Novel Oral RORγ Agonists Demonstrate Anti-Tumor Efficacy in the 4T1 Breast Cancer Model

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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
- Lycera has identified selective small molecule RORγ agonists with good oral pharmacokinetic properties

CONCLUSIONS

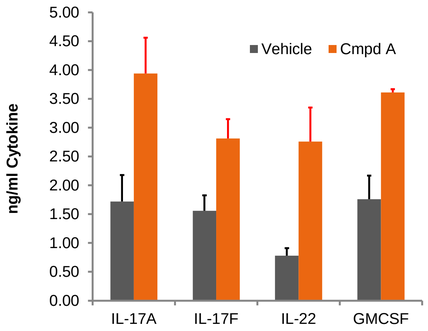
RORγ small molecule agonists:

- 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

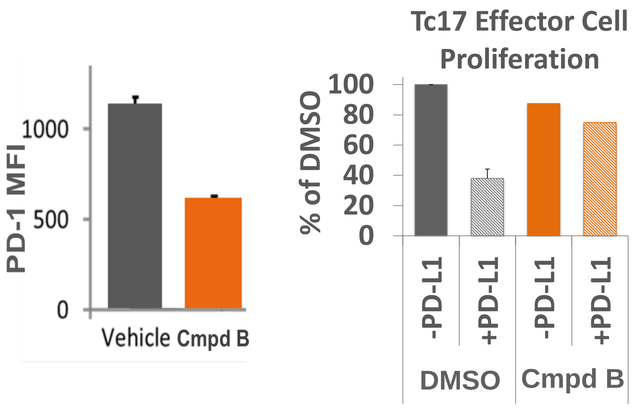
High potency, bioavailable compounds are rapidly advancing as a promising immunotherapy approach

RESULTS

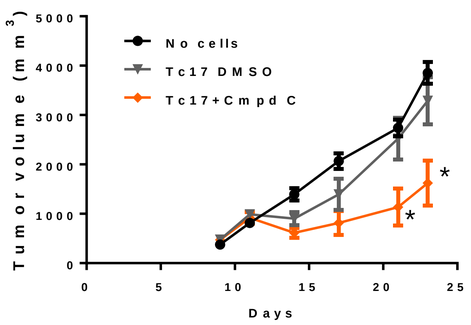
RORγ Agonists Enhance Tc17 Differentiation and Anti-Tumor Effector Function



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

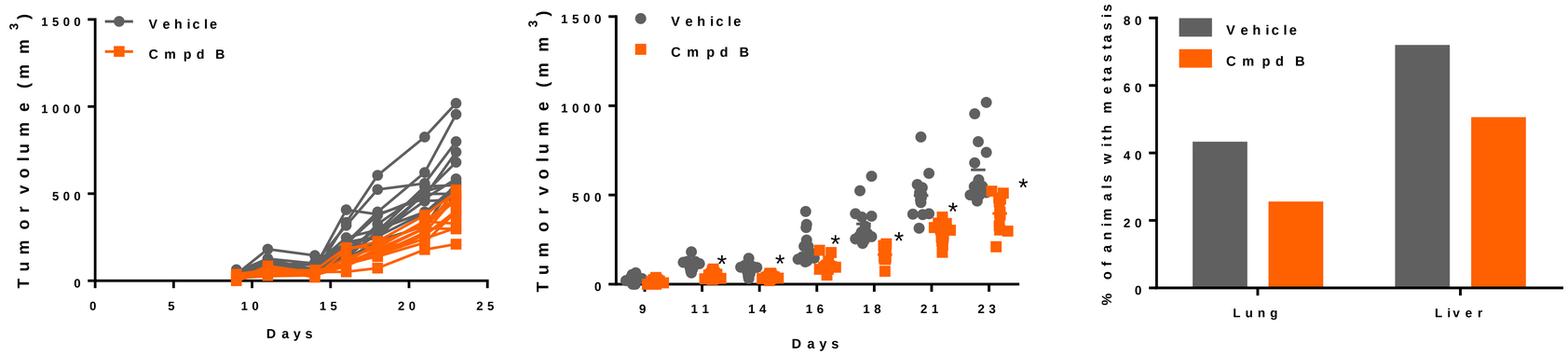


OT-1 splenocytes activated for 4 days with OVA peptide, TGFβ, IL-6 +/- compound B (5μM). Cells were washed and rested for 24 hrs then restimulated with anti-CD3 or anti-CD3/PD-L1.Fc beads for 6 days or assessed for PD-1 expression by flow cytometry (MFI = mean fluorescent intensity)

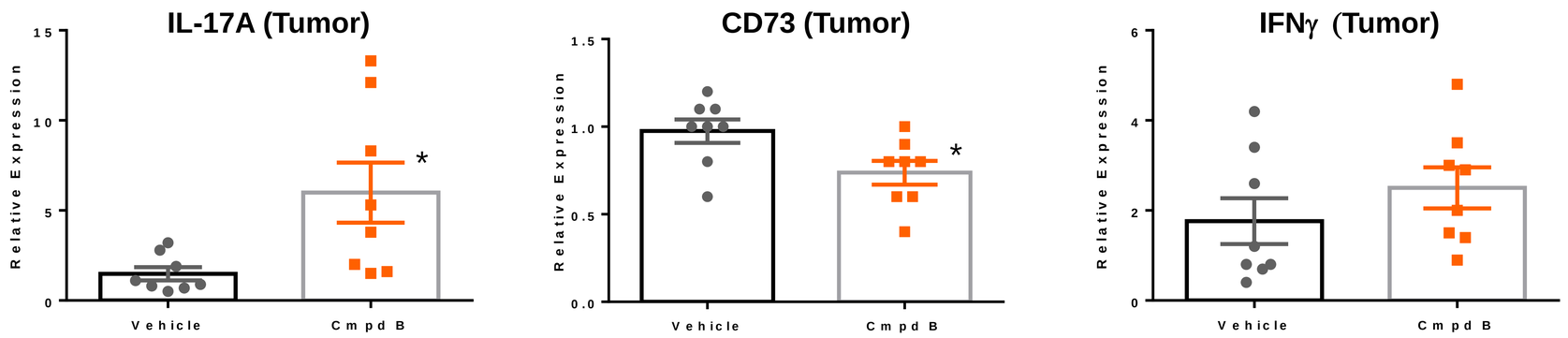


EG7 (EL4.OVA) cells implanted subcutaneously in C57BL/6 mice (Day -7). OT-1 splenocytes activated *in vitro* for 5 days with OVA peptide, IL-6, TGFβ +/- compound C (5 μM). On Day 0, 5 x10e6 Tc17 cells were transferred. Tumor size monitored by caliper. Statistics for tumor volume were calculated using multiple t test. *p<0.01

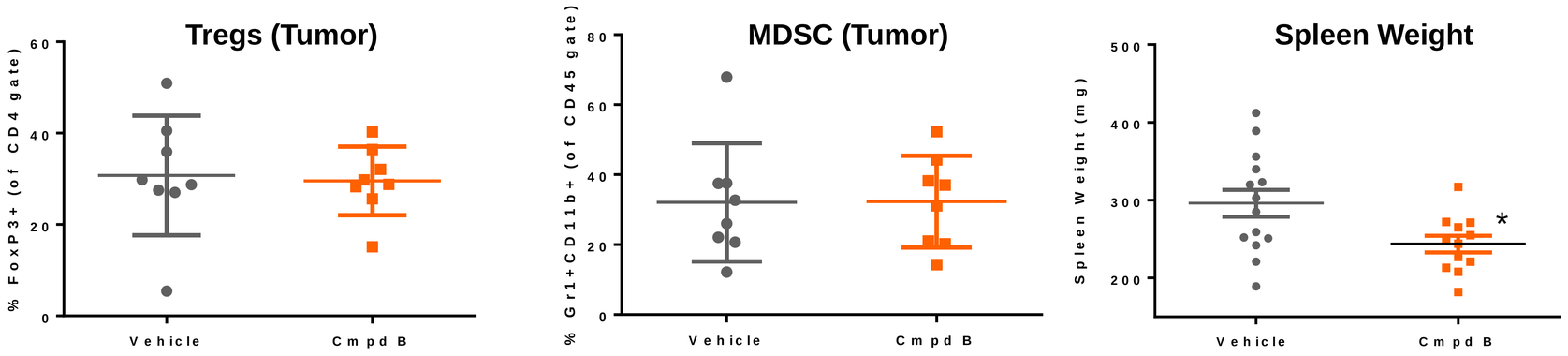
Oral administration of RORγ agonist inhibits 4T1 tumor growth



4T1 breast cancer cells (0.5 x10e6) were implanted subcutaneously in Balb/c mice. Dosing of compounds began on Day 3 (100 mg/kg PO BID). Tumor size was monitored by caliper starting on Day 9. Tumor volume was calculated using the formula 0.5x(lengthx(width)^2).Tumor growth statistics calculated using multiple t-tests. Assessment of presence of lung metastasis was done on paraffin-fixed tumor samples stained with H&E and scored by a blinded pathologist. *p<0.01



4T1 breast cancer cells (0.5 x10e6) were implanted subcutaneously in Balb/c mice. Dosing of compounds began on Day 3 (100 mg/kg PO BID). On day 24 after cell implantation, tumors were removed and digested using collagenase I and DNase I to obtain a single cell suspension. QPCR analysis was performed on RNA extracted from these cell suspension. Statistics were calculated using Mann Whitney test. *p<0.05



4T1 breast cancer cells (0.5 x10e6) were implanted subcutaneously in Balb/c mice. Dosing of compounds began on Day 3 (100 mg/kg PO BID). On day 24 after cell implantation, tumors and spleens were removed. Tumors were further digested using collagenase I and DNase I to obtain a single cell suspension. Flow cytometry analysis was done using a panel of antibodies against CD45, CD4, FoxP3, Gr-1 and CD11b. Treatment with RORγ agonist led to a significant reduction in spleen weight. Spleen weight statistic calculated using Mann Whitney test. *p<0.05