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Bertil RR Persson

Table of contents

A story about combining immunotherapy with ultrahypo-fractionated radiation therapy

This story is dedicated to the memory of my 60 years of participation in the John and Augusta Persson Foundation for Scientific Medical Research, especially concerning cancer research, mainly at Lund University. Since the foundation's formation in 1964, I participated in its activities with my mentor, laborator and later professor Kurt Lidén.

After succeeding Kurt Lidén as professor of medical radiophysics and head of hospital physics in Lund, I became a member of the foundation's scientific council in 1984 and, from 1998, its vice-chairman. I remained active in the foundation until my departure in 2024.

During the first years, I produced Technetium-99m radiopharmaceuticals for the Nordics' first Gamma camera, which the foundation donated to Nuclear Medicine Research at Lund University.

Later, the foundation supported the operation of microwave treatment of breast cancer recurrence in combination with low-dose radiotherapy, which, among other things, initiated this story.

In connection with the foundation's 20th anniversary, IBM generously supported my activities in building the Nordics' first Magnetic-Resonance camera in Lund by providing computing power.

I now leave the foundation with this story of immunotherapy combined with ultra-hypo-fractionated radiation therapy, which I hope will inspire someone to further clinical evaluation as a therapeutic option for patients with recurrent cancer after undergoing a conventional treatment regimen.

Lund on 31 December 2024

Bertil RR Persson Fil.Dr. jub., Med.Dr. h.c.
Professor Emeritus Medical Radiation Physics

Summary:

This story about immunotherapy combined with ultra-hypo-fractionated radiation therapy has its origins in the clinical study called "Brain Immune Gene Tumour Therapy" (BRIGTT), which Professor Leif G. Salford in Lund initiated just before the turn of the 2000s. He produced a specific tumour vaccine based on tumour cells extracted from tumour tissue from the glioma patient he previously operated on. The survival time of the patients treated with the vaccine was prolonged, but none of them fully recovered.

In an attempt to improve the vaccination effect, the present author used the same tumour model as used in the preclinical tumour immunological research that was the basis for the Salford clinical study.

Fischer 344 rats with N29glioma tumours inoculated into the brain exposed to combined vaccine treatment with radiation therapy:

- Radiotherapy alone with a single dose of 5 Gy resulted in no survivors.
- In contrast, if immunization therapy with 3 rounds of vaccine was combined with only one fraction of 5 Gy radiation therapy, six out of eight treated animals survived (approx. 75%).

This dramatic result for a previously incurable tumour spurred to try to work for a new tumour treatment regimen with immunotherapy in combination with hypofractionated radiotherapy in only a few fractions.

Now after 20 years, clinical studies have begun to be reported showing that immunotherapy also works well with drugs, so-called immune checkpoint inhibitors, which are used to block CTLA-4 and PD1 receptors, so that the killer cells, the cytotoxic T-lymphocytes CTL more effectively can eliminate the cancer cells.

Over the years, attempts to spread knowledge about the combination of immunotherapy with hypofractionated radiation therapy have proven difficult to break the use of conventional clinical radiation therapy with upwards of 30-40 daily fractions of 2 Gy. However, the clinical trials carried out so far with combining immunotherapy with conventional radiation therapy have resulted in modest effects.

Recently, a Greek study by Koukourakis and coworkers has shown that localized hypofractionated radiation therapy works well as an adjunct to immunotherapy with checkpoint PD-1 inhibitors.

They treated cancer patients with recurrent inoperable head and neck tumours after previous conventional radiotherapy and chemotherapy. Hypofractionated radiotherapy (HFRT) of 8 Gy was administered in one, two, or three fractions, with one fraction per week, together with *Nivolumab* anti-PD1 immunotherapy. Early and late radiotherapy toxicities were minimal, and the immunotherapy showed excellent tolerance, with only three patients discontinuing immunotherapy.

In patients who received anti-PD1 immunotherapy in combination with 2–3 fractions of HFRT à 8 Gy, the objective tumour response was over 80%, while 57% was noted in combination with only one 8 Gy fraction of radiation.

Patients with non-small cell lung cancer who had locally recurrent tumours after conventional radical chemo-radiotherapy were also treated with anti-PD1 immunotherapy in combination with one or two 8 Gy fractions of hypo-fractionated radiotherapy. The results of that immuno-radiotherapy regimen in a group of NSCLC patients were 27.2% complete remissions and 81.8% objective response rates. The 22-month locoregional recurrence-free rate was 54.5%, while the estimated 2-year disease-specific overall survival was 62%.

Their findings inspired the presentation of this story about immunotherapy combined with ultra-hypo-fractionated radiation therapy:

Chapter 1 describes the research and progress in immunotherapy and radiotherapy to treat glioblastoma and other cancers.

Brain Immune Gene Tumour Therapy **(BRIGTT)** was a project led by Professor Leif Salford at Lund University to develop a vaccination method against glioblastoma (GBM). A vaccine was produced from tumour cells extracted from patients. After infecting the tumour cells with an adenovirus containing the gene for human interferon-gamma (IFNγ), they irradiated to prevent cell division.

Eight patients were treated with this specific tumour vaccination, which resulted in significantly longer survival (16 months compared to 9 months for the control group).

Immunotherapy with tumour vaccine and radiation therapy

The combination of radiation therapy and glioma vaccination showed significantly improved treatment efficacy in a preclinical model. Experiments on rats with brain tumours showed that survival was increased with combined treatment compared to single treatments (up to 75% survival for a single 5 Gy fraction).

Clinical studies

Conventional radiation therapy reduces the number of T cells in the target volume that inhibits the effects of combination with immunotherapy. However, other clinical trials of combination immunotherapy with localised hypo-fractionated radiation showed promising therapeutic results and reduced relapse and side effects.

Chapter 2 discusses the relationship between radiotherapy and the immune response to cancer, focusing on the tumour microenvironment and different immune cells and molecules

The tumour microenvironment coexists with blood vessels, immune cells, and fibroblasts, influencing cancer development.

Radiation therapy kills cancer cells and releases tumour antigens, which activate dendritic cell and cytotoxic T lymphocyte (CTL) production and tumour infiltration.

Effects of radiotherapy on the microenvironment:

High doses of radiation cause necrosis and inflammation, which suppress the immune system, while low doses can stimulate it.

Proteins such as HMGB1 released from dying cancer cells act as "danger signals" and activate dendritic cells.

Hyper-fractionated radiotherapy can enhance the immune response by increasing CTL infiltration and stimulating antigen uptake.

Immunotherapy with immunosuppressants that block CTLA-4 and PD-1/PD-L1 interactions activate CTL cells and fight tumours.

PD-L1 is often overexpressed in tumours, which allows for immune system evasion.

Role of radiotherapy in immunosuppression:

Combining radiotherapy and immunotherapy can reduce immunosuppressive cells such as MDSCs and Treg cells. Radiation dose and fractionation balance is crucial to promote antitumour immunity.

Hyper-fractionated radiotherapy combined with PD-L1 blockade effectively reduces MDSCs and enhances CD8+ T-cell infiltration into the tumour.

The text highlights the importance of combining immunotherapy with radiotherapy to optimise cancer treatment by both stimulating immune reactions and overcome tumour immunosuppressive mechanisms.

Chapter 3 summarises preclinical studies combining radiotherapy and immunotherapy, focusing on CTLA-4 blockade, anti-PD1, dendritic cell vaccines and CAR T-cell-based therapies.

CTLA-4 Blockade:

In animal models, combined radiotherapy and CTLA-4 blockade can induce tumour regression and reduce metastases (e.g., lung metastases).

Hypo-fractionated radiation (e.g. 8 Gy × 3 fractions) was particularly effective in inducing abscopal effects, which involve immune responses even outside the radiation field.

Mechanisms such as the cGAS-STING pathway and the enzyme Trex1 influence the immune response to radiation.

PD1 Blockade:

Anti-PD1 in combination with Ultra-Hypo-fractionated radiation (8 Gy × 3) increased antitumour immunity and abscopal effects.

The combination activates T-cells and reduces immunosuppressive myeloid-derived suppressor cells (MDSCs).

Dendritic Cell Vaccines:

Dendritic cell vaccines can improve immune responses, but their clinical efficacy is still limited.

However, single case studies (e.g., terminal small bowel cancer) have shown improved survival and tumour shrinkage when vaccines were combined with radiation therapy.

CAR T-cell-based Immunotherapy:

CAR T-cell therapies are successful in haematological cancers but have limited efficacy in solid tumours.

Combination therapy with radiation may potentially improve outcomes for solid tumours. Studies of ultra-hyperfractionation and CAR T-cell therapies are limited.

In summary:

Proper dosing and radiation fractionation are crucial to optimise immune responses and reduce side effects.

Components of the immune system, such as CD8+T-cells and Dendritic cells, play central roles in treatment response.

Chapter 4 summarises clinical trials with immuno-radiotherapy

Immunotherapy and Conventional Radiotherapy

Several studies have investigated the combination of immunotherapy and conventional radiotherapy, particularly in locally advanced head and neck cancer.

Avelumab (anti-PD-L1) combined with chemoradiotherapy showed no significant improvements in progression-free survival.

Pembrolizumab or *Cetuximab* in combination with radiotherapy also did not result in significant survival gains, although *Pembrolizumab* had lower toxicity.

The negative effect of radiation on tumour-draining lymph nodes is thought to affect the effectiveness of immunotherapy.

Hypofractionated radiotherapy

Hypofractionation, where fewer but higher doses of radiation are used, was compared with conventional fractionation for various cancers:

Prostate cancer: Studies show similar results for disease-specific survival and toxicity between hypofractionation and conventional treatment.

Breast cancer: Hypofractionation is associated with fewer complications associated with implant reconstruction.

Rectal cancer: Hypofractionated treatment produced stronger immuno-stimulatory responses compared to conventional fractionation.

Immunotherapy and Ultra-hypofractionated radiotherapy

Ultra-hypofractionated radiotherapy, with only one 8 Gy fraction per week, can potentially enhance the effect of immunotherapy by reducing the tumour's immunosuppressive environment and stimulating anti-tumour immunity.

Head and neck cancer: Combination therapy with *Nivolumab* and 2-3 fractions ultra-hypofractionated radiation showed high objective response rates (80%) and good tolerability.

Non-small cell lung cancer: Ultra-hypofractionated radiotherapy combined with anti-PD-1 immunotherapy produced an objective response rate of 81.8% and long-term local control.

Conclusions

For patients with limited options after previous treatments, *Ultra-Hypo-fractionated Radiotherapy* shows promising results in combination with Immunotherapy. The increased tolerability and improved immune responses support further studies to optimise treatment protocols.

I hope that this story about immunotherapy combined with ultra-hypo-fractionated radiation therapy will motivate further clinical evaluation as a therapeutic option for patients with recurrent cancer after undergoing a conventional treatment regimen.

Chapter I
Prologue

1.1 "Brain *Immune Gene Tumour Therapy*" (BRIGTT).

The immune response against the brain tumour malignant glioma (GBM) is mainly mediated by the tumour-killing function of activated cytotoxic T cells (CTL). Thus, a vaccination regimen that enhances the effector functions of CTL and increases the number of lymphoid cells infiltrating the glioma should be able to provide an effective therapy.

At the turn of the millennium, Professor Leif Salford at Lund University initiated a clinical study named "Brain Immune Gene Tumour Therapy" (BRIGTT) to study a vaccination therapy regimen (Salford et al., 2001a, Salford et al., 2002, Salford et al., 2001b, Salford et al., 2022).

