Can Oncolytic Adenovirus Be Implemented as Therapeutic Strategies for DIPGs?

DIPG EUROPEAN MEETING
Barcelona 23-24 February 2012
He ain’t digging no tyrosine kinase inhibitor
Oncolytic Adenovirus

(Fotos obtenidas de: “The Universe in a Nutshell” por Stephen Hawking)
CAPSID
DNA
Strategies to improve cancer selectivity and potency of oncolytic adenovirus

- Functional deletion in critical viral genes
- Modifying the tropism of the adenovirus
- Using tumor or tissue-specific promoters to restrict the expression of essential viral genes
Oncolytic adenovirus Delta-24-RGD

Adenovirus genome

Delta-24 E1A

RGD-4C modified fiber
Adenovirus WT

Adenovirus DNA

Rb

E1A Viral replication

E2

E2F

Delta-24

No viral replication

mE1A

Viral replication

Cancer cells

Normal cells

Adenovirus Delta-24
<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>

**Graph:**
- **X-axis:** Time from tumor implantation (days)
- **Y-axis:** Fraction of mice surviving
- **Legend:**
  - Delta-24-RGD
  - Delta-24

**Statistical Significance:**
- $P < 0.001$ for both Delta-24-RGD and Delta-24 compared to PBS.
PHASE I TRIAL OF CONDITIONALLY REPLICATION-COMPETENT ADENOVIRUS (Delta-24-RGD) FOR RECURRENT MALIGNANT GLIOMAS

CO-CHAIRMEN:

Frederick F. Lang, Jr., M.D. / Charles A. Conrad, M.D.
The University of Texas M. D. Anderson Cancer Center
Departments of Neuro-Oncology and Neurosurgery
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

Biological Studies
Toxicity Studies
Follow up
Cocktail Time: adenoviruses and chemotherapy

(gene&tonic)
Delta-24-RGD/TMZ displays a synergistic cytotoxic effect in vitro

U87 MG
- D24R (1 MOI)
- D24R (0.1 MOI)
- TMZ

T98G
- D24R (1 MOI)
- D24R (5 MOI)
- TMZ

MGMT expression
Delta-24-RGD inhibits MGMT expression
Hypothesis: E1A mediates MGMT shut off

p300 is a positive transcriptional regulator of MGMT expression

Viral protein E1A binds and inhibits p300
E1A disrupts p300 interaction with MGMT promoter

**ChIP ASSAY**
The combination Delta-24-RGD/TMZ increased the median survival and nr of long-term survivors in brain tumor xenografts.

Graphs showing survival fractions over days for different treatment groups (PBS, TMZ, D24R, D24R/TMZ) with P < 0.0001 for both experiments.
Conclusions:

• Delta-24-RGD in combination with temozolomide induce a synergistic antitumor effect in gliomas

• This effect is mediated by the capacity of the E1A to bind p300 and thus abrogate its positive effect in the transcription of MGMT
Adenovirus and DNA damage

- Temozolomide
  - O(6)-meG or N(7)-meG
  - Base mismatch
  - Futile MMR
  - Double-strand breaks

- Survival
  - DNA repair

- G2/M arrest
  - Failure of DNA repair

- Cell Death

- Double base repair
- Mismatch repair

- MGMT
  - Transcription

- PARP
  - Cleavage
  - Degradation

- MRN
  - E1A
  - E4orf3 & E4orf6-E1b55K

- p300

- E3
  - E4orf6-E1b55K

- D24-RGD
RAD001

- Derivative of Rapamycin
- Potent anticancer effect
- Immunosuppressive and antiangiogenic properties
- Inhibits mTOR

Gene transcription
↓ VEGF production
↓ Cell Growth
↓ Cell proliferation
Delta-24-RGD antitumoral activity is enhanced by combination with RAD001
Combination treatment with Delta-24-RGD and RAD001 enhanced autophagy in vitro

Increases in the % of AVOs

**AVO positive (%)**

<table>
<thead>
<tr>
<th></th>
<th>mock</th>
<th>RAD</th>
<th>D24R</th>
<th>D24R/RAD</th>
<th>Ad-WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**P<0.01**

Increases the expression of ATG5

**ATG5**

**Actin**

Time (hrs) 24 48 72 24 48 72 24 48 72 24 48 72
Inhibition of autophagy induced a decrease in the citotoxic effect exerted by the virus alone or in combination with RAD001.
RAD001 enhanced the antiglioma effect of Delta-24-RGD in vivo.

