Co-operative Radio-Immune-Stimulating Cancer Therapy

Bertil R.R. Persson PhD, MDh.c

Professor emeritus of medical radiation physics
1990-1992

My history of discovering the Radio-Immune-Stimulating effect began in the early 1990s with attempts to use Electroporation in combination with Bleomycin.

Electro-Chemotherapy "ECT"
Electrical pulses increase cell membrane permeability and allows Bleomycin access to the cytosol.

No Electric Field

Intact Cell Membrane

Bleomycin molecules Outside the tumour cell

Applied Pulsed Electric Field

Perforated Cell Membrane permeable to molecules

Bleomycin molecules inside the tumour cell
Cell Membrane electroporation

Theoretical cell membrane before and after Electroporation
The rat glioma model

In vitro culture of rat glioma cells

Stereotactic implantation in the brain (caudate n.)

1 week later, a tumour (diam. 4-6 mm) giving symptoms, has developed
Fig. 1. Inoculation of RG 2 cells in the right caudate nucleus and electropermeabilization 10-12 days later with electrodes enclosing the growing tumour.
Fig 2. Kaplan-Meyer plot for the survival of 17 animals treated with bleomycin and electropermeabilization (400 V, 8 pulses) and 13 untreated controls.
1995

Elektroporation in combination with Radiation therapy

Electro-Radioterapi “EPX”
The tumour was Treated with PEF, RT and RT+PEF
Figure 4. CD8+ infiltration in tumours of various treatment: Controls, Pulsed electric fields PEF (1400 V/cm), radiation therapy RT (4x5 Gy) and the combination PEF+RT.
Figure 3. CD4+ infiltration in tumours of various treatment: Controls, Pulsed electric fields PEF (1400 V/cm), radiation therapy RT (4x5 Gy) and the combination PEF+RT
1998-2001

Immunotherapy

Brain-Immuno-Gene-
Tumour-Therapy
"BRIGHTT"
The BRIGTT study

A clinical Phase I study of immuno-gene therapy of brain tumours "BRIGTT" (Brain Immuno Gen Tumour Therapy) was started by Neuro-surgery professor Leif G Salford 2001 in Lund. The primary aims of this study were to ascertain safety, feasibility and efficacy of immunotherapy in patients with glioblastoma multiforme with a vaccine of autologous IFN-γ transfected tumor cells
1998-2002

Elektro-Immuno Radioterapi

"EPIX"
Combination of 5Gy×4 Radiation Therapy, Pulsed Electric Fields (PEF) and Immunization with syngeneic Interferon-gamma IFNg gene transfected tumour cells.

Tumour growth rate (TGR, black), the specific therapeutic effect (STE, red) of all the experiments performed and Tumour enhancement ratios (TER, blue) of the combined treatments.
First preclinical experience of co-operative radio-immune therapy

Combination of 5Gy×4 Radiation Therapy, Pulsed Electric Fields (PEF) and Immunization with syngeneic Interferon-gamma secreting tumour cells.

At the SNO meeting in San Diego. Nov 21-24. 2002 Persson et al. presented a summary of results from tumour treatment experiments performed during 1998-2002 in Lund by using Pulsed Electric Fields (PEF) combined with Radiation Therapy (RT) and Immunization with syngeneic Interferon-gamma (IFNγ) secreting tumour cells.  

Combination of 1×15Gy RT and Immunization with syngeneic cellular tumour vaccine

Martin Graf et al. (2002) treated rats bearing a 5-day intracranial (i.e.) syngeneic glioma with a subcutaneous (s.c.) vaccination consisting of irradiated glioma cells or a multimodality approach composed of radiotherapy plus s.c. vaccination (Graf et al., 2002).

B Advancing edge of the 5-day glioma showing secondary tumor structure of short interwoven bundles ( ), tumor infiltration into the parenchyma (ł) with accompanying edema (× 200·).

