Preclinical Studies of IL-21 Plus Rituximab Combination Therapy for B-Cell Lymphoma

Wayne Kindsvogel
ZymoGenetics, Inc.
Seattle, WA
Cytokines that utilize the $\gamma$–Common Cytokine Receptor

- **IL7**: $\gamma_c$ IL7R
  - Lymphocyte survival and Development

- **IL2**: $\gamma_c\beta\alpha$
  - Th1; T- cell Homeostasis
  - Cell-mediated Immunity

- **IL15**: $\gamma_c\beta\alpha$
  - Th1; T-, NK cell Homeostasis
  - Cell-mediated Immunity

- **IL21**: $\gamma_c$ IL21R
  - Th2; Humoral Immunity
  - B-cell regulation

- **IL4**: $\gamma_c$ IL4R$\alpha$
  - Th2; Mast Cell Biology

- **IL9**: $\gamma_c$ IL9R

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IL-21 is a CD4+ “T-Helper” cytokine that acts on most leukocyte subsets.
1. IL-21 is a potent regulator of B-cell differentiation (plasma cells) and can stimulate apoptosis of “bystander” B-cells.

- IL-21 inhibits the proliferation of many B cell tumor lines in vitro and in vivo.
IL-21 inhibits the proliferation of B lymphoma cell lines in vitro.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment duration (d)</th>
<th>Percentage Change (Mean ± sd)</th>
<th>Number of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSU-NHL</td>
<td>12</td>
<td>-82.3 ± 6.1</td>
<td>4</td>
</tr>
<tr>
<td>IM-9</td>
<td>8</td>
<td>-77.1 ± 18.1</td>
<td>5</td>
</tr>
<tr>
<td>MC116</td>
<td>11</td>
<td>-62.7 ± 6.2</td>
<td>5</td>
</tr>
<tr>
<td>HS-Sultan</td>
<td>9</td>
<td>-49 ± 38.2</td>
<td>4</td>
</tr>
<tr>
<td>Raji</td>
<td>8</td>
<td>-13 ± 2.7</td>
<td>2</td>
</tr>
<tr>
<td>Ramos</td>
<td>12</td>
<td>-7.4 ± 7.1</td>
<td>3</td>
</tr>
<tr>
<td>DOHH2</td>
<td>10</td>
<td>-10.1 ± 17.8</td>
<td>7</td>
</tr>
</tbody>
</table>
IL-21 prolongs survival of mice with disseminated human B lymphoma

**IM-9**

- hIL-21 (12ug/day)
- hIL-21 (1.2ug/ml)
- hIL-21 (.012ug/ml)
- cont.

**Raji**

- hIL-21 (12ug/day)
- cont.
Rationale for testing IL-21 in the context of B-cell lymphoma

1. Direct actions on B cells and NHL

2. IL-21 enhances Rituximab-mediated anti-tumor activity.
IL-21 enhances antibody dependent tumor cell killing by NK cells in vitro

IL-21 enhances antibody dependent tumor cell killing by NK cells in vitro.
IL-21 enhances rituximab-mediated survival of lymphoma-bearing mice

**Legend:**
- mIL21+rituximab
- rituximab
- mIL21
- PBS

**Graph:**
- X-axis: Days After Tumor Injection
- Y-axis: Fraction survival

**Title:**
IL-21 enhances rituximab-mediated survival of lymphoma-bearing mice
IL-21 enhances rituximab activity in the absence of functional NK cells
Survival of tumor-bearing mice treated with IL-21+ rituximab is partially regulated by Granulocytes
Survival of tumor-bearing mice treated with IL-21 +Rituximab is partially regulated by Macrophage.
1. Direct actions on B cells and NHL
2. Stimulation of anti-tumor immunity
3. IL-21 enhances rituximab-mediated anti-tumor activity in mice.

4. **IL-21 prolongs rituximab-mediated B-cell depletion in non-human primates.**
<table>
<thead>
<tr>
<th>Group</th>
<th>Rituxan</th>
<th>rIL-21</th>
<th>Animals Terminal Sac</th>
<th>Animals Recovery Sac</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 mg/kg</td>
<td>0 mg/kg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Once weekly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10 mg/kg</td>
<td>0.3 mg/kg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Once weekly</td>
<td>Once weekly</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg</td>
<td>1.5 mg/kg</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Once weekly</td>
<td>Once weekly</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Peripheral Activated Monocytes
CD45+/CD14+/CD64+

Study Day

# activated Monocytes/μL whole blood

- rituximab (10 mg/kg)
- rituximab + IL-21 (0.3 mg/kg)
- rituximab + IL-21 (1.5 mg/kg)
Group Mean B cells in Blood

- rituximab (10 mg/kg)
- rituximab + IL-21 (0.3 mg/kg)
- rituximab + IL-21 (1.5 mg/kg)

# B cells/μL whole blood

Study Day

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Lymph Node: CD20 IHC

CD20-stained B cells in Mesenteric Lymph Nodes

Rituximab only group (4-week Recovery)

