Novel RORγ Agonists Enhance Anti-Tumor Activity of Adoptive T Cell Therapy

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Type 17 T Cells Mediate Potent, Durable Anti-Tumor Responses

Murine Transfer Model
- pMEL-1 CD8 T cells + B16F10 Melanoma

![Graph showing tumor area over time with different treatments.](image)

Human Transfer Model
- Th17 + Tc17 Meso CAR-T cells + M108 Tumor

![Graph showing tumor area over time with different treatments.](image)

Ovarian Cancer Patients
- Correlation of RORγ pathway with improved patient prognosis

![Graph showing survival rates with different IL-17 conditions.](image)

References:
- Blood 2009 114, 596-9
- Sci Transl Med 2011 2, 55ra78
- Blood 2009 114, 1141-49
Can RORγ agonists be used to enhance Type 17 responses and anti-tumor T cell responses?

Transcription Factor agonist will activate genetic program
- Multiple mechanisms
- Not equivalent to overexpressing IL-17
Synthetic Agonists Enhance RORγ Activity

Gal4-RORg Reporter Cell Assay

Graph showing the concentration response of Gal4-RORγ reporter activity.

Concentration Log [µM] vs. % Basal Activity graph with different concentrations of Lycera compounds (LYC-51264, LYC-53772, LYC-53793, LYC-53789) and an endogenous agonist.
**RORγ Agonists Enhance Cytokine Production by Murine Th17 and Tc17 Polarized T Cells**

**CD4 T Cells**
- Th17 conditions

<table>
<thead>
<tr>
<th>Cytokine (ng/ml)</th>
<th>DMSO</th>
<th>Agonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17A</td>
<td>3.2</td>
<td>4.4</td>
</tr>
<tr>
<td>IL-17F</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>IL-22</td>
<td>1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>GMCSF</td>
<td>1.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

**CD8 T Cells**
- Tc17 conditions

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</tr>
<tr>
<td>IL-17F</td>
<td>1.5</td>
<td>2.7</td>
</tr>
<tr>
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</tr>
</tbody>
</table>

*OT-I or OT-II splenocytes activated 4 days with peptide, IL-6, TGFβ*
**RORγ Agonists Enhance Cytokine Production by Unpolarized Murine T Cells**

**Th0 Condition (IL-2)**

- **IL-17A**
  - DMSO: 2 ng/ml
  - Agonist: 8 ng/ml

- **IFNγ**
  - DMSO: 12 ng/ml
  - Agonist: 8 ng/ml

**IL-17A Production from Th0 vs Th17 Cells**

- Th0: DMSO 20 ng/ml, Agonist 60 ng/ml
- Th17: DMSO 100 ng/ml, Agonist 80 ng/ml

**RORγ-dependent Effect**

- **IL17A**
  - WT: DMSO 10 ng/ml, Agonist 15 ng/ml
  - RORg (-/-): DMSO 5 ng/ml, Agonist 10 ng/ml

*Trp-1 splenocytes activated 5 days with peptide*

*B6 splenocytes activated 4 days with anti-CD3/CD28, IL-6, TGFβ*
RORγ Agonists Shift the Balance of Co-stimulation over Co-inhibition

B6 splenocytes activated 4-5 days with anti-CD3/CD28, IL-6, TGFβ +/- agonist → flow cytometry, qPCR
RORγ Agonists Desensitize Cells to PD-1 Checkpoint Inhibition

RORγ agonist Decreases PD-1 Expression and Percentage PD-1+ Cells

OT-1 splenocytes activated 4 days with peptide, IL-6, TGFβ +/- agonist; washed, rested then restimulated for 6 days

RORγ Agonist Desensitizes Cells to PD-L1 Inhibition of Proliferation

Similar restoration of cytokine production observed (IL-17, IFNγ)
EG7 Tumor Growth is Inhibited Following Adoptive Transfer of RORγ Agonist Treated Tc17 Cells

Inject EG7 cells s.c. → 14 days → Inject OVA-specific Tc17 cells → Tumor Volume

Significant inhibition of tumor growth and improved survival
Adoptive Cell Therapy Model

Tumor is established

B16F10
*most challenging line of melanoma to treat.
Treat post 10 days of development

Th0

pMEL-1 CD8 or TRP-1 CD4+ T cells (1e6)

Th17

IL-2

IL-6, TGF-β, IL-21, IL-1B

Anti-IFN/4

Ablate host immune system

Adoptively transfer T cells

Tumor regression?

dr Chrystal Paulos, Dr Kinga Majchrzak Medical University of South Carolina
RORγ Agonist Improves Effectiveness of Transferred T Cells vs Established B16F10 Tumors

Trp-1 CD4 Th17 cells

Trp-1 CD4 Th0 cells

PMEL-1 CD8 Tc17 cells

Dr Chrystal Paulos, Dr Kinga Majchrzak Medical University of South Carolina
RORγ Agonist Improves Effectiveness of co-Transferred CD4+ CD8+ T Cells vs Established B16F10 Tumors

Trp-1 Th17 + pMEL-1 Tc17

No Cells
Th17 + Tc17
Th17 + Tc17 + Agonist

Tumor-specific TIL Day 71

Enhanced persistence of agonist treated cells in tumor draining lymph node and spleen

Dr Chrystal Paulos, Dr Kinga Majchrzak Medical University of South Carolina
RORγ Agonist Treated Cells Maintain Enhanced Cytokine Production 71 Days After Transfer

Splenocytes (day 71) ex vivo Trp-1 Stimulation of CD4 T cells

Similar data observed for IL-2 and IL-21 and for pMEL-1 CD8 T cells

Dr Chrystal Paulos, Dr Kinga Majchrzak Medical University of South Carolina
Increased PD-1-Negative TILs in Mice Receiving Agonist Treated Th17 + Tc17 Cells

Day 71

Dr Chrystal Paulos, Dr Kinga Majchrzak Medical University of South Carolina
Systemic Administration of RORγ Agonist Effective Against Established MC38 Colon Carcinoma

Inject tumor cells (SC) → 3-7 days → Begin daily dosing → 14-48 days → Tumor Volume

- Tumor Growth Control
- Improved Survival
- Increased plasma cytokines

Graphs showing:
- Tumor Volume vs. Days
- % Survival vs. Days
- Cytokine Levels (IL-17A, GMCSF, IL-6, IL-21, IL-17F, TNF, MIP3a) with Vehicle and Agonist comparisons.
**RORγ Agonists**

- Increase production of pro-inflammatory cytokines and chemokines
- Increase co-stimulatory and decrease co-inhibitory receptor expression
- Desensitize cells to PD-L1-mediated inhibition
- Decrease Treg development
- Addition of RORγ agonists to in vitro T cell expansion cultures significantly enhances anti-tumor responses
  - Effective in CD4 and CD8 model systems
  - Effective with Type17 cytokine cocktails or IL-2
  - Observe long-lasting effects consistent with RORγ biology
Thank You!

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• Xiao Hu
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