C Higher magnification of the preceding section of the infiltrative border showing the presence of a glioma cell in anaphase (ł) and an abnormal tri-mitotic figure (× 1,000·).
F Higher magnification of a glioma from a moribund vaccinated rat revealing a cluster of mononuclear cells within the glioma; ×1000

G Evaluation of the implantation site of a rat treated with combined radiation and vaccination 2 months after glioma implantation showing the presence of hemosiderin-laden macrophages and mononuclear cells in the residual lesion; ×1000
Fig. 2. Vaccination of glioma-bearing rats results in decreased survival. Rats bearing a 5-day T9 glioma were vaccinated s.c. with $5 \cdot 10^6$ irradiated T9.F cells (p; n=9) or were sham-treated (d; n=9). Vaccinated rats showed a significant reduction of survival ($P<0.0001$).

Fig. 5. Rats bearing a 5-day T9 glioma treated with 15 Gy of whole-head irradiation (j; n=9) had a significant extension of survival ($P=0.015$) compared to rats receiving no treatment (d; n=9).

Rats that received combined radiation and vaccination had an even greater extension of survival compared to controls ($P=0.004$), and 45% of the rats remained tumor-free (p;n=11).
Myeloid-derived suppressor cells (MDSC) are also enriched at the tumor site. They express the inducible nitric oxide synthase (iNOS) allowing them to produce high amounts of nitric oxide (NO), which inhibits T cell migration as well as T cell receptor (TCR) and cytokine signalling.
Laboratory Investigation

Tumor infiltration by myeloid suppressor cells in response to T cell activation in rat gliomas

Martin R. Graf ¹, Jeremy T. Sauer ² and Randall E. Merchant ¹,²
¹Department of Neurosurgery, Virginia Commonwealth University Medical Center, Richmond, VA, USA;
²Department of Anatomy and Neurobiology, Virginia Commonwealth University Medical Center, Richmond, VA, USA

Immunoregulatory MSC present in gliomas can suppress the function of tumor-infiltrating, activated T cells, and therefore play a role in brain-tumor associated immunosuppression. The inadvertent generation of MSC in clinical trials involving vaccine-based strategies may represent a significant obstacle to successful brain tumor immunotherapy. However, we believe that modulating MSC populations or their
2002-2003

Radio - Immune-Gene-Tumour -Therapy

"RIGTT"
The rat glioma models N29

6-7 weeks later, a tumour (diam. 4-6 mm) giving symptoms, has developed.
Immunization against experimentally induced brain tumour

N29 brain tumour cells

Stereotactic injection of 5000 cells

IMMUNIZATION
Day 7, 21, 35
By ip injection of 10 million cells

Day 100 untreated control
6 out of 7 dead

Day 7
Radiation therapy

Day 100
2 out of 8 dead!
Number of Survivals and mean survival time of intra cerebral tumours treated with IFNγ cell immunization, radiation therapy and their combination.

<table>
<thead>
<tr>
<th>Date</th>
<th>Num. Survival / Num. Animals at day 100</th>
<th>Median Survival time days</th>
<th>Significance t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1/6</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Immunization with IFN-γ</td>
<td>2/6</td>
<td>147</td>
<td>0.08</td>
</tr>
<tr>
<td>RT 5 Gy</td>
<td>0/8</td>
<td>45</td>
<td>0.2</td>
</tr>
<tr>
<td>RT 15 Gy</td>
<td>2/8</td>
<td>88</td>
<td>0.4</td>
</tr>
<tr>
<td>RT 5 Gy + Immunization 3x</td>
<td>6/8</td>
<td>154</td>
<td>0.01</td>
</tr>
<tr>
<td>RT 15 Gy + Immunization 3x</td>
<td>5/8</td>
<td>118</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Survival of rats with intracerebral N29 tumors

Panel A: Control rats.

Panel B: Rats given immunization with syngeneic IFN-γ gene-transfected N29 tumor cells.

Panel C: Rats given 5 Gy or 15 Gy only.

