ESRF	**Experiment title:** Imaging of imobilised magnetic target-nanoparticles: liposomes and ferrofluids entrapping target for magneto-photodynamic X-ray therapy of cancer M-PXT	**Experiment number**: MD163-1 (part1)
Beamline:	**Date of experiment**: (part1) (part2 : November 2005) from: 26.9.2005 to: 30.9.2005	**Date of report**: 9.10.2005
Shifts: 9 (+9)	**Local contact(s)**: PD. Dr. Alberto Bravin	*Received at ESRF:*

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Report: preliminary report : for part1 of the **MD163** experiment (including MD128 & MD118 results in part), due to urgent success , important for cancer treatment.

Abstract: Biocompatible heavy metal complexes for **photon activation therapy of cancer** PAT in free form and as bound to nanoparticles where examined for treatment and imaging. The imaging used as an indicator for X-ray absorption, sufficient for later cancer treatment.
Basing on the results of the successful earlier ESRF-experiments **MD118** (L-edge detection & localization of heavy metals in bio-nanoparticles, magnetic liposomes at **ID1**) and **MD128** (first K-edge detection, absorption and imaging of metal- bio-nanoparticles, at **ID17**) we made in the first part of the **MD163** experiment a tremendous **progress towards cancer treatment**:

The **biocompatible** lanthanide complex **Lutetium-DTPA**, in a novel highly concentrated formulation, was successfully applied for **X-ray phase contrast imaging** after injection into the brain of a living rat. The X-ray experiment was done exactly at that energy, which will be later used for **cancer treatment** (63.9 keV). The **animal survived** the treatment with 10 µl of 0.253 M LuDTPA (0.44 mg Lutetium) until the end of the experiment (no brain oedema was observed). The agent was visible during 3.5 h, while the complex diffused slowly into an area of 5-10 mm size. This covers the area of a typical brain tumor in rat (Glioblastome, 3 – 4 mm). Thus we are **ready for a first cancer treatment** pre-study with brain tumors. This shall be the focus of the further experiments, in parallel to necessary work on metal nanoparticle detection and imaging. It has to be noticed that the current success offers opportunities for novel cancer treatment with concentrated **Lanthanide complexes of (Lu, … Gd)** with Synchrotron X-ray as well as with Neutron radiation.

1. Strategy for Cancer treatment by indirect radiation therapy (IRT)

Radiation therapy benefits of the enhanced radiation sensitivity of many cancer cells as compared to healthy tissue. Indirect radiation therapy (IRT) reduces the unfavorable side effects evolving from radiation absorption in the healthy tissue outside the tumor by application of target materials, which upon specific absorption of external radiation, develop secondary radiation of short range or tumor-toxic products, e.g. free radicals.

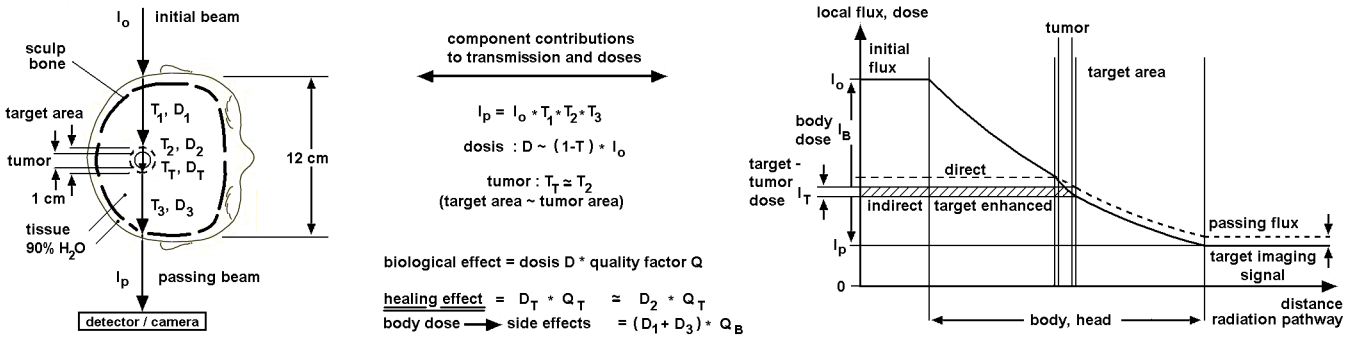

Fig.1: Scheme of indirect radiation therapy of brain cancer and imaging for the enhancer target Lutetium.