A specific tumour vaccine based on tumour cells of tumour tissue from the glioma patient he previously operated. After separating the tumour cells, they were infected with an Adenovirus that had a gene for human interferon-gamma (IFNγ). The transfected cells were irradiated with gamma radiation so that the tumour cells would not be able to multiply after being administered to the patient (Baureus-Koch et al., 2004). After irradiation, the cells were examined for the degree of IFNγ production using ELISA measurements.

The vaccine was administered to the patient in the skin to activate the immune system and produce specifically activated T-lymphocytes (CTL) that pass through the blood-brain barrier. In the brain parenchyma, the activated T cells seek to eliminate both regrowths from the original resected tumour and migrating 'guerrilla' cells in the surrounding brain (Salford et al., 2001a; Salford et al., 2002; Salford et al., 2001b, Salford et al., 2022).

The clinical study included eight treated patients, between 50 and 69 years of age, and nine matched controls. They were all previously treated according to the program with surgery and radiation therapy without side effects or toxicity but

received indications of recurrent tumour growth. They were immunized between eight and 14 times with their specific tumour vaccine.

Neurological status and quality of life remained unchanged during immunotherapy. The study also included a control group of nine similar glioma patients who were also primarily treated according to the program with surgery and radiotherapy.

After the patients were immunised with their vaccine, the median overall survival time of the group was 488 days (16.1 months), which was significantly longer ($p < 0.05$) compared to 271 days (9.0 months) in the patients in the matched control group. The survival time of the immunised patients was also significantly longer than that of all GBM patients treated in the same clinic during the same period, and with other control groups within the same age cohort (Salford et al., 2022).

1.2 Combination with radiation therapy

However, Roszman and co-workers showed in 1991 that eliminating all glioma cells does not occur even when CNS tumours are exposed to large populations of T- lymphocytes (Roszman et al., 1991). This raised the question of whether immunisation with tumour vaccines could be combined with any other therapy. The closest to hand was radiation therapy, which we had previously successfully used in combination with hyperthermia to treat breast cancer recurrence.

One of my doctoral students, Per Nilsson, studied in the 1980s together with oncologist Lisa Kjellén how a combination of microwave-induced hyperthermia and low-dose (30 Gy) conventional radiotherapy at 2 Gy per day could be used to treat breast cancer recurrence.

Figure 1-1

My presentation of our first hyperthermia treatment equipment with the IMSAI computer for H.M. King Carl XVI Gustav at Research Day 1983, in Lund.

In my left hand, I hold the patient applicator that Per Nilsson and Lisa Kjellén used when treating patients.

With a special microwave applicator, tumours implanted on the flanks of mice were first treated. After the tumours disappeared in mice treated with a combination of hyperthermia and radiation therapy, the method developed to treat patients.

For Research Day 1983, I was commissioned to demonstrate the hyperthermia equipment to Sweden's King H.M. Carl XVI Gustav and H.M. Queen Silvia. Patients with local breast cancer recurrence were treated with the waveguide applicator shown in Figure 1-1 connected to the microwave equipment (Nilsson et al., 1982b, Nilsson and Persson, 1985). The treatment of breast cancer recurrence in combination with radiotherapy was successfully carried out during the 1980s at the oncology clinics in both Malmö and Lund (Lindholm et al., 1982, Lindholm et al., 1983, Nilsson et al., 1982a, Lindholm et al., 1985, Lindholm et al., 1987, Lindholm et al., 1995).

In 1995, Lindholm and colleagues summarised the experiences of prognostic factors for tumour response and skin damage in combined radiotherapy and hyperthermia in superficial recurrent breast carcinoma (Lindholm et al., 1995).

Prognostic factors for complete tumour response and acute skin damage in combined hyperthermia and radiotherapy were analysed from patients with recurrent breast cancer in previously irradiated areas. Radiotherapy was given daily at 2 Gy to a total absorbed radiation dose of 30 Gy in 2 weeks or 34.5 Gy in 3 weeks.

- Schedule A: The first radiation treatment schedule with applied heat twice a week for two weeks.
- Schedule B: The second radiotherapy schedule was combined with hyperthermia either once or twice a week, resulting in a total of three heat treatments, or
- Schedule C: with six heat treatments.

Hyperthermia was induced with microwaves (2450, 915 or 434 MHz) via external applicators and was always given after radiotherapy. The complete response (CR) rate in 49 of 69 evaluable patients was 71%. There was no significant difference in CR frequency between the three hyperthermic regimens. For each schedule, the CR frequencies were:

- Schedule A: 74 % (14/19),
- Schedule B: 65 % (15/23),
- Schedule C: 74 % (20/27) . (Lindholm et al., 1995).

Peeken, discussed in 2017 the use of hyperthermia in modern radiation oncology (Peeken et al., 2017).

In addition to enhancing the effect of radiation, the hyperthermia treatment causes changes in perfusion, oxygenation, and inhibition of DNA repair mechanisms. There are also indications for immune stimulation and induction of systemic immune responses.

However, despite the increasing number of solid clinical studies, only a few oncology clinics have included hyperthermia therapy in their repertoire. Over the years, however, abundant prospective and randomised clinical data have emerged showing a clear benefit of combined hyperthermia and radiation therapy for superficial breast cancer recurrence, cervical cancer, or cancer in the head and neck region.

In summary, Peeken believed that hyperthermia treatment for local control might gain clinical significance in conjunction with targeted systemic therapy and improved chemotherapy for micrometastatic disease (Peeken et al., 2017).

1.3 Immunotherapy with tumour vaccine and radiation

In 2003, Graf and coworkers carried out immunisation by s.c. injection of irradiated syngeneic tumour cells to induce localisation of T-cells (CTL) into intracranially implanted T9-glioblastoma tumours of Fischer-344 rats. However, this procedure did not correlate with a curative effect but instead appeared to enhance tumour progression (Graf et al., 2002).

However, they found that combining immunisation with radiation exposure in rat models showed an increased therapeutic response in T9 glioblastoma and that total remission of tumours occurred in almost 50% of the animals (Graf et al., 2002). In addition, the animals developed glioma-specific immunity through radiotherapy combined with cellular vaccination.

Thus, co-administration of cellular immunization and radiotherapy inhibited the tumour from producing immunosuppressive factors, favouring an antitumour response (Graf et al., 2002).

Lumniczky and co-workers reported 2002 that combining cytokine-producing cancer cell vaccines with local conventional radiotherapy increased the therapeutic effect in a mouse glioma brain tumour model (Gl261) (Lumniczky et al., 2002).

They treated brain tumour-bearing mice with various cytokine-producing vaccines, prepared by in vitro transfection of GL261 mouse glioma cells with corresponding adenoviral vectors (IL-4, IL-6, IL-7, GM-CSF, TNFα). The vaccine that produced IL-4 or GM-CSF caused 20-40% elimination of the tumours in the mice.

However, by combining local tumour irradiation and vaccination of syngeneic tumour cells transfected with GM-CSF, IL-4, or IL-12 vectors, 80-100% of the tumours in the glioma-bearing mice are eliminated. High efficacy of combined treatment occurs even under suboptimal conditions where neither modality alone cured any of the mice (Lumniczky et al., 2002).

These experimental findings in rat and mouse models suggest that vaccination therapy may open a new potential in the clinical treatment of high-grade glioma when applied as an adjuvant to existing treatment modalities such as radiation therapy (Graf et al., 2002, Lumniczky et al., 2002 42).

This story about immunotherapy combined with ultra-hypo-fractionated radiotherapy originates in our studies in Lund of immunisation with interferon-gamma-secreting syngeneic tumour cells combined with radiotherapy. The trials began in 2003 with the tumour model used in the pre-clinical research that was the basis for the Salford clinical study reported above.

In 2004, an experiment at Lund University involved inoculating 5000 N29 tumour cells into the brains of Fischer 344 rats, which were arranged in groups according to Table 1-1.

Table 1-1

The animals with intracerebral tumours were arranged in the following groups:

Group number	Treatment	Number of animals
B1	Controls without treatment	6
B2	Radiation treatment 5 Gy	8
B3	Radiation treatment 15 Gy	8
B4	Immunization	6
B5	Radiation 5 Gy + Immunization	8
B6	Radiation 15 Gy + Immunization	8

One week after inoculation, when the animals developed intracerebral tumours, they received radiation treatment, as shown in Figure 1-2, by delivering

an absorbed radiation dose of either 5 or 15 Gy as measured by a TLD chip placed next to the tumour during field bolus.

Within one hour after radiation treatment, immunization occurred in half of the animals by i.p. injection of three million radiation-sterilized (70 Gy) syngeneic IFN□-secreting N29 cells. The vaccination of the animals repeated after 14 and 28 days.

When daily observations showed signs of symptoms of growing tumours, their brains were examined histopathologically.

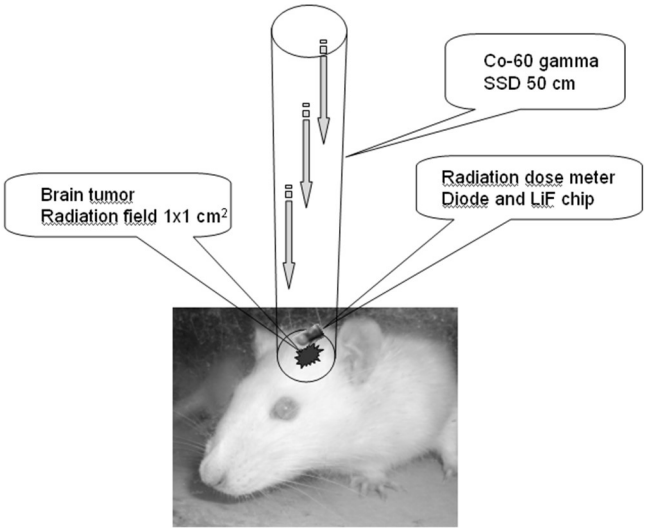

Figure 1-2

Irradiation of intracerebral rat tumour with a radiation field size collimated to cover the brain (1x1 cm^2).

With the ionisation chamber, the adsorbed dose of either 5 or 15 Gy was measured by TLD dose sensors.

To build up the radiation, a 5 mm thick sheet of tissue equivalent bolus was placed over the head

The animals were observed daily for symptoms of growing tumours. When the tumour severely affected the animal, they were resected, weighed, and examined histo-pathologically.

Figures 1-3 show diagrams of the survival time after the inoculation of rats in the different groups with different treatments.

Of the untreated controls, 1 in 8 survived, which was extremely rare. With only immunotherapy and without radiation, 2 out of 6 treated animals survived (about 33%).

Radiation treatment alone with 5 Gy did not result in any long time survivors, but with 15 Gy, 2 out of 8 animals survived (about 25%).

In contrast, with three rounds of immunization therapy combined with one fraction of 15 Gy radiation therapy, five out of eight animals survived (63%), and with 5 Gy, six out of eight treated animals survived (75%).

These noteworthy results were first presented at "The International Conference on Biomedical Engineering and Informatics" (BMEI 2008) in Sanya, Hainan, China (Persson et al., 2008).

The study also published in the journal of "Radiation Research" with the willing assistance of Silvia C Formenti, and Sandra Demaria. They observed similar results in their studies immune-mediated inhibition of metastases after tumour treatment with local radiation and CTLA-4-blocked alternative vaccination (Demaria et al., 2005, Newcomb et al., 2006, Persson et al., 2010).

