Radiation immunomodulatory gene tumor therapy

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瑞典
Brain Immuno Gene Tumour Therapy

Immunization with autologous interferon-gamma secreting glioma cells in patients with Glioblastoma Multiforme

A phase 1-2 clinical trial

Purpose: 目的 mu4 di4:

The primary aims of the BRIGTT study were to ascertain

• **Safety**, 安全 an1 quan2

• **Feasibility** 可行性 ke3 xing2 xing4

• **Efficacy** 效力 xiao4 li4

of immunotherapy with autologous IFN-γ transfected tumor cells in patients with glioblastoma multiforme

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Grows with arms as an octopus

Sends migrating “guerilla-cells” into the surrounding brain where the BBB is intact
Timeline of immunization and monitoring procedures.

Fig. 1
Autologous tumor cells were cultured and transfected with the human IFN-γ gene by the use of an adenoviral vector.

After irradiation with 100 Gy the cells were administered as intradermal immunizations in the upper arm every 3rd week.

Endpoints
- for safety were: records of toxicity and adverse events,
- for feasibility percent treated patients out of eligible patients and time to treatment and
- for clinical efficacy overall survival (OS) and progress free survival (PFS).
Results

8/17 (47%) of eligible patients, aged between 50 and 69 years, were immunized between 8-14 times after surgery and radiotherapy.

- No adverse events or toxicity were recorded.
- There was no deterioration in neurological status of the patients during treatment.
- The treated patients had a significantly longer overall survival (p<0.05) than the control group of 9 patients (525 days, 17.4 months vs 325 days, 10.4 months).

The treated group and control groups did not differ in terms of age, extent of tumor resection or performance.

The prolongation of survival was also significant when compared to historical and published controls within the same age group.
Conclusions

Immunizations with autologous, irradiated tumor cells transfected with the gene for IFN-\(\gamma\) in patients with glioblastoma multiforme is

- **Safe,**
- **Feasible** in slightly less than 50% of eligible patients
- **Show signs of clinical efficacy.**

The small number of patients warrants further studies in larger cohorts.
Kaplan-Maier graph showing overall survival of immunized (included) and control patients. The survival was analyzed with the log-rank test.

Fig. 2
MRI (T1 with gadolinium) images from non-responding and responding patients

preoperatively, postoperatively and at the 6th immunization.

The postoperative image of the non-responding patient shows a dense area, which constituted a haemorrhage also seen on non-gadolinium enhanced images (not shown).
"RIGTT" Radiation ImmunoGene Tumor Therapy

- After the "BRIGTT" study has shown that the immunotherapy is safe we wish to improve the efficacy by combining it with radiation therapy,

- We wish to use a single low dose fraction in order to be able to treat previously irradiated patients.
Investigation of Rats with intracerebral implanted N29 Brain Tumors after Single fraction 5 or 15 Gy Radiotherapy combined with Immunotherapy.

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Groups of animals with various treatments in the various experiments with N29 and N32 tumors.

Table 1.
Groups of animals with various treatments in the various experiments with N29 and N32 tumors.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Number of N29 Animals Experiment A</th>
<th>Number of N32 Animals Experiment B</th>
<th>Number of N32 Animals Experiment C</th>
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<tbody>
<tr>
<td>1</td>
<td>Controls with no treatment</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Radiation 5 Gy</td>
<td>8</td>
<td>7</td>
<td></td>
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<tr>
<td>3</td>
<td>Radiation 15 Gy</td>
<td>8</td>
<td>6</td>
<td>6</td>
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<tr>
<td>4</td>
<td>Immunization</td>
<td>6</td>
<td>7</td>
<td>6</td>
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<tr>
<td>5</td>
<td>Radiation 5 Gy + Immunization</td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Radiation 15 Gy + Immunization</td>
<td>8</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>
Animals were given a single radiation treatment using a $^{60}$Co radiotherapy unit.
Inoculation at day 0 and treatments were performed at day 7 after inoculation.