Indirect radiation therapy is possible with synchrotron X-rays (photon activation therapy PAT, PXT) and neutrons (neutron capture therapy NCT). In both cases the healing tumor dose ($\mathbf{I_T}$, $\mathbf{D_T}$) has to be significant as compared to the non-specific body dose ($\mathbf{I_B}$, $\mathbf{D_B}$), as this causes unfavorate side effects, which may reduce life quality unbearably, or even may cause secondary cancer years after the treatment. As shown in fig.1 this is difficult because of the high body absorption of neutron and X-ray radiation (energy dependent). A further requirement arising from tumor biology is the high effectivity of tumor treatment: a tumor, which is incompletely inactivated by the treatment would desintegrate, and release residual cancer cells, which subsequently form metastases. Consequently, two treatment concepts are usual:

a) **Adjuvant** treatment = complete healing (no cancer cell of 10^7-10^8 in the tumor survives the treatment)

b) **Palliative** treatment=treatment with the aim of life prolongation and quality improvement (with metastasis)

We have developed a stringent strategy for the adjuvant cancer treatment, With the above calculations, this leads us to an "imaging-treatment theorem" for indirect radiation therapy:

An effective (adjuvant) cancer therapy target should be visible by *in vivo* contrast imaging !

The desired high target absorption can be obtained by biocompatible Lanthanide complexes and nanoparticles. Additionally we plan the application of double therapy by double-entrapping nanoparticles: radiation therapy by metal content and chemotherapy by drugs included in the same coctail (epirubicine, doxorubicine etc.). The sensitive detection, imaging and treatment is achieved by complementary experiments at ESRF-ID17 and ID1.

2. Preliminary work important for cancer treatment

- Lanthanide- complexes (DTPA): By liposome- and protein- structure investigations at DESY-HASYLAB (report) and ESRF (ID1: LS1718) we developed biocompatible targets (level-1, 50 mM). In cooperation with industry (FerroMed), the metal complexes were improved twice for the MD128 and MD63 experiments (level-2 = 0.1 M; level-3 = 0.25 M Lanthanide in biocompatible DTPA-formulation, pH7.2)
- Liposome structure and dynamics was a) investigated by neutron scattering at ILL-D22 (TR-SANS); b) incorporation of membrane proteins in liposomes → important for antibody-target-liposomes (later).
- Magnetic liposomes were developped and investigated at ILL-D22 (report, Nawroth et al. 2004). In a second experiment series (2003/04) those liposomes contained Boron-targets for indirect radiation therapy.
- Ferrofluids of selectable size for cancer therapy (magnetic drug targeting) were developed, investigated (dynamic light scattering, electron microscopy) and applied (with Dr. Ch. Alexiou, Univ. clinics Erlangen).

3. Result - abstract of ESRF experiment MD118 at target *L-edges* (ID1 : 4/2005)

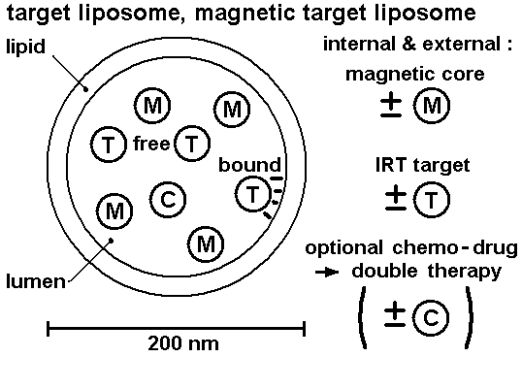

Fig.2: Lipsomes were investigated with metal targets **T** and magnetic cores **M**. The chemo-drug option **C** is important for the planed cancer double treatment.

