Induction of CD8+ T Cell Responses Against Novel Glioma-
Associated Antigen Peptides and Clinical Activity by
Vaccinations with α-Type-1-Polarized Dendritic Cells and
Poly-ICLC in Patients with Recurrent Malignant Glioma

Hideho Okada
University of Pittsburgh Cancer Institute

iSBTC 25th Annual Scientific Meeting  Oct 3, 2010
Conflicts of Interests (COI)

Hideho Okada is one of the inventors of the IL-13Rα2 (345-353:1A9V) and EphA2 (883-891) peptides, for which an exclusive licensing agreement has been executed with Stemline, Inc.

Per the University of Pittsburgh COI policy, interpretation of presented data was not performed solely by Hideho Okada, but by the investigator team.
Malignant Gliomas
• WHO grade 3 anaplastic glioma
• WHO grade 4 glioblastoma multiforme (GBM)
Critical Aspects/Factors for Potent Dendritic Cell Vaccines (05-115)

- Type of DCs
  - Type-1 DCs (alphaDC1)
- Target Antigens
  - Multiple CTL epitopes from 4 GAAs
- Administration Route
  - Intranodal administration (superior to s.c)
- Adjuvant
  - Poly-ICLC as a ligand for intracellular dsRNA receptors
Background
Glioma Vaccines with Type 1 DCs

Cytokines modulate the IL-12 production in DC and promote the development of stable, polarized DC (DC1).

Kalinski P and Okada H. Seminars in Immunology 2010

Type-1 “Polarizing” cocktails
IFN-γ
IFN-α
TNF-α
IL-1β
Poly-IC
Poly-ICLC administration enhances the infiltration of OVA-Specific T cells and therapeutic efficacy

Brain-Infiltrating T cells

These cells express CXCR3 and VLA-4

Anti-Tumor Effect

Mock Tx Alone
Poly-ICLC Alone
Vaccine Alone
Vaccine plus Poly-ICLC

Zhu X. et al.
Objectives (UPCI 05-115)

- **Primary Objective**
  - Safety - to determine the maximal tolerated dose of DC1 and evaluation of toxicities

- **Secondary Objectives**
  - Assess immunological response against GAAs using ELISPOT and tetramer assays
  - Assess the *preliminary anti-tumor clinical activity* of the vaccines as measured by radiological response (MRI), overall survival as well as 6 month-progression free survival (PFS).
Eligibility

- Adult patients with recurrent GBM or WHO grade 3 AG
- HLA-A2+ based on flow-cytometry
- Minimum corticosteroid (4 mg/day or less for Dexamethasone)
- Maximum 2 previous recurrences
Treatment

- Ultrasound-guided intranodal injections of type-1 DC1 (1 or 3 x 10^7 /injection with dose escalation) loaded with 4 glioma-associated antigen (GAA)-derived HLA-A2-restricted CTL epitopes (IL-13Ra2_{345-353:1A9V}, gp100_{209-217:2M}, EphA2_{883-891} and YKL-40_{201-210})
- Intramuscular injections of poly-ICLC (20 mcg/kg; Twice/week)

Recurrent High Grade Glioma (WHO III or IV)

<table>
<thead>
<tr>
<th>Vaccines (Q2W)</th>
<th>Booster Vaccine Phase I (Q4W)</th>
<th>Booster Vaccines Phase 2 (Q3M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly-ICLC (twice/week)</td>
<td>Poly-ICLC (twice/week)</td>
<td>Poly-ICLC (once/week)</td>
</tr>
</tbody>
</table>