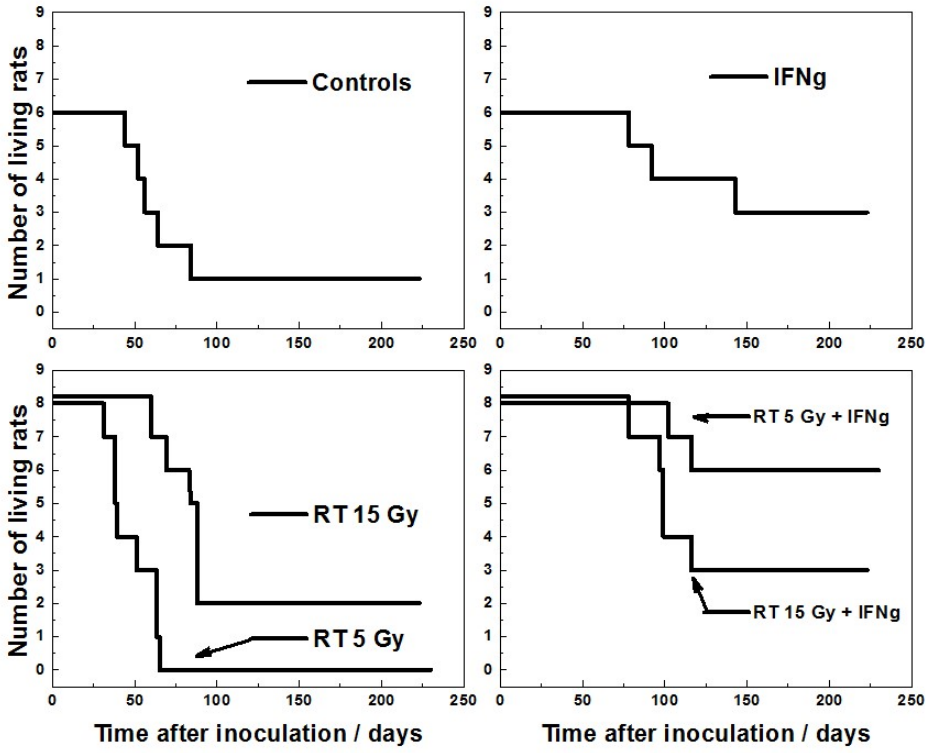

Figure 1-3

Survival plot of the number of rats with intracerebrally implanted N29 tumour controls (upper left), Immunization with syngeneic N29 tumour cells (upper right); radiotherapy RT (lower left) and a combination of radiotherapy RT and immunization IFNg (lower right) (Persson et al., 2010).

1.4 Clinical studies

Conventional radiation therapy for cancer is delivered in daily low-dose fractions (2 Gy) until a high target dose (60-70 Gy) is achieved. This treatment regimen aims to eliminate the tumour through radiation-induced cancer cell death. However, traditional fractionated radiotherapy also reduces the number of radiation-sensitive T cells (CD3+, CD4+, and CD8+) in the tumour and thus prevents immunogenic cell death.

Over the past years, we have tried to spread knowledge about radiotherapy in combination with immunotherapy and the effects of different fractionation methods for radiation on the tumour and various immune cells: CD4+ and CD8+

T cells, Treg, natural killer cells (NK) and dendritic cells (DC) (Persson, 2011, Ceberg and Persson, 2013).

Recently, clinical trials in Greece have successfully tested localized hypofractionated radiation therapy as an adjunct to immunotherapy with immune-checkpoint PD-1 inhibitors (Koukourakis et al., 2023).

This inspired me to present this story of immunotherapy combined with ultra-hypo-fractionated radiation therapy. Perhaps it may stimulate someone to conduct clinical trials of combining established immunotherapy regimens with intermittent 5 to 8 Gy ultra-hypo-fractionated radiation therapy iHFIRT. This therapeutic combination regimen can also open up the possibility of reducing the likelihood of side effects and relapse.

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Chapter II
Radiation therapy and the immune response

2.1 The tumour microenvironment

Cancer progression closely relates to the tumour microenvironment, where the tumour coexists with surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells (MDSCs), signalling molecules and the extracellular matrix. Tumours can affect the microenvironment by releasing extracellular signals that promote tumour angiogenesis and induce immune tolerance, which stimulates tumour growth, while immune cells in the microenvironment try to inhibit the growth and development of cancer cells (Zagardo et al., 2024).

Radiation therapy kills cancer cells, which increases the release of endogenous tumour antigens that activate dendtritic cells and other antigen-presenting cells (APCs). This favours the production of tumour-killing Cytotoxic Tumour Lymphocytes (CTL), which are CD8+T-lymphocytes (Gupta et al., 2012; Formenti and Demaria, 2009; Formenti, 2017).

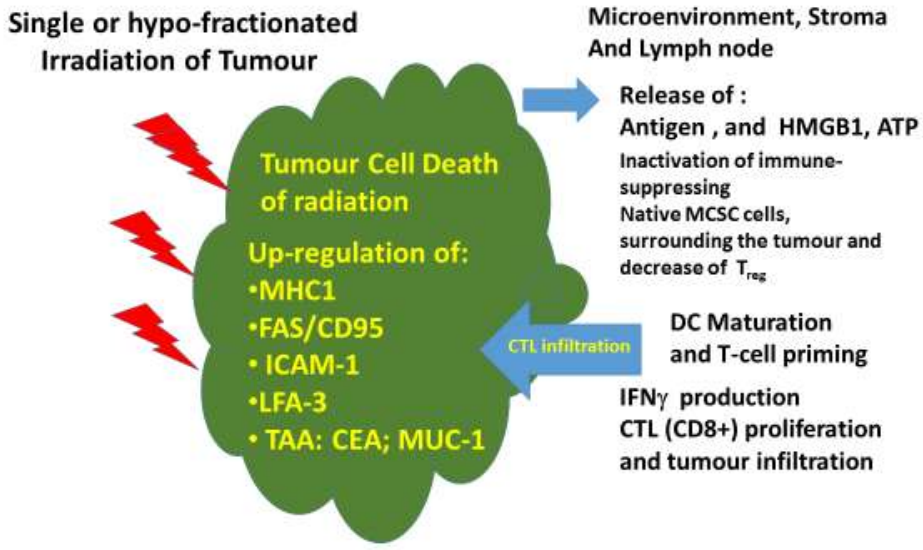


Figure 2-1
Radiation effects on the tumour microenvironment

The behavior of the tumours depends on the interaction of the tumour cells with various immune cells, such as:

- T-cells: CTL($CD8^+$) , T_H ($CD4^+$), Treg ($CD4^+$, $CD25^+$)
- Natural killercells (NK),
- Dendritic cells (DC),
- Myeloid-Derived-Suppressor-Cells (MDSC)
- Tumour-associated macrophager (TAM).

Myeloid-derived suppressor cells (MDSC) contribute to angiogenesis and vasculogenesis and stimulate regulatory T-cells, T_{reg}(CD4+, CD25+), which are potent suppressors of the CTL cells' antitumour effect.

Dendritic cells are aimed at presenting antigens, but vascular endothelial growth factor (VEGF) from tumour and stromal secretion, as well as interleukin-10 and TGF-ß, inhibit their maturation into effective antigen-presenting cells.

Tumour-associated macrophages (TAMs) promote tumour progression by secreting various factors, including matrix metalloproteinases and immunosuppressive cytokines.

M1 macrophages are pro-inflammatory cells that can secrete pro-inflammatory cytokines, activate endothelial cells and induce the recruitment of other immune cells to inflamed tissue.

M2 macrophages inhibit inflammation by releasing anti-inflammatory mediators and ensure tissue integrity.

In addition, a vascular component exists that includes blood and lymphatic endothelial cells, which communicate with each other and with the cancer cells.

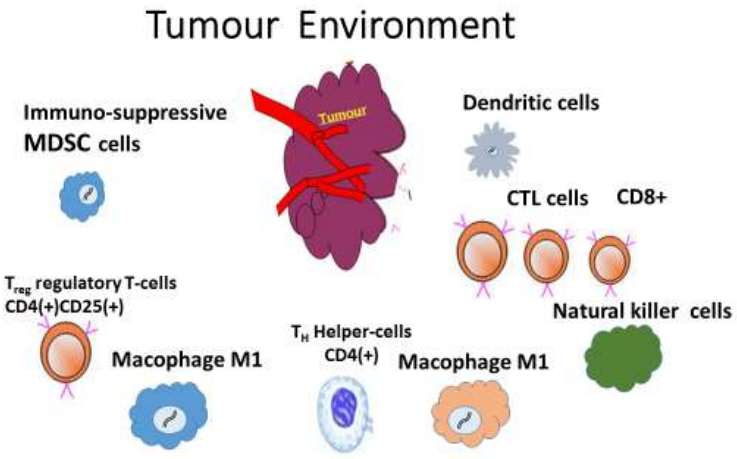


Figure 2-2

Cells in the tumour microenvironment

2.2 The effects of radiation on the tumour microenvironment

Radiation therapy can affect tumour cells by damaging DNA and modifying the tumour microenvironment (TME), with different effects depending on the delivered absorbed dose. Irradiation with an absorbed dose larger than 10 Gy induces necrosis and massive inflammation, giving rise to immunosuppressive effects. However, intermittent low-absorbed dose irradiation (5–8 Gy per fraction) provides conditions for lower immunosuppression and immunological death of tumour cells (Zagardo et al., 2024).

Gupta and collaborators reported in 2012 that Radiotherapy promotes tumour-specific effector CD8+T cells via dendritic cell activation (Gupta et al., 2012).

The main mode of Radiotherapy action in cancer treatment is usually thought to be irreversible damage to tumour cell DNA. However, they showed that irradiation mobilises tumour-specific immunity and that the efficacy of radiotherapy as a single dose crucially depends on dendritic cells and CD8+T-cells. They propose that the radiation activation status of dendritic cells rather than the amount of tumour-derived antigen is the bottleneck of efficient anti-tumour immunity (Gupta et al., 2012).

Lymphocytes are among the most radiosensitive white blood cells, and acute lymphopenia often occurs after high-dose focal radiation therapy, probably because the irradiation kills lymphocytes as they pass through the radiation field.

Conventional fractionated radiation therapy of 2 Gy results in a loss of active lymphocytes in the whole-body volume, with an average loss of approximately 11% (Ellsworth et al., 2020). After completing conventional radiation therapy, some patients may exhibit persistent lymphopenia for long periods.

Hyperfractionated radiation can promote dendritic cell uptake of antigens from dying cancer cells and the release of antigens (Gupta et al., 2012).

Also, the "High mobility group box 1" protein (encoded by the HMGB1 gene) is released from dying cancer cells. It is a so-called danger signal that activates dendritic cells and is proposed as a DNA vaccine adjuvant.

In surviving cancer cells, the irradiation can contribute to increased expression of adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, the cell death receptor PD-1 and its ligand PD-L1, Fas (apoptosis receptor) and major histocompatibility complex class I (MHC-I). In addition, antigen-presenting molecules (APC) are activated, which stimulate the production of cytotoxic tumour lymphocytes CTL with the ability to identify and kill tumour cells.

The mTOR pathway activates several hours after exposure to radiation, releasing radiation-specific peptides that can induce a tumour-specific immune response.

Ionizing radiation also generates chemokines, such as CXCL16, which attract CTL cells to the irradiated tumour volume.

Hyperfractionated radiotherapy favours immunological effects that further enhance the effect of immunotherapy. These mechanisms involve up- or down-regulation of membrane molecules, such as PD-L1, HLA class-I, CD80/86, CD40, CD47 and Fas/CD95, which play an important role in immune checkpoint pathways and increased cytokine expression (e.g. Interferon INFγ,β,α, IL1,2 and TNFα) by cancer or immune cells. To complete the action of all these biological processes initiated by a single radiation fraction of 8Gy, the time interval between additional radiation fractions should be long enough, at least one week (Koukourakis et al., 2023b).

Using only one fraction of hypo-fractionated radiation therapy also induces immunogenic modulation, altering the biology and environment of surviving tumour cells, making them more susceptible to T-cell-mediated immunogenic cell death (Ahmed et al., 2013).