The **MD118** experiment gave important information of magnetic target liposome structure and target entrapping for the later medical experiments at ID17. The results yielded a) the target entrapping and enrichment at membranes (direct use in MD128 & MD163) and b) target localization (important for later improvements of biological effect, quality factor Q in fig.1). The nanoparticles were :

- pure Liposomes ± metal chelates (Gd, Tm, Lu, Hf, cis-Pt)
- Ferrofluid cores (15 nm) ± metal chelates, entrapped & out
- Metal targets –DTPA, -Citrate: in & out: Gadolinium (Gd) Thulium (Tm), Lutetium (Lu), Hafnium (Hf), Platinum (Pt)

Results: At the L-edges the detection limit was 0.2 mM metal (Gd, Tm, Lu). Lanthanide-DTPA did not disturb liposome structure (1M KCl inside). Ferrofluid cores were entrapped, removal outside not required. Cis-Pt was 10x enriched at PE-membranes (Soy bean PL).

4. Results of ESRF experiments MD128 (7/2005), MD163-1 (9/2005) at target *K-edges*

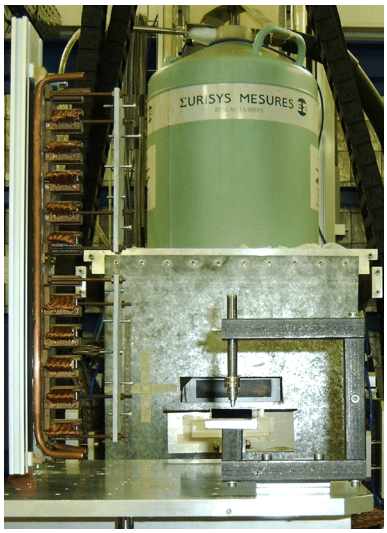

Fig.3: Sample environments for Teflon-sealed 10 mm Quartz-cells, setup at ID17

4.1. General & experiment setup

- **setup of multi-sample environment** with precise Quartz-cells (as for neutron scattering at FRJ-2, Jülich) ± magnets: fig.3
- **setup for tomography** and treatment for animals test with rat sculp and permanent magnets: see fig.4
- **chemical test** of late target radiaton products: H_2O_2-analysis by chemo-luminescence (Luminol + photon counter)
- **biological tests** for target radiation action:
  a) bacteria (*Micrococcus luteus*);
  b) rat Glioblastoma **cell culture** 9L-cells
- animal **dummy tests** (rat sculp + cup)
- **animal test with living rat:** injection of LuDTPA and time-resolved tomography (phase contrast), pharmaco-kinetics

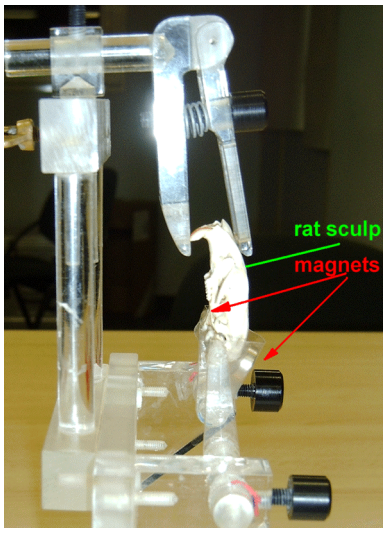

Fig.4: Tomography and treatment setup with rat sculp and permanent magnets

4.2. Absorption spectra - the key for an efficient target IRT application (target dose ratio $R_{TB} = I_T/I_B$)

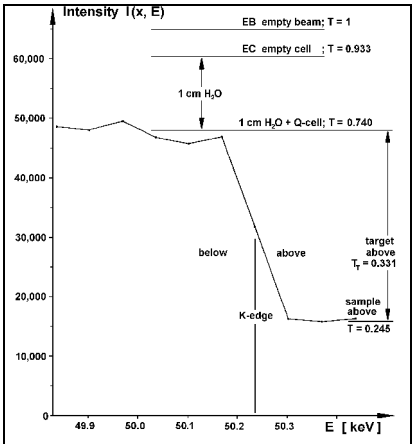

Fig.5: Abs.: 0.7 M Gd-Citrate

For the estimation of target efficency a set of 5 measurements in precise Q-cells was taken, as usual in neutron and X-ray scattering:

