Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD-4C) for Recurrent Malignant Gliomas

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NIH Recombinant DNA Advisory Committee Review (March 10, 2004)
Goals

• Rationale/ Background
  – General concepts: biology of gliomas
  – Experience with Ad-p53 clinical trial
• Development of Delta-24
• Enhancing tumor cell tropism: RGD-4C
• Clinical trial
Glioblastoma Multiforme
Molecular Alterations in Gliomas

**Self-sufficiency in growth signals**
- EGF/EGFR
- PDGF/PDGFR
- Ras

**Insensitivity to Growth inhibitory signals**
- pRb
- p16
- TGFβ

**Evading apoptosis**
- p53 mutation
- bcl-2
- PI3 Kinase-AKT
- PTEN
- c-Myc

**Sustained Angiogenesis**
- VEGF/VEGFR
- bFGF
- HIF-1α
- EGF/EGFR

**Tissue Invasion**
- Matrix Metalloproteases

**Limitless Replicative Potential**
- Telomerase reactivation
Replication Incompetent Adenoviral Vectors
Ad-p53 Trial: Objectives

- To determine the qualitative and quantitative **toxicity** of Ad-p53 administered by intratumoral injection.

- To determine the **maximum tolerated dose** (MTD) of Ad-p53 administered by intratumoral injection in patients with recurrent malignant gliomas.

- To determine the **biological effects** at the molecular level of intratumoral administration of replication-deficient adenovirus vector containing wild-type p53 gene (Ad-p53) in human malignant gliomas by analyzing the expression and distribution of exogenous p53 protein.
Ad-p53 Clinical Trial for Recurrent Malignant Gliomas: Treatment Schematic

Procedure 1

Day 0

Stereotactic injection of Ad-p53 via catheter

Procedure 2

Day 3

A

‘En bloc’ Tumor Resection with catheter

B

Intramural injection of Ad-p53

Follow-up

Biological Studies

Toxicity Studies
Clinical Studies: Toxicity

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade</th>
<th>Relationship Possible (No. of events)</th>
<th>Relationship Probable/definite (No. of events)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS hemorrhage</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Confusion</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>5</td>
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<td></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Fever without neutropenia</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>4</td>
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<tr>
<td>Granulocytopenia</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<td>Headache</td>
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<td>1</td>
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<td>Leukopenia</td>
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<td>2</td>
<td>0</td>
<td>2</td>
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<td>Motor dysfunction</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Nausea alone</td>
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<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Pyramidal tract dysfunction</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Seizure</td>
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<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>Speech impairment</td>
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<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>3</td>
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<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>Viral-like syndrome</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Vomiting</td>
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<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>41</td>
<td>4</td>
<td>45</td>
</tr>
</tbody>
</table>

\(^1\) Results are reported as independent events. More than one event may have occurred per patient or the same event may have occurred more than once in a given patient.
Ad-p53 Phase I Trial: Biological Studies
### Biological Studies: Summary

**Table 2. Biological Analysis**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose Level</th>
<th>p53 IHC</th>
<th>p53 mutational status</th>
<th>Pretreatment biopsy</th>
<th>Post injection specimen</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nuclear staining</td>
<td>Cytoplasmic staining</td>
</tr>
<tr>
<td>1</td>
<td>I</td>
<td>Rare</td>
<td>ND</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>+</td>
<td>Wt</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>I</td>
<td>-</td>
<td>Wt</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
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<td>ND</td>
<td>ND</td>
<td>+</td>
<td>-</td>
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<tr>
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<td>II</td>
<td>+</td>
<td>Wt</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>II</td>
<td>ND</td>
<td>ND</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>7</td>
<td>III</td>
<td>-</td>
<td>Wt</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>III</td>
<td>+</td>
<td>Wt</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>III</td>
<td>-</td>
<td>Wt</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
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<td>ND</td>
<td>ND</td>
<td>NE</td>
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<td>14</td>
<td>IV</td>
<td>-</td>
<td>Wt</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>IV</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemistry; ND, not done; Wt, wild-type; NE, not evaluable; Intracytic injection

1 Patients 10, 11, 12 (see Table 1) were also analyzed for p53 mutation using the pretreatment biopsy specimen. Mutations were identified in patient 10 [codon 234 (tyr/stop) and codon 157 (val/phe)] and patient 11 [codon 248 (Arg/Gln)].
Ad-p53 Clinical Trial: Lessons Learned

• No significant toxicities were observed
• Maximum Tolerated Dose (MTD) was reached at $1 \times 10^{12}$ viral particles
• Two-stage design was well tolerated and without complications
• No Ad-p53 virus was detected systemically
  – (blood, urine, sputum, or feces)
Ad-p53 Clinical Trial: Lessons Learned

Selectivity: Ad-p53 can infect and transduce exogenous p53 in both normal and tumor cells.