Summary of the effects of radiation on the tumour's microenvironment

- Release of the tumour antigen and:
  CDAMP = CellDeathAssociated Molekylar Pattern
  TLR = Toll Like-receptors on naive T-cells (TLR 4)
  HMGB1 = ”High *Mobility Group1*” a non-histonchromatinbinding nuclear protein
- Release of Adenin-Tri-Fosfat (ATP)
- Stimulates maturation of Dendritic cells (DC) and T-cells with IFN production
- Increases proliferation and tumour infiltration of CTL= Cyto-Toxic Leucocytesr (CD8+T-cells)

The immunosuppressive properties of the tumour mostly prevail, which often eliminates the effect of the immunotherapy. However, hyperfractionated radiation therapy reduces the suppressive properties of the tumour through inactivation of immune-suppressive immature MCSC cells and reduction in the population of immune-suppressive regulatory T-cells Treg in the tumour's surroundings.

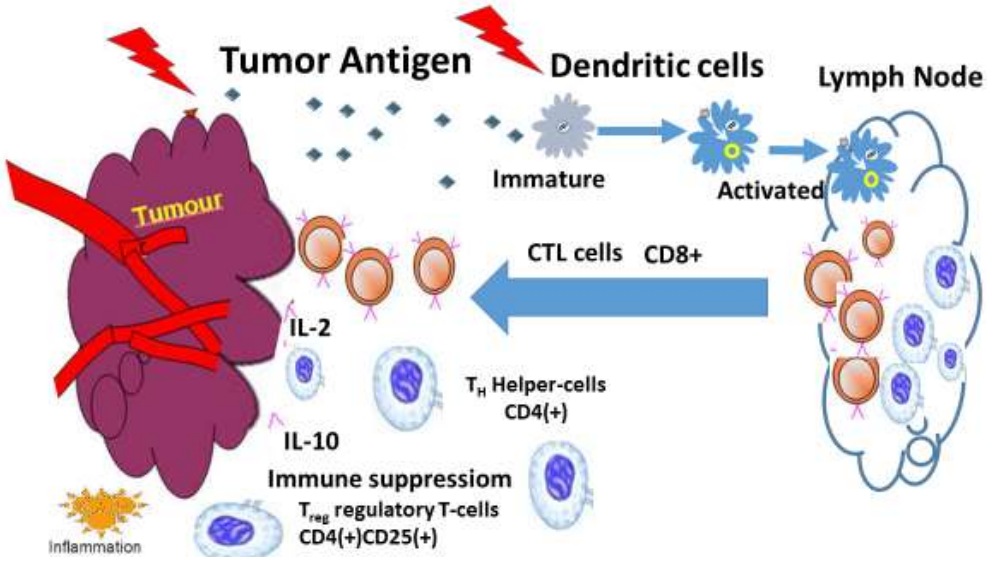


Figure 2-3
The effects of radiation on the tumour microenvironment.

2.3 Cancer immunotherapy

As can be seen from the above description of T cell function, they play a prominent role in the cell-mediated adaptive immune response against foreign pathogens and malignant cells (Rudolph et al., 2006).

However, to prevent the immune system from targeting normal host tissue, it is equipped with regulatory mechanisms to modulate and downregulate the immune response.

***Cytotoxic T-lymphocyte associated protein 4*, CTLA-4**, is a protein found on T-cells that helps control the body's immune response. When CTLA-4 binds to another protein called B7, found on both normal and tumour cells, the CTL killer cells are prevented from killing both normal and tumour cells.

Figure 2-4 shows how the CTLA-4 receptor on the CTL cell connects to the B7 ligand, thereby suppressing the immune response from the CTL. In this way, CTL with the body's antigen can show immune tolerance against the body's tissues and cells. Figure 2-5 shows how blocking the CTLA-4 interaction with anti-CTLA-4 monoclonal antibodies can be applied for treatment against tumours.

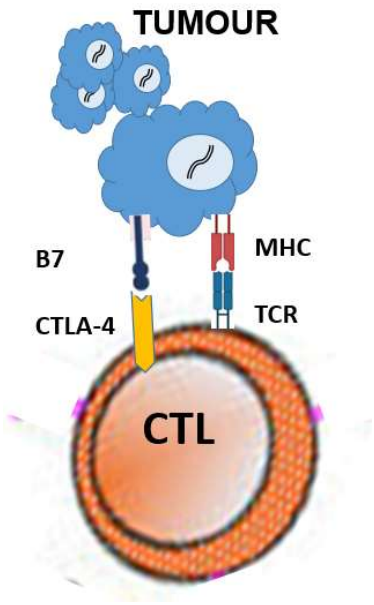


Figure 2-4

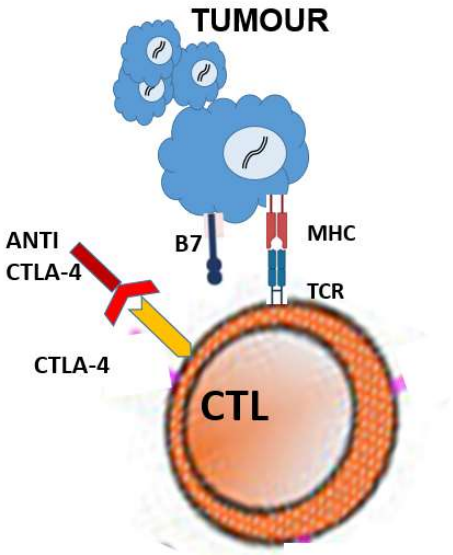
When the CTLA-4 Receptor on the CTL cell connects to the B7 ligand on the Cancer cell or any other cell, the CTL cell's killing response is suppressed.

In this way, the attack of CTL cells against the host's tissues and cells can also be prevented

Pharmaceuticals known as immune checkpoint inhibitors block CTLA-4 so that the killer cells' CTL immune response can eliminate the tumour cells.

Figure 2-5

Therapeutic interventions against different types of tumours performs by blocking the CTLA-4 /B7 interaction with anti-CTLA-4 monoclonal antibodies.



Programmed cell death receptor-1 (PD-1) is another important immune modulator. It is a transmembrane protein of the CD28 family, with 288 amino acids, expressed in the membrane of activated T cells (CTL), B cells and tumour-associated macrophages (Xu., 2020). When PD-1 binds to the endogenous ligand PD-L1 on the cancer cell or any other cell, the immune response of activated T cells (CTL) is suppressed, thereby maintaining immune tolerance against the body's normal tissues and cells (Hamid et al., 2013).

However, PD-L1 is often overexpressed in various tumours, such as melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancer, and

Hodgkin lymphoma, which enables these cancers to escape an attack by immune system CTL (Kataoka et al., 2016).

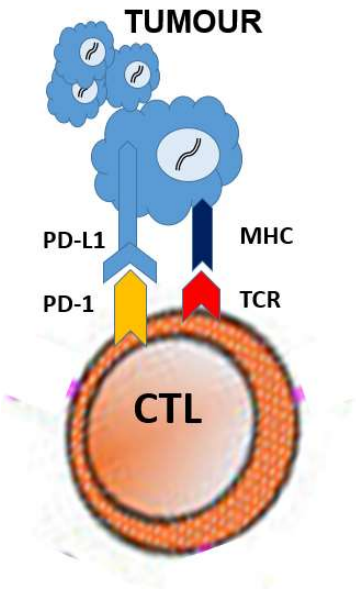


Figure 2-6

When the PD1 receptor on the CTL cell connects to the PDL1 ligand on the cancer cell or any other cell, the immune response against the body's own antigens is suppressed, maintaining immune tolerance against the host's normal tissues and cells.

Therapeutic interventions against different types of tumours take place by blocking the PD-1/PD-L1 interaction with monoclonal antibodies against these receptors (Tomasiak et al., 2021). Figure 2-6 shows how the PD1 receptor on the CTL cell connects to the PD-L1 ligand on the cancer cell, which suppresses the immune response and prevents the CTL from killing the cancer cell. In figure 2-7, blocking of PD-1 or PD-L1 shows the interaction with monoclonal antibodies.

However, the PD-L1 ligand is also expressed in antigen-presenting cells, which can affect the response of T-cells and the tumour's ability to survive (Kleffel et al., 2015; Wen et al., 2024). It is thus important to consider the choice of drug for optimal response.

In PD-L1 negative advanced non-small cell lung cancer, dual PD-1/CTLA-4 blockade exhibit to have clinical significance (Wang et al., 2024).

However, immunotherapy with these immune checkpoint inhibitors appears to fight a wide range of tumour types and is associated with lower levels of toxicity than other immunotherapies, with durable responses. Therefore, PD1/PD-L1 inhibitors are considered a promising drug category for many different cancers. But if the effect is too strong, normal tissue is also attacked, with serious side effects.

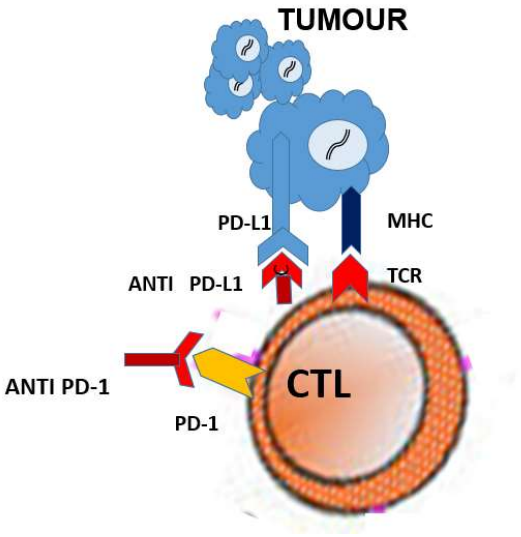


Figure 2-7

The PD-L1 ligand is often overexpressed in various tumours, enabling these cancers to escape an attack by the immune system's CTL.

Therapeutic interventions against tumours block the PD-1 or PD-L1 interaction with monoclonal antibodies.

2.4 Effects of radiation on tumour immunosuppression

Yin and coworkers discussed in 2019, the interaction between Myeloid-derived suppressor cells (MDSC) in the tumour microenvironment and radiotherapy.

Myeloid-derived suppressor cells (MDSCs) are one of the tumour microenvironment's most important cellular components, promoting tumour growth and metastasis. MDSCs are shown to be essential regulators of immune responses in cancer and are important for the effectiveness of cancer therapy with radiotherapy (Graf et al., 2002, Poschke and Kiessling, 2012, Poschke et al., 2012).

In addition to releasing tumour antigens, radiation therapy can promote local and systemic antitumour immunity by affecting myeloid-derived cells (MDSCs), including tumour-associated macrophages (TAMs) in the tumour. However, depending on radiation dose and fractionation, radiation therapy can have both a pro-tumour or anti-tumour immune effect.

The optimal radiotherapy regimen combined with immunotherapy effectively counteracts the inhibitory function of MDSCs, which promotes the infiltration of killer CD8+ T cells (CTL) into the tumour. Targeting to reduce MDSC and strengthen antitumour immunity is crucial for the therapeutic effect of immunotherapy combined with hypofractionated radiotherapy HFRT.

In combination with anti-PD-L1 immunotherapy, HFRT is potent for cancer treatment. By reducing the number of MDSCs, HFRT favours the infiltration of CD8+ T cells into the tumour. Recent studies have shown that the combination of anti-PD-L1 with HFRT radiation therapy effectively reduces the accumulation of

MDSC in the tumour microenvironment, which inhibits tumour growth. However, radiation dose fractions that are too high in HFRT may increase the level of Treg-regulatory T-cells, suppress radiation-induced immune responses, limit the full expression of antitumour immunity, and facilitate relapse. Therefore, the effect of MDSC cells in the tumour microenvironment is dependent on dose level and fractionation of the radiation therapy.