Weeks: 1 3 5 7 9 13 17 21 25 29 33 Up to 3 Years

Brain MRI
## SL-701 Adverse Events

No grade 3 or 4 toxicities observed

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade 1</th>
<th>Grade 2</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Blood/Bone Marrow</td>
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<td></td>
</tr>
<tr>
<td>Leukocytopenia</td>
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<td>5</td>
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<tr>
<td>Injection site reactions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Redness, induration, pruritis, pain</td>
<td>17</td>
<td>77</td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue (lethargy, malaise, asthenia)</td>
<td>16</td>
<td>73</td>
</tr>
<tr>
<td>Fever</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>Chills/Rigors</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Nausea</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Headache</td>
<td>5</td>
<td>23</td>
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<tr>
<td>Insomnia</td>
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<td>5</td>
</tr>
<tr>
<td>Light headed/dizziness</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Myalgia</td>
<td>7</td>
<td>32</td>
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<tr>
<td>Body ache</td>
<td>6</td>
<td>27</td>
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<tr>
<td>Dermatological</td>
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<tr>
<td>Skin rash</td>
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<tr>
<td>Dry skin</td>
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<tr>
<td>Bruising</td>
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<td>9</td>
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<tr>
<td>Urticaria</td>
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<td>5</td>
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<td>Pulmonary/Upper Respiratory</td>
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<td>Rhinitis/Runny nose</td>
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<td>5</td>
</tr>
</tbody>
</table>
IL-12 production levels positively correlated with PFS ($p=0.0255$ based on Cox regression test)

Closed circles indicate patients who have already progressed, whereas closed diamonds represent patients who have not recurred to date.
Promotion of type-1 responses detected in post-vaccine PBMCs by RT-PCR

**IFNα1 (p=0.0306)**

Relative Expression

Pre- vs. Post-first vac/poly-ICLC

**TLR3 (p=0.0303)**

Relative Expression

Pre- vs. Post-first vac/poly-ICLC

**CXCL10 (p=0.0098)**

Relative Expression

Pre- vs. Post-first vac/poly-ICLC

**CCL22 (p=0.0105)**

Relative Expression

Pre- vs. Post-first vac/poly-ICLC
Complete Radiological Response in A Patient with Recurrent GBM (Pt #20)

Pre-Vaccine
Week 0

Post-Vaccine
Week 9

Post-Vaccine
Week 17
PR Patient with subsequent pseudo-tumor progression

Biopsy demonstrates intratumoral infiltration of macrophages and CD8+ T cells

- Reactive gliosis, possible residual glioma
- Numerous CD68+ macrophages
- CD8+ T cells

Pre-Vaccine | Post-Vaccine (wk 9)
Summary 05-115; Phase I/II Vaccine Study in Adult Recurrent Malignant Glioma (JCO In Press)

- The regimen was well tolerated in 22 patients.
- Immune responses against at least one of the vaccination-targeted GAAs were detected in post-vaccine PBMC in 11 of 19 patients.
- Analyses of PBMC demonstrated significant up-regulation of type-1 cytokines and chemokines, including IFN-α and CXCL10.
- Nine (4 GBM, 2 AA, 2 AO and 1 AOA) achieved progression free status lasting at least 12 months. One patient with recurrent GBM demonstrated sustained complete response.
- IL-12 production levels by aDC1 positively correlated with progression-free survival.
Primary vs. Secondary GBM

**Primary glioblastoma**
- *de novo*
- mean = 55 yrs

**Secondary glioblastoma**
- mean = 45 yrs

**Low grade astrocytoma**
- **p53** (17p13) mutation (>65%)
- **PDGF, FGF2** overexpression (~60%)
- **CDK4** (12q13) amplification
- **RB** (13q13) alteration (~25%)
- **LOH 19q** (~50%)

**Anaplastic astrocytoma**
- **PTEN** (10q23) loss (~4%)
- **PDGFR-α** amplification (<10%)
- **EGFR** (7p12)
  - amplification (~40%)
  - overexpression (~60%)
- **MDM2** (12q14)
  - amplification (~8%)
  - overexpression (~50%)
- **CDKN2A** (9p21) loss (~50%)
- **PTEN** (10q23) loss (~70%)

**PDGF, FGF2** overexpression (~60%)

**CDK4** (12q13) amplification

**RB** (13q13) alteration (~25%)

**LOH 19q** (~50%)

**EGFR** (7p12) amplification (~40%)

**MDM2** (12q14) amplification (~8%)

**CDKN2A** (9p21) loss (~50%)

**PTEN** (10q23) loss (~70%)
Contributors

Co-Investigators
Pawel Kalinski – development of alphaDC1
Ryo Ueda, Aki Hoji and Gary Kohanbash – cytokine and tetramer assays
Frank S. Lieberman and Teresa E. Donegan – patient management
Todd A. Reinhart – in situ hybridization
Theresa L. Whiteside and Lisa H. Butterfield – immuno-monitoring
Ronald L. Hamilton – neuro-pathology
Douglas M. Potter – biostatistics
Andres M. Salazar – provision of poly-ICLC

Brain Tumor Program
Clinical Research Services
IMCPL of the UPCI

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Musella Foundation
Pittsburgh Foundation
The Brain Tumor Society

Participants and their families

iSBTC 25th Annual Scientific Meeting Oct 3, 2010
I am stopping my talk

- though our work will never stop at any time!

