

# Development of Thulium-170 Brachytherapy Sources and Application in Rats Treatment

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## Abstract

Experimental work where Tm-170 LDR seeds and one HDR source were used to treat cancer on rats is described. Experiments were done with Lewis rats, carrying tumor developed from implantation of CNS-1 Rat Brain Tumor Astrocytoma cells, under the thigh skin. 75% of both HDR and LDR treated rats were completely cured. I-125 seeds experiments were done as a control, only 8.3% were cured with a similar photon dose. The dose due to beta radiation is very significant and was the main reason for the treatment success.

**Keywords:** Thulium-170; Brachytherapy; Beta radiation; Tumor treatment; Dose to the tumor; Rats

**Abbreviations:** LDR: Low Dose Rate; HDR: High Dose Rate; MDR: Medium Dose Rate; CNS: Central Nervous system; Sv: Sievert; Sv/h: Sievert per hour; Gy: Gray; Gy/h: Gray per hour; keV: kilo-electron Volt; DNA: Deoxyribonucleic Acid; MCNP: Monte Carlo N-Particle; GEANT: Geometry And Tracking; AAPM TG: American Association of Physics in Medicine Task Group; Ci: Curie; mCi: milliCurie; FBS: Fetal Bovine Serum; DMSO: Dimethyl Sulfoxide; RPMI: Roswell Park Memorial Institute; NRG: Nuclear Research Group; TLD: Thermoluminescence Dosimetry

## Introduction

Tm-170 seeds and a source were developed and tested for brachytherapy. The two common brachytherapy methods were used in the present work; permanent implantation, or low dose rate, LDR, where low activity seeds are implanted and remain inside the tumor, and temporary implantation of a high dose rate (HDR) source, where a high activity source stays inside the tumor for a short time and then removed [1].

## Tm-170 characteristics

- Half-life: 128.6 days; Mode of Decay 1: Beta to Yb-170 (the significant mode due to its branching ratio); Branching ratio: 99.87% beta emission; Decay energy: 1) 884 keV, 24% decays to an excited state of Yb-170, causing photons emission of energy of 84 keV; 2) 968 keV, 76% decays to ground state of Yb-170. The most probable beta energy is about 400 keV.
- Mode of Decay 2: Electron Capture to Er-170; Branching ratio: 0.13%; Decay energy: 314 keV.
- The range of the beta radiation in tissue is about 3 mm.
- Tm-170 emits x-ray photons in four main energies – 7.4, 49.7, 50.9, 52.3, 57.3 and 59.16 keV, and gamma ray photons of energy 84.25 keV.

In addition to Tm-170 for the LDR experiments, I-125 seeds were also used as a control group. The I-125 seeds used in the experiment were purchased from Best Medical model #2301.

## Benefits of Tm-170 in Brachytherapy

### For HDR

- The use of the relatively low energy photons enables performance of HDR in a minimally shielded environment.

- A significantly longer half-life that enables convenience of production and longer use, 128.6 days compared to 74 days of Ir-192.
- Production of Tm-170 involves neutron activation and packaging, while production of Ir-192 is much more complex and therefore more expensive.
- Causing less damage to surrounding healthy tissue due to lower photon energy (84 keV compared to 383 keV and above for Ir-192).
- Beta radiation delivers a very high dose near the source, without damaging the surrounding healthy tissue.
- Suitable for treating more sensitive tissues such as prostate, gynecological tumors, eyes, brain, and children's tumors.

### For LDR

- Ability to treat relatively large prostate glands of 50 cc and more, with a smaller number of seeds due to larger photon range compared to I-125 seeds.
- Use of a beta-radiation source for sensitive tissues and shallow tumors.
- Gamma ray source is useful for relatively big tumors while the beta ray source (3 mm range) is more suitable for small tumors in sensitive tissues/organs such as ophthalmic, hepatic, tongue, skin, and brain tumors.
- Due to the unique construction of the new source, the emission of the beta or gamma rays can be controlled by gold coating.

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## Literature Survey

A number of papers were published regarding the use of Tm-170 for brachytherapy. All of them described theoretical work. Munro et al. [2] used MCNP Monte Carlo code to investigate the dose distribution around a hypothetical HDR source; their final conclusion was that "Thulium-170 has great promise as a low energy (soft gamma) source". Enger et al. [3] reported modeling of hypothetical Tm-170 source for brachytherapy, using GEANT4 Monte Carlo code. Their conclusion was that Tm-170 is primarily a bremsstrahlung emitter with mean photon energies well above 10 keV. Granero et al. [4] investigated the broad beam transmission from Tm-170 and Yb-169. They used GEANT4 Monte Carlo code for their calculations. Their conclusions were regarding the transmission of the photons through a number of shielding materials. They did not discuss the suitability of Tm-170 for brachytherapy, although mentioned that both Tm-170 and Yb-169 are suitable for brachytherapy. A thorough and more comprehensive study of Tm-170 as a potential isotope for brachytherapy was published by Ballester et al. [5]. Their study included all aspects of brachytherapy source, including calculations of the parameters requested by the AAPM TG-43 report. They found that with a stainless steel capsule, the Tm-170 dose rates were about 100 times smaller than for Ir-192 and treatment times were much longer than for Ir-192.