Combination therapy with PD-L1 blockade and properly balanced HFRT eliminates MDSC through increased production of T-cell-derived TNF.

Koukourakis and coworkers reported in 2023 a summary of the molecular basis for the interaction between immunotherapy and radiation therapy (Koukourakis et al., 2023b).

Preclinical research shows the importance of exploiting the interaction between radiation therapy's cytotoxic effects and the immune system. Their review provides the basics of antitumour immunity and focuses on the mechanisms underlying the molecular interaction in immuno-HFRT, which can be considered radio vaccination.

In addition, the interactions of the radiation with the tumour microenvironment's fibroblasts, tumour-infiltrating lymphocytes, monocytes, and dendritic cells are also important components of the radio vaccination process. Thus, properly balanced HFRT can be used as an aid in immunotherapy to effectively treat different cancer subtypes (Koukourakis et al., 2023b, Koukourakis et al., 2023a).

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Chapter III
Preclinical trials

3.1 Preclinical trials with CTLA-4

Demaria and coworkers reported in 2005 on immune-mediated inhibition of metastases after local radiotherapy combined with CTLA-4 blockade in a mouse model of breast cancer (Demaria et al., 2005).

They were the first to test the hypothesis that local radiotherapy to the primary tumour in combination with CTLA-4 blockade can induce tumour regression and inhibit metastasis.

Mice were injected s.c. with 4T1 breast cancer cells, and 13 days later, when the primary tumours measured 5 mm in mean diameter, treatment was started. The mice were divided randomly into the following four treatment groups:

(1) Control IgG (IgG),

(2) Radiation therapy RT + IgG,

(3) 9H10 monoclonal antibody against CTLA-4,

(4) Radiation therapy RT + 9H10-antibody against CTLA-4.

Radiotherapy (RT) is delivered to the primary tumour with one or two fractions of 12 Gy given at 48-hour intervals.

Monoclonal antibody against CTLA-4 (9H10) and immunoglobulin IgG as placebo, administered by intraperitoneal injections (IP) into the peritoneal cavity three times after radiotherapy (RT).

In group 2 with radiotherapy alone, growth of the primary irradiated tumour was delayed, but survival time was equivalent to that of control mice in group 1 (Demaria et al., 2005).

Consistent with the fact that 4T1 breast cancer is poorly immunogenic, 9H10-monoclonal antibody to CTLA-4 alone, as in group 3 had no effect on primary tumour growth or survival.

In contrast, mice in group 4 that were treated with Radiotherapy in combination with 9H10-monoclonal antibody against CTLA-4 had a statistically

significant increased survivaltime compared to the controls in group 1. The increased survival also correlated with inhibition of lung metastasis formation.

In conclusion, local Hypo-Fractionated radiotherapy combined with CTLA-4 blockade appears to be a promising new immunotherapeutic strategy against poorly immunogenic metastatic cancers (Demaria et al., 2005).

Dewan and coworkers showed in 2009 that properly fractionated radiotherapy induces an immune-mediated abscopal effect in combination with an anti-CTLA-4 antibody (Dewan et al., 2009).

They tested the hypothesis that the type of dose fractionation regimen affects radiation therapy's ability to act synergistically with immunotherapy.

Mouse TSA mammary carcinoma cells were injected subcutaneously s.c. in syngeneic mice at two separate sites. One was defined as a "primary" site, which was irradiated, and the other as a "secondary" site outside the radiation therapy area simulating metastasis. When the tumours started to grow, the mice were randomly divided into the following eight groups:

1 Control without radiation treatment
2 Radiation treatment 20 Gy × 1 fraction
3 Radiation treatment 8 Gy × 3 fractions with one fraction daily
4 Radiation treatment 6 Gy × 5 fractions with one fraction daily
5 Alone treatment with 9H10-antibody against CTLA-4
6 Radiation treatment 20 Gy × 1 fraction + antibody against CTLA-4
7 Radiation treatment 8 Gy × 3 fraction + antibody against CTLA-4
8 Radiation treatment 6 Gy × 5 fraction + antibody against CTLA-4

In each radiotherapy regimen 2, 3, and 4, comparable delays in the growth of the primary tumours were observed, but no effect was noted on the secondary tumours outside the radiation field.

When treated in regimen 5 with 9H10-antibody against CTLA-4 alone, no detectable regression of the tumour was observed.

In contrast, in groups 6, 7 and 8 with the combination of radiotherapy and the 9H10-antibody against CTLA-4, significant ($p<0.0001$) regression of the primary tumours was observed.

In addition, in groups 7 and 8, the combination treatment caused abscopal effect with significant ($p<0.01$) reduction in the growth of the secondary tumours outside the field. The frequency of CD8+CTL cells exhibiting tumour-specific IFN-γ production was proportional to the inhibition of the secondary tumour.

In contrast, regimen 6, which involves a single 20 Gy radiotherapy in combination with an anti-CTLA-4 antibody, does not produce an abscopal effect.

The best therapy effect occurred in group 7 with the hypofractionated radiotherapy regimen: eight gray (Gy) × 3 fractions, one fraction per day, combined with 9H10 antibody against CTLA-4 (Dewan et al., 2009).

Vanpouille-Box and colleagues in 2017 reported that DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity (Vanpouille-Box et al., 2017).

They analysed how radiotherapy combined with immunotherapy (anti-CTLA4) can induce immune responses directed both at irradiated and distant tumours (so-called abscopal effects).

The effect of different radiation doses shows that Hypo-fractionated radiation (8 Gy given in three fractions) was effective in inducing abscopal effects in combination with anti-CTLA4. Hypo-fractionated radiation-induced expression of interferon-stimulated genes (ISG) and production of IFNβ in tumour cells. This response is necessary to recruit dendritic cells and activate tumour-specific CD8+ T-cells (CTL).

However, in contrast, high single doses (20 Gy and 30 Gy) were ineffective in inducing abscopal effects.

The enzyme Trex1 regulates double-stranded DNA (dsDNA) levels in the cytoplasm, which signals IFN-β production. High radiation doses induced Trex1, which reduced cytoplasmic dsDNA levels and attenuated the IFNβ-response.

However, the knockdown of Trex1 allowed high-dose radiation (20 Gy) to induce abscopal effects.

The pathway of cyclic GMP-AMP synthase (cGAS) and Stimulator of Interferon Genes (STING) is a component of the innate immune system that detects the presence of cytosolic DNA and, in response, triggers the expression of inflammatory genes. They found this cGAS-STING signalling pathway crucial in mediating IFN-β production in tumour cells in response to Hypo-fractionated radiation. Inhibition of cGAS or STING prevented abscopal effects and tumour-specific immune responses.

In summary, by comparing radiation therapy of 20 Gy in a single fraction with 24 Gy in 3 fractions, they found the ideal dose fractionation that exerted systemic immune effects to be 8 Gy per fraction.

Their results from patient-derived tumour models showed that Hypo-fractionated radiation (8 Gy x 3) induced robust IFN-beta responses, while high

single doses of radiation increased Trex1 and attenuated the immune response (Vanpouille-Box et al., 2017).

Hemphill and collaborators suggested in 2021 that Trex1 is a Novel Immunotherapeutic Target (Hemphill et al., 2021).

They proposed that Trex1 degrades tumour-derived DNA in the tumour environment that would otherwise activate cGAS-STING. If tumour-derived DNA were not degraded, the cGAS-STING pathway would be activated to promote IFNβ-dependent antitumour immunity. Thus, they hypothesise that Trex1 exonuclease inhibition is a novel immunotherapeutic strategy. They demonstrate antitumour immunity in a mouse model and discuss the theory surrounding the best strategy for Trex1 inhibition and the potential applications of Trex1 inhibition as a therapeutic strategy (Hemphill et al., 2021).

Rudqvist and colleagues reported in 2023 on preclinical studies of immunotherapy directed at different immune compartments that, in combination with radiotherapy RT, induce regression of resistant tumours (Rudqvist et al., 2023).

Radiation therapy with 8 Gy per day for 3 consecutive days (RT) increases the tumour response by inhibiting CTLA-4 (CTLA4i) in mice, and complete remissions are rare in some patients.

To identify rational combinations of immunotherapy to improve responses, models of triple-negative breast cancer, which is highly resistant to immunotherapy, when used in female mice.

Combination therapy (CTLA4i+RT) decreases regulatory CD4+T-cells, increases effector memory, and activates CD8+T-cells. A combined gene signature comprising three CD8+T-cell clusters is associated with survival.

They show that CD40 agonist therapy contributes to resistant tumours responding to the combination of RT and CTLA4i, indicating the need to target different immune compartments (Bullock, 2022).

The authors provide immunological insights into the response to RT and CTLA4 inhibition in tumour-bearing mice and show that agonistic CD40 therapy further improves the response to the combination of RT and immune checkpoint inhibition.

While the addition of anti-CD40 dramatically enhanced the rejection of the irradiated tumour, it didn´t further enhance the inhibition of lung metastases achieved in 4T1-bearing mice by RT+CTLA4i. Their results suggest that

therapeutic CD40 agonism enhances the efficacy of RT+CTLA4i in established tumours dominated by an immunosuppressive myeloid infiltrate.

In summary, when CTLA4i is used together with radiation therapy with 8 Gy per day for 3 consecutive days, it leads to the emergence of CD8 functional subsets of T.cells associated with increased patient survival, accompanied by a decrease of CD4 regulatory T-cells (Rudqvist et al., 2023).

3.2 Preclinical trials with anti-PD1

Schaue and coworkers studied in 2012 how tumour immunity could be maximised with fractionated radiotherapy and antibodies against PD1 (Schaue et al., 2012).

C57BL/6hPD-1 knock-in mice bearing two syngeneic contralateral MC38 murine colon cancer tumours were treated to evaluate the abscopal effect.

Radiotherapy with three fractions of 8 Gy on consecutive days was chosen in combination with anti-PD-1. Anti-PD1 administration enhanced local and systemic cytotoxic T cell (CTL) antitumour immunity. During the combination treatment, the spleen showed reduced myeloid-derived suppressor cells (MDSC) levels.

Furthermore, RNA sequencing revealed significantly increased tumour necrosis factor (TNF) receptor levels and cytokines associated with lymphocyte infiltration in the combined group's tumours.

The results show that hypofractionated radiotherapy with eight Gy in 3 fractions was the optimal fractionated dose to maximize the immune effect, and the combination with anti-PD-1 showed promising results to increase the abscopal effect.

Underlying mechanisms appear to include both activation of T cells and reduction of Myeloid-derived suppressor cells (MDSCs), which is achieved through the action of Tumour Necrosis Factor (TNF) and related cytokines (Schaue et al., 2012).

Teng and coworkers confirmed in 2023 the optimal effect of 8 Gy fractionation of radiotherapy combined with PD1 blockade (Teng et al., 2023).

Their study aimed to investigate fractionated radiotherapy to maximize immunity during combination therapy. The abscopal effect in C57BL/6hPD-1 knock-in mice bearing two syngeneic contralateral MC38 murine colon cancer

tumours was evaluated after treatment with the following four different radiotherapy regimens:

1. A single dose of 20 Gy (20 Gy × 1 fraction),
2. 3 fractions á 8 Gy (8 Gy × 3 fractions with one fraction per day),
3. 15 fractions á 2 Gy (2 Gy × 15 fractions), 30 Gy totally
4. A single fraction of 8 Gy followed by 10 fractions á 2 Gy on consecutive days (8 Gy + 2 Gy × 10 = 28 Gy in total).