IL-21 + Rituximab (4-week Recovery)
Spleen: CD20 IHC

RTX 48 h post treatment

RTX + IL-21 48 h post treatment

RTX 4 wk recovery

RTX + IL-21 4 wk recovery
Lymphoid Atrophy in Spleen

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grade of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab (10 mg/kg)</td>
<td>Terminal</td>
</tr>
<tr>
<td>Rituximab + IL-21 (0.3 mg/kg)</td>
<td>Minimal / Slight</td>
</tr>
<tr>
<td>Rituximab + IL-21 (1.5 mg/kg)</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

Grade of Change
- None
- Minimal / Slight
- Moderate

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Summary

- IL-21 has direct antiproliferative activity on B lymphoma cell lines in vitro and in vivo.
- IL-21 enhances rituximab mediated killing of B lymphoma cell lines in vitro and in vivo.
- Prolongation of survival by rituximab + IL-21 is dependent on granulocytes and macrophage.
- IL-21 causes monocyte activation and prolonged B cell depletion in nonhuman primates.
Acknowledgements

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IL-21 inhibits growth of RenCa.2 tumors

The diagram shows the mean tumor volume (mm³) over time (Days after tumor inoculation). The orange line represents the Vehicle group, while the purple line shows the IL-21 group. IL-21 was administered at Day 19, as indicated by the upward arrows. The data points and error bars indicate the variability in tumor volume over the course of the experiment.
Establishment of memory in Renca.2tumor-bearing mice

![Graph showing tumor volume (mm³) over days after tumor rechallenge for different groups labeled C1 to C5 and IL-21-1 to IL-21-4.](graph.png)
IL-21 Induces and Sustains CD8+ T-cells Responses to E.G7

% Tetramer positive CD8+ T cells

Day's post tumor challenge

- PBS
- IL-2
- IL-21
- IL-15
IL-21 Enhances Tumor Killing Cytotoxic T Cells

Target = EG.7 mouse thymoma
## Antitumor activity of IL-21

<table>
<thead>
<tr>
<th>Tumour</th>
<th>IL-21 treatment</th>
<th>Effect on tumour</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>B16 melanoma or MCA205 fibrosarcoma</td>
<td>Injection of IL-21-encoding plasmid at day 5 and day 12 after tumour initiation</td>
<td>Increases survival rate (40%)</td>
<td>NK-cell-mediated killing, partially CD8+ T-cell mediated</td>
</tr>
<tr>
<td>B16 melanoma or MethA fibrosarcoma</td>
<td>Expression of IL-21 by tumour</td>
<td>Prevents initiation of tumour (for 41 weeks, protects from later tumour challenge (results in delayed growth)</td>
<td>Tumour-specific CTL and NK-cell activity, perforin dependent</td>
</tr>
<tr>
<td>Mouse colon carcinoma or human pancreatic carcinoma</td>
<td>Expression of IL-21 by tumour</td>
<td>Prevents initiation of tumour</td>
<td>NK-cell-mediated killing</td>
</tr>
<tr>
<td>TS/A mammary adenocarcinoma</td>
<td>Expression of IL-21 by tumour</td>
<td>Prevents initiation of tumour, protects from later tumour challenge (results in delayed growth)</td>
<td>CD8+ T-cell- and granulocyte-dependent mechanism, partially IFN-γ dependent</td>
</tr>
<tr>
<td>RLmale1 lymphoma</td>
<td>Injection of IL-21-encoding plasmid before or after lymphoma injection</td>
<td>No effect on the number of metastatic foci, synergistic protective effect when in combination with IL-15</td>
<td>CD4+ T-cell-, CD8+ T-cell- and NK-cell-dependent mechanism</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>Injection of IL-21-encoding plasmid before tumour injection</td>
<td>Reduces lung and liver metastases</td>
<td>NK-cell-mediated killing, perforin but not IFN-γ dependent</td>
</tr>
<tr>
<td>E.G7 thymoma (ovalbumin expressing)</td>
<td>Injection of IL-21 (20 μg intraperitoneally) early, during days 2–12 after tumour initiation</td>
<td>Increases survival compared with administration of IL-2 or IL-15</td>
<td>CD8+ T-cell-mediated mechanism</td>
</tr>
<tr>
<td></td>
<td>Injection of IL-21 (20 μg intraperitoneally) late, during days 12–22 after tumour initiation</td>
<td>Protects from later tumour challenge</td>
<td>Increased persistence of tumour-specific CD8+ T cells</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>Injection of IL-21 (5–10 μg intraperitoneally twice daily), and adoptive transfer of tumour-specific CD8+ T cells at day 8–10 after tumour initiation</td>
<td>Cures established tumours, synergistic effect when in combination with IL-15</td>
<td>CD8+ T-cell-mediated killing, IL-21 and IL-15 mediate functional and proliferative changes in CD8+ T cells</td>
</tr>
</tbody>
</table>

CTL, cytotoxic T lymphocyte; IFN-γ, interferon-γ; IL, interleukin; NK, natural killer.
Rationale for Testing IL-21 in the Context of B-cell Lymphoma

1. Direct actions on B cells and NHL

2. IL-21 is a potent regulator of anti-tumor immunity
IL-21 Elevates Myeloid/Monocyte Cells in Blood of Cynomologous Monkeys (CD11c+, per mL)
Summary

(1) direct anti-tumor responses

(2) anti-tumor immune response

(3) Antibody-mediated tumor killing