# Novel, Synthetic RORγ Agonist Compounds as a Potential Anti-Cancer Approach

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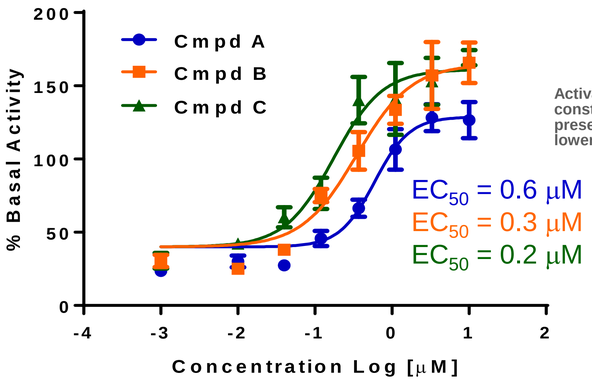
## BACKGROUND

- **RORγ isoforms are nuclear receptor transcription factors that modulate gene expression**
  - **RORγ modulates expression of genes operating in pathways that enhance immunity and decrease immune suppression**
  - **RORγt is the master transcription factor for Th17/Tc17 differentiation**
  - **Th17/Tc17 cells have demonstrated stemness and plasticity which contribute to durable anti-tumor efficacy**
- **IL-17 is associated with good prognosis in some cancers**
- **Although IL-17 is the signature cytokine of Th17/Tc17, RORγ-expressing cells are polyfunctional effectors with multiple anti-tumor mechanisms**
- **Agonists enhance RORγ-dependent reporter activity**



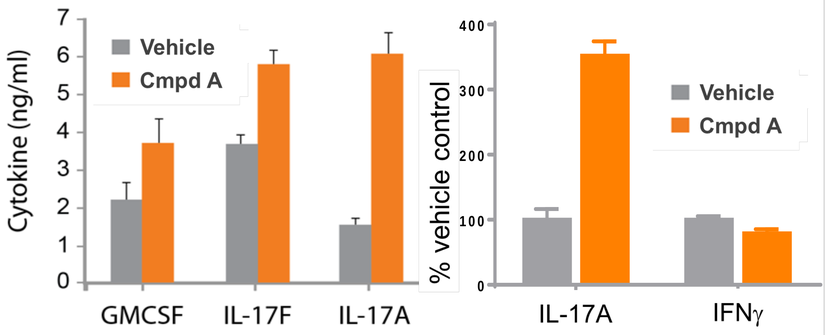

Activation of Gal4 reporter construct in HEK 293T cells in presence of ursolic acid to lower constitutive activity

- **Selective against closely related nuclear receptors RORα and RORβ**
- **Non-promiscuous on receptor binding panel**
- **Active across species**
- **Excellent ADME properties**
- **Good oral PK profile suitable for QD dosing**

## RESULTS

### *RORγ Agonists Increase Immune Activation Mechanisms*

#### (1) Enhanced cytokine production from murine and human T cells

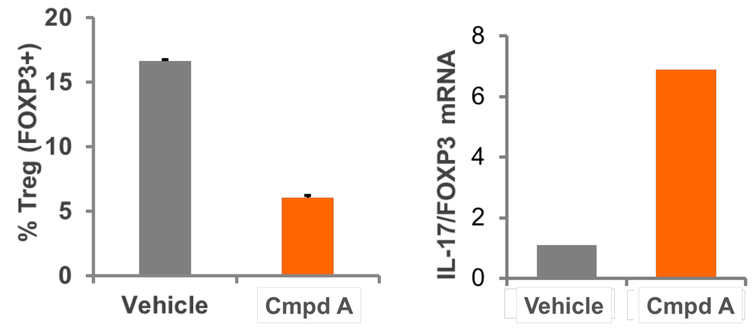



OT-1 splenocytes activated for 4 days with OVA peptide, TGFβ, IL-6 +/- compound A (10μM). Cytokine titers determined by ELISA

PBMC from 2 healthy donors were incubated with IL-1β, IL-23 +/- compound A (0.2 μM) for 48 hours. Cytokine titers determined by ELISA

