Co-operative Radio-Immune-Stimulating Cancer Therapy

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Co-operative Radio-Immune-Stimulating Cancer Therapy by Bertil R.R. Persson PhD, MDh.c Professor emeritus of medical radiation physics at Lund University, 221 85 LUND Sweden. E-mail: bertil_r.persson@med.lu.se
My history of discovering the Radio-Immune-Stimulating effect began in the early 1990’s by attempts to use electroporation and electro-chemotherapy in combination with Bleomycin for tumor therapy.
By applying electric pulses to the tumour, pores or channels are formed in the cell-membranes, through which pharmaceuticals e.g. Bleomycin which otherwise don’t penetrate the cell membrane, can migrate into the cell and affect it.
This is a schematic illustration of how the pores is formed in the cell membranes after application of the electric field which is named "electroporation".
The first in vivo experiments were performed in the early 1990’s by using Leif G Salford’s tumour model with intra-cerebral implanted RG2 GLIOMA tumours into the right caudate nucleus of Fischer rats. After about one week a tumour has developed in the brain.
After 10-12 days two electrodes were introduced on opposite sides of the tumour and then treated with Bleomycin and electroporation which is named Electro-chemotherapy.
After 6 days approximately 50% of the untreated animals got symptoms of the growing tumour and were sacrificed, while 50% of those treated with electro-chemotherapy lived symptom-free for 11 days and two of them lived for more than 20 days without symptoms.
Since I'm engaged with radiotherapy, I was curious to examine the effect of electroporation in combination with radiotherapy ie Electro-Radio-Therapy "EPX" instead of chemotherapy.
N32 Tumor cells were inoculated subcutaneously in the flank of Fischer rats. Different groups of rats were treated with pulsed electric fields only, with Cobalt-60 gamma radiation only, or a combination of both radiation and pulsed electric fields.
We found that the infiltration of cytotoxic T cells, (ie CD8 +T cells) in the tumour increased most in the tumours treated with the combination of electroporation and radiation. This indicated that this combinational therapy initiated an increase of immuno-genic cell-death.
Also the infiltration of helper T-cells (i.e. CD4+ T-cells) increased the most in the tumours treated with a combination of electroporation and radiation.
During the 1990s, Leif G. Salford, professor and head of Neurosurgery in Lund, initiated a project which intended to use a vaccine based on endogenous tumor cells for immunotherapy of brain tumors.
Leif.G Salford started a clinical Phase I study of immunotherapy in patients with brain-tumours (malignant glioma). The study was called the BRIGTT study (Brain-Immuno-Gene-Tumour-Therapy). My involvement was mainly to radiation sterilize the vaccine made from patients' own tumour cells transfected with Interferon-gamma gene. The vaccine was then injected subcutaneously into the arm of the patients.
I was curious to study the combination of immune-therapy in combination with both electroporation and radiotherapy. During 1998-2001, preclinical studies with subcutaneously inoculated tumours were performed in order to combine the tumour vaccine treatment with both electroporation and radiotherapy.
The figure shows the cumulative results of the various experiments conducted during 1998-2002. Tumour growth rate (TGR) is displayed with black bars, the specific therapeutic effect (STE) with red stack and Tumour enhancement ratios (TER) with blue bars.

We found that Immunotherapy alone showed none or even a negative therapeutic effect which was quite unexpected.

Also electroporation alone was ineffective, but in combination with the Vaccine of Interferon-gamma transfected tumour cells the therapeutic effect (red bars) defined as reduction in the growth rate of the tumours, increased to 30%.

By Combining the Vaccine of Interferon-gamma transfected tumour cells with radiation therapy the therapeutic effect increased further, up to 50%.

But the greatest therapeutic effect of 80% was recorded with a combination of both immunization, electroporation and radiotherapy.
These results were presented at the 2002 Society of Neuro-Oncology (SNO) annual meeting in San Diego, and the abstract was published in the journal of Neuro-Oncology, 4 (Supplement 2), 68
At the 2002 SNO conference in San Diego, I met Dr. Martin Graf who had studied immunotherapy of brain tumours with a vaccine of endogenous non-transfected tumour cells in rats with tumours implanted in the brain, in combination with radiation therapy. He had observed the same phenomenon as we, that immunotherapy with vaccines increased tumour growth. But when immunotherapy is combined with radiation the tumour’s growth considerable decreased.
He also observed that in the immunized rats there was a vigorous infiltration and accumulation of immature monocytes (myeloid derived suppressor cells MDSC) in the tumour. But after a single radiation treatment with 15 Gy the presence of immature monocytes was considerably sparse.
This is Graf’s results presented as survival diagrams. The left diagram show that just vaccination with tumor cells caused decreased survival of the rats compared with the untreated controls.

