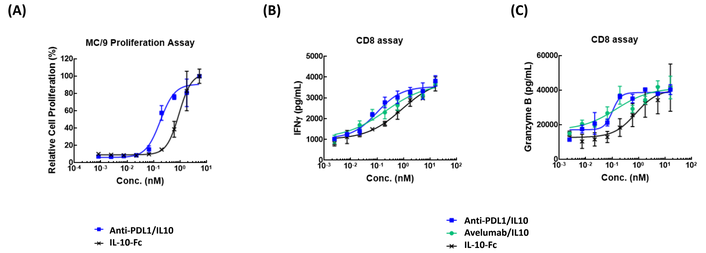


Figure 2. (A) Anti-PDL1/IL10 fusion proteins promote of MC/9 cell proliferation. MC/9 cells were co-cultured for 3 days with IL10-Fc or anti-PDL1/IL10 fusion proteins. Cell proliferation was measured by CellTiter-Glo assay. The potentiation of **(B)** IFNγ and **(C)** granzyme B production from activated CD8 T cells by anti-PDL1/IL10 fusion proteins. CD8 T cells were isolated from PBMC and activated with anti-CD3 plus anti-CD28 for 3 days. Activated T cells were treated with anti-PDL1/IL10 fusion proteins for 3 days and restimulated with anti-CD3 (BioLegend) for 4 hours. Level of IFNγ and granzyme B were measured by ELISA (BioLegend).

Anti-PDL1/IL10 Treatment Controls Tumor Growth in a Mouse Model

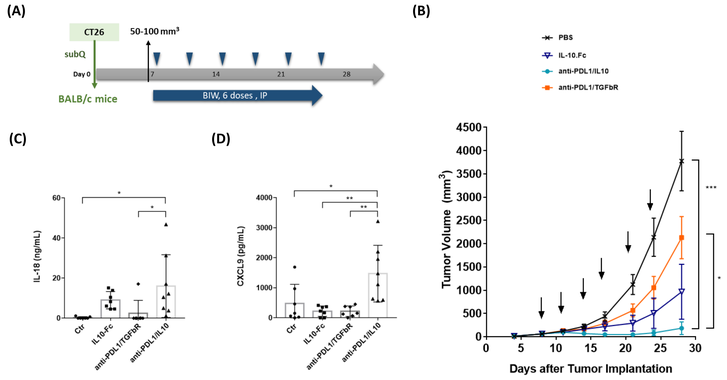
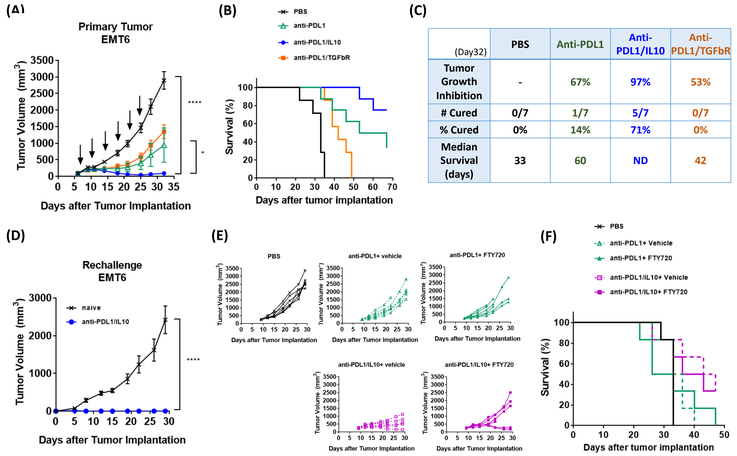


Figure 3. Anti-PDL1/IL10 enhances anti-tumor response. **(A)** Scheme of treatment in CT26 tumor-bearing mice. Syngeneic CT26 tumor-bearing mice (n=7) were randomized 7 days post-implantation and then treated with IL10-Fc (3 mg/kg), anti-PDL1 (5 mg/kg) plus anti-CSF1R/IL10 (36 mg/kg), anti-PDL1/IL10 (6 mg/kg), and anti-PDL1/TGFbR (6 mg/kg) twice weekly for 3 weeks. **(B)** Tumor growth was measured twice per week. Serum level of **(C)** IL-18 and **(D)** CXCL9 after treatment (day 29) were measured by ELISA (BioLegend). Mean ± SEM is shown. *p < 0.05, **p < 0.01, ***p < 0.001

Anti-PDL1/IL10 Promotes Tumor Rejection After Rechallenge, Indicating Durable T Cell Memory Responses



(Day32)	PBS	Anti-PDL1	Anti-PDL1/IL10	Anti-PDL1/TGFbR
Tumor Growth Inhibition	-	67%	97%	53%
# Cured	0/7	1/7	5/7	0/7
% Cured	0%	14%	71%	0%
Median Survival (days)	33	60	ND	42

Figure 4. Anti-PDL1/IL10 has anti-tumor efficacy and promotes long-term memory protection. **(A-D)** Syngeneic EMT6 tumor-bearing mice (n=7) were randomized 7 days post-implantation and treated with anti-PD-L1 (5 mg/kg), anti-PDL1/IL10 (6 mg/kg) or anti-PDL1/TGFbR (6 mg/kg) twice weekly for 3 weeks. **(A)** Primary tumor growth curves, **(B)** survival curves and **(C)** summary table for tumor growth inhibition, numbers of cured mice and median overall survival. **(D)** 5 weeks after tumor 'cure' (day 67), cured and naïve Balb/c mice (n=5) were implanted with EMT6 tumor cells and growth monitored for 4 weeks. **(E-F)** EMT6 tumor-bearing mice (n =6) were randomized on day 10 and treated with FTY720 (2 mg/kg, PO) daily for 2 weeks. Anti-PDL1 (10 mg/kg) or anti-PDL1/IL10 (10 mg/kg) were administered twice weekly from day 11 for 4 weeks. **(E)** Tumor growth was measured twice weekly. **(F)** survival curves. Mean ± SEM is shown. *p < 0.05, **p < 0.01, ***p < 0.001

CONCLUSIONS

- Anti-PDL1/IL10 blocked PD-1 signaling through PD-1/PD-L1 interaction and enhanced IL-2 and IFNγ production in MLR.
- Anti-PDL1/IL10 induced IL-10-dependent cell proliferation and enhanced IFNγ and granzyme B production from activated CD8 T cells.
- In syngeneic CT26 and EMT6 tumor models, anti-PDL1/IL10 fusion protein showed effective anti-tumor activities and superior efficacy than anti-PDL1/TGFbR (M7824).
- Anti-PDL1/IL10 treatment allowed for subsequent, secondary tumor rejection in mice 'cured' of their primary tumor, indicating durable T memory responses.
- The ability of anti-PDL1/IL10 to enhance tumor control was in part dependent on increased activity of tumor-resident cells as co-treatment with FTY720, which inhibits lymphocyte egress from secondary lymphoid organs, did not reduce anti-tumor activity in some albeit not all mice.
- Taken together, our findings support this dual mechanism strategy of targeting exhausted T cells to potentiate anti-tumor immunity for the treatment of colon and breast cancer.

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Contact Us: Hung-Kai Chen (hkchen@elixiron.com) or Pandelakis Koni (pkoni@elixiron.com)

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