Radiobiology Research with Laser Driven Ionizing Radiation (LDIR)

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EUCALL Workshop: Biology at Advanced Laser Light Sources European X-ray FEL, Schenefeld, Germany, 2017
Complex tumor therapy

Advanced diagnostics

Improved outcome

Targeted therapy

Immuno therapy

Radiotherapy

Chemotherapy

Surgery
Ionizing radiation effects in space and time

From atomic-, molecular level to whole body

Immuno-activation
Increase of the therapeutic ratio

Timing
- Fractionation
- Hypo-, Hyperfract.
- Acceleration (CHART)
- Prolongation
- Flash

Combined treatment
- sensitisation/protection
  - Chemo-, hormon, biol.m., hypoxic RT-sensit., immunth.

Technical development
- Conformal RT, IMRT, IGRT,
  - dose painting
- HadronRT, MRT, BNCT, BPF
Hadron therapy
new gen. part. acc

120 years
photons/electrons
3DCRT, IMRT, SRS/ SBRT/ SABR

Selectivity, accuracy (mm), beam quality divergence, dose rate (10Gy/min)

IGRT
Motion control

Hadron therapy
3DCRT, IMRT, SRS/ SBRT/ SABR

Selectivity, accuracy (mm), beam quality divergence, dose rate (10Gy/min)
Dose depth curves

Electrons (21 MeV)

Carbon (270 MeV/u)

Photons

Protons

Relative dose [%]

Depth [mm]

Dose depth curves

Isolated lesions

Clustered lesions

Very dense ionisation

High LET

Low OER

High RBE

Low indirect action
Proton Therapy Scientific Milestones

1931 First cyclotron (E.O. Lawrence, LBNL)
1946 Biomedical advantages of Bragg-peak (R.R. Wilson, LBNL)
1947 184-Inch Synchrocyclotron (E.O. Lawrence, LBNL)
1948 First biology experiments using protons (C.A. Tobias, LBNL)
1955 Human therapeutic exposure (LBNL)
1993 Bevalac closed (LBNL)


LBNL 30  LBNL (helium ion)  2054
Uppsala, Sweden  73
MGH, Cambridge, MA
Moscow
Dubna  84
St. Petersburg
Chiba, Japan
Tsukuba, Japan  700
Villigen, Switzerland  3712 +
Clatterbridge, UK  1201
Loma Linda  7176
Louvain, Belgium  21
Nice, France  1951
Orsay, France  2157
Bloomington, IN  34
Faure, S Africa  398
Davis, CA  448
Vancouver  77
Berlin  317
Catania  24
Kashiwa  161
Hyogo  60
Wakasa  2
Boston  229
Tsukuba  145

1st Hospital based facility

Range in mm of ocular tissue
Mainly indirect action - OH

High LET
- Very dense ionisation
- Clustered lesions
- High RBE
- Low OER

Low LET
- Mainly indirect action - OH
- Isolated lesions
- Very dense ionisation
High-LET Particle Therapy Milestones

- **1956**: Scientific justification of using HCP for therapy
- **1971**: Heavy ions in Bevatron
- **1971**: Radiobiology using neon ions
- **1975**: Bevalac completed
- **1976**: HCP therapy trial at Bevalac
- **1993**: Bevalac stand down

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1950</td>
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<td>1960</td>
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<td>1990</td>
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**Clinical Trials**
- LBNL, Berkeley
- BHIC, Berkeley, design study
- HIMAC, Chiba, Japan, 917
- GSI, Darmstadt, Germany, 84
- EULIMA, EU, feasibility study
- ITEP, Moscow, design study
- Hyogo, Japan, commissioned
- Heidelberg, Germany, gb
- Stockholm, Sweden
- ADROTERAPIA, Italy
- MedAustron, Austria

- >25000 patients
- Hospital-Based Medical Accelerators
Compact superconducting synchro-cyclotrons (IBA, Varian, Mevion) provide a KHz proton source with nanoampere current with 34 to 250 MeV.
Hadron centers

54 centers are in operation and further 40 is planned

<2% of all RT
FLASH irradiation: <500ms pulses of >40 Gy/s

A 17 Gy conventional irradiation induced pulmonary fibrosis and activation of the TGF-beta cascade in 100% of the animals 24-36 weeks post-treatment, as expected, whereas no animal developed complications below 23 Gy flash irradiation, and a 30 Gy flash irradiation was required to induce the same extent of fibrosis as 17 Gy conventional irradiation.