Delivery: With the injection technique used, p53 distribution is limited to 5-6 mm from injection site.
Glioblastoma Multiforme

Tumor cells

Tumor cells and normal neurons
Conditionally Replication-Competent Adenoviruses

- **dl1520** (ONYX-015)
  - Contains a deletion in E1b 55kD gene
  - Replication depends on p53 status

- **Delta-24**
  - Contains a partial deletion in E1a gene
  - Replication depends on Rb/p16 status of the cell
Replication Competent Adenovirus

A. Delta 24 virus injection

B. Delta 24 virus replication by host cell

C. Release of more Delta 24 and infection of other tumor cells

D. Lysis and further release of Delta 24 virus

Astrocytic tumor cell

CELL LYSIS
A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo


Oncogene. 2000;19:2-12
Construction of Delta-24

Delta-24 has a deletion of 24 DNA base pairs within the E1A gene. This deletion renders the E1A protein incapable of binding to and inactivating Rb.

(Oncogene. 2000;19:2-12)
Selective Mechanism of Delta-24 Tumor Cell

- **Normal Cell**
  - E1a
  - Rb
  - E2F
  - Cell cycle control
  - Free E2F
  - Forced Cell Cycle Entry (S-phase)

- **Delta-24 E1a**
  - E1a
  - Rb
  - E2F
  - Cell cycle control
  - Bound E2F
  - Controlled Cell cycle

- **Tumor Cell**
  - E1a
  - Rb
  - E2F
  - Uncontrolled Cell Cycle
  - Free E2F
  - Uncontrolled Cell Cycle (S-phase)
Glioma cell lines are permissive to replication of Delta-24 and are therefore killed (lysed) by this agent.

Crystal violet staining of cell culture plates demonstrating “cytopathic effect” (CPE)

Glioma cell lines are permissive to replication of Delta-24 and are therefore killed (lysed) by this agent.

Oncogene. 2000;19:2-12
Infection of 293 cells with the supernatant from infection cell lines

Titration values of virus progeny produced from the infection of Delta-24 in three cell lines

Oncogene. 2000;19:2-12
Delta-24 Effect: Rescue by Rb

Treatment with either Ad5CMV-pA or Ad5CMV-Rb

Protection against cell lysis in Rb+ cells

OncoGene. 2000;19:2-12
Cell Surface Expression of CAR and αV Integrins

Adapted from Journal of the National Cancer Institute, 2003
A Conditionally Replicative Adenovirus with Enhanced Infectivity Shows Improved Oncolytic Potency

Kaori Suzuki, Juan Fueyo, Victor Krasnykh, Paul N. Reynolds, David T. Curiel and Ramon Alemany

Δ24-RGD Adenovirus

RGD

Δ24

E1

L1 → L2 → L3 → L4 → L5

E3

E2B E2A E4

p3602

ITR

0 10 20 30 40 50 60 70 80 90 100 mu (1 mu = 0.36 Kb)
Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway


Delta-24 Vs. Delta-24-RGD: Effect of Low CAR Expression

Low CAR expression
Kaplan-Meier Survival Curves for Delta-24 and Delta-24-RGD

![Kaplan-Meier Curves](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>mean (days)</th>
<th>95% CI (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta-24-RGD</td>
<td>25</td>
<td>131</td>
<td>100 to 162</td>
</tr>
<tr>
<td>Delta-24</td>
<td>26</td>
<td>50</td>
<td>30 to 70</td>
</tr>
<tr>
<td>UV-i Delta-24-RGD</td>
<td>26</td>
<td>18</td>
<td>18 to 19</td>
</tr>
<tr>
<td>PBS</td>
<td>20</td>
<td>19</td>
<td>18 to 20</td>
</tr>
</tbody>
</table>
Predicted Spread of $\Delta 24$ within Gliomas

- Necrosis
- $\Delta 24$
- Tumor
“Late” Viral Protein Expression (Hexon)
## Effect of Delta-24 and Delta-24-RGD on Normal Vs. Tumor Cells:

