Preclinical RBE for normal tissue damage established in in vivo models

Aims and Perspectives:

The focus of this application is to study the biological effects of proton irradiation, a key issue in particle therapy, and determine the impact of these. We will deliver the supporting biological data in order to provide patients with the most optimal proton therapy. This proposal addresses the biological effect in normal tissue and the increased effect of the distal edge of the Spread Out Bragg Peak (SOBP). This enlarged effect in part of the beam has given rise to increasing concern in the use of proton therapy, as clinical examples of unexpected normal tissue toxicity has emerged in patients treated with proton therapy [1,2]. To be able to comply with these issues and avoid late effects, it is crucial to have in vivo biological studies to determine the extent and magnitude of this increased biological effect.

The project aim to test the hypothesis that increased LET in the distal edge of the SOBP translates into an increased biological effect, which can be quantified in biological systems. The proposed project aims to *in vivo* determine the relative biological effectiveness (RBE) of protons in a in vivo model of normal tissue damage, to clarify the increased effects of the distal edge of the SOBP, and to enlighten the influence on fractionation on these factors.

Introduction:

Particle therapy as cancer treatment provide a more favorable dose distribution compared to x-rays, effectively reducing unwanted dose to healthy tissue and achieving higher conformal dose distributions [3]. However, there is still a range of unresolved radiobiological questions that need to be answered in order to fully exploit the advantages of particle therapy [4,5]. While the physical characteristics of particle radiation have been the aim of intense research, less focus has been on the actual biological responses to particle irradiation.

Relative biological effectiveness (RBE) is a concept which has been introduced to account for an increased efficiency of protons and other particles relative to conventional radiotherapy. This efficiency is due to an increased linear energy transfer (LET), a measure of the quality of different types of radiation [6]. RBE is defined as the ratio of a dose from photons to a dose from any other particle to produce the same biological effect. This is complicated by the fact that RBE is not a constant, but depends, among others, on the specific tissue, on the particle energy, and on the dose [7].

Currently, an RBE value of 1.1 is used in clinical situations to calculate the equivalent biologic dose for proton therapy relative to photon therapy [8]. However, the LET increases drastically in the last few microns of the particle track [9], which in vitro studies have shown to translate into a higher RBE [10–14] (fig 1), and there is an increased concern that the RBE of 1.1 is an oversimplification, which may have clinical implications [1,2].

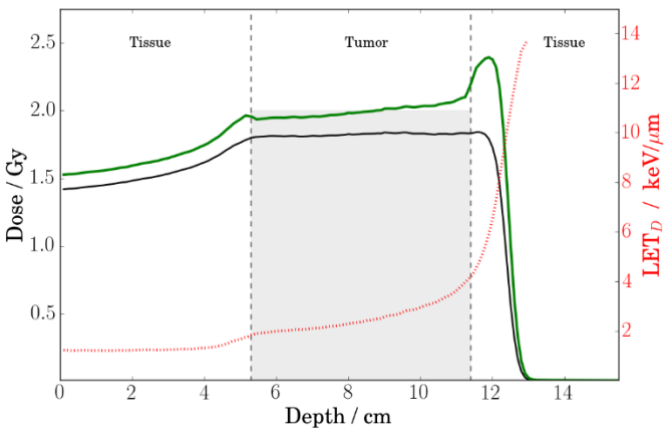

Fig 1. Dose depth curve showing the physical dose (black line) of a 6cm Spread Out Bragg Peak (SOBP), potentially covering a tumor. The LET (Linear Energy Transfer) is shown as the read line, and as can be seen, the LET is increasing towards the end of the SOBP. This has in in vitro data shown to induce an increased RBE, so that the biological effect is increased in the distal edge of the SOBP (green line). In vitro data is from [14]. *Figure by Armin Lühr, OncoRay*

The in vivo data to support is very limited, with a study on rat spinal cords confirming this distal edge effect [15]. We have in vivo data on acute skin reactions of proton irradiated mice in different positions of a proton beam [16] showing an increased effect in the last part of the SOBP. This study demonstrates the feasibility of delineating the distal edge effects in vivo. However, to fully understand the extent of the varying RBE and the clinical impact, factors as fractionation and different tissue types must be taken into account. To be able to comply with these issues and avoid late effects, it is crucial to have experimental biological studies to determine the extent and magnitude of these biological effects.
The aim of this project is to establish the RBE of acute skin damage and radiation induced fibrosis in vivo. This will include simulation of clinical treatment and different positions in the beam.