Culture of N29 or N32 brain tumour cells

Day 0
Inoculation by Stereotactic Injection of 5000 cells in the brain

Syngeneic tumour cells Transfected with IFN-γ gene

Day 7
Radiationtherapy + Immunization

Day (7) 21, 35
IMMUNIZATION
By ip Injection of 10 million cells

Controls
†
Day 40-60 +

Complete remissions 75 %

Day 40-60 +

Immuneresponse

2010-11-13
Number of Survivals versus total number of rats in each group with intra cerebral implanted N29 tumors treated with IFN-g cell immunization (IMU IFNγ), single fraction radiation therapy (RT) and their combination at 7 days after inoculation. Immunization was repeated for at least two more times at days 21 and 35.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Number Surv. &gt;200 d / Num. animals</th>
<th>Fisher exact probability test, two-tailed</th>
<th>Fisher exact probability test, two-tailed</th>
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<tr>
<td>Controls</td>
<td>1/6</td>
<td>vs Control</td>
<td>P=9.43 NS</td>
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<tr>
<td>IMU IFNγ 3x</td>
<td>2/6</td>
<td>p=0.24 NS</td>
<td>p=0.06 NS</td>
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<tr>
<td>RT 5 Gy</td>
<td>0/8</td>
<td>p=0.35 NS</td>
<td>vs RT 5 Gy</td>
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<tr>
<td>RT 15 Gy</td>
<td>2/8</td>
<td>p=0.70 NS</td>
<td>p=0.47 NS</td>
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<tr>
<td>RT 5 Gy + IMU IFNγ 3x</td>
<td>5/8</td>
<td>p=0.026*</td>
<td>p=0.007 **</td>
</tr>
<tr>
<td>RT 15 Gy + IMU IFNγ 3x</td>
<td>3/8</td>
<td>p=0.38 NS</td>
<td>p=0.025*</td>
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</table>
Survival plot of intra cerebral implanted N29 tumors Controls (Lower panel). Immunization with syngeneic N29 tumor cells (2nd panel); radiation therapy (3rd panel) and a combination of radiation therapy and immunization (upper panel).
In the lower panel is given the primary survival rate in %. The percentage of the challenged animals that didn’t develop any tumor, given in the middle panel, multiplied with the fraction of primary survival gives the percentage of cured animal. that is displayed in the upper panel.
Conclusion

The most effective therapeutic regime for N29 tumors is one fraction of radiation therapy of 5 Gy combined with immunization. The immunization repeated for at least two more times at days 21 and 35.

This regime resulted in a significant prolonged survival and 75% complete remissions ($p<0.05$).

Corresponding combination with 15 Gy RT resulted in 50% complete remissions. Neither immune therapy nor radiation therapy alone with 5 or 15 Gy resulted in any significant therapeutic effect.
Subcutaneously implanted
N29 Brain Tumors
N29 tumours inoculated
Subcutaneously on both sides

Only the right tumour was irradiated
Investigation of Rats with subcutaneously implanted N29 Brain Tumors after 4 fractions 5 Gy Radiotherapy combined with Immunotherapy.

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Controls

Control Group 1

Left side

Time after inoculation / days

Tumor Volume / mm$^3$

Right side

Time after inoculation / days

Tumor Volume / mm$^3$

Average

Exp Growth fit

r$^2$ 0.98

.rs 2009

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Immunotherapy

**Left side**

- IFN at days 15; 29; 43;
- 1000 \( \text{u/m} \) m

**Right side**

- IFN at days 15; 29; 43;
- 100 \( \text{u/m} \) m

**Tumor Volume / mm\(^3\)**

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<tr>
<th>Time after inoculation / days</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
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</table>

**Average Exp Growth fit**

- \( r^2 = 0.999 \)

- \( r^2 = 0.997 \)

RRS 2009

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Radiation therapy

Radiation therapy treatment at days 29; 30; 32; 33;

RT

Time after inoculation / days

Tumor Volume / mm³

Untreated

Left side

RT

Right side

Tumor Volume / mm³

Time after inoculation / days

Average

Exp. Growth fit

r² 0.90

Exp. Growth fit

r² 0.89

s.c. glioma tumor

collimated radiation field

Unaffected tumor

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Radiation and Immunotherapy

Immuno therapy + RT

Untreated Left side

IFNγ at days 27; 42; 47; 55;

RT right tumor

10000

1000

100

10

0 10 20 30 40 50 60 70 80 90 100 110

Time after inoculation / days

Tumor Volume / mm³

rat 5328
rat 5371
rat 5331
rat 5335
rat 5339
rat 5342
rat 5364
rat 5365
Average
Exp Growth fit
$r^2 = 0.90$

Immunotherapy + RT Right side

IFNγ at days 27; 42; 47; 55;

RT 29; 30; 32; 33

10000

1000

100

10

0 10 20 30 40 50 60 70 80 90 100 110

Time after inoculation / days

Tumor Volume / mm³

rat 5328
rat 5371
rat 5331
rat 5335
rat 5339
rat 5342
rat 5364
rat 5365
Average
Exp Growth fit
$r^2 = 0.93$

Exp Growth fit

Average

RT

i.p injection of IFNγ tumor cells

Time after inoculation / days

s.c. glioma tumor
Exponential Tumour Growth Model

Tumour growth rate “TGR” is estimated from the tumour volume measurements by fitting the data of each individual tumour to a model of exponential growth

\[ TV_t = TV_0 \cdot \exp[TGR \cdot t] \]

where

“TV_t” is Tumour volume at time \( t \)

“TV_0” is Tumour volume at time \( t = 0 \),

“TGR” is tumour growth rate constant (% per day)
Tumour growth rate of subcutaneous N29 tumours: Controls and after treatment with RT, IFNγ immunization or their combination.
Tumour Growth rate "TGR" of subcutaneous tumours implanted on both Right and Left hind leg.