Group 2 with three fractions of 8 Gy proved optimal when combined with anti-PD-1 to maximize immunity. Anti-PD-1 administration enhanced local and systemic antitumour immunity in a cytotoxic T cell-dependent manner. In addition, the spleen showed reduced myeloid-derived suppressor cells (MDSCs) levels during combination therapy. Furthermore, RNA sequencing revealed significantly increased tumour necrosis factor (TNF) receptors and cytokines associated with lymphocyte infiltration in the combined group.

The study confirms that hypofractionation with 8 Gy in 3 fractions was optimal for maximizing immunity when combined with anti-PD-1.

The results showed an increase in the abscopal effect. Underlying mechanisms may include activation of T cells and reduction of MDSCs, which occur through the action of TNF and related cytokines. Their study indicates that combining immunotherapy with hypofractionated radiotherapy is an effective approach to overcome the current limitations of tumour immuno-suppression in immunotherapy alone.

Their studies confirm the hypothesis that reduction of MDSC is an integral part of overcoming tumour immunosuppression that reduces the effect of immunotherapy (Graf et al., 2005; Persson et al., 2010; Ceberg and Persson, 2013).

The moderate total radiation dose during ultra-hyperfractionation also enables combined immunotherapy treatment of tumours in radiation-sensitive organs such as colon cancer. It also opens up the possibility of reducing the immunotherapy dose of checkpoint inhibitors to reduce the rate of serious side effects.

3.3 Dendritic cells and immunotherapy

Dendritic cell vaccination enriches the tumour environment and potentiates the patient's systemic antitumoural response. So far, however, dendritic cell

vaccines (DCV) have induced immune responses in patients without any significant impact on treatment outcome.

Hata and coworkers reviewed the role of dendritic cell vaccine in various solid tumours with its strengths and weaknesses, in an attempt to improve the effectiveness of this immune strategy (Hato et al., 2024).

Nagai and coworkers presented in 2024 the results of a combination of radiotherapy, with dendritic cell vaccine therapy for end-stage small bowel cancer (Nagai et al., 2024).

They performed intensity-modulated radiotherapy with 8 Gy for pain relief in a 40-year-old male patient with end-stage small bowel cancer who had been diagnosed with a life expectancy of two months after chemotherapy had been ineffective. Subsequent administration with seven doses of dendritic cell vaccine recognizing Wilmstumour-1 (WT1) and β-galactosyl-ceramide antigens resulted in significant tumour reduction and marked improvement in the patient's general condition.

Combination therapy of 8 Gy radiotherapy and dendritic cell vaccine therapy can suppress cancer progression and prolong survival, even in patients with chemotherapy-refractory terminal cancer. In particular, dual dendritic cell vaccine therapy with WT1 and β-galactosylceramide pulsed dendritic cells can produce an antitumour immune effect superior to the respective monotherapies (Nagai et al., 2024).

3.4 CAR T-cell-based immunotherapy

Hovhannisyan and coworkers discussed potential opportunities for treating solid tumours with CAR T-cell-based immunotherapy combined with radiation therapy in 2023 (Hovhannisyan et al., 2023).

CAR T-cell-based therapies have revolutionised the treatment of haematological malignancies such as leukaemia and lymphoma. In contrast to the success in haematological cancers, treating solid tumours with CAR T-cells is still unsuccessful.

As reported above, radiation-immune checkpoint inhibitor combinations have proven successful. Therefore, a combination of radiotherapy should also have the potential to overcome the limitations of CAR T-cell therapy in solid tumour entities. However, so far, research in the area of CAR T cells and radiation has been sparse (Hovhannisyan et al., 2023).

Some studies with CAR-T and ultra-hyperfractionation with optimal radiation dose and fractionation (8 Gy per fraction in one to three fractions) do not appear current.

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Chapter IV
Clinical Trials with Immuno-Radiation Therapy

4.1 Immunotherapy and Conventional Radiation Therapy

Lee and colleagues investigated in 2021 whether a combination of the drug *Avelumab* (anti-PD-L1) with conventional chemo-radiotherapy could improve treatment results (Lee et al., 2021).

Chemoradiotherapy is the standard for non-surgical treatment of locally advanced squamous cell carcinoma in the head and neck region. In a randomised, double-blind, placebo-controlled phase 3 trial, patients were recruited from 196 hospitals and cancer treatment centres in 22 countries.

In the *Avelumab* group, patients were administered every other week with 10 mg/kg *Avelumab* intravenously in combination with chemo-radiotherapy according to:

- Immunotherapy *Avelumab* intravenously 10mg/kg every two weeks
- Chemotherapy (100 mg/m² *Cisplatin*) every three weeks and
- Intensity-modulated radiotherapy with standard fractionation à 2 Gy over 7 weeks with 35 fractions to a total of 70 Gy.

As a comparison group, placebo immunotherapy was applied with the same chemoradiotherapy treatment.

Radiation treatment is preceded by a 7-day administration of 10 mg/kg Avelumab or placebo. After radiotherapy is completed, 10 mg/kg *Avelumab* or placebo is given every two weeks as maintenance treatment for up to 12 months.

The median follow-up for progression-free survival was 14.6 months (8.5–19.6) in the *Avelumab* group and 14.8 months (11.6–18.8) in the placebo group.

Serious treatment-related adverse events occurred in

- 124 (36 %) patients in the *Avelumab*-group
- 109 (32 %) patients in the placebogroup.

Treatment-related deaths occurred with

- Two (1 %) patients in the *Avelumab*-group due to general disorders and vascular rupture
- One (<1%) in the placebo group due to acute respiratory failure.

In conclusion, the primary objective of prolonging progression-free survival in patients with locally advanced squamous cell carcinoma of the head and neck with immunotherapy plus standard chemo-radiotherapy followed by Avelumab maintenance was not met (Lee et al., 2021).

Tao, and colleagues reported in 2023 the results of conventional radiotherapy combined with *Pembrolizumab* (PD1 inhibitor) or with *Cetuximab* (inhibits the binding of epidermal growth factor EGF) in a randomised phase II study of patients with locally advanced squamous cell carcinoma of the head and neck unsuitable for treatment with *Cisplatin* chemotherapy (Tao et al., 2023).

The standard treatment for patients with locally advanced squamous cell carcinoma of the head and neck (LA-SCCHN) is radiation therapy with 2 Gy once daily up to 70 Gy in combination with *Cetuximab*. In the study, a group treated with 200 mg of *Pembrolizumab* combined with the same radiation therapy regimen (RT), but no placebo Immuno-drug group was included.

The primary endpoint was the occurrence of a locoregional tumour 15 months after the completion of radiation therapy. The study showed no significant difference in progression-free or overall survival between the *Cetuximab* and *Pembrolizumab* groups. The toxicity in patients with at least one grade 3 side effect, such as mucositis, radiodermatitis, or skin rash, was 74% in the *Pembrolizumab*+RT group, compared to 92% in the *Cetuximab*+RT group (Tao et al., 2023).

Danish and coworkers reported in 2024 the results of a study of non-Hodgkin lymphoma patients who received bridging radiotherapy after CAR T-cell therapy (Danish et al., 2024).

About 47% (38/81) of patients classified as having a high risk of relapse after CART, received bridging radiation therapy with 1.80 Gy in 23 fractions for a total dose of 41.4 Gy. However, not significantly, bridging radiation therapy may have improved response to provided locoregional disease control in this setting (Danish et al., 2024).

The above-mentioned studies, carried out with conventional fractionated radiation therapy at daily 2 Gy fractions, indicate that no significantly improved treatment results are achieved when combined with immunotherapy.

Radiation therapy initially initiates a certain radio vaccination effect through the release of tumour antigens that contribute to the production of active CD8 T-cells. However, subsequent daily radiation fractions progressively kill the tumour-infiltrating T-cells even with extra immunotherapy stimulation.

Another reason conventionally fractionated radiotherapy fails to show a survival benefit in combination with immunotherapy may be the irradiation of the tumour-draining lymph nodes (TDLN) (Lee et al., 2021).

Conventionally fractionated radiotherapy irradiation of the tumour-draining lymph nodes (TDLN) is often standard in radical radiotherapy of head and neck tumours (HNSCC). The TDLN are the central station for activating cytotoxic T-cells (CTL) by tumour-antigen-primed dendritic cells. However, the daily repeated irradiation of the TDLN eliminates the CTL, blocking the radio-vaccination effect of the T cells (Koukourakis and Giatromanolaki, 2022).

Koukourakis, and Giatromanolaki showed 2022 the importance of the tumour-draining lymph nodes' immune response during radiotherapy (Koukourakis and Giatromanolaki, 2022).

The *tumour-draining lymph nodes* (TDLN) are the primary sites for developing anti-tumour immunity. Primary tumour irradiation increases the release of tumour antigens and activates the interferon type-I pathway. Intratumoral dendritic cells (DCs) activated with tumour antigens migrate to the TDLN, where adaptive antitumour immune responses are developed. The dendritic cells DC will there present tumour-related antigens to activate the production of CD4+ and CD8+CTL T-cells (See Figure 2-3 Effects of radiation on the tumour's microenvironment).

Their experimental results suggest that tumour clearance after irradiation strongly depends on the accumulation of such cytotoxic T cells in the tumours.

During conventional radiation therapy, the TDLN is exposed, and the critical population of immune cells present in the TDLN is progressively reduced, blocking the immune response and compromising the effectiveness of immunostimulatory combinations.

Since TDLNs are essential for incoming dendritic cells previously activated in the tumour environment to produce active CTL cells, the following should be considered (Koukourakis and Giatromanolaki, 2022):

- TDLN irradiation or the removal of nodes should be avoided if the immunology during or after radiotherapy is to have therapeutic significance.
- The TDLN represents the main site of formation and production of tumour-specific cytotoxic immune cells CTL that influence the results of the local radiotherapy and the manifestation of abscopal effects.

The biological and clinical role of TDLN is thus a critical factor to consider in designing clinical trials of combination immunotherapy and radiotherapy aimed at eliminating cancer cells at the local and systemic levels (Koukourakis and Giatromanolaki, 2022).

4.2 Hypo-fractionated radiotherapy

Prostate cancer

Hickey and colleagues reported in 2019 the results of searches for controlled comparison studies that included men with clinically localised prostate adenocarcinoma. They compared external beam radiotherapy to the prostate with Hypo-Fractionated (greater than 2 Gy per fraction) with conventional fractionated radiotherapy to the prostate delivered with standard fractionation (1.8 Gy to 2 Gy per fraction) (Hickey et al., 2019).

They included 10 studies with 8,278 men in their analysis that compared hypo-fractionation with conventional fractionation to treat prostate cancer.

In summary, their findings suggest that moderate hypofractionation (up to a fraction size of 3.4 Gy) results in similar oncologic outcomes in terms of disease-specific symptoms, metastasis, and overall survival. There also appears to be little or no increase in both acute and late toxicity (Hickey et al., 2019).

Widmark and colleagues reported 2019 five-year results of Ultra-hypo-fractionated versus conventionally fractionated radiotherapy for prostate cancer (Widmark et al., 2019).

Hypo-fractionated radiotherapy for prostate cancer has received increased attention due to reports of studies comparing moderately hypo-fractionated and

conventional fractionated radiotherapy supporting the clinical use of moderate hypofractionation.

Widmark and colleagues, initiated studies comparing ultra-hypofractionated radiotherapy with conventional fractionated radiotherapy. Their report presents the results of the Scandinavian HYPO-RT-PC phase 3 study with the aim of showing the effect of ultra-hypofractionation versus conventional fractionation. This open, randomised, phase 3 study was conducted at 12 centres in Sweden and Denmark. Men up to 75 years of age with intermediate to high-risk prostate cancer and WHO performance status between zero and two were recruited and randomly grouped according to:

- Ultra-hypo-fractionation (42.7 Gy in seven fractions of 6 Gy, 3 days per week for 2.5 weeks) or
- Conventional fractionated radiotherapy (78.0 Gy in 39 fractions of 2 Gy, 5 days per week for 8 weeks).