Panel D: Rats treated with a combination of 5 Gy or 15 Gy plus immunization.
These results was presented at Society of Neuro-Oncology SNO's annual meeting in 2003 and the abstract was published in the journal of *Neuro-Oncology*, 5(4) 305

In October 2003 I reached retirement age and got no additional research funding to continue these experiments and the experimental files ended up in my archive.
"A 2nd CHANCE"

But 5 years later my former graduate student Crister Ceberg told me that Silvia Formenti at the ESTRO meeting 2008 in Gothenburg presented a study of breast cancer with the combination radiation therapy and immunotherapy that showed a considerable increase in the therapeutic effect similar what I previously found for brain tumours. She even stressed that radiation therapy combined with immunotherapy represents a paradigm shift for cancer treatment.
Radiation Immunomodulatory Gene Tumor Therapy of Rats with Intracerebral Glioma Tumors

Bertil R. R. Persson, Catrin Bauërëus Koch, Gustav Grafström, Crister Ceberg, Per Munck af Rosenschöld, Henrietta Nittby, Bengt Widegren and Leif G. Salford

a Department of Medical Radiation Physics, Lund University, SE-221 85 Lund, Sweden; b Department of Neurosurgery, Lund University, SE-221 85 Lund, Sweden; c Department of Tumor Immunology, Lund University, SE 220 07, Lund, Sweden; and d Rausing Laboratory, Biomedical Centre, Lund University, 221 85 Lund, Sweden
CTLA-4

CD8+ T-cell

Ipilimumab Blocking CTLA-4

B7 1/2

DC ; Macrophages, B-Cells

Release of tumour antigens

Curiel 2013
Cancer Immunotherapy
Invariant Natural Killer T Cells Regulate Breast Cancer Response to Radiation and CTLA-4 Blockade

Clin Cancer Res 2009;15(2)

**Graph:**
- **Y-axis:** Survival (%)
- **X-axis:** Days post-tumor inoculation

**Legend:**
- **IR + anti–CTLA-4 mAb 9H10**
- **IR 2x12Gy + IgG 9H10**
- **Control IgG**
The 4-dimensions in Co-operative Radio-Immune stimulating Therapy

1\textsuperscript{st} Dimension - Radiation Cell killing release of Tumour antigens activate DC

2\textsuperscript{nd} Dimension - Repealing Immune suppression by decrease of $T_{\text{reg}}$, TAM-M2, and MDSC

3\textsuperscript{rd} Dimension - Stimulating Immune Cell killing by Immune Therapy (anti-CTLA-4)

4\textsuperscript{th} Dimension - Vaccination-Immunization no relaps of the tumour
**Review article:**

**Co-operative Radio-Immune-Stimulating Cancer Therapy**

Crister Ceberg, and Bertil R.R. Persson  
Medical Radiation Physics, Lund University, S-22185 Lund Sweden

**ABSTRACT**

Radiation therapy for cancer treatment is delivered more or less in the same mode during the past 100 years. Low dose (2 Gy) fractions are given daily until a high target dose (60-70 Gy) is achieved. This treatment regime aims at eradicating the tumour by radiation induced cancer cell death. But traditional fractionated radiation therapy also decreases the number of radiation sensitive T-cells (CD3+, CD4+, and CD8+) in the tumour and thus prohibits immunogenic cell death. Several pre-clinical studies show that radiation therapy given by hypo-fractionation dramatically enhances the effect of otherwise non-effective immune-therapy. This opens up the possibility for an alternate cancer therapy regime using radiation in co-operation with immune therapy, instead of counteracting as in conventional fractionated radiation therapy regimes. This review summarizes the effects of various fractionation modes of radiation on the tumour and various immune cells: CD4+ and CD8+ T-cells, T\(_{\text{reg}}\), natural killer (NK) cells and dendritic cells (DCs). A number of pre-clinical studies which demonstrate the enhanced therapeutic response of malignant tumours to various combinations of immunotherapy (IMU) with single fraction or hypo-fractionated radiation therapy (RT) are reviewed. The clinical trials of combining immune therapy and radiation therapy carried out so far have been performed by using conventional radiation therapy with sparse effect. Clinical studies of combining established IMU regimes with a single 8 Gy fraction RT could open up the possibility for a deeper co-operation between biology and physics. This therapeutic co-operative regime may also reduce the probability of relapse, and if relapse occurs the treatment can be repeated.

bertil_r.persson@med.lu.se  
WCC_2014

33
The various steps in the process of **radio-immune tumour cell killing**. A single fraction of radiation therapy cause release of antigen from dying cells which are phagocytised by DCs which also become activated by the irradiation. **High-mobility group cromatin protein B1** also released from dying cells, binds to TLR4 on DCs which favour antigen processing and up-regulation of pro-IL-β. Dying cells also release ATP which binds to the receptor P2RX7 on DCs. This activates NLRP3 inflammasome which secrete IL-1-β polarizing CD8+ T cells to produce IFNγ and proliferate. The CD8+ T cells the infiltrate and eradicates the tumour.
The concept of Co-operative Radio-Immune-Stimulating Therapy.