Research Plan:
Study 1: RBE of acute and late effects in vivo.
This study of normal tissue effects will be performed on an already established in vivo model in mice, where the right hind leg of the mice is irradiated. This model contain tissue endpoints representing both early and late radiation induced reactions, respectively acute skin reactions (moist desquamation of irradiated areas of the skin [17]) and radiation induced fibrosis, a late reaction of tissue to radiation [18].
For the irradiation, a setup already established at tested for both photon and proton irradiation is used [16] See fig 2. In short, the mice are restrained an a small jig, which is placed on a plate on a waterbath, allowing the target leg only to be submerged in the water and in the radiation field. The mouse body is shielded with lead (for photon irradiation) or brass (for proton irradiation). The mice are irradiated without anesthesia.

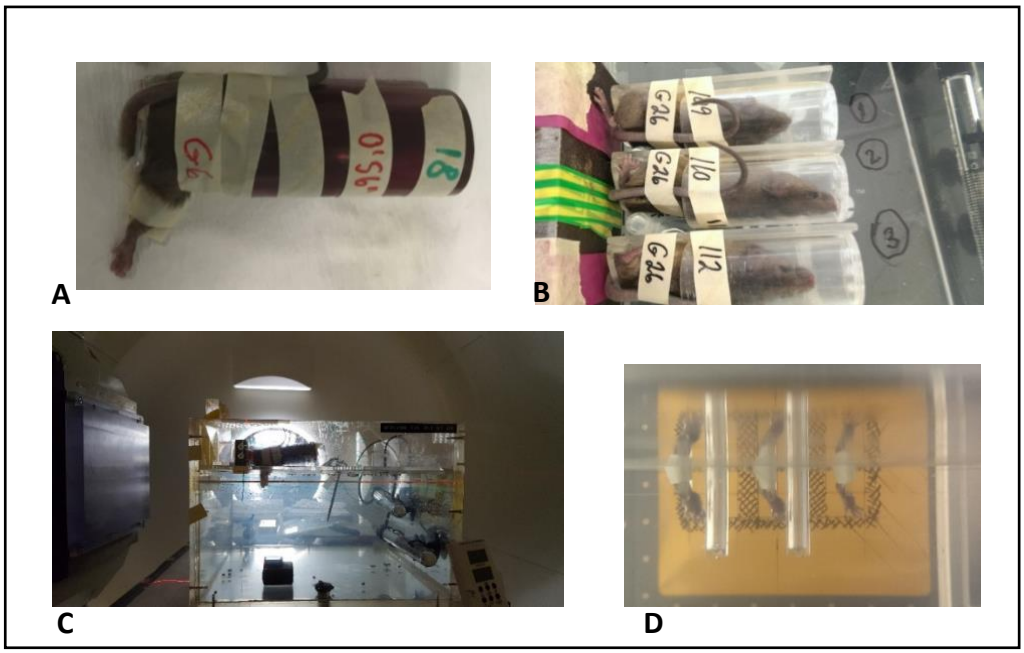

Fig 2. Setup of mice for irradiation. ***A.*** *The are irradiated without anesthesia, but are restrained in a jig, with the target leg fixed.* ***B.*** *The mice are placed on a waterbath, and three mice at the time are irradiated. The mouse body is shielded to avoid irradiation of internal organs.* ***D.****View of the mice placed on the waterbath.* ***C.*** *Only the target leg is submerged in water. Here is a view in through the waterbath, and the legs are placed in the radiation field.*

After irradiation, the acute skin reactions are scored blindly by evaluating the irradiated skin. Mice will be observed on daily basis from day 1 to day 30, and the percentage of animals in each treatment group showing moist desquamation of the treated foot are recorded [34]. The late reaction of tissue to radiation will be

assessed using the leg contracture model. The difference in extensibility of the irradiated legs of untreated and treated animals is measured every second week from day 1 to day 356 [35].