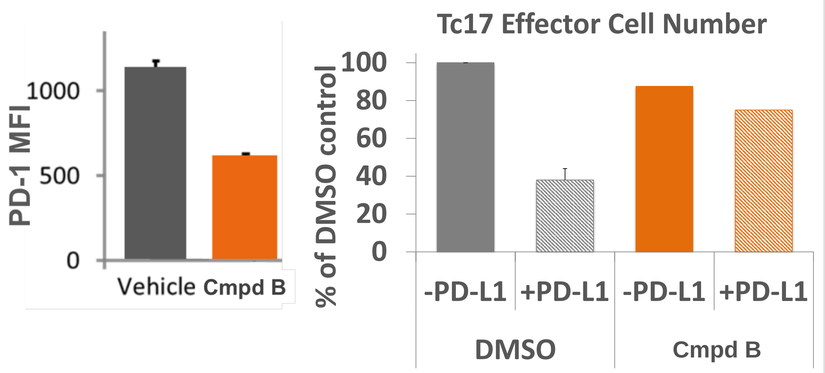
### *RORγ Agonists Decrease Immune Suppression Mechanisms*

#### (2) Shifted $T_{effector}$:$T_{reg}$ ratio




OT-2 splenocytes activated for 4 days with OVA peptide, TGFβ, IL-2 +/- compound A (10μM). %FOXP3+ determined by FACS (left). qPCR of mRNA from naïve CD4+ T cells stimulated for 4 days with anti-CD3/CD28 beads, IL-1β, IL-23, IL-6, TGFβ +/- compound A (10μM) (right)

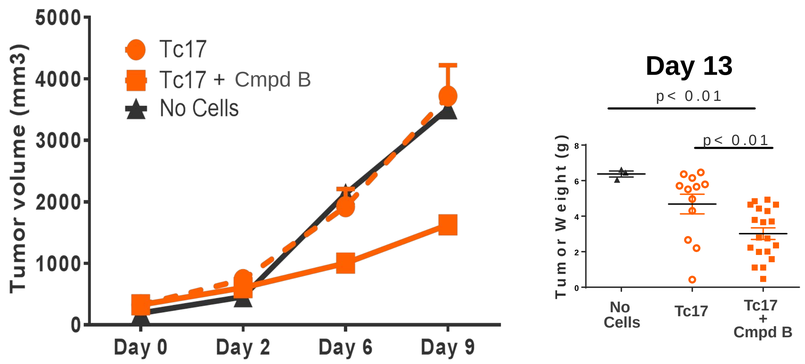
#### (3) Reduced PD-1 expression and desensitization to checkpoint inhibition




OT-1 splenocytes activated for 4 days with OVA peptide, TGFb, IL-6 +/- compound B (5μM). Cells were washed and rested for 24 hrs then restimulated with anti-CD3 or anti-CD3/PD-L1.Fc beads for 6 days or assessed for PD-1 expression by flow cytometry (MFI = mean fluorescent intensity)

### *RORγ Agonists Inhibit Tumor Growth*

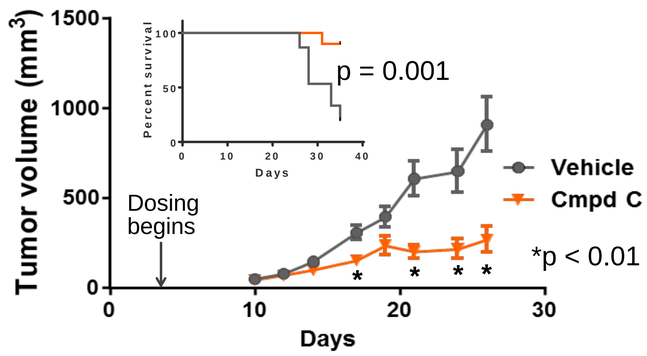
#### (4) *In vitro* treatment with RORγ agonist improves anti-tumor Tc17 responses




EG7 (EL4.OVA) cells implanted subcutaneously in C57BL/6 mice (Day -12). OT-1 splenocytes activated *in vitro* for 5 days with OVA peptide, IL-6, TGFβ +/- compound B (5 μM). On Day 0, 5 x10e6 Tc17 cells were transferred. Tumor size monitored by caliper. Statistics were calculated using Mann Whitney test in Prism

**Mice receiving Tc17 + agonist cells:**
- More donor cells recovered from spleen and tumor
- Tc17 express less PD-1

#### (5) Oral administration of RORγ agonist inhibits MC38 tumor growth leading to long term survival




MC38 colon cancer cells (0.5 x10e6) implanted subcutaneously in C57BL/6 mice. Dosing of compounds begins on Day 3 (100 mg/kg PO BID). Tumor size monitored by caliper starting on Day 10. Tumor growth statistics calculated using multiple t-tests; survival statistics calculated using Mantel-Cox log rank test.

## CONCLUSIONS

**RORγ small molecule agonists:**
- **Have activities consistent with established RORγ biology**
- **Combine multiple anti-tumor mechanisms into a single therapeutic**
- **Demonstrate single agent activity in several models without evidence of enhanced tumor growth**

***High potency, bioavailable compounds are rapidly advancing as a promising immunotherapy approach***