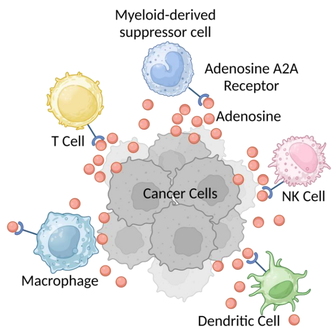
Selective A2AR Blockade Synergizes with Immune Checkpoint Therapy by Potentiating Proinflammatory Th1 Helper Cell Responses

Dan Li, Poorva Ghosh, Lih-Yun Hsu, Richard A. Miller

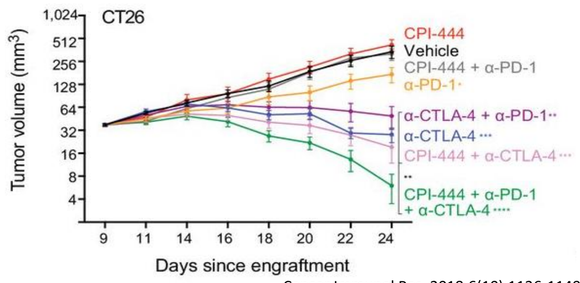
Corvus Pharmaceuticals, Inc., Burlingame, California, United States

BACKGROUND

Adenosine Signaling Through A2AR Limits the Efficacy of Immunotherapy

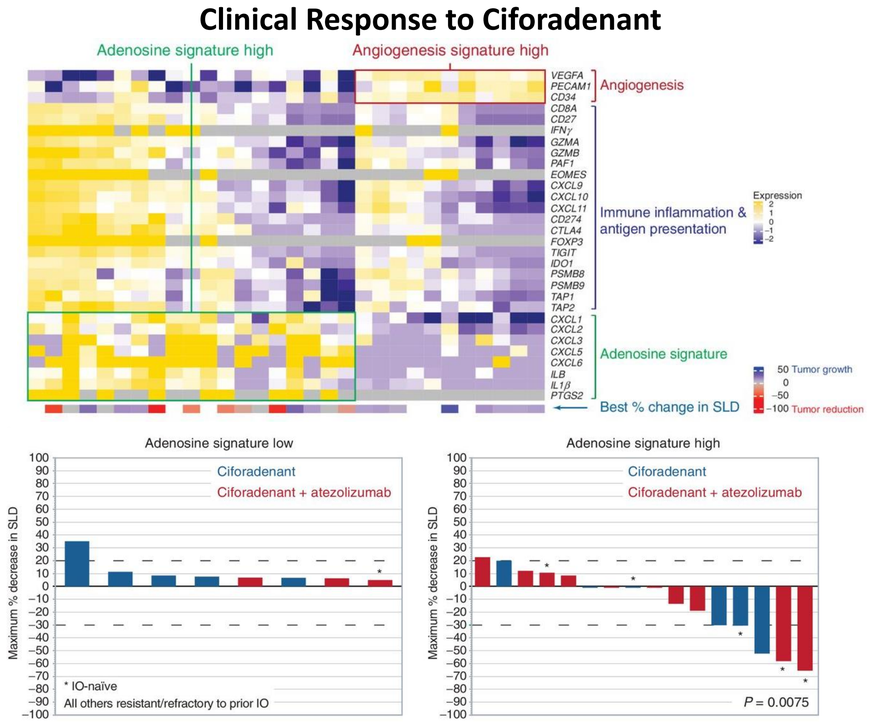


Ciforadenant Augments the Efficacy to Immune Checkpoint Blockade (ICB)



Cancer Immunol Res. 2018;6(10):1136-1149.

Adenosine Signature in Pretreatment Biopsies Predicts Clinical Response to Ciforadenant



Cancer Discov. 2020;10(1):40-53.

MATERIALS AND METHODS

Syngeneic murine tumor models: CT26 (colorectal carcinoma) and B16F10-OVA (melanoma) were subcutaneously implanted into Balb/c and C57BL/6 mice, respectively. Caliper measurement was performed by a digital caliper (Flwer-Sylvac). Tumor volume was calculated by the formula (length x width^2)/2. Mice with established tumors (~50 mm^3) were treated with anti-PD1 (clone RPM1-14, 25 μg/mouse) and anti-CTLA4 (clone 9H10, 25 μg/mouse) and Ciforadenant (30 mg/kg).