As mentioned, all these publications reported Monte Carlo calculations; they all ignored the dose due to beta radiation. The only experimental work done was published by Ayoub et al. [6] and Ayoub et al. [7]. They used Tm-170 seeds of activity of 2.5 mCi, to treat tumor on mice. The results were: 60% of the mice completely cured, on 20% the tumor growth was delayed and in 20% the tumor continued to grow. In a control group treated with 0.5 mCi I-125 seeds only 25% of the tumors were cured.

In the present study we intended to show the efficacy of using Tm-170 as a radiation source for tumor treatment with bigger tumors on larger animals.

The present article reports the development and the use of a new radiation source for brachytherapy, both LDR and HDR. It shows the result of cancer treatment on rats and demonstrates the benefits of using Tm-170 for brachytherapy.

## Materials and Methods

### Maintenance of cell line – CNS-1

The CNS-1 Rat Brain Tumor Astrocytoma (gift from Prof L. Eisenbach, Department of Immunology, Weizmann Institute of Science, Rehovot, Israel; originally given by W. Hickley, Department of Pathology, Dartmouth Medical School, Lebanon, New Hampshire, USA) was selected as the tumor model because of its therapy resistance [8].

The CNS-1 cell line was maintained with RPMI medium which was supplemented with L-Glutamine solution (2.0%), Fetal Bovine Serum (FBS) (15%), and Penicillin–Streptomycin–Neomycin antibiotic solution (2%) (from Beit Haemek Ltd.). The CNS-1 cell line was grown in cell culture flasks and petri dishes, and maintained by regular cell replantation protocol: cells were removed from petri dishes with Trypsin 0.25% solution (Beit Haemek Ltd.) and transferred to petri dishes containing new RPMI complete medium. This process was performed every two-three days.

The CNS-1 cell line was also frozen for preservation with freezing solution and maintained at -76°C. The freezing solution, containing 90% FBS and 10% DMSO, was passed through a 0.2 µm filter for sterilization after the DMSO was completely dissolved.

All experiments were done with the same cell line; for every experiment cells from the cell line were taken from the frozen stock. All experiments were done with Lewis rats.

### Implantation of CNS-1 tumors in Lewis rats

As preparation for implantation trials, a suitable volume of tumor cells was calibrated for those trials. A number of rats were implanted with CNS-1 cells in different quantities ( $0.5 \times 10^6$ ,  $1 \times 10^6$ ,  $2 \times 10^6$  cells injected) and grown tumors were examined in several volumes in order to find which volume of tumor is big enough to be implanted with several seeds and still preserve the solid texture of tissue. The findings of those calibration trials pointed to  $\sim 600 \text{ mm}^3$  to  $800 \text{ mm}^3$  as the optimal tumor volume that allows implantation of 3-4 seeds and retains a solid internal texture.

Tumors were measurable 5-7 days after injection. The measurements were performed by a digital caliper. Three parameters were measured: length (l), width (w) and height (h); the volume was calculated according to ellipsoid volume equation:

$$V=1/6 \{ \pi \times l \times w \times h \}$$

### Preparation of Tm-170 seeds

Tm-170 seeds were made of 10 mg  $\pm$  0.1 mg for LDR and 13 mg  $\pm$  0.1 mg of thulium for HDR procedures, each seed contains thulium wire of 0.6 mm diameter, and 4.2 mm or 5.0 mm lengths for LDR and HDR procedures, respectively. The thulium was inserted into titanium capsules of 0.8 mm outer diameter, 0.7 mm inner diameter, and 7 mm long. Seeds were sealed with titanium plugs, inserted to the capsules on both ends, using mechanical force. Seed impermeability was checked by comparing their weight before and after staying for 15 min in a water bath (after removal from water the external surface was dried). If the weight increased, the seeds were not sufficiently sealed. The seeds (source) were then sent for neutron activation.

The neutron capture cross-section for Tm-169 to become Tm-170 is 115 barn (b) or  $115 \times 10^{-24} \text{ cm}^2/\text{atom}$  [9].

The seeds designated for the LDR procedure were irradiated for 12 h with a neutron flux of  $1 \times 10^{13} \text{ n/cm}^2.\text{sec}$ , at the Soreq Nuclear Research Center in Israel.