For both endpoints, a range of doses will be used to produce full radiation response curves, for both protons and x-rays. For x-rays, 6 MV photons from the clinical LINAC will be used, as there is a biological difference between orthovoltage and megavoltage xrays [16]. The RBE will be calculated as the ratio of the isoeffect dose for x-rays to that of protons. The doses and the number of animals per dose-point is determined based on previous data, with sufficient animals per dose group to allow for meaningful statistics. To ensure sufficient power for the statistical analysis, we have simulated the dose-response curve with the expected increase in effect for the proton irradiation, and confirmed that this would result in statistical significance.

After follow up, the mouse legs are removed and fresh frozen for additional histological analysis, which are performed in collaboration with Professor Verena Jendrossek in Essen, Germany. Here the mouse legs will be sectioned analysed with staining for Trichome, CD31/34, SMA and TGFb.

Study 2- Distal edge effects

There has been reported both in vivo and in vitro an increased RBE in the distal edge of the SOBP. To take this factor into account in a clinical setting, the effects needs to be thoroughly mapped in regards to magnitude and tissue specific effects. We will in this study conduct full dose response curves in different positions of the SOBP.

The endpoints for normal tissue damage (both acute and late damage) will be assessed in different positions of the beam, to measure the enhancement ratio (ER) from the middle of the SOBP to the distal edge. This is possible to measure with our setup, because we monitor the damage in the mouse leg. This ensures a high enough resolution to see the changes induced by an increase of LET that occurs within very few millimetres. Previous in vivo experiments addressing the distal edge problematic have irradiated either whole body or the mouse thorax in the middle of the SOBP and in the distal edge, and found only a small increase in effect in the distal part of the beam, compare to in vitro data [19,20]. Due to the size of a mouse body, this is averaging over a large part of the SOPB, diluting the effect. In a clinical situation, an enhanced effect even only in a few millimetres might have severe impact.

Study 3 – Fractionation effects

In the clinic, radiotherapy is delivered fractionated due to fewer toxic effects on the healthy tissue. As RBE in vitro has been demonstrated to increase with decreasing dose, fractionation of proton radiation can lead to a higher RBE, both for tumor and normal tissue [21,22]. To mimic this, all experiments will therefore not only be conducted with single doses, but also in a clinical relevant fractionated scheme.

To establish an appropriate fractionation level using the clinically relevant dose of 2Gy, the first step will be to conduct a study with varying number of fractions (25-40 fractions) to establish at which level the mice will start to develop radiation induced fibrosis with this fractionated scheme. When a suitable fractionation level has been determined, full dose response curves will be performed with both protons and xrays. For the protons, the position in the beam will be determined based on the outcome of study 2, so both the position with the highest RBE and the lowest RBE will be examined for fractionation effects. This will demonstrate the impact of fractionation on the distal edge effect.

The fractionation studies will be based on radiation induced fibrosis, as the mice are not expected to develop severe acute damage due to the fractionated. Any acute effects will be monitored and reported.

Time Line:
This is planned as a three year phd project, starting early 2019. The time plan is as follows:

Year 1:

- Dose-response curves for clinical relevant fractionated radiation (2 Gy pr fraction) will be established on reference radiation (6MV photons)
- In the middle of 2019, the experimental beam room DCPT will be ready, and we will start proton radiation. First part will be perform dose response curves for one fraction in different parts of the beam to investigate the impact of LET on radiation induced normal tissue damage

Year 2:

- When the dose-response curves for fractionated radiation are established, these will be repeated with proton irradiation in different positions in the beam
- Follow up of irradiated animals
- The data on Acute damage will be ready
- Histology analysis on sections of mouse legs

Year 3:

- Follow up of irradiated animals
- Histology analysis on sections of mouse legs
- Data on late damage will be ready
- Data will be analyzed and published

Clinical and national perspectives:
With the establishment of the Danish Centre for Particle Therapy, we have the platform and the expertise to perform internationally recognised, substantial, clinically implementable research in the biology of proton radiation; a new, upcoming research area.
The knowledge gained by the proposed research will uncover the biological potential and improve our understanding of the radiobiological properties of proton radiation. This is needed to secure treatment with proton radiation. This will fully unlock the biological potential of particle therapy and provide data for the development of biological models and implementation of these in treatment planning systems for particle therapy.