No androgen deprivation therapy was applied. The primary endpoint was time to biochemical or clinical relapse, analysed in the per-protocol population.

Physician-recorded toxicity was measured according to the *Radiotherapy Oncology Group* (RTOG) *morbidity scale* and patient-reported outcome measures using the *Prostate Cancer Symptom Scale* (PCSS) questionnaire.

Between July 1, 2005, and November 4, 2015, 1200 patients were randomised to conventional fractionation (n=602) or ultra hypofractionation (n=598). Of the participants, 89%mwere at intermediate risk, and 11% were at high risk. The median follow-up time was 5 years. The estimated failure-free survival at 5 years was 84% (95% CI=80-87) in both treatment groups, with an adjusted HR of 1.002 (95% CI 0.758-1.325; log-rank p= 0.99).

There was a weak indication of an increased rate of acute physician-reported RTOG grade 2 or worse ureteric toxicity in the ultra-hypofractionation group at the end of radiotherapy. There were no significant differences in grade 2 or worse urinary or bowel toxicity between the two treatment groups at any time point after radiotherapy. However, the ultra hypofractionation group showed an increase in urinary toxicity compared with the conventional fractionation group at 1-year follow-up.

They observed no between-group differences in rates at 5 years of RTOG grade 2 or worse urinary and bowel toxicity. However, patient-reported results revealed significantly higher acute urinary and bowel symptom levels in the ultra-hypofractionation group. Still, no significant increases in late symptoms occurred,

except for increased urinary symptoms at 1-year follow-up, consistent with physician-assessed toxicity.

In conclusion, Ultrahypofractionated radiation therapy is neither better nor worse than conventional fractionated radiation therapy for intermediate- to high-risk prostate cancer in terms of error-free survival.

Ultra-hypofractionation caused more pronounced early side effects than conventional fractionation, while late toxicity was similar in both treatment groups.

The results support radiotherapy with ultra-hypofractionation for the treatment of prostate cancer (Widmark et al., 2019).

Wieslander and colleagues presented a treatment planning study based on Ultrahypofractionated Radiation Therapy for Prostate Cancer in 2024 (Wieslander et al., 2024).

When treating patients with a high Gleason score, that is, a high PSA/T3 ratio, seminal vesicles (SV) are routinely included in the target volume. Thus, they studied the feasibility of implementing ultra-hypofractionated integrated boost (UHF-SIB) treatment for prostate cancer RT, including seminal vesicle SVs. The treatment was based on the Scandinavian HYPO-RT-PC-phase-3 prostate cancer study results with the fractionation schedule: 7 fractions of 6 Gy over 2.5 weeks (Widmark et al., 2019).

A second objective was to analyse the unintended dose coverage of seminal vesicle SVs in volumetric modulated arc therapy (VMAT) treatments of the prostate gland only in the clinical target volume.

Their results indicate that Ultrahypo-fractionated radiotherapy UHF-RT based on the HYPO-RT-PC fractionation scheme with a simultaneous integrated boost SIB technique to prostate and seminal vesicles SVs could be planned with generally lower doses (EQD2) to organs at risk, compared to conventionally fractionated á 2Gy radiation therapy based on a sequential boost technique.

However, the unintended dose to the proximal parts of the seminal vesicles in prostate-only VMAT treatment can be significant and should considered when designing ultra-hypofractionated simultaneous integrated boost SIB treatment regimens (Wieslander et al., 2024).

Breast-cancer

Park and colleagues presented a systematic review and meta-analysis of postoperative complications with hypofractionated (HF) or conventional fractionated radiotherapy (CF) in patients with implant-based breast reconstruction in 2024 (Park et al., 2024).

Radiotherapy after mastectomy is an important component of adjuvant therapy for high-risk patients. However, radiation to reconstructed breasts can cause various complications. Recently, several countries have begun using hypofractionated (HF) treatment protocols.

Seven articles with 924 implant reconstructions, 506 (54.8%) of which underwent HF, were included in the study. HF patients received an average of 43.8 Gy, while CF patients received 51.2 Gy. Follow-up times ranged from 10.6 to 35 months.

The results indicate that compared to CF groups, HF groups had a significantly lower risk observed ratio (OR) of:

- Capsular contracture (OR ≈ 0.25; 95% CI 0.11-0.55),
- Major revision surgery (OR ≈ 0.19; 95% CI 0.05-0.80) and
- Wound resolution (OR≈ 0.24; 95% CI 0.07- 0.707).

Their study indicates that HF protocols are associated with fewer complications than CF protocols in implant-reconstructed patients, providing support for the use of hypofractionation in the radiotherapy of implant-reconstructed breast cancer patients (Park et al., 2024).

Rectal Adenocarcinoma

Koukourakis and colleagues presented in 2024 the results of IFN-Type-I response and systemic immunity in patients with rectal adenocarcinoma after treatment with conventional-fractionated “CFRT” or hypo-fractionated “HFRT” radiation therapy (Koukourakis et al., 2024).

Patients with advanced rectal adenocarcinoma were treated with hypofractionated HFRT-5Gy for 5 days or conventional fractionated CFRT-1.8Gy in 28 days of radiation therapy. The study uses this model to compare the immunostimulatory properties of radiation therapy at a systemic level.

They prospectively analyzed IFN-β plasma levels and lymphocyte counts (LCs) in rectal adenocarcinoma patients before and after treatment with hypofractionated radiotherapy HFRT: 5× 5Gy <n = 22) respectively conventional

radiotherapy CFRT: 28×1.8Gy (n = 40). Flow cytometry performed to assess the effects on lymphocytic subpopulations in a subset of 20 patients.

A statistically significant($p = 0.004$) increase in interferon-(IFN-β) plasma levels was noted post-RT in patients who underwent HFRT: 55Gy, which was associated with significantly($p = 0.003$) improved pathological tumour regression compared to CFRT:28×1.8Gy.

Although all patients experienced significant post-treatment lymphopenia, the lymphocyte count value LC for patients treated with HFRT:5× 5Gy was significantly($p = 0.001$) higher than that for CFRT:28×1.8Gy.

Patients who underwent hypofractionated HFRT: 5× 5Gy showed significantly lower percentages of regulatory CD4+/CD25+T-cells ($p = 0.02$).

The results show that hypofractionated HFRT:5×5Gy radiation therapy enables more effective radiation stimulation of the IFN-type-I pathway at the systemic level, provides reduced lymphocytic cytotoxicity, and lowers the level of immunosuppressive regulatory T-cells compared to CFRT:28×1.8Gy (Koukourakis et al., 2024).

Although there is no clear evidence regarding the superiority of either fractionation regimen for the radiotherapy of locally advanced rectal carcinoma, the presented results indicate that hypofractionated radiotherapy HFRT:5×5Gy produces more potent anti-tumour immune responses through the IFN-type I pathway compared to standard fractionation (CFRT). In addition, hypofractionated HFRT:5×5Gy causes lower lymphotoxicity and limits the immunosuppressive effects of regulatory T-cells and MDSCs.

Hypofractionation means fewer but higher doses of radiation per daily treatment session over a shorter period. Since the results of conventional radiotherapy for prostate and breast cancer are comparable, radiotherapy with hypofractionation could free up existing resources for the treatment of additional cancer patients, according to a Lancet Oncology Commission led by the International Atomic Energy Agency (IAEA).

(https://www.thelancet.com/commissions/radiotherapy-theranostics)

Patients appreciate the hypofractionated approach since they often are prepared for at least 6 weeks of radiation and are then very happy to hear they might only need it for half that time (Otto Alexander, 2024).

4.3 Immunotherapy and Hypofractionated radiation therapy

Initial preclinical studies of immunotherapy and combined hypofractionated radiotherapy were mostly performed with tumour vaccines (Graf et al., 1999; Lumniczky et al., 2002; Persson et al., 2003; Persson et al., 2010; Persson, 2011; Ceberg and Persson, 2013).

Ahmed and coworkers presented an overview of radiation-induced modulations of the immune system in 2013, which could be exploited for clinical cancer therapy (Ahmed et al., 2013).

The conventional 2 Gy fractionated radiation therapy for local tumour control with high radiation doses (60-80 Gy) in the primary tumour generally does not create an immunostimulatory environment that could treat systemic disease.

In contrast, radiation-induced tumour destruction with Hypo-Fractionated radiation therapy with 1-3 fractions of 8 Gy with one fraction every week should be a strategy where tumour antigens released from dying tumour cells can be presented for a time in a more immunostimulating environment. Namely, hypofractionated radiotherapy usually reduces the immunosuppression in the tumour microenvironment. Hypo-fractionated radiation therapy also induces immunogenic modulation in different tumour types, altering the biology and environment of surviving tumour cells and making them more susceptible to T-cell-mediated immunogenic cell death.

These properties of hypofractionated radiotherapy should work in combinatorial immunotherapies to enhance systemic antitumour immunity (Ahmed et al., 2013).

Nowadays, clinical studies of the effect of combining hypofractionated radiation therapy with immunotherapy are mostly carried out with drugs based on antibodies that block CTLA-4 and PD1/PD-L1 receptors, as previously described (Demaria et al., 2005; Demaria et al., 2021; Galluzzi et al., 2023).

Recently, intermittent hypofractionated radiotherapy with one fraction per week combined with immunotherapy was successfully tested in patients with recurrent tumours after conventional therapy (Koukourakis et al., 2023a; Filippatos et al., 2023).

Breast cancer

Koukourakis reported in 2023 on new concepts in breast cancer treatment, such as anti-tumour immunity and preoperative radio vaccination (Koukourakis et al., 2023b).

Neoadjuvant chemotherapy (NACT) for certain subtypes of breast cancer (BC) provides significant tumour regression and a survival benefit for patients with a complete pathologic response.

However, clinical and preclinical studies have shown that immune-related factors could provide even better treatment outcomes, and therefore neoadjuvant immunotherapy has emerged as a way to further improving survival.

Modern stereotactic irradiation techniques targeting the primary tumour could combine with chemotherapy and or immunotherapy. Their review discusses the basic biology, clinical experience, and ongoing research underlying the interplay between neoadjuvant chemotherapy, antitumour immune responses, and the emerging role of radiotherapy as a preoperative adjunct with immunologic therapeutic implications for breast cancer (Koukourakis et al., 2023b).

Single 8 Gy fractions of radiotherapy (RT) significantly interact with the immune system and promote antitumour immunity for breast cancer (Demaria et al., 2005, Formenti and Demaria, 2009). This "radio-vaccination" effect could be exploited further in the treatment of breast cancer recurrence with weekly intermittent ultra-hypo-fractionated radiation therapy to enhance the effects of already established clinical practice significantly.

Head- och Neck-cancer

Koukourakis and coworkers presented in 2023, a clinical study of patients with locoregional recurrence of head and neck cancer who were treated with anti-PD-1 immunotherapy and ultra-hypofractionated radiotherapy (Koukourakis et al., 2023a).

The study included 17 patients who, after conventional radiotherapy and chemotherapy, had recurrent inoperable squamous cell carcinoma of the head and neck (HNSCC), as well as one patient with melanoma. They evaluated the efficacy and tolerability of ultra-hypofractionated immuno-radiotherapy (hypo-IRT).

- Seven patients received 1 fraction of 8 Gy to the tumour.
- Seven patients received 2 fractions of 8 Gy to the tumour with one per week.
- Four patients received 3 fractions of 8 Gy to the tumour with one per week.

Nivolumab anti-PD1 immunotherapy was administered concurrently with radiation therapy and thereafter for a maximum of 24 cycles, until tumour progression or manifestation of immune-related adverse events (irAEs) occurred.