Gene profile analysis: Tumor treated with Ciforadenant alone by oral gavage or in combination with anti-PD1 and anti-CTLA4 were harvested and stored in RNAlater Solution (ThermoFisher) at 4ºC. RNA was extracted using Rneasy Mini Kit (Qiagen). Nanostring mouse Myeloid Panel along with a custom codeset consisting of probes to genes were used on nCounter Sprint Profiler (NanoString). Data were analyzed by nSolver Analysis Software.

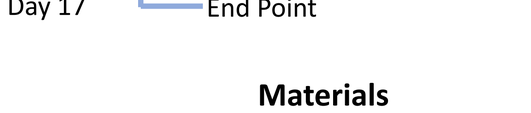
Analysis of surface and intracellular marker in tumor: Single-cell suspension of tumors were prepared by tumor dissociation kit and gentleMAC dissociator (Miltenyi Biotec). Cell surface markers were stained for 20-30 min in 4ºC, and then fixed and permeabilized using the Foxp3 Fix/Perm buffer set (ThermoFisher). Intracellular proteins or cytokines were stained after permeabilization. Flow cytometry was performed on the Cytoflex cytometer (Beckman Coulter) and data were analyzed using FlowJo software (TreeStar).

Measurement of intratumoral cytokines/chemokines: 50 mg of tumors were resuspended with PBS and 1% Triton and 1% protease inhibitor cocktail (Sigma-Aldrich). The tissue was then homogenized with a Bullet Blender Blue (Next Advance). Intratumoral cytokines were measured by MSD (Meso Scale Diagnostics). The cytokine concentrations were normalized to protein concentration of the same tumor lysates.

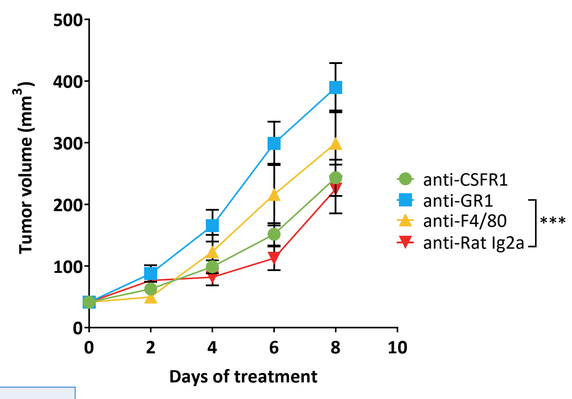
RESULTS

Depletion of Myeloid Cells Abolishes Synergy of Ciforadenant & ICB in Murine B16 Ova Melanoma Model

Study Design



Tumor Growth Curve



Materials

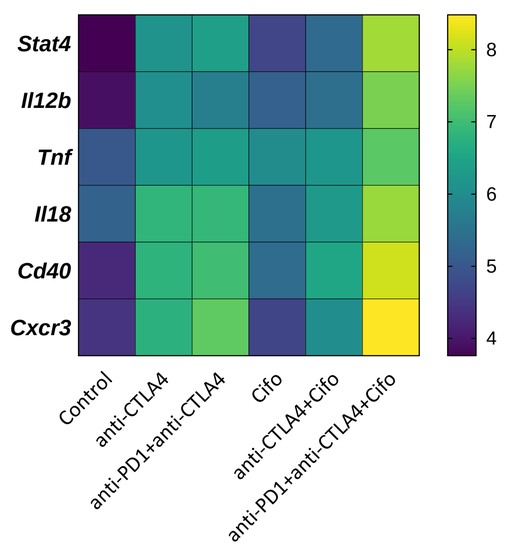
ICB+Ciforadenant				
Depletion antibody	anti-CSFR1	anti-GR1	anti-F4/80	anti-Rat Ig2a
Clone	AFS98	RB6-8C5	CI: A3-1	2A3
Depleting Cell types	TAM & DC	MDSC	M2 TAM	Control Isotype

CSF1R: colony-stimulating factor 1 receptor
TAM: tumor-associated macrophage
DC: dendritic cell
GR1: gamma response 1
MDSC: myeloid-derived suppressor cell
F4/80: adhesion G protein-coupled receptor E1
M2 TAM: M2-polarized tumor-associated macrophage

