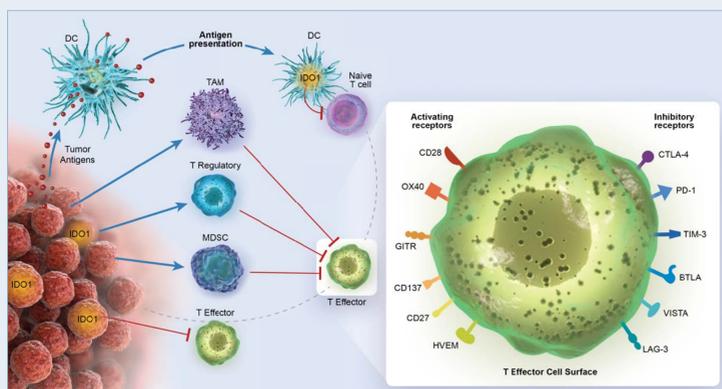


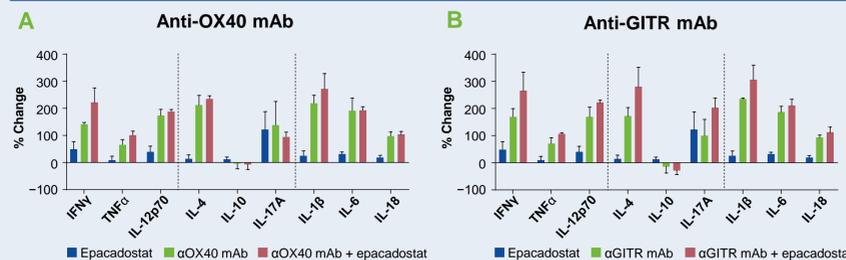


Abstract

The majority of immunotherapeutic agents developed thus far either attempt to stimulate a more productive anti-tumor immune response or to inhibit key proteins in the immunosuppressive tumor milieu. PD-1/PD-L1 axis blockade, CTLA-4 blockade and IDO1 inhibition are examples of the latter approach and have been utilized to reverse the suppressive tumor microenvironment, resulting in clinical benefit for cancer patients. Recent clinical and preclinical data have also demonstrated that combining these approaches results in enhanced therapeutic benefit. Notably, the IDO1-selective inhibitor epacadostat has been shown to increase the efficacy of two checkpoint inhibitors, the anti-CTLA-4 antibody ipilimumab and the anti-PD-1 antibody pembrolizumab, in patients with melanoma and other cancers at well-tolerated doses. Because both checkpoint receptors and IDO1 serve as negative regulators of the immune response, we also explored the ability of IDO1 inhibition to combine with agents that directly activate T cells through costimulatory receptors of the tumor necrosis factor receptor (TNFR) superfamily. Rodent active surrogate agonist antibodies to 4-1BB, OX40 and GITR were tested with epacadostat in multiple preclinical models. In the B16-SIY melanoma model that does not express IDO1 in tumor cells, both epacadostat and anti-OX40 had little effect, but the combination resulted in enhanced efficacy. This was associated with increased infiltrates of CD8⁺ T cells and decreased numbers of FoxP3⁺ TILs. Increased numbers of SIY-reactive T cells were found in both the tumor and the TDLN post-therapy. Clear combinatorial effects were seen with anti-GITR and epacadostat in the more inflamed, IDO1-expressing PAN02 pancreatic cancer model. These data suggest that IDO1 inhibition can be effective in combination with agents that agonize T cell costimulatory receptors as well as with agents that block coinhibitory receptors.

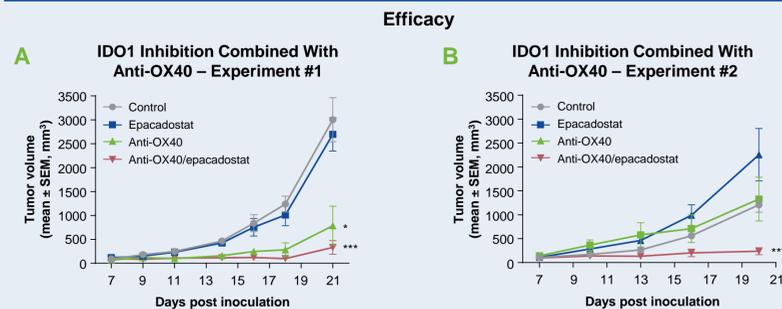


Epacadostat Enhances the Activity of Anti-OX40 and Anti-GITR in Splenocytes From Tumor-Bearing Mice