- sample (target) in solvent, Q-cell (10 mm)
- solvent (H_2O) in Q-cell (10 mm)
- empty cell EC (air), Q-cell (2.5mm Quartz
- empty beam EB (air), no cell
- noise of detector (dark, = black sample)

The results were the precise contributions of the components, as indicated in fig.1.
The K-edge absorption spectra of conentrated Lanthanide complexes are shown in fig.5 & 6

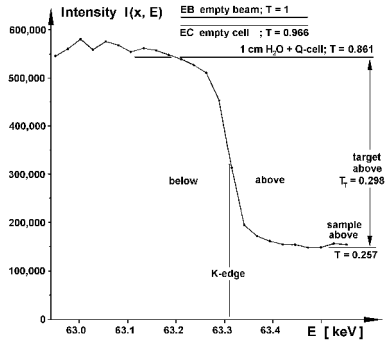

Fig.6: K-edge absorption spectrum of 1.14 M Lu-Citrate

An extract of the results is shown in table1 with data for the estimation of the unfavorate body dose by tissue/water absorption, which reversely depends on the penetration depth $d_{1/2}$ of the radiation at the target K-edge (~ $1/E_K^3$), and the target absorptions of some important elements, but for the physiologically possible (!) concentration. The comparison shows that, 1) Iodine (usual x-ray contrast agent) leads to high body dose by the low K-edge energy; 2) Platinum (cis-Pt) has a favorate K-energy, but fails by the low physiological concentration (additionnaly it is *strongly toxic and cancerogenic* !!); 3) Hafnium has sufficient K-energy, but the toxic properties are unknown until now; 4) the Lanthanide-DTPA complexes can be applied in high concentration (up to 0.5 M in biocompatible formulation); the K-energy increases from Lanthanum (40 keV, too low), over Gadolinium (50.3 keV, moderate, 3 cm water penetration), to Lutetium (63.3 keV, 6 cm water penetration), which at present looks best. At the head dose level, Lutetium is 1.6x as efficient as Gadolinium.

Table1: **Element**	**Z**	**E_K [keV]**	**$d_{1/2}$ [cm] in H_2O**	**1-T_{H2O} 1 cm @ K-edge**	**1-T_{H2O} 12 cm ~ head**	**Physiol. possible concentr.**	**1-T_T target-tumor d=1 cm, metal-c= $c_{physiol}$**	**$R_{TB} = I_T/I_B$, (6+6) cm H_2O-head dummy, 1 cm tumor-target**
J Iodine	**53**	**33.17**	**0.93**	**0.525**	**0.9998**	**1 M**	**~ 0.6**	**0.007; before tumor!**
Pt cis-Platinum	**78**	**78.39**	**11.4**	**0.059**	**0.518**	**0.001 M**	**~ 0.0006**	**~ 0.0008; conc. low**
Hf Hafnium	**72**	**65.35**	**6.6**	**0.100**	**0.716**	**? (0.2 M)**	**? (~ 0.12)**	**? (0.1) tox. unknown**
La Lanthanum	**57**	**38.92**	**1.4**	**0.370**	**0.996**	**0.25 M +**	**~ 0.2 (calc.)**	**0.013 // 2x6 cm H_2O**
Gd Gadolinium	**64**	**50.24**	**3.0**	**0.206**	**0.938**	**0.25 M +**	**0.239, see fig.5**	**0.063 // 2x6 cm H_2O**
Lu Lutetium	**71**	**63.31**	**6.0**	**0.109**	**0.750**	**0.25 M +**	**0.154, see fig.6**	**0.098 // 2x6 cm H_2O**

+ Note: The present Lanthanide-DTPA concentration is max.0.253M, a level-4 improvement may yield 0.5 M.