Taking into account the available preclinical and experimental data (Koukourakis et al., 2023d), it was suggested that three radiotherapy fractions of 8 Gy, one fraction per week, are optimal for inducing the interferon type I response and improving the efficacy of immunotherapy. However, only one fraction has a significant effect.

In the current study, the above hypothesis was tested in a group of head and neck cancer patients with recurrent inoperable disease after previous conventional radiotherapy and chemotherapy.

Re-irradiation with conventional radiation therapy was not considered due to the radiation dose required to predict an acceptable effect exceeding 60 Gy, which is inevitably linked to high rates of severe fibrosis and necrosis, often with a fatal outcome (Dionisi et al., 2019).

However, in their study, three cohorts of patients were treated with 1, 2, or 3 fractions of 8 Gy, one fraction per week, together with *Nivolumab* anti-PD1 immunotherapy. Early and late radiotherapy toxicities were minimal, and immunotherapy showed excellent tolerability, with only 3 patients discontinuing immunotherapy.

The high overall objective response rate (OR) averaged 70.6%, of which 41.2% were complete remissions (CR). The proportion of patients with Objective response (OR= CR+PR) is the sum of the proportion with Complete remissions (CR) and the proportion with Partial response (PR). In patients treated with 2 or 3 fractions of 8 Gy, the OR was over 80%, and 57% was noted after administration of only one 8 Gy fraction of radiation.

Most responders showed an increase in peripheral lymphocyte counts. The median time to tumour progression was 10 months. The 3-year predicted locoregional progression-free survival was 35%, while the 3-year disease-specific overall survival was 50%.

In summary, the study shows that Anti-PD1 in combination with ultra-hypo-fractionated radiotherapy at 8 Gy results in high objective response rates and increased survival without signs of disease, which supports further trials with ultra-hypo-fractionated radiotherapy.

The excellent tolerability profile justifies large-scale evaluation of weekly intermittent ultra-hypo-radiotherapy as a primary treatment, where the number of 8 Gy fractions can be increased, and the immunotherapy dose reduced (Koukourakis et al., 2023a).

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Non-Small Cell Lung Cancer

Filippatos and colleagues reported in 2023, the results of Ultra-hypo-fractionated 8 Gy re-irradiation with anti-PD-1 immunotherapy in patients who received conventional radical chemoradiotherapy for locally recurrent non-small cell lung cancer (Filippatos et al., 2023).

The study included a cohort of 11 patients with locoregionally recurrent non-small cell lung cancer (NSCLC) after radical chemoradiotherapy. Between 2019 and 2021, they treated patients with one or two fractions of ultra-hypo-fractionated radiotherapy at 8 Gy and concomitant administration of anti-PD-1 immunotherapy (*Nivolumab* or *Pembrolizumab*).

The overall response rate of complete remissions was 27.2%, and the objective response rate of this immuno-radiotherapy regimen was 81.8%. In 54.6% of the patients, partial tumour regression occurred with more than 80% of initial tumour dimensions. The average response (% Change of tumour dimensions) after one fraction was about 50% in 8 cases, 97 % after 2 fractions in 3 cases, and 100% after 3 fractions in 3 cases of metastases

The 22-month locoregional recurrence-free rate was 54.5%, while the estimated 2-year disease-specific overall survival was 62%. These encouraging results provide the basis for continuing trials of immune-radiotherapy with ultra-hypofractionated radiotherapy schedules in this group of NSCLC patients.

Radiotherapy-related toxicities were negligible, while immune-related adverse events forced immunotherapy discontinuation in 36% of patients but results were independent of PD-L1 status.

This observation warrants further evaluation of ultra-hypo-radiotherapy with intermittent 5-8 Gy fractions and reduced immunotherapy dose to reduce side effects of immune intolerance (note BRP)

The results of their study provide encouraging evidence that radiotherapy with one or two 8 Gy fractions is feasible and can be safely combined with anti-PD-1 immunotherapy.

Despite the low number of patients, the significant tumour regression achieved and the long-term locoregional control and overall progression-free intervals provide a basis for continuing immuno-radiotherapy trials with intermittent ultrahypo-fraction schedules in this group of NSCLC patients with poor prognosis (Filippatos et al., 2023).

4.4 Summary:

Head and neck cancer patients with recurrent inoperable disease after previous conventional radiotherapy and chemotherapy can be re-treated with 1, 2 or 3 fractions hypofractionated radiotherapy (HFRT) of 8 Gy with one fraction per week together with *Nivolumab* anti-PD1 immunotherapy. Early and late radiotherapy toxicities were minimal, and the immunotherapy showed excellent tolerance, with only 3 patients discontinuing immunotherapy. In patients who received anti-PD1 immunotherapy treatment in combination with 2–3 fractions of HFRT à 8 Gy, the objective tumour response was over 80%, and 57% was noted with only one 8 Gy fraction of radiation.

In summary, the study shows that patients with locally recurrent non-small cell lung cancer after conventional radical chemoradiotherapy can be treated with anti-PD-1 immunotherapy in combination with one or two 8Gy fractions (Filippatos et al., 2023).

The response rate of complete remissions was 27.2%, while the partial tumour regression with more than 80% of initial dimensions was noted in 54.6% of the patients. The outcome of this immuno-radiotherapy regimen was 81.8% objective response rates.

The 22-month locoregional recurrence-free rate was 54.5%, while the estimated 2-year disease-specific overall survival was 62%. These encouraging results provide the basis for continuing trials of immuno-radiotherapy with intermittent ultra-hypo-fractionated radiotherapy schedules in this group of NSCLC patients.

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Kapitel 5
Epilogue

5 Epilogue

In the 1980s, we showed in both Malmö and Lund that microwave-induced hyperthermia treatment of tumour recurrence could be successfully combined with radiation therapy (Lindholm et al., 1987; Lindholm et al., 1995).

This also led to trying to combine radiation therapy with the syngeneic tumour vaccine that Leif G Salford, in the BRIGTT project, successfully developed. In a patient study, this vaccine significantly extended the survival time (Salford et al., 2022).

Using the same rat glioma model used in the BRIGTT project to develop a syngeneic tumour vaccine, gliomas inoculated in rats with syngeneic radiation-sterilized IFNγ-transfected tumour cells were treated with the tumour vaccine and only one fraction of radiotherapy with 5 or 15 Gy.

The results with 5Gy showed a surprisingly high percentage of 75% complete remissions of implanted brain tumours, which had not previously been achieved with any other therapy regimen against glioma tumours. However, due to the sparse number of individuals in the study, the result was perceived as too uncertain to be published.

However, with the help of Silvia Formenti and Sandra Demaria, who, in their immunotherapy experiments with the checkpoint inhibitor CTLA 4, observed the same phenomenon, the results after 5 years in the drawer could be published in the journal Radiation Research (Formenti and Demaria, 2009, Persson et al., 2010).

In 2023, Koukourakis and coworkers in Greece presented a clinical trial with anti-PD1 immunotherapy and radiation therapy with 8 Gy intermittent ultra-hypo-fractionated treatment of patients with locoregional recurrent head and neck cancer (Koukourakis et al., 2023). Three cohorts of patients were treated with only

one fraction of 8 Gy, or 2-3 fractions of one fraction per week, together with *Nivolumab* anti-PD1 immunotherapy.

The results show that after administration of only one 8 Gy fraction of radiation, objective response rates of 57% were observed. In patients who received 2 or 3 fractions of 8 Gy with one fraction weekly, objective response rates (OR) above 80% were noted. With 3 fractions of 8 Gy, 66% complete remissions (CR) of primary tumour and CR 75% of metastases were obtained (Koukourakis et al., 2023).

They also treated patients with locally recurrent non-small cell lung cancer after conventional radical chemoradiotherapy. They were treated with anti-PD-1 immunotherapy in combination with one or two 8 Gy fractions with one fraction per week. This immuno-radiotherapy regimen was safe and produced 81.8% objective response rates. The complete response (CR) rate was 27.2%, while tumour regression of 80–100% of initial dimensions was noted in 63.5% of patients (Filippatos et al., 2023).

The conclusion is that anti-PD1 immunotherapy combined with ultra-hypo-fractionated radiation therapy with 5-8 Gy per fraction repeated weekly up to 3 times is a promising therapeutic regimen for patients with relapse after conventional treatment that cannot be addressed with surgery or other conventional treatment.

The Greek grip on one radiation fraction per week is completely in line with my vision of how immuno-radiotherapy could be implemented.

I also describe this in my publication "A Tale of a Promising Immuno-therapeutic Strategy for Malignant Glioma" (Persson, 2023). "*Thus a single irradiation fraction of eight gray(Gy) suggested being optimal for enhancing the effect of immune therapy by cell-vaccination or electro-chemotherapy, and could be applied in repeated sessions until complete remission occurs".*

Koukourakis et al.'s findings inspired this story about immunotherapy combined with intermittent ultra-hypo-fractionated radiation therapy. Hopefully, it will motivate further clinical evaluation as a therapeutic alternative for the treatment of patients with recurrent cancer after undergoing a full conventional radiotherapy regimen.

With clinical experience with different radiation dose levels of 5-8 Gy, different numbers of fractions and intermittent time intervals depending on the degree of recurrence and type of tumour, one can eventually hope for a randomized study of primary tumours with Immunotherapy and intermittent ultrahypo-fractionated radiation therapy versus conventional treatment.

It is also not entirely unthinkable that intermittent ultra-hypo-fractionated radiation therapy of 5-8 Gy and microwave-induced hyperthermia, when combined with immunotherapy, would bring about further improvements (Logghe et al., 2024).

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A story of immunotherapy combined with Ultra-hypo-fractionated radiation therapy

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Born : October 12, 1938 in Malmoe, Sweden.
1970 PhD, 2004 MD h.c.
From 1980 to 2005 professor of medical radiation physics
From 2005 to present: Professor emeritus at
Lund University, Sweden

Published : >400 scientific publications, >20 extensive reports and books.

Tutor of 40 doctoral dissertations on the faculties of medicine and science.

This story originated in a clinical study called "Brain Immune Gene Tumour Therapy" (BRIGTT) by Professor Leif G, Salford in Lund, which was initiated shortly before the turn of the millennium. He produced a specific tumour vaccine based on tumour cells extracted from tumour tissue from the glioma patient he previously operated on. The survival time of the vaccine-treated patients was prolonged, but none fully recovered.

In an attempt to improve the effect of vaccination, the tumour model was used in the pre-clinical tumour immunological research that was the basis for Salford's clinical vaccination study with malignant gliomas.

The vaccine treatment was combined with radiation therapy of Fischer 344 rats with N29glioma tumours inoculated into the brain, and the results showed that:

- Radiotherapy alone with a single dose of 5 Gy resulted in no survivors.
- In contrast, if immunization therapy with 3 rounds of vaccine was combined with only one fraction of 5 Gy radiation therapy, six out of eight treated animals survived (approx. 75%).

This unexpectedly positive result for a previously incurable tumour spurred me to try to work for a new tumour treatment regimen with immunotherapy in combination with hypofractionated radiotherapy in only a few fractions.

Now after 20 years, clinical trials have begun to be reported showing that immunotherapy combined with hypofractionated radiation therapy works well with immune checkpoint inhibitors, which are used to block CTLA-4 and PD1, so that the killer cells, the cytotoxic T lymphocytes CTL, do not prevented from eliminating the cancer cells.

Koukourakis et al.'s results inspired this story about immunotherapy combined with intermittent ultra-hypo-fractionated radiation therapy, which I hope will motivate further clinical evaluation as a therapeutic option for patients with recurrent cancer after undergoing a conventional treatment regimen.

Lund Januar 2, 2025, Bertil RR Persson PhD, MDhc, professor emeritus