Synchrotron-based Microbeam radiation therapy (MRT) Under preclinical evaluation

MRT: spatially fractionated, planar x-ray (50-600keV) /proton/electron/ 25-75 micron-wide beams, with a very sharp penumbra, separated by several times of their beam width.

Dose profiles of alternating peaks and valleys with high peak-to-valley-dose-ratios (PVDR)

Synchrotron-based MRT resulted in 10 fold prolonged survival of the treated animals with brain tumor xenograft

Peak entrance doses of several hundreds of Gy are extremely well tolerated by normal tissues and at the same time provide a higher therapeutic index for various tumor models in rodents.

E. Brauer-Krisch a, J-F.s Adam et al. Medical physics aspects of the synchrotron radiation therapies:Microbeam radiation therapy (MRT) and synchrotron stereotactic radiotherapy (SSRT) Physica Medica 31 (2015) 568e583
Donzelli et al.: Conformal image-guided MRT at the ESRF

With the implementation of conformal image-guided MRT, the treatment of deep-seated tumors in large animals will be possible for multiple port irradiations.

Physiologically gated microbeam radiation using a field emission x-ray source array

Daniele Pelliccia, Jeffrey C Crosbie, and Kieran G Larkin
Phase contrast image guidance for synchrotron microbeam radiotherapy
Physics in Medicine & Biology
Boron Neutron Capture Therapy (BNCT)

Thermal neutrons captured by high probability by $^{10}\text{B}$ desintegrates into two particles.

The two particles $\alpha$ and $^{7}\text{Li}$ absorption ranges in tissue (~9 mm and ~5 mm respectively). All the energy is released inside the tumor cell.
BNCT
Selective, cell-targetted energy deposition

- High LET, dense ionization
- High RBE
- Low OAR
- Binary approach
Requirements on the $^{10}$B carriers

- Low systemic toxicity
- Selective uptake into the tumour cells
- Rapid clearance from normal tissues
- High intratumoural concentration (20 μg/gTumor)/
  >100 ppm)
- Favourable intracellular distribution (preferably in the cell nucleus)
Neutron beam requirements for BNCT

- e\textit{pithermal neutron flux} \quad \cong 10^9 \text{ neutrons/cm}^2\text{s} \\
  \text{(at the therapy position)}

- neutron energy \quad \sim 1 \text{ eV to } \sim 10.0 \text{ keV}

- gamma dose rate \quad \leq 1.0 \text{ Gy/hr}

- fast neutron dose rate \quad \leq 0.5 \text{ Gy/hr}

- current:flux (J/\Phi) ratio \quad > 0.8

- the parameter J/\Phi \text{ reflects the forward directionality (degree of collimation) of the beam of neutrons, which equals 0.5 for a completely isotropic beam and 1.0 for a purely parallel beam}
Dose components

D$_{\text{Boron}}$
D$_{\text{Nitrogen}}$
D$_{\text{Photon}}$
D$_{\text{neutron}}$
Clinical application of BNCT N>200

Malignant melanoma
10B carrier: BPA+BSH

Extracorporeal liver BNCT

10B carrier: BPA

Recurrent H&N tumors

10B carrier: Boro-phenylalanine

HFR Research reactor
10B carrier BSH
Na$_2$B$_{12}$H$_{11}$SH

50 years
Neutron sources for BNCT

Nuclear reactors
Charged particle accelerators
Compact neutron generators
LINAC based neutron source

High power laser facilities may provide via (p, n) reaction intense epithermal neutron beam
BPF $^{11}\text{B}$ $(p, 3\alpha)$ reaction occurs between protons and boron-11, without producing high-energy neutrons.