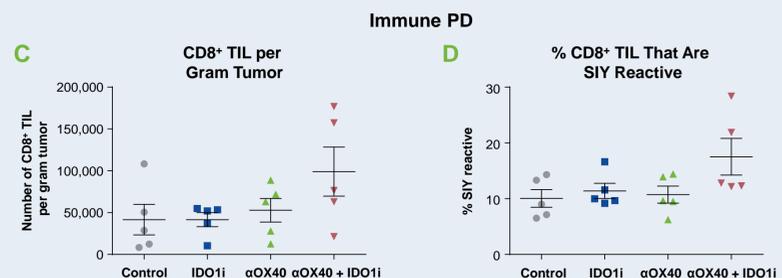


Epacadostat enhances the activity of both anti-OX40 and anti-GITR *in vitro*. Splenocytes from Pan02 tumor-bearing mice were isolated and stimulated for 3 days using soluble anti-CD3 mAb (2 µg/mL). The cells were stimulated in presence of anti-OX40 mAb (OX86 clone – 3.3 µg/mL), anti-GITR mAb (DTA1 clone – 3.3 µg/mL), epacadostat (100 nM), or a combination of epacadostat with either mAbs. After 3 days of culture, cell supernatants were collected and cytokine levels in the supernatants were analyzed using Luminex technology. Results for 9 different cytokines are depicted as percentage of changes compared with vehicle-treated cells (n = 3 per group).

Epacadostat Enhances Efficacy and Immune PD Measures of Anti-OX40 in the B16-SIY Tumor Model



Epacadostat demonstrates enhanced anti-tumor activity when combined with anti-OX40 treatment (A & B). Efficacy of 300 mg/kg epacadostat administered orally once daily, 100 µg per animal murine anti-OX40 antibody administered intraperitoneally Q3D x 4, or the combination in C57BL/6 mice bearing B16-SIY melanoma tumors was assessed. The mean tumor volume (± SEM) for groups of 5 animals are shown for 2 replicate experiments. * P<0.05, *** P<0.001.



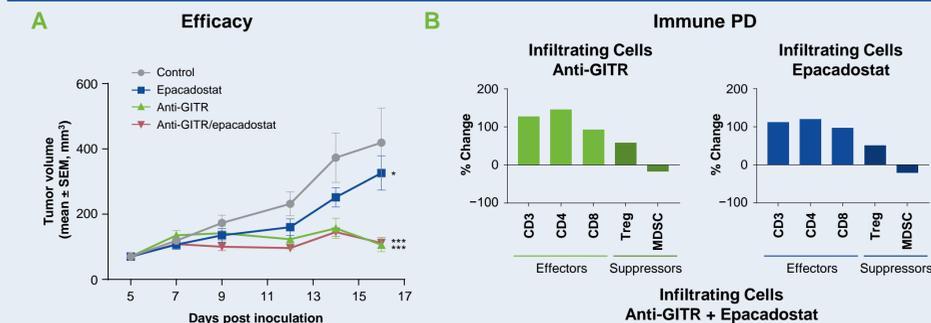
The combination of epacadostat and anti-OX40 enhances total CD8⁺ cells (C) and the percentage of antigen reactive cells (D). C57BL/6 mice bearing B16-SIY tumors were treated for 6 days with epacadostat, anti-OX40, or the combination as in A & B. Tumors were harvested and processed for tumor-infiltrating lymphocytes (TIL) that were subsequently analyzed by flow cytometry.

Epacadostat Does Not Affect the Efficacy of Anti-4-1BB or Anti-GITR in B16-SIY Tumor Model



Epacadostat does not demonstrate enhanced anti-tumor efficacy when combined with anti-4-1BB (A) or anti-GITR (B) in the B16-SIY model. Efficacy of 300 mg/kg epacadostat administered orally once daily, anti-4-1BB (100 µg intraperitoneally Q3D x 4) or anti-GITR (500 µg intraperitoneally once), or epacadostat in combination with these antibodies in C57BL/6 mice bearing B16-SIY tumors. The mean tumor volume (± SEM) for groups of 4 to 5 animals is shown. *** P< 0.001.

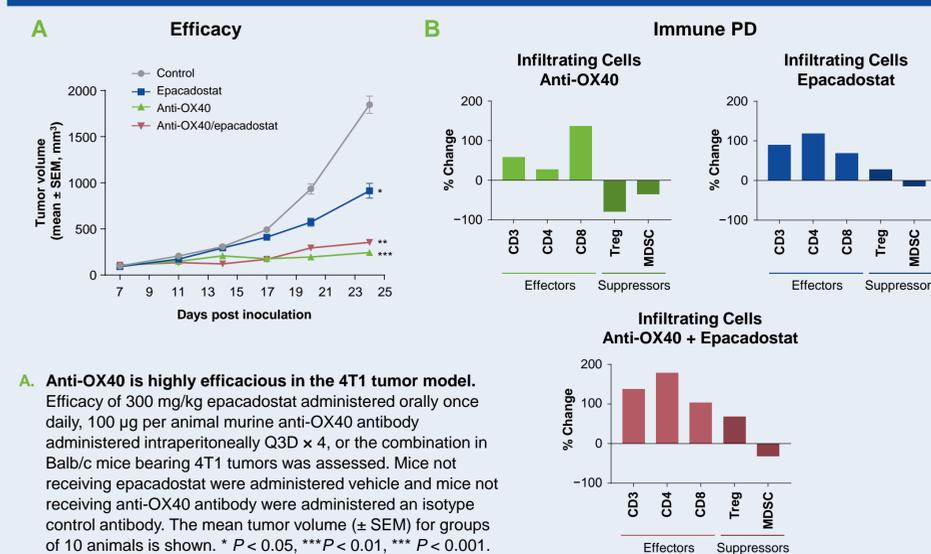
Epacadostat Enhances Pharmacodynamic Measures of Anti-GITR in 4T1 Model