- Immune suppressing MCSC cells surrounding the tumour are deactivated by RT.
- RT also down regulate generation of regulatory CD4+ T-cells (T_{reg}) secreting immune suppressive IL10.
- RT up-regulates tumour antigens, co stimulatory molecules MHC-1 complex and FAS which makes tumours more susceptible to immune mediated attack.
- RT up-regulates chemokine CXCL16 that promote CD8+ T-cell migration and infiltration which promote immune cell death (ICD) of the tumour.

These processes can be further promoted by various immune therapies.
Co-operative radio-immune-stimulating cancer therapy

But still there seems to be no clinical study in progress fully adopting the co-operative concept of an 8 Gy single fraction external RT combined with an effective established immune therapy regime.
We conclude that most data about the immunogenicity of RT are based on single high-radiation doses which are mostly not used in clinical practice. Current and future work should focus on the immunogenicity of distinct fractionated RT schemes and result in the determination of innovative combinatory treatments consisting of Chemotherapy and Immune Therapy.
Combining a Recombinant Cancer Vaccine with Standard Definitive Radiotherapy in Patients with Localized Prostate Cancer

James L. Gulley, Philip M. Arlen, Anne Bastian, et al.


ORIGINAL ARTICLE

Long-term follow-up of prostate cancer patients treated with vaccine and definitive radiation therapy

M Kamrava¹, AH Kesarwala², RA Madan², E Lita², A Kaushal³, K-Y Tsang³, DJ Poole³, SM Steinberg⁵, T Ferrara³, W Dahut⁴, J Schlo⁴ and JL Gulley³,⁴
## Table 2. Long-term clinical outcomes of patients

<table>
<thead>
<tr>
<th></th>
<th>S-IL-2</th>
<th>M-IL-2</th>
<th>Placebo</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median follow-up (range)</td>
<td>98 m (16 - 115)</td>
<td>76 m (61 - 86)</td>
<td>79 m (56 - 86)</td>
<td></td>
</tr>
<tr>
<td>Actuarial 5-year PSA failure-free probability</td>
<td>78%</td>
<td>82%</td>
<td>86%</td>
<td>0.58</td>
</tr>
</tbody>
</table>

**PSA failure^a** by NCCN risk group

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% (0/2)</td>
<td>0% (0/3)</td>
<td>0% (0/1)</td>
</tr>
</tbody>
</table>

Actuarial 5-year OS probability

<table>
<thead>
<tr>
<th></th>
<th>S-IL-2</th>
<th>M-IL-2</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>94%</td>
<td>100%</td>
<td>NA</td>
</tr>
</tbody>
</table>

Actuarial 5-year PCSS probability

<table>
<thead>
<tr>
<th></th>
<th>S-IL-2</th>
<th>M-IL-2</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>94%</td>
<td>100%</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Abbreviations:** IL, interleukin; m, months; M-IL-2, metronomic dose IL-2; NCCN, National Comprehensive Cancer Network; NA, not applicable; OS, overall survival; PCSS, prostate cancer-specific survival; S-IL-2, standard dose IL-2.

^aPhoenix definition.
Conclusion

It is a great challenge to achieve a clinical study confirming whether single fraction radiation therapy combined with immuno-therapy is the right path for future cancer treatment.

Who dares to break from established methods?
Co-operative radio-immune-stimulating cancer therapy

But still there seems to be no clinical study in progress fully adopting the co-operative concept of an

8 Gy single fraction external RT combined with an effective established immune therapy regime.
Thank You for your attention

Do You want to know more
Contact
bertil_r.persson@med.lu.se