The right diagram shows that the combination of the immunization with radiotherapy resulted in a survival of 45% after more than 70 days, whereas the untreated controls and rats treated with radiotherapy alone all died within 40 days.
The results of recent immunological research show that immature monocytes called "Myeloid Derived Suppressor Cells" MDSC are attracted and enriched at the tumor site through secretion of Vascular epithelial growth factor “VEGF” by the tumor cells. The "Myeloid Derived Suppressor Cells" 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 signaling.
Thus the cooperative effects of radiation and immunotherapy could be explained by effect of radiation killing the myeloid suppressor cells which infiltrate the tumour, that repeal the suppression of immunogenic tumour cell killing by the T-cells.
In the fall of 2002 after returning from the SNO conference I started pre-clinical studies in Salford’s rat model of brain tumours by combining immunotherapy and radiation therapy. This study was called Radio-Immune-Gene-Tumour-Therapy "RIGTT"
5000 N29 glioma cells were injected stereo-tactically into the brains of Fischer rats and after a few weeks, a tumour was established in caudate nucleus. After further 6-7 weeks, the untreated tumour has grown to 4-6 mm in the brain, resulting in symptoms in the rat.
A vaccine was prepared from syngeneic N29 cells transfected with interferon gamma genes, cultured and finally radiation sterilized. Immunotherapy was performed by injecting with this vaccine into the abdomen of the rats at 7, 21, and 35 days after the inoculation of the N32 tumour cells into the brain. At 100 day after the inoculation of the tumour 6 out of 7 untreated controls were dead while of those treated with a combination of radiation and immunotherapy only 2 out of 8 animals were dead.
The results of just immunization causes no significant change in survival time or in the number of survivors after 100 days. Also single radiation therapy sessions with 5 or 15 Gy resulted in no significant changes (p=0.08). But, the combination of immunization and radiation therapy resulted in a noticeable change in the number of survivors after 100 days. A single Radiation therapy session of 5 Gy combined with immune-therapy resulted in a significant increase (p<0.01) of survival animals compared with the controls.
These are the results in the number of survival animals versus the number of days after inoculation of tumor cells in the brain. The best result with 75% of survival animals was achieved with the combination of immune-therapy and 5 Gy single session radiotherapy.
These results of the brain tumour study 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.

But 5 years later I got a 2\textsuperscript{nd} chance by my former graduate student Crister Ceberg who 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.
When I contacted Silva Formenti and told her about my unpublished results of combining vaccine immune-therapy with radiation, she became very enthusiastic and helped me to get the results published in a special issue of Radiation Research Journal in 2010.
An alternative to tumour vaccine therapy is manipulating co-stimulatory/inhibitory molecules, which are present on the surface of highly activated effector T cells. Such inhibitory ligand-receptor pairs include B7.1/2-CTLA-4. The pharmaceutical Ipilimumab is Anti-CTLA-4 mAb that is blocking CTLA-4 which increased the population of CD8+T-cells. A single radiation exposure of about 8 Gy inhibit the action of suppressing cells which gives a possibility for the increased population CD8+T-cells initiated by Ipilimumab to infiltrate and kill the tumour cells.
Formenti presented in 2008 the results of combining anti-CTLA-4 mAb (9H10) and radiation on breast cancer, that resulted in a statistically significant improvement with 6 out of 9 long term survival animals as compared with control (IgG) mice with none survival animals. Treatment with radiotherapy alone resulted in only 2 out of 9 long time survival animals.
The 4-dimensions in Co-operative Radio-Immune stimulating Therapy are thus summarized as follow:

In the 1st Dimension - Radiation Cell killing release of Tumour antigens activate DC

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

In the 3rd Dimension - Stimulating Immune Cell killing by Immune Therapy (anti-CTLA-4)

In the 4th Dimension - Vaccination-Immunization no relaps of the tumour
More details about Co-operative Radio-Immune-Stimulating Cancer Therapy is found in the review which is written along with Crister Ceberg who brought me back to the track. We recently published the overview article in the journal “Trends in Cancer Research”.

Co-operative Radio-Immune-stimulating Cancer Therapy


This article explains the background and can serve as an introduction for those whom are interested.
This picture illustrates the various steps in the process of **radio-immune tumour cell killing**. A single fraction of radiation therapy causes 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-1β. Dying cells also release ATP which binds to the receptor P2RX7 on DCs. This activates NLRP3 inflammasome which secretes IL-1β polarizing CD8+ T cells to produce IFNγ and proliferate. The CD8+ T cells then infiltrate and eradicate the tumour.

1. 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.
2. The immune suppression is repealed by decreased populations of T_{reg}, TAM-M2, and MDSC.
3. The CD8+ T cells can then without hindrance infiltrate and eradicate the tumour.
Another view of the concept of Co-operative Radio-Immune-Stimulating Therapy.

- RT up-regulates tumour antigens, co stimulatory molecules MHC-1 complex
- 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 like
- Ipilimumab or tumour vaccine
By compiling the results from our own and other published preclinical reports on the effects of combining immunotherapy with radiation therapy, we concluded that the combination of an established immune therapy with a single 8 Gy fraction of radiotherapy should be the way forward for a clinical study.
In a recently published overview of the immune response by ionizing radiation, established oncologists conclude that although most data on immunogenicity are observed in preclinical trials when the radiation therapy is given in single fractions with moderate radiation doses around 8 Gy this is not what is commonly used in clinical practice. Thus, future research should focus on the immunogenicity of fractionated radiotherapy and combinations of chemotherapy and immunotherapy.
But a clinical study conducted 2005 in patients with localized prostate cancer with a combination of cancer vaccine therapy in combination with standard radiation therapy, resulted on no significant differences to established fractionated radiation treatment.
A long-term follow-up of 36 patients with prostate cancer treated with vaccine in combination with a full radiation therapy shows no significant differences in PSA control or late toxicity compared with radiotherapy alone of 11 patients. The effect of long-term immune response after vaccine therapy was also limited. Thus conventional fractionated radiation therapy does not cooperate with immunotherapy.
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?**
By compiling the results from our own and other published preclinical reports on the effects of combining immunotherapy with radiation therapy, we concluded that the combination of an established immune therapy with a single 8 Gy fraction of radiotherapy should be the way forward for a successful clinical study.
Thank You for Your attention.
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