The source designated for the HDR procedure was irradiated for 24 days with a neutron flux of  $2.5 \times 10^{14} \text{ n/cm}^2.\text{sec}$  at the NRG Reactor in Petten, Holland.

The activity of the seeds was calculated using equation:

$$A=N \times \sigma \times \Phi \times (1-e^{-\lambda t_0})$$

Where: N=number of thulium atoms,  $\sigma$ =cross section for activation,  $\Phi$ =neutron flux,  $\lambda$ =decay constant,  $t_0$ =time of irradiation.

The thulium mass in the LDR seeds was  $m_{(LDR)}=10 \times 10^{-3} \text{ gr}$  or  $N=3.55 \times 10^{19}$  atoms and  $m_{(HDR)}=13 \times 10^{-3} \text{ gr}$  or  $N=4.62 \times 10^{19}$  atoms.

The activity at the end of activation was:

$A=0.6 \text{ mCi}$  for the LDR seeds (about 0.5 mCi during the experiments) and

$A=5.4 \text{ Ci}$  for the HDR source (about 2.3 Ci during the experiments).

Ten seeds were prepared as described, for the LDR experiments.

## LDR Experiments

Tm-170 and I-125 seeds were implanted into rats bearing the CNS-1 tumors. Seed implantations were performed using an implantation system developed and built in the laboratory, taking into account radiation safety instructions.

In each tumor 3-4 seeds were implanted, depends on its dimensions.

The implantation system included a platform on which the anesthetized animal was laid. It was shielded by a 5 mm thick lead tube, with the tumor carrying rat's leg outside the tube, fixed and stabilized. The seeds were implanted through a set of 3 trocars, which were held by a device capable of adjusting the height and the angle of the trocar.

The Iodine-125 and Thulium-170 seeds of activity in the order of  $1.85 \times 10^7$  Bq were kept inside the tumors until the tumors had disappeared, at which time seeds were surgically removed (under anesthetization), or tumors allowed to continue growing to upper limit volume ( $\sim 1600 \text{ mm}^3$ ), when rats were sacrificed and seeds removed.

There were four LDR experimental groups:

1. Control group 1, no treatment.
2. Control group 2, implantation of dummy seeds.
3. Experimental (control) group, implantation of I-125 seeds.
4. Experimental group, implantation of Tm-170 seeds.

The number of rats in this experiment was determined based on a number of limitations: the number of Tm-170 seeds (9+1 reserve) produced (the number of Tm-170 seeds was limited by the radiation safety officer according to the university license), the number of Tm-170 seeds planned to be inside tumors (3-4, according to tumor volume), the decay time of the I-125 and the Tm-170 seeds (60 days and 128.6 days, respectively) and radiation safety certification for a definite number of implanted tumors. Therefore, each group contains 12 animals.

Rats with radioactive seeds implanted into their tumors, were kept in regular cages, placed in special lead boxes, to shield them from each other.

The numbers of seeds (3 or 4) implanted in each tumor varied depending on the tumor volume and seed activity at that time. This, and the fact that seed activity varied during the research, were taken into account in dose calculation.

The total dose to the tumor on the various rats, according to the number of seeds and dwell time was in the range 8 Sv to 48 Sv.

## HDR Experiments

High dose brachytherapy with Tm-170 seeds was performed with Lewis rats carrying CNS-1 tumors. Tumor implantation method and place was the same as described in previous sections about the LDR procedure. Only one source was implanted for a short period of time, in the tumor of each rat.

In the HDR procedure, tumor irradiation was done for calculated periods of time (10-20 min), several times. Those periods are a function of the desired dose for the individual rat, depending on tumor measurements and source activity.

Each animal was treated at least three times; the extension of each fraction was determined based on tumor volume and source activity (source decay was taken into account).

Tm-170 HDR source dose rate was 30 Sv/h 4 mm from the source for Gammas and  $2.46 \times 10^3$  Sv/h for betas 2 mm from the source, based on dosimetry data (presented in Results section).

There were Three HDR Experimental Groups:

1. Control group 1, no treatment.
2. Control group 2, dummy implantation similar to HDR procedure.
3. Experimental group, implantation of Tm-170 source in HDR procedure.

The anesthetized rats were placed in a 5 mm thick lead shield to protect the rest of its body from radiation. The tumor extended out of the lead shield through a hole in the lead. A 2 mm diameter plastic tube inserted along the main axis of the tumor, used as a catheter for placing the source, which moved in 5 mm steps along the tumor axis.

The source was fixed at the end of a 20 cm long and stainless steel wire of 1.5 mm diameter. The experiment was done in a sealed lead box. The source positioning during procedures was done manually with a precise measurement of the source location. No radiation above natural background was measured outside the sealed box.

The experiment area was sterilized with 70% ethanol solution before and after the treatment.