<table>
<thead>
<tr>
<th>Adenovirus</th>
<th>NHA (pfu/mL)</th>
<th>U-251 MG (pfu/mL)</th>
<th>U-87 MG (pfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Delta-24</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td>1.9 x 10^2</td>
<td>6.3 x 10^5</td>
<td>3.1 x 10^2</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>0</td>
<td>7.9 x 10^4</td>
<td>7.9 x 10^2</td>
</tr>
<tr>
<td><strong>Delta-24-RGD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td>2.5 x 10^3</td>
<td>4.0 x 10^7</td>
<td>1.0 x 10^7</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>5.0 x 10^3</td>
<td>3.2 x 10^6</td>
<td>2.0 x 10^7</td>
</tr>
<tr>
<td><strong>Ad300</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td>1 x 10^6</td>
<td>2.5 x 10^7</td>
<td>1.5 x 10^5</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>5 x 10^6</td>
<td>3.1 x 10^6</td>
<td>1.5 x 10^5</td>
</tr>
</tbody>
</table>

*Data represent the final viral titer in plaque-forming units (pfu)/mL from two independent experiments. Viral titers were determined by the tissue culture infection dose50 method (15) from lysates of cells plated in low confluence, maintained in serum-starved conditions (0.5% fetal bovine serum, no growth supplements), and infected with 2 x 10^4 pfu/mL of Delta-24, Delta-24-RGD, or Ad300 (a wild-type adenovirus used as a control). Exp. = experiment.

Journal of the National Cancer Institute, 2003
The expression of alpha-V integrins is common in gliomas and uncommon in normal brain


Pasqualini et al., Alpha(v) integrins as receptors for tumor targeting by circulating ligands Nat. Biotechnol. 15:542-546, 1997


“Normal brain astrocytes expressed alpha 2, alpha 3, alpha 6, beta 1, and beta 4 chains … but they were consistently negative for other integrins examined (alpha 4, alpha 5, alpha V, alpha L, alpha M, alpha Z, beta 2, and beta 3)”
Conclusion of Pre-clinical Data

Delta-24-RGD infects glioma cells with low expression of CAR, a common feature of human tumors, by binding alpha-V integrins.

Importantly the expression of alpha-V integrins is common in gliomas and uncommon in normal brain.
Clinical Trial

Phase I Trial of Conditionally Replication-Competent Adenovirus (Delta-24-RGD-4C) for Recurrent Malignant Gliomas

Charles Conrad, M.D. – Co-P.I.
Frederick Lang, M.D. – Co-P.I.
Objectives

- To determine the qualitative and quantitative toxicity of Delta-24-RGD administered by intratumoral injection.

- To determine the maximum tolerated dose (MTD) of Delta-24-RGD-4C administered by intratumoral injection in patients with recurrent malignant gliomas.

- To determine the biological effects at the molecular level of intratumoral administration of replication-competent adenovirus Delta-24-RGD in human malignant gliomas by analyzing; viral distribution, expression of “late” viral genes, evidence of oncolysis, characterization of immune responses, evaluate for the presence of viral shedding.
## Dose escalation

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Dose Delta-24-RGD (viral particles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$1 \times 10^9$</td>
</tr>
<tr>
<td>2</td>
<td>$3 \times 10^9$</td>
</tr>
<tr>
<td>3</td>
<td>$1 \times 10^{10}$</td>
</tr>
<tr>
<td>4</td>
<td>$3 \times 10^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>$1 \times 10^{11}$</td>
</tr>
</tbody>
</table>
Dose Dependence Experiments

RGD Dosing Experiment, 10 Animals per Dose

HR = 0.56, 95% CI = (0.46, 0.67), p < 0.0001
Treatment Plan: Group A

Procedure 1: Stereotactic Tumor Injection

Day 0

Stereotactic injection of Delta-24-RGD via catheter

→ Follow

Toxicity Studies
Treatment Plan: Group B

Procedure 1: Stereotactic Tumor Injection
- Day 0
  - Stereotactic injection of Delta-24-RGD via catheter

Procedure 2: Craniotomy with Tumor Resection
- Day 14
  - A: ‘En bloc’ Tumor Resection with catheter
  - B: Intramural injection of Delta-24-RGD

Follow up

Biological Studies

Toxicity Studies
**Additional Components Regarding the Clinical Trial**

1. IRB approval has been obtained with MDACC for this phase I protocol.

2. The current clinical trial has plans to perform pre- and post-treatment serum antibody titers for adenovirus.

3. The clinical trial will also perform biodistribution studies on serum, sputum, urine and feces.

4. Specific studies to identify local immune reactions around the injection site within the tumor (in Group B patients) will be further defined.

5. A pre-IND meeting with the FDA has been completed.
   - Suggested studies with Cotton rats are in the planning stages.
   - A discussion of the suggested toxicology, pharmacokinetic, and biodistribution studies is ongoing.

6. The Manufacture and quality control of the clinical-grade (GMP-virus) is being provided through the RAID program.

7. Formal plans for the animal toxicology, pharmacokinetics, biodistribution, and specific neurotoxicity studies is being provided through the RAID program with concurrent discussions with the FDA.