THANK YOU!
A Bi-Institutional Pilot Study of Vaccinations with GAA-peptides in Adult Patients with High-Risk LGG – University of Pittsburgh and Wake Forest University

Primary Objectives – To determine safety and glioma-associated antigen (GAA)-specific immune responses of the regimen.

Rationale –

1) The slow growth rate of LGG should allow sufficient time to repeat multiple immunizations, and the induction of high levels of GAA-specific immunity
2) minimal toxicity allows for maintenance of high quality of life
3) SL-701 could delay the use of RT in this patient population

Eligibility - HLA-A2+ adult patients WHO grade II astrocytoma or oligoastrocytoma with “high-risk” factors - defined as at least one of the following conditions: 1) age ≥ 40 with any extent resection
2) age 18-39 with incomplete resection (post-op MRI showing >1cm residual disease) or
3) the tumor size is ≥ 4 cm
Treatment Schema

- No corticosteroid will be allowed within 4 weeks prior to the first vaccine. Baseline MRI and other screening procedures will be done within 4 weeks prior to the 1st vaccination.

- Vaccines: Peptide-vaccines Q3W (Wk 0-21) and i.m. poly-ICLC (on days 0 and 4 following each vaccination).

- PBMC for immune studies (Q3W: Wk 12-24); MRI (Wks 12 & 24).

- Additional vaccines and poly-ICLC (Q12W) if applicable.

Weeks:
-4 0 3 6 9 12 15 18 21 24
Robust Induction of GAA-Specific CD8+ cell Response in a Subject with WHO Grade 2 Low Grade Glioma

% of EphA2 tetramer+ cells in CD8+ cells
% of IL13Ra2 tetramer+ cells in CD8+ cells
% of Survivin tetramer+ cells in CD8+ cells
% of WT1 tetramer+ cells in CD8+ cells

WT1

Pre-Vaccine  WK12  WK18  WK24

0.019  0.772  5.63  5.99

SSC

CD27

CD45RA

BL  wk12  wk18  wk21  wk24

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Type-1 effector function of CD8+ T cells in response to brief *ex vivo* stimulation with the WT-1 peptide

No Peptide

<table>
<thead>
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<th>IFN-γ</th>
<th>TNF-α</th>
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<tr>
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<td>0.0282</td>
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<td>0.453</td>
<td>0.359</td>
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With WT-1

<table>
<thead>
<tr>
<th>IFN-γ</th>
<th>TNF-α</th>
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<tr>
<td>0.0076</td>
<td>0.0583</td>
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<tr>
<td>0.362</td>
<td>0.187</td>
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</table>

<table>
<thead>
<tr>
<th>IFN-γ</th>
<th>CD107a</th>
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<td>0.0583</td>
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<tr>
<td>0.362</td>
<td>0.187</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TNF-α</th>
<th>CD107a</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0076</td>
<td>0.0583</td>
</tr>
<tr>
<td>0.362</td>
<td>0.187</td>
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</tbody>
</table>
Acknowledgement

Co-Investigators
Frank S. Lieberman, Ryo Ueda, Aki Hoji,
Pawel Kalinski, Arlan H. Mintz, Johnathan A. Engh, David L. Bartlett, Herbert Zeh,
Teresa E. Donegan, Theresa L. Whiteside,
Lisa H. Butterfield, Walter J. Storkus,
Douglas M. Potter, Ronald L. Hamilton,
Regina Jakacki and Ian F. Pollack

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Clinical Research Services
IMCPL
Of the UPCI

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Michael E. Scheurer (Baylor)
John H. Ohlfest (U. Minnesota)
Andres M. Salazar (Oncovir, Inc)

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iSBTC 25th Annual Scientific Meeting Oct 3, 2010
University of Pittsburgh Medical Center
University of Pittsburgh Cancer Institute
Central Nervous System (CNS) immunology