4.3. Biological and chemical target enhanced radiation effect tests

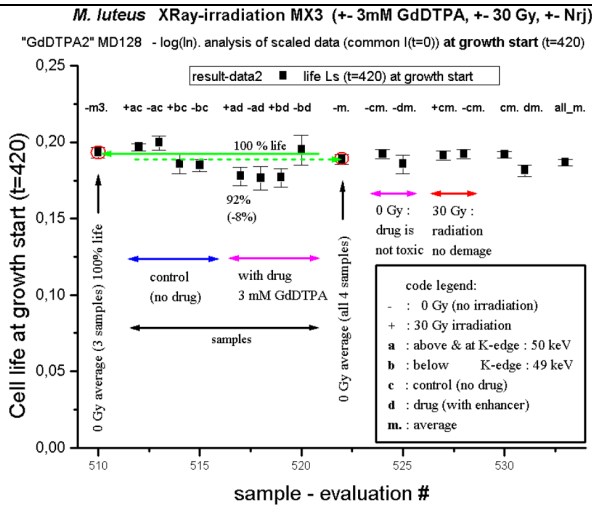

Fig.7: Precise fast biological target test with bacteria

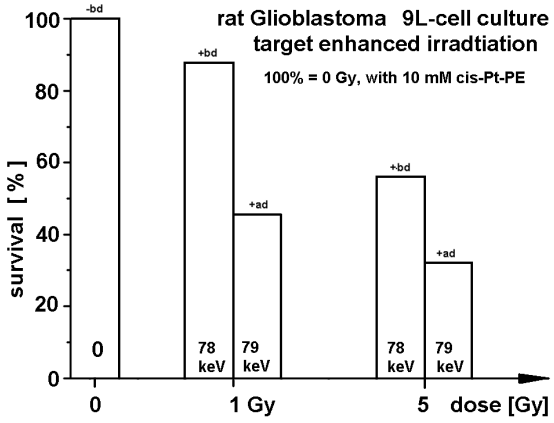

Fig8: 9L-cell culture test with magnetic Pt-liposomes

- **ChemoLuminescence tests** of radiation products were difficult: hydrogen (H_2) is present in irradiated H_2O, Lanthanides are quenchers. We succeded in the 1-3 mM Lu + 1 Gy range/ 63.9 keV: 0.1mM
- **Biological tests with bacteria** of target enhanced radiation effects on *Micrococcus luteus* ATCC 4698 in sealed Q-cells yielded *fast& precise* results (only 2% error) in 2 d (delayed growth curves): with pure targets (Gd-, Lu-DTPA) at 30 Gy successful (-8%, fig.7), while GdDTPA without X-ray reduced growth speed slightly, but did not lead to a reduced survival. Disadvantage: less sensitive with nanoparticles (bacteria have no endocytosis), high dose required, > 30 Gy (hours beam time/ sample !)
- **Biological tests with cell culture** of target enhanced radiation effects on 9L cancer cells are currently under investigation. The part ready now (fig.8, MD128) yielded: With the novel cis-Platinum-lipid (cis-Pt-Phosphatidylethanolamine, cis-Pt-PE) the target above-K reduced the survival to 46% @ 1Gy and 32% @ 5 Gy at 79 keV (controls 88%, 57%).

No severe cell toxicity was observed for Gd-, Lu-DTPA and Gd-, Lu-Liposomes. The high systematic error (~15% for single test) of the time consuming procedure (3 weeks) shall be reduced by methodical improvements (cell-ATPanalysis: bioluminescence).