![Graph showing survival fraction over time with different treatments: mock, D24-RGD, RAD, and D24R/RAD. The graph indicates a statistically significant difference with a p-value of <0.0001. The survival time is measured in days (0 to 100).]
ADVANTAGES:
• Oncolytic Adenoviruses do not work as usual drugs do
• Not known resistances
• Low toxicity and robust antitumor potency
• Potential to become a good therapeautic candidate

DISADVANTAGES:
• We do not have good experimental models
• Lack of cell lines and animal models
Thank you
Acknowledgments

Lab:
Enric Xipell
Leire Urquiza Cirauqui
Patricia Jauregi
Marisol Gonzalez

Collaborators
Juan Fueyo, Candelaria Gomez-Manzano and Fred Lang (MD Anderson Cancer Center, Houston, USA)
Ramón Alemany (ICO, Barcelona)
Ricardo Diez-Valle y Sonia Tejada (CUN, Pamplona)
Jaume Mora`s Group (HSJD, Barcelona)
Teresa Tuñon and Idoya Zazpe (Hospital de Navarra, Pamplona)
Delta-24-RGD exhibit a robust effect against Pediatric Glioma cell lines

**Res 186**

![Graph showing viability for Res 186 cell lines with different concentrations of Delta-24-RGD.]

**Res 259**

![Graph showing viability for Res 259 cell lines with different concentrations of Delta-24-RGD.]

**KNS42**

![Graph showing viability for KNS42 cell lines with different concentrations of Delta-24-RGD.]

**SF188**

![Graph showing viability for SF188 cell lines with different concentrations of Delta-24-RGD.]

Viability (%) vs Concentration of Delta-24-RGD for different cell lines.
Delta-24-RGD replicates efficiently in pediatric glioma cell lines
Can we enriched these cell lines for BTSCs?

<table>
<thead>
<tr>
<th></th>
<th>Parental/NSC Media (log2)</th>
<th></th>
<th>Parental/NSC Media (log2)</th>
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<tbody>
<tr>
<td>Res 259</td>
<td></td>
<td>KNS42</td>
<td></td>
</tr>
<tr>
<td>BMP4</td>
<td>2,3</td>
<td>BMP4</td>
<td>1.7</td>
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<tr>
<td>β-Tubulin</td>
<td>2.71</td>
<td>β-Tubulin</td>
<td>2.91</td>
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<tr>
<td>GFAP</td>
<td>2,2</td>
<td>GFAP</td>
<td>2.5</td>
</tr>
<tr>
<td>Nestin</td>
<td>2,9</td>
<td>Nestin</td>
<td>3.3</td>
</tr>
<tr>
<td>Sox2</td>
<td>5,3</td>
<td>Sox2</td>
<td>2.3</td>
</tr>
<tr>
<td>REST</td>
<td>3,1</td>
<td>REST</td>
<td>3.4</td>
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</table>
Mitoxantrone is excreted more effectively by the BTSCs.
Effect of TMZ and Salinomycin in BTSCs Self-renewal

A

Neurosphere Formation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DMSO</th>
<th>100</th>
<th>10</th>
<th>100</th>
<th>10</th>
<th>1</th>
<th>0.1</th>
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</thead>
<tbody>
<tr>
<td>TMZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Salinomycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

P = 0.09
* P < 0.001

B

Control

TMZ 100 µM

TMZ 10 µM

Sal 10 µM

Sal 1 µM

Sal 0.1 µM

Neurosphere size (Diameter µM)

<table>
<thead>
<tr>
<th>UNTREATED</th>
<th>DMSO</th>
<th>TMZ 100</th>
<th>TMZ 10</th>
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<tbody>
<tr>
<td>Salinomycin</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Box plots showing neurosphere size for each condition.
Effect of TMZ and Salinomycin in “Stemness” markers

- Musashi
- Sox2
- Nestin

mRNA expression (folds)

- Control
- TMZ (10 µM)
- Sal (1 µM)
Effect of TMZ and Salinomycin in BTSCs cell cycle
**Top Canonical Pathways**

<table>
<thead>
<tr>
<th>Name</th>
<th>p-value</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ephrin Receptor Signaling</td>
<td>5.7E-04</td>
<td>13/191 (0.068)</td>
</tr>
<tr>
<td>CXCR4 Signaling</td>
<td>8.39E-03</td>
<td>9/160 (0.056)</td>
</tr>
<tr>
<td>TGF-b Signaling</td>
<td>8.74E-03</td>
<td>6/80 (0.075)</td>
</tr>
<tr>
<td>Rac Signaling</td>
<td>9.03E-03</td>
<td>7/113 (0.062)</td>
</tr>
<tr>
<td>Regulation of Actin-based Motility by Rho</td>
<td>1.14E-02</td>
<td>6/85 (0.071)</td>
</tr>
</tbody>
</table>
Effect of Delta-24-RGD and TMZ or Salinomycin in BTSCs

**KNS42 (10% FBS)**
- TMZ IC50 = 2.1 µM
- Sal IC50 = 0.1 µM

**KNS42 (SC Media)**
- TMZ IC50 = 1373 µM
- Sal IC50 = 0.5 µM

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Sal+D24RGD (5 MOI) = 0.001 µM
Sal+D24RGD (5 MOI) = 0.007 µM
Res 259 (10% FBS)

- TMZ IC50 = 3.7 µM
- Sal IC50 = 0.1 µM

Res 259 (SC media)

- TMZ IC50 = 54 µM
- Sal IC50 = 0.06 µM

Sal+D24RGD (5 MOI) = 0.01 µM

Sal+D24RGD (5 MOI) = 0.001 µM