Concept of CNS as immunologically privileged

1. Blood Brain Barrier/No lymphatic system

2. No dendritic cell (DC) distribution

3. Roles of microglia/brain macrophages as antigen-presenting cells (APC) are not clear
Central Nervous System (CNS) immunology
- “Privileged” status has been revised -

- Autoimmune diseases in the CNS
  (Experimental Autoimmune Encephalitis, Multiple Sclerosis, Paraneoplastic Cerebellar Degeneration [PCD] against cdr2)

- A-beta1-40/42 formulated in QS-21 as a vaccine for Alzheimer’s disease- a few treated patients developed signs of aseptic encephalitis/meningitis following the second administration of the vaccine.
Figure 4

A

Week 0

Week 17

Week 33

B

Weeks

% of Tetramer+ CD8+ Cells

- IL-13Ra2 tetramer+ cells
- EphA2 tetramer+ cells
- gp100 tetramer+ cells

0 3 5 7 13 17 21 29 33
IFN-inducible protein (IP)-10/CXCL10 plays a critical role in the recruitment of Tc1 cells to the CNS tumor site

IP-10 deficient mice bearing day 7 i.c. M05 received i.v. adoptive transfer of $3 \times 10^6$ Tc1 cells and anti-IP-10 i.p. ($250 \mu g$ on day 6 and $100 \mu g$ on days 7, 8, and 9). On the same day (day 7), $50 \mu g$ anti-IP-10 was i.t. co-injected with $1 \times 10^5$ DC-IFN-α. BILs were harvested 3 days after adoptive transfer.

High level expression of VLA-4 (heterodimer of α4-integrin [CD49d] and β1-integrin [CD29]) on Tc1

Pittsburgh Night View
Representative ELISPOT and Tetramer Responders

Patient 10 (GBM)

- IL-13Ra2
- EphA2
- YKL-40
- gp100
- PADRE

Spots/10^5 CD4+ or CD8+ Cells vs. Weeks

Weeks: 0, 3, 5, 7, 9

Patient 6 (AOA)

- IL-13Ra2 tetramer+ cells
- EphA2 tetramer+ cells
- gp100 tetramer+ cells

% of Tetramer+ CD8+ Cells vs. Weeks

Weeks: 0, 3, 5, 7, 9, 13, 17, 21, 25, 29, 33

Week 0 vs. Week 25:
- IL-13Ra2 tetramer+ cells: <0.01
- gp100 tetramer+ cells: 1.14
Time course for IFN-γ ESLIPOT assays for all evaluated patients with box plots
TTP and OS for Each of the Two Tumor Grades (WHO Grade 3 vs. 4)

**Time to Progression (TTP)**

- Median TTP:
  - 4 months for GBM
  - 13 months for AG

**Overall Survival (OS)**

- Median OS:
  - 12 months for GBM
  - Undefined for AG (as 5 of 9 patients are still alive with the median follow-up period for 23 months)
<table>
<thead>
<tr>
<th>Pt ID</th>
<th>Age/ Gender</th>
<th>Tumor Histol.</th>
<th>Location of Tumor</th>
<th>Prior Therapy</th>
<th>No. Prev Rec.</th>
<th>DC IL-12 (pg)</th>
<th>ELISpot</th>
<th>Tetramer</th>
<th>RR at Week 9</th>
<th>TTP (Mo)</th>
<th>OS (Mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57/M</td>
<td>GBM</td>
<td>Rt. Temp/Pa</td>
<td>Res/RT/TMZ/ Mol</td>
<td>1</td>
<td>10</td>
<td>I E Y G Pa</td>
<td></td>
<td>P P P</td>
<td>PR</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>52/M</td>
<td>GBM</td>
<td>Rt. Temporal</td>
<td>Res/RT/TMZ</td>
<td>1</td>
<td>25</td>
<td></td>
<td></td>
<td>P P P</td>
<td>PD</td>
<td>&lt;2</td>
</tr>
<tr>
<td>3</td>
<td>77/M</td>
<td>AA</td>
<td>Rt. Parietal</td>
<td>Resx2/RT/ TMZ/Mol</td>
<td>2</td>
<td>25</td>
<td></td>
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<td>N N N</td>
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<td>5</td>
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<tr>
<td>4</td>
<td>38/M</td>
<td>AA</td>
<td>Rt. Frontal</td>
<td>Res/RT/TMZ</td>
<td>&lt;10</td>
<td>Not Tested</td>
<td></td>
<td></td>
<td>ND*</td>
<td>&lt;2</td>
<td>&gt;35</td>
</tr>
</tbody>
</table>