The highest cross-section of this reaction occurs with protons having energies around 600-700 keV corresponding to the Bragg peak.
D. Adam and B. Bednarz, SU-F-T-140: Assessment of the proton boron fusion reaction for practical radiation therapy applications using MCNP6, Med. Phys. 43 (2016) 3494

Jung JY, Yoon DK, Barraclough B, Lee HC, Suh TS, Lu B Comparison between proton boron fusion therapy (PBFT) and boron neutron capture therapy (BNCT): a Monte Carlo study. Oncotarget. 2017 Feb 25


Both chromosoma abberation analysis and colony forming assay confirmed the enhanced effectivity of BPR in cell cultures using natural (80% \(^{11}\text{B}\) containing) BSH at a 62 MeV proton source
PBR Enhanced Proton Therapy
PBREPT

In addition to selective proton therapy

- High spatial resolution
- High LET,
- High RBE
- Low OAR

Binary approach
Simultaneous dose and LET optimisation has a potential to achieve higher tumour control and/or reduced normal tissue control probability.

**LET-painting increases tumour control probability in hypoxic tumours**

N. BASSLER J. TOFTEGAARD et al.
Acta Oncologica

Figure 3. Dose and dose-averaged LET profiles shown in left and right column, respectively. First row is a carbon-12 ion plan using four conventional fields with homogeneous dose. The highest LET is then found at the rim of the SOBP as seen in the upper right figure. LET-painting, as shown in the middle row, allows for redistributing LET to cover the assumed hypoxic structure, depicted as the black entity, with increased LET. The energy fluence budget for the amount of particles used is the same for both plans in the upper two rows. The last row shows LET-painting again, but now with oxygen-16 ions, resulting in a pronounced increase of LET in the HTV, HTV, hypoxic target volume; LET, linear energy transfer; SOBP, spread out Bragg peak.
High LET radiation in combination with immunotherapy is a highly promising new approach.

RT Paradigm shift: local → systemic effect
# Exploring the potential of LDIR

**RADIOBIOLOGY**

<table>
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<td>As reference radiation</td>
<td>Ultra short pulse - time resolution</td>
<td>Ultrahigh dose rate</td>
<td>Extreme small beam – spatial resolution</td>
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<td>for comparison</td>
<td>High repetition rate</td>
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**SETUP DESIGN, DOSIMETRY, DOSE CALCULATION,**

- Effects on normal tissue / tumor response
- RBE of pulsed, ultraintense beams
- BNCT, BPREPT $^{10}$B/$^{11}$B carriers
- MRT Flash

**Effective, safe application**
Exploring the potential of LDIR

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- Effects on normal tissue / tumor response
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**Classic In vitro and in vivo biological systems**

- Development of novel vertebrate model

**Assessment of morphologic, functional, cellular, molecular changes of different normal tissue and tumor models**
Traditonal in vitro model cell cultures

Colony forming assay

MTS assay

Rat model for focal brain injury

**Anaesthesia:** i.p. chloral-hydrate

**Positioning:** special bunk-bed

**Source:** 1,26 MeV energy Cobalt

**Dose:** 40 Gy (2x20Gy)

**Irradiation:** 10 mm diameter collimator, homogen irradiation of hippocampus (at both hemispheres)
Mouse experiments with electron beam

**Anaesthesia:** i.p. chloral-hydrate

**Positioning:** on one side

**Source:** 6 MeV energy Siemens linear accelerator

**Dose:** 40 Gy

**Irradiation:** 5 mm diameter collimator
Research on RBE using RT modifying agents

**Anaesthesia:** i.p. chloral-hydrate

**Positioning:** special bunk-bed

**Source:** 6 MeV energy Siemens linear accelerator

**Dose:** 40 Gy

**Irradiation:** 10 mm diameter collimator, homogen irradiation of hippocampus (at one hemisphere)
Neurofunctional tests

Morris Water maze

Open-field test

Passive avoidance
Histopathological evaluation of effects of focal brain irradiation
Exploring the potential of LDIR