4.4. Rat head dummy experiments – feasability tests with Lu-DTPA by imaging

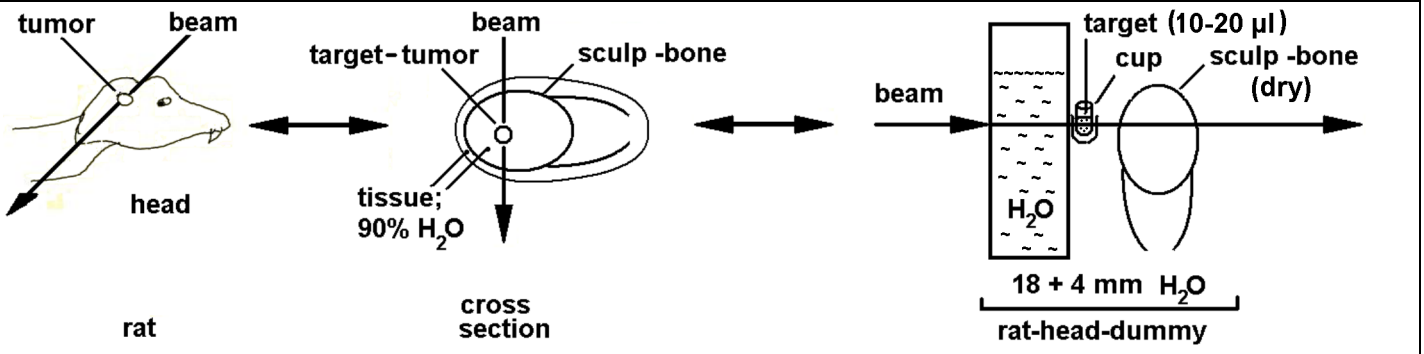

Fig.9: A rat head was simulated by a set of its major components: water, sculp-bone and target-cup (20 µl)

According to fig.9, a rat head in side projection was simulated by its main components in series: rat sculp-bone, water (18 mm), and a cup (3.8 mm diameter) for the target to be injected (10-20 µl = 2-4 mm fill hight). As depicted in fig.10, 0.25 M LuDTPA was visible directly, with K-edge phase contrast the limit was 25 mM.

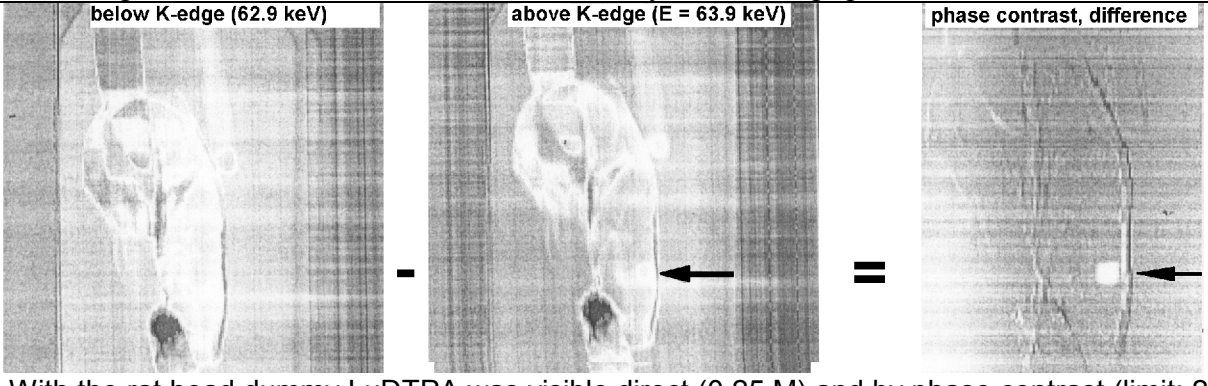

Fig.10: With the rat head dummy LuDTPA was visible direct (0.25 M) and by phase contrast (limit: 25 mM)