Please, do not read – I will summarize these findings for you –
Poly-ICLC as the Key Adjuvant

- Polyinosinic-polycytidylic acid (poly-IC) stabilized with poly-lysine and carboxymethylcellulose (Poly-ICLC), has been used as a single agent or combo with TMZ for treatment of malignant glioma (provided by Dr. Andres Salazar [Oncovir]).
- Poly-ICLC stimulates TLR3 RIG-I and MDA-5
- Among the TLRs, TLR3 is most abundantly expressed in the CNS.
### Demographics and Clinical Characteristics of Participating Patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DC Dose Level (No. of DC/dose)</th>
<th>Total (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (1 x 10^7)</td>
<td>2 (3 x 10^7)</td>
</tr>
<tr>
<td>Received at least one vaccine</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Completed at least 4 vaccines</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Female (received at least 4 vaccines)</td>
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<td>4</td>
</tr>
<tr>
<td>Median age, years</td>
<td>52</td>
<td>46</td>
</tr>
<tr>
<td>Range</td>
<td>37-71</td>
<td>28-63</td>
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<tr>
<td>Tumor Histology</td>
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<tr>
<td>AA</td>
<td>3</td>
<td>2</td>
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<tr>
<td>AO</td>
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<td>2</td>
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<tr>
<td>AOA</td>
<td>1</td>
<td>0</td>
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<tr>
<td>GBM</td>
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<td>7</td>
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<tr>
<td>No. of Previous Recurrences</td>
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<td>0</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
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</table>

(Statistical data and analysis not included in the image.)
Upregulated expression of CXCL10 mRNA in murine GL261 glioma treated with GAA-vaccines and i.m. poly-ICLC 
(In situ hybridization)

A  Vaccine Plus Poly-ICLC  B  Vaccine Alone

C  Poly-ICLC Alone  D  Mock-Treatment

Zhu X.  Et al  
2010
**RT-PCR (Pre-vac vs. 24-hr Post 1\(^{st}\) Vac)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pre-Vac</th>
<th>24-hr Post 1(^{st}) Vac</th>
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</thead>
<tbody>
<tr>
<td>IFNα1</td>
<td>.031</td>
<td></td>
</tr>
<tr>
<td>CXCL10</td>
<td>.010</td>
<td></td>
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<tr>
<td>CCL5</td>
<td>.007</td>
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</tbody>
</table>

**Comparison of pre- vs. 24-hr post 1\(^{st}\) vaccine**

- Case# 10
- Case# 11
- Case# 16
- Case# 19
- Case# 22

**RT-PCR (Pre-vac vs Post-4\(^{th}\) Vac)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pre-Vac</th>
<th>Post-4(^{th}) Vac</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNα1</td>
<td>.056</td>
<td>.029</td>
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<tr>
<td>CXCL10</td>
<td>.029</td>
<td>.025</td>
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</tbody>
</table>

**Comparison of pre-1\(^{st}\) vs. post-4\(^{th}\) vaccine**

- Case# 9
- Case# 10
- Case# 12
- Case# 16
- Case# 18
- Case# 19
- Case# 20

**C Luminex**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pre-Vaccine</th>
<th>24-hr Post 1(^{st}) vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNα</td>
<td>&lt;.001</td>
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</tr>
<tr>
<td>CXCL10</td>
<td>.031</td>
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<tr>
<td>IL-15</td>
<td>.021</td>
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<tr>
<td>MCP-1</td>
<td>.004</td>
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<tr>
<td>MIP-1β</td>
<td>.025</td>
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</tbody>
</table>

**Comparison of pre-vaccine vs. post 1\(^{st}\) vaccine (pg/ml)**

- Case# 2
- Case# 10
- Case# 11
- Case# 15
- Case# 19

**D In Situ Hybridization**

- CXCL10 (Pre-Vaccine)
- CXCL10 (Post-Vaccine)
- Sense control