The HDR source dwell time in each position was initially 10 min. In later treatments, as the source decayed, the dwell time was longer accordingly. At the end of the dwell time in each location the source was moved 5 mm to the next position along the tumor axis. Therefore, for a 20 mm long tumor, the treatment lasted for 40 min and the source was moved forward along the tumor length three times.

At the end of the procedure antiseptic ointment was carefully applied to the animal's wounds.

After the HDR brachytherapy treatment, the animals were given extra attention until their recovery from anesthetization and for the next few days. Antiseptic ointment was applied to the wounds if necessary.

## Tumor Measurement

Tumors of every animal (in the control and experimental groups) were measured with a digital caliper 3 times a week. Tumor length, width, and height were measured, and tumor volume was calculated based on the ellipsoid model.

In rats bearing radioactive seeds, the measurement procedure was done behind lead glass, accordingly to safety instructions.

A summary of the statistical analysis is presented following the results presentation, it includes: tumor volume distribution, Chi-square test, Kolmogorove-Smirnove test, T-test and Mann-Whitney test.

## Dose Measurements and Calculation

Dosimetry of the LDR seeds was performed in a cylindrical Perspex phantom with a suitable hole at the center, where every seed was placed for dose distribution measurements. Six LiF (Mg, Ti) TLD crystals with dimensions of 3.2 mm  $\times$  3.2 mm  $\times$  0.9 mm were placed at various distances (4, 6, 8, 12, 16 and 20 mm) and angles in six slots around the source [10].

Dosimetry of the HDR source was done as that of the LDR, with the source inserted into the central hole in the Perspex phantom.

Figure 1 describes dose distribution due to photons only. The dose due to beta was calculated.

Only beta particles from the outer 0.13 mm thick layer of the Thulium cylindrical wire are emitted from the source while the source-core beta emission, is self-absorbed [11].

The ratio between this ring and the whole circle (Thulium wire cross-section) is given by:

$$\pi[0.6^2 - (0.6 - 0.26)^2] / \pi 0.6^2 = 0.244 / 0.36 = 0.679$$

Therefore, only 68% of beta particles are emitted from the source.

The average energy of photon emission from Tm-170 source is 60 keV. Absorption coefficient in tissue for photons at that energy is  $\mu = 0.2 \text{ cm}^{-1}$ , therefore photon fraction absorbed in tissue within 2 mm distance from the source is:

$$1 - e^{-\mu r} = 1 - e^{-0.2 \times 0.2} = 0.039$$

Therefore, 3.9% of photons emitted will be absorbed in the range of 2 mm from the source.

Photon spectrum measurements showed that the photons of energy in the range 48 keV to 84 keV comprise about 12% of the total emission, therefore the beta intensity from Thulium-170 is 8.3 times higher than that of the photons. The most probable beta energy is about 400 keV [12]. The ratio between beta and photons dose is therefore:

$$\beta / \gamma = \frac{400 \text{ keV} \times 8.3 \times 0.679}{60 \text{ keV} \times 0.039} = 963$$

Beta emission is also absorbed in titanium capsules of thickness 0.005 mm. Exponential like absorption of beta emission from radioactive source in tissue can be assumed, while absorption coefficient can be expressed [13]:

$$\mu = [16 / E_{\text{max}}^{1.14}] \times \rho_{\text{Titanium}} = 16 / [0.9^{1.14}] \times 4.5 = 81.19 \text{ cm}^{-1}$$

$$e^{-\mu r} = e^{-81.19 \times 0.005} = 0.666$$

Therefore, only 67% of Beta emission passes through titanium.

According to that, the ratio between beta and photons is:

$$0.67 \times 963 = 645.5$$

The total dose to the various rats according to the number of seeds and dwell time is therefore in the range; 8 Sv to 35 Sv due to photons and  $5 \times 10^3 - 2.26 \times 10^4 \text{ Sv}$  at 2 mm from the seeds due to beta.

The measured dose rate is due to gamma and X-rays. Beta rays are absorbed in the Perspex within the 3.5 mm diameter [14].

The HDR source was sealed in a stainless steel tube with side wall 0.1 mm thick, which must be taken into account in dose calculation.

Fraction of dose that had left the tube is given by:

$$e^{-\mu r} = e^{-144 \times 0.01} = 0.24$$

That means that 24% of Beta particles had passed to the tissue.

Due to plastic tube presence in addition to stainless steel tube around the source, another calculation is needed:

$$e^{-\mu r} = e^{-18 \times 0.035} = 0.53$$

Therefore, 53% of 24% fraction of Beta particles reached the tissue, meaning only 0.127 of total Beta emission.

Dose rate in HDR treatments was 30 Sv/h at 4 mm from the source due to photons, as shown in Figure 2. The dose due to Beta particles is calculated accordingly to aforementioned calculation of Beta/Gamma ratio and consideration of an above calculated fraction.