Workplace, research team, Collaborators and Resources
The project presented here is a collaboration project of The Department of Experimental Clinical Oncology (ECO), and at The Danish Centre for Particle Therapy (DCPT). DCPT will feature unique, state-of-the-art experimental facilities, including dedicated experimental beam room with full access to beam time, and both animal - and in vitro facility. The biology research will be integrated with the physics research at the particle facility. ECO has a longstanding experience in experimental radiation oncology, and in radiation induced normal tissue damage in animal models. ECO's facilities include all the necessary equipment for radiobiological studies including mouse facilities.

The supervisor team will consist of:
Brita Singers Sørensen is PI of the project and will be main supervisor. She will lead the animal experiments. Niels Bassler, Medical Radiation Physics, Department of Physics, Stockholm University, is collaborating physicist on the project, and will be responsible on the dosimetry and physical validations.

Professor Cai Grau, Head of research at DCPT.
Professor Dr. Verena Jendrossek, Head of Molecular Cell Biology, University of Duisburg-Essen, Germany, is collaborator on the project. The histological analysis of the samples will be performed in her laboratory.

Ethical considerations

All experiments will be performed according to current national and European guidelines, and with the appropriate licenses. Animal experiments will be performed under licenses issued by the Danish Animal Supervisory Committee; these have already been issued (license no. 2015−15−0201−00624).

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BRITA SINGERS SØRENSEN - Curriculum Vitae

Birth date: 181077
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Current position: Associate Professor, Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark

Education: PhD in Medicine, 2009, University of Aarhus, Denmark
M.Sc. in Molecular Biology, 2004, University of Aarhus, Denmark
B.Sc in Biology, 2002, University of Aarhus, Denmark

Total number of Publications and citations (Scopus): 40/1010, **H-index:** 16
Total number of scientific presentations: >35

Scientific Focus Areas: The radiobiological effect of particle therapy
Experimental radiobiology
Biomarkers of the tumor microenvironment

Leadership: -Leading the National Infrastructure for Experimental Radiation Oncology (NIERO), WP10 under DCCC – National Research Center for Radiotherapy
-Co-organizer of EPTN (European network on Particle Therapy), WP6: Radiobiology and RBE.
-Leading the planning of the radiobiology research at DCPT (Danish Centre for Particle Therapy)
-Leader of WP1, Particle Radiobiology, in "Research in Particle Therapy at the Danish Centre for Particle Therapy"

International Relations: 2006-2018: Experiments performed at CERN, the European Organization for Nuclear Research, at GSI, Darmstadt, Germany and at Institute of Nuclear Physics in Krakow, Poland
2012-2013: Visiting Scientist, British Cancer Research Institute, Vancouver, Canada
2002- 2004: Master project performed at MCRI, Melbourne, Australia.

Academic awards and honours (Selected):
2015: VARIAN-Juliana Denekamp Award
2012: Poster Award, ICTR-PHE 2012, Geneve, Schwitzerland
2010: Junior Investigator Award, The Tumor Microenvironment, Hypoxia, Angiogenesis & Vasculature, 12th International Workshop, Toronto, Canada.

Major Grants: **2017: Danish Cancer Society. 2.220.000 dkr** to the project: Biologiske effekter af partikelterapi
2014: Danish Cancer Society. Knæk Cancer – Talentfulde unge kræftforskere (Talented Young Cancer Scientists), 3.030.000 dkr to the project: Radiobiologi i Partikelterapi
2013: Dansk Kræftforskningsfond, 150.000 dkr to the project: Particle Therapy – Radiobiological Optimization in Hypoxic Tumors

Supervision: From 2011: Two Phd projects, 5 Master projects and 1 Bachelor project

PhD assesments: University of Copenhagen, Denmark; University of Oslo, Norway; Aarhus University (Chairman)