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Classic *In vitro* and *in vivo* biological systems

**Development of novel vertebrate model**

Assessment of morphologic, functional, cellular, molecular changes of different normal tissue and tumor models
Zebrafish (*Danio rerio*):
- Easy to handle, good reproduction captivity
- Embryo and larva body transparency
- External fertilization
- Rapid embryonic development
- Genomic similarity to the human genome
  - The complete genome sequence was published (2013)
- Availability of several transgenic lines
- High resilience.
- Size of the embryos and larvae is 0.5-2 mm
- Can be kept in standard plates (4-96 wells) and plastic bags, eppendorf tubes

- Rapid development

Embyo size is corresponding to a 3D cell culture size (4-500um)
Irradiation of zebrafish embryos

Wild type zebrafish embryos (24 hpf) were irradiated with photons at 5 - 20 Gy dose and reactor/cyclotron neutron-photon mixed beam at 1-8 Gy dose.
End points of zebrafish embryo experiments

detection of malformation and survival

Control

10 Gy

5 Gy

15 Gy

20 Gy
Survival and malformation depending on the age of the embryo

6 hpf

24 hpf
Survival, malformation of different beams for RBE definition

**Photon**

- Survival %
  - Time (dpf)
  - CO
  - 5 Gy
  - 10 Gy
  - 15 Gy
  - 20 Gy

- Distortion %
  - Time (dpf)
  - CO
  - 5 Gy
  - 10 Gy
  - 15 Gy
  - 20 Gy

**Neutron**

- Survival %
  - Time (dpf)
  - CO
  - 1.25 Gy
  - 1.875 Gy
  - 2 Gy
  - 2.5 Gy

- Distortion %
  - Time (dpf)
  - CO
  - 1.25 Gy
  - 1.875 Gy
  - 2 Gy
  - 2.5 Gy

**RBE** = \( \frac{LD_{50\text{ph}}}{LD_{50\text{n}}} = \frac{20}{2} = 10 \)
Macroscopic morphological abnormalities of the zebrafish embryos
Distance between the two ends of the embryo
Diameter of the Yolk sac
Diameter and circumference of the eye
Feasibility study at the proton source of the LION Facility Munnich

36+ 18 hours transport, 10 hours at the laser facility was well tolerated

The system is highly adaptable to the industrial conditions, and to the limitations of the beam (direction, energy, size)
30-24/6 bad shots

micro-opthalmia
Microscopic lesions of the zebrafish embryos

Control

After 10 Gy
Inflammatory cytokine expression (RT-PCR)

Tissue interleukin-1β levels

Tissue NFκB levels

1 h after irradiation
2 hrs after irradiation

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<th>GPC</th>
<th>RT</th>
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<tr>
<td>1 h</td>
<td>1.0</td>
<td>2.5</td>
<td>3.0</td>
<td>2.5</td>
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<tr>
<td>2 hrs</td>
<td>1.5</td>
<td>2.0</td>
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* p<0.05 vs GPC + RT
# p<0.05 vs GPC

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* p<0.05 vs Control
The development of high power laser driven particle acceleration (VHEE, proton, neutron) with ultrahigh time and space resolution, could accelerate the implementation of promising novel methods - energy modulation, high resolution IMRT - multi particle beam radiation, - Microbeam radiotherapy MRT, - Flash RT - BNCT, BPF enhanced proton therapy

Intensive radiobiology research is essential to explore, and optimize these techniques for clinical application.

We propose a new vertebrate model (zebrafish embryo) for preclinical radiobiological research at laser facilities.
Zebrafish embryo model is proposed for radiobiology research at laser driven ionizing radiation sources and for intercomparison on the biological effectiveness of the laser accelerated particle beams at different centres.
THANK YOU FOR YOUR ATTENTION!

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