4.5. Animal test & first therapeutic imaging after Lu-DTPA injection in a living rat

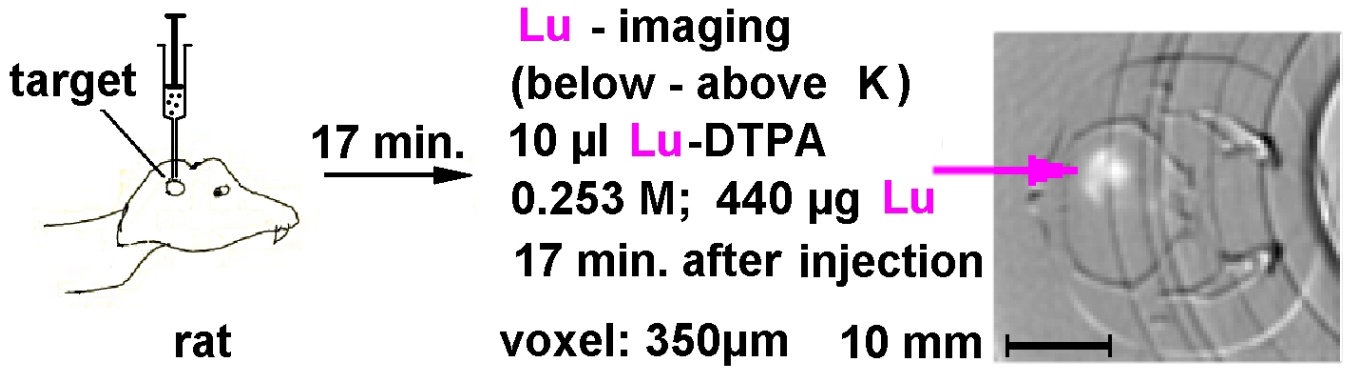

Fig.11: 10 µl LuDTPA physiological solution (0.253 M , pH7.2) was injected in the brain of a living rat after anestesia. The first therapeutic imaging was obtained 17 min later, and followed for 3.5 h (drug kinetics).

In cooperation with inhouse research (ESRF-BMF, ID17), the biocompatible lanthanide complex **Lutetium-DTPA**, in a novel highly concentrated formulation (level 3), was successfully applied for X-ray phase contrast imaging after injection into the brain of a living rat. The X-ray experiment was done exactly at that energy, which will be later used for **cancer treatment** (63.9 keV). The animal survived the treatment with 10 µl of 0.253 M LuDTPA (0.44 mg Lutetium) until the end of the experiment (no brain oedema was observed, but the animal was sacrificed in order to avoid pain by the applied high dose during > 40 tomographies).
The agent was visible during 3.5 h, while the complex diffused slowly into an area of 5-10 mm size. This covers the area of a typical brain tumor in rat (Glioblastome, 3 – 4 mm). The first difference image (below-above K-edge; 62.7 // 63.9 keV), obtained 17 min. after injection (offline) is shown in fig.11 (a slice of a tomography).
Conclusion of results of therapeutic imaging:

- animal survived the enhancer application (10 µl LuDTPA, 0.253 M)
- the LuDTPA was clearly visible, as predicted (table 1, fig.1)
- the Lanthanide has distributed during 3.5 h into an area of 5-10 mm size, that remained for hours
- the imaging was recorded just at the energy of the later cancer treatment = therapeutic imaging
- the temporal concentration in the first tomography was ~ 0.24 mg/ml, enough for treatment
- due to the progress, we are ready for the next step, a first cancer treatment pre-study (rat brain tumor)

5. Discussion and perspectives

The surprisingly fast and successful progress of the project indicates the concept is right and feasible at the ESRF. The improved Lanthanide complexes (level3) and nanoparticles are biocompatible. Lutetium appears to be optimal for photon activation therapy at the radiation level. The fast progress to therapeutic imaging became possible by tight collaboration with ESRF-BMF, ID17. The rat in the first animal test survived upon application of LuDTPA in an amount suitable for cancer therapy by PAT above the K-edge (63.9 keV). Thus we are earlier (~ 1 y) as expected for the next step: starting a pre-study with tumor-rats, in parallel to the necessary studies of toxity, particle structure (L-edges, ASAXS), and imaging / tomography, especially with the further improved targets (level 4 : reduced ionic strength and improved metal montent).

Last not least our project strategy of therapy has to be discussed: It differs from the conventional project / poroposal strategy, where everything is developped step by step in a sequential manner. With the target nanoparticle project we follow by speed reasons a different strategy: parallel-start at several points (). These are currently: (target nano- chemistry), (structure of nanoparticles / L-edges at ID1), (K-edge spectroscopy and dummy- imaging / ID17); (biological tests with cell cultures & bacteria); (tomography in animal tests). Now tumor treatment trials have to be started in animal ests. This strategy is, while more laborious, the result of the highest priority criterium: development of a new cancer treatment technique for urgent human application as fast as possilble (speed first !!). If this can be held, no significant problems should appear, and clinical partners can be found as soon as possible, the first human application could be in three years.