= The right tumour was treated with radiation (RT).

Average of all experiments

<table>
<thead>
<tr>
<th>Resultat</th>
<th>LEFT</th>
<th>SE</th>
<th>Right</th>
<th>SE</th>
<th>N</th>
<th>t ctrl L</th>
<th>t Ctrl /R</th>
<th>t R/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>9.1 ± 0.3</td>
<td></td>
<td>8.5 ± 0.3</td>
<td></td>
<td>40</td>
<td>NS</td>
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<tr>
<td>INFγ</td>
<td>9.2 ± 0.8</td>
<td></td>
<td>7.6 ± 0.6</td>
<td></td>
<td>19</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>RT</td>
<td>6.1 ± 0.4</td>
<td></td>
<td>4.5 ± 0.3</td>
<td></td>
<td>15</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RT +INFγ</td>
<td>6.4 ± 0.5</td>
<td></td>
<td>5.9 ± 0.5</td>
<td></td>
<td>7</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

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Specific Therapeutic Effect “STE”

is defined as follow.

\[
STE = \frac{TGR_C - TGR_E}{TGR_C};
\]

\[
TGR_E
\]

The average of the individual Tumour growth rate constant in the group of exposed rats. day\(^{-1}\)

\[
TGR_C
\]

The average of the individual Tumour growth rate constant in the group of control rats. day\(^{-1}\)
The STE is equal to 0 when the average of tumour growth rate constant of the exposed group, is equal to the average of the tumour growth rate constant of the control.

The STE is equal to 1 when the average tumour growth rate constant of the exposed group, is equal to 0.
Specific therapeutic effect of subcutaneous N29 tumors after RT, Immunization with IFNγ and their combination RT + IFNγ

Type of treatment

Radiation + IFNγ
- Left: None
- Right: Irradiated

IFNγ
- Left: None
- Right: Irradiated

Radiation
- Left: Specific Abscopal Effect “SAE”
- Right: Specific Therapeutic Effect “STE”

p < 0.0001

2010-11-13 Bertil_R.Persson@rmed.lu.se
CONCLUSION:

Significant **Abscopal effect** was confirmed on *subcutaneously* implanted N29 rat glioma tumours, from contra-lateral treatments with radiation therapy alone and in combination with immunization by using syngeneic interferon-gamma secreting tumor cells.
Mechanism of Abscopal effect?

Ionizing radiation??

IMMATURE DC
- High intracellular MHCII (MHCs)
- Endocytosis, including FcR
- Low CD54, 58, 80, 86
- Low CD40, CD25, IL-12
- Low CD83, p55
- Low granule antigens
- Actin cables

MATURE DC
- High surface MHCII
- Low endocytosis and FcR
- High CD54, 58, 80, 86
- High CD40, CD25, IL-12
- High CD83, p55
- High M342, 2A1, MIDC-8 antigens
- No actin cables
Dendritic Cells- Maturation

Secretion of cytokines - recruits monocytes from blood
Macrophage and more dendritic cell precursors

Increased MHC expression on cell surface

Increased expression of co-stimulatory molecules on cell surface

Migration to Lymph nodes
Conclusion:

Based on the findings that immunization combined with 5 Gy radiation therapy increased the survival time 87% (p=0.003) with 75% complete remissions, new regimes of glioma treatment might be developed.
In view of the finding that radiation enhance the antitumor effect of Immune-therapy,

The combination approach should be studied further for clinical translation.
Conclusion:

For example, single fraction radiation therapy sessions with a target absorbed dose in the order of 5 - 10 Gy combined with clinically proven immunotherapy.
Conclusion:

Due to the moderate absorbed dose, relapse patients previously treated to full dose might benefit from a single fraction radiation therapy of 6 Gy combined with immunotherapy.
Conclusion:

Another benefit of the moderate absorbed dose is that, if the response in not complete after the first treatment, additional combined treatments with single fraction radiation therapy and immunotherapy sessions could be given with a few weeks interval.
In view of the finding that radiation enhances the antitumor effect of Immune-therapy, the combination approach should be studied further for clinical translation.
Conclusion:

Other alternatives than the presently used immunization by vaccination with the patient’s own tumor cells might be used, such as dendritic cell vaccines or other clinically proven methods of immunization.
RIGTT History?

- Experiment at Lund 2001: INF-γ cells
- Lumniczky Safrani 2002: GM-CSF cells
- Prins and Graf 2002: Autol. Tum cells
- Sandra De Maria 2005: CTLA-4 blockade
- Newcomb 2006: GM-CSF
- Sharp NCI 2007: AntiFAS mAb
- Teitz-Tennenbaum 2008: Dendritic cells
- Newcomb 2010: Anti-CD137 Therapy