A. Anti-GITR is highly efficacious in the 4T1 tumor model. Efficacy of 300 mg/kg epacadostat administered orally once daily, 500 µg per animal murine anti-GITR antibody administered intraperitoneally once, or the combination in Balb/c mice bearing 4T1 mammary carcinoma tumors was assessed. Mice not receiving epacadostat were administered vehicle and mice not receiving anti-GITR antibody were administered an isotype control antibody. The mean tumor volume (± SEM) for groups of 10 animals is shown. * P< 0.05, *** P< 0.001.

B. Epacadostat enhances PD immune parameters of anti-GITR. Balb/c mice bearing 4T1 tumors were treated for 10 days with epacadostat, anti-GITR, or the combination regimen as indicated above. Tumors were harvested and infiltrating cells analyzed by flow cytometry.

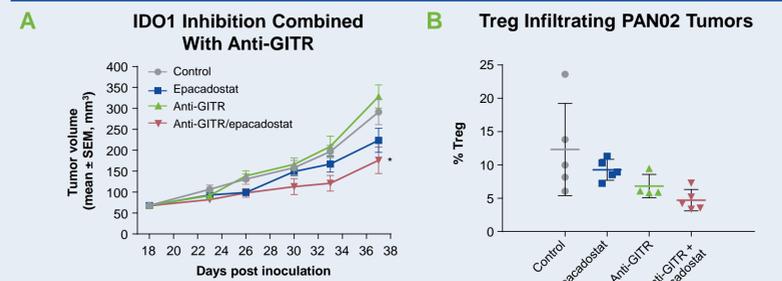
Anti-OX40 Is Highly Efficacious in the 4T1 Tumor Model and Combination With Epacadostat Further Enhances Immune PD Measures



A. Anti-OX40 is highly efficacious in the 4T1 tumor model. Efficacy of 300 mg/kg epacadostat administered orally once daily, 100 µg per animal murine anti-OX40 antibody administered intraperitoneally Q3D x 4, or the combination in Balb/c mice bearing 4T1 tumors was assessed. Mice not receiving epacadostat were administered vehicle and mice not receiving anti-OX40 antibody were administered an isotype control antibody. The mean tumor volume (± SEM) for groups of 10 animals is shown. * P< 0.05, ***P< 0.01, **** P< 0.001.

B. Epacadostat enhances PD immune parameters of anti-OX40. Balb/c mice bearing 4T1 tumors were treated for 10 days with epacadostat, anti-OX40, or the combination regimen as indicated above. Tumors were harvested and infiltrating cells analyzed by flow cytometry.

Enhanced Anti-tumor Effect and Treg Reduction Observed With Epacadostat Combined With Anti-GITR in PAN02 Model



A. Epacadostat demonstrates enhanced anti-tumor activity when combined with anti-GITR treatment in PAN02 tumors. Efficacy of 300 mg/kg epacadostat administered orally once daily, 500 µg anti-GITR administered intraperitoneally once, or epacadostat in combination with anti-GITR in C57BL/6 mice bearing PAN02 tumors was assessed. Mice not receiving epacadostat were administered vehicle and mice not receiving anti-GITR antibody were administered an isotype control antibody. The mean tumor volume (± SEM) for groups of 10 animals is shown. * P< 0.05.

B. Combination of epacadostat and anti-GITR reduces percentage of Tregs. PAN02 tumor bearing mice were treated for 7 days with anti-GITR, epacadostat, or the combination regimen as indicated above. TILs were harvested and analyzed by flow cytometry.

Epacadostat Combined With Anti-OX40 and Anti-GITR Results in Enhanced Anti-tumor Effects

Model	PAN02	4T1	B16-SIY
Epacadostat	Moderate	Moderate	No effect
Anti-GITR	No effect	Strong (3 PR, 7 CR, n = 33; ORR = 30%)	Moderate
Anti-GITR + epacadostat	Enhanced effect	Enhanced effect (6 CR, n = 10; ORR = 60%)	No enhancement
Anti-OX40	Moderate	Strong (1 PR, 3 CR, n = 20; ORR = 20%)	Moderate
Anti-OX40 + epacadostat	No enhancement	No enhancement (2 CR, n = 20; ORR = 10%)	Enhanced effect (1 CR, n = 10)

Conclusions

- Epacadostat enhances the activity of several agents that modulate the tumor microenvironment
- Preclinical data support clinical testing of epacadostat in combination with agents that activate T cells
- Further investigation into mechanism of action in models where agonist antibodies are strongly efficacious independently are needed
- Syngeneic models and surrogate antibodies may be limited and more sophisticated models should be explored

Author Disclosures
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