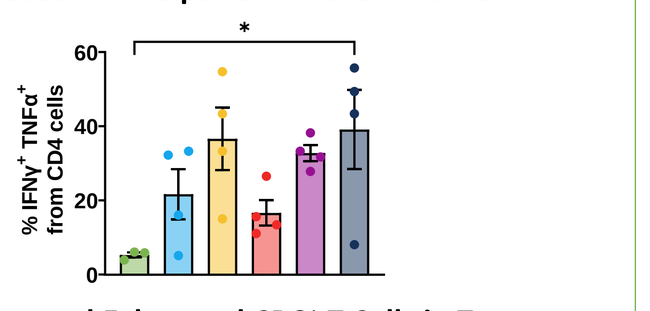
Figure 1. ***Depletion of MDSC induces tumor progression in mice.*** 1 million of B16F10-Ova tumor cells were engrafted to B6 mouse. Anti-CSFR1 (400 μg/mouse) or anti-GR1 (400 μg/mouse) or anti-F4/80 (200 μg/mouse) or anti-Rat Ig2a (400 μg/mouse) plus ICB, anti-PD1 and anti-CTLA4 and Ciforadenant were injected when the tumor was around 50 mm^3. Data are expressed as mean±SEM, 2-way ANOVA, ***p<0.001.

Combination of Ciforadenant & ICB Upregulates Genes in IL-12 Signaling Axis and Proinflammatory Th1 Helper Cell Responses

Upregulated Genes in IL-12 Signaling Pathway



Increased Th1 Helper CD4$^+$ Cells in Tumor



Decreased Exhausted CD8$^+$ T Cells in Tumor

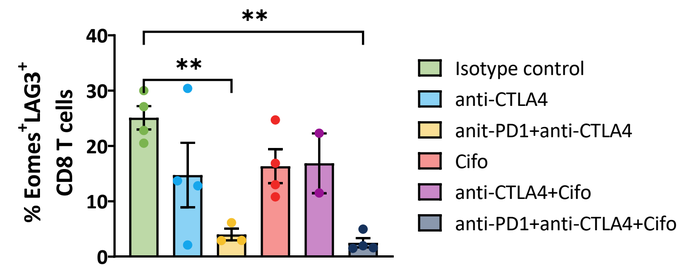
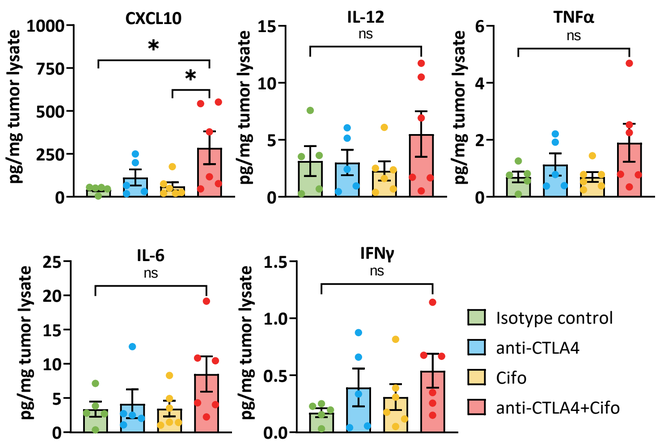


Figure 2. ***Proinflammatory state in the TME of CT26 mouse tumor with Ciforadenant and ICB.*** Genes in IL-12/STAT4 signaling axis in combination of Ciforadenant with ICB in CT26 mouse tumor model were identified using the NanoString mouse myeloid panel and shown with Log2 fold changes. The production of Th1 effector cytokines from CD4$^+$ T cells marked by IFNγ and TNFα and exhausted CD8$^+$ T cells marked by Eomes (Eomesodermin) and LAG3 (Lymphocyte Activating Gene 3) from tumor were detected by Flow cytometry. Data are expressed as mean±SEM, 1-way ANOVA, *p<0.05, **p<0.01.

Combination of Ciforadenant & ICB Increases CXCL10 for Recruiting Proinflammatory Th1 Helper Cell Responses to Hot and Cold Tumors

CT26 Colorectal Carcinoma



B16F10 OVA-expressing Melanoma

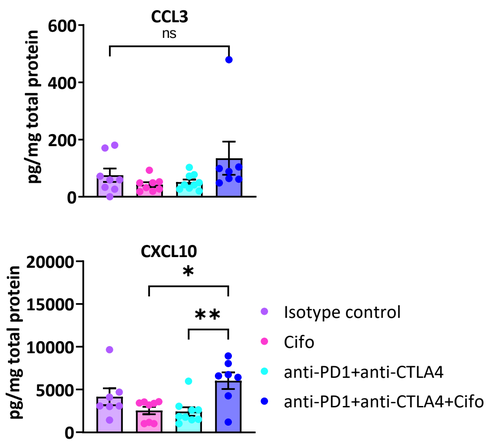
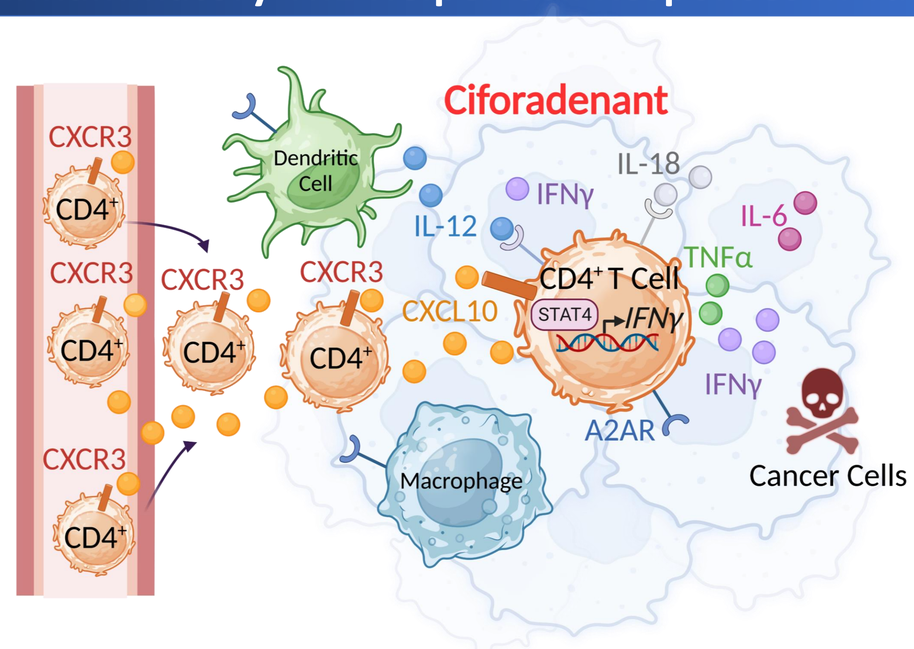


Figure 3. ***Ciforadenant & ICB increase proinflammatory cytokine production in tumor.*** Ciforadenant with ICB treatment increased production of CXCL10, ligands for recruiting CXCR3+ T cells into the tumor from CT26 and B16F10 OVA detected by MSD. Data are expressed as mean±SEM, 1-way ANOVA, *p<0.05, **p<0.01.

Mechanism of Ciforadenant in Potentiating Proinflammatory Th1 Helper Cell Responses in Tumor



CONCLUSIONS

- ❑ Depletion of myeloid cells abolishes the synergy of Ciforadenant and ICB in the murine melanoma model.
- ❑ Combination of Ciforadenant with ICB upregulates the genes involved in IL-12/STAT4 signaling axis which leads to the development of CXCR3$^+$ IFNγ-producing Th1 helper cells.
- ❑ Ciforadenant treatment increases production of chemokine CXCL10, a ligand for recruitment of CXCR3$^+$ Th1 helper cells into the tumor.
- ❑ Ciforadenant modulates antitumor responses by turning the tumor microenvironment into the proinflammatory state.
- ❑ Combining Ciforadenant with ICB promotes production of proinflammatory cytokines such as IL-6, TNFα, and IFNγ.
- ❑ Ongoing Phase II clinical trial together with nivolumab plus ipilimumab (NIVO+IPI) is in collaboration with Kidney Cancer Research Consortium.

CORVUS PHARMACEUTICALS

Disclosure: Dan Li is a full-time employee at Corvus Pharmaceuticals, Inc. and holds stock options of Corvus.