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
  - RORγ 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

<table>
<thead>
<tr>
<th>Concentration Log [µM]</th>
<th>% Basal Activity</th>
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<tbody>
<tr>
<td>Cmpd A</td>
<td>Activation of Gal4 reporter construct in HEK 293T cells in presence of uracil acid to lower constitutive activity</td>
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<tr>
<td>Cmpd B</td>
<td>EC50 = 0.6 µM</td>
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<tr>
<td>Cmpd C</td>
<td>EC50 = 0.3 µM</td>
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- 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-17A, IL-17F, compound A (10 µM): Cytokine titers determined by ELISA

<table>
<thead>
<tr>
<th>Cytokine (ng/ml)</th>
<th>Vehicle</th>
<th>Cmpd A</th>
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<tbody>
<tr>
<td>GMCSF</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>IL-17F</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>IL-17A</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

2) Shifted Teffector:Treg ratio

OT-2 splenocytes activated for 4 days with OVA peptide, TGFβ, IL-17A, IL-17F, compound A (10 µM): IL-17A, IL-17F, IFNγ mRNA levels determined by qPCR

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

OT-1 splenocytes activated for 4 days with OVA peptide, TGFβ, IL-17A, compound A (10 µM): %FOXP3+ cells

<table>
<thead>
<tr>
<th>% FOXP3+</th>
<th>Vehicle</th>
<th>Cmpd A</th>
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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-4, TGFβ, compound B (0.2 µM). On Day 8, 5 x 10^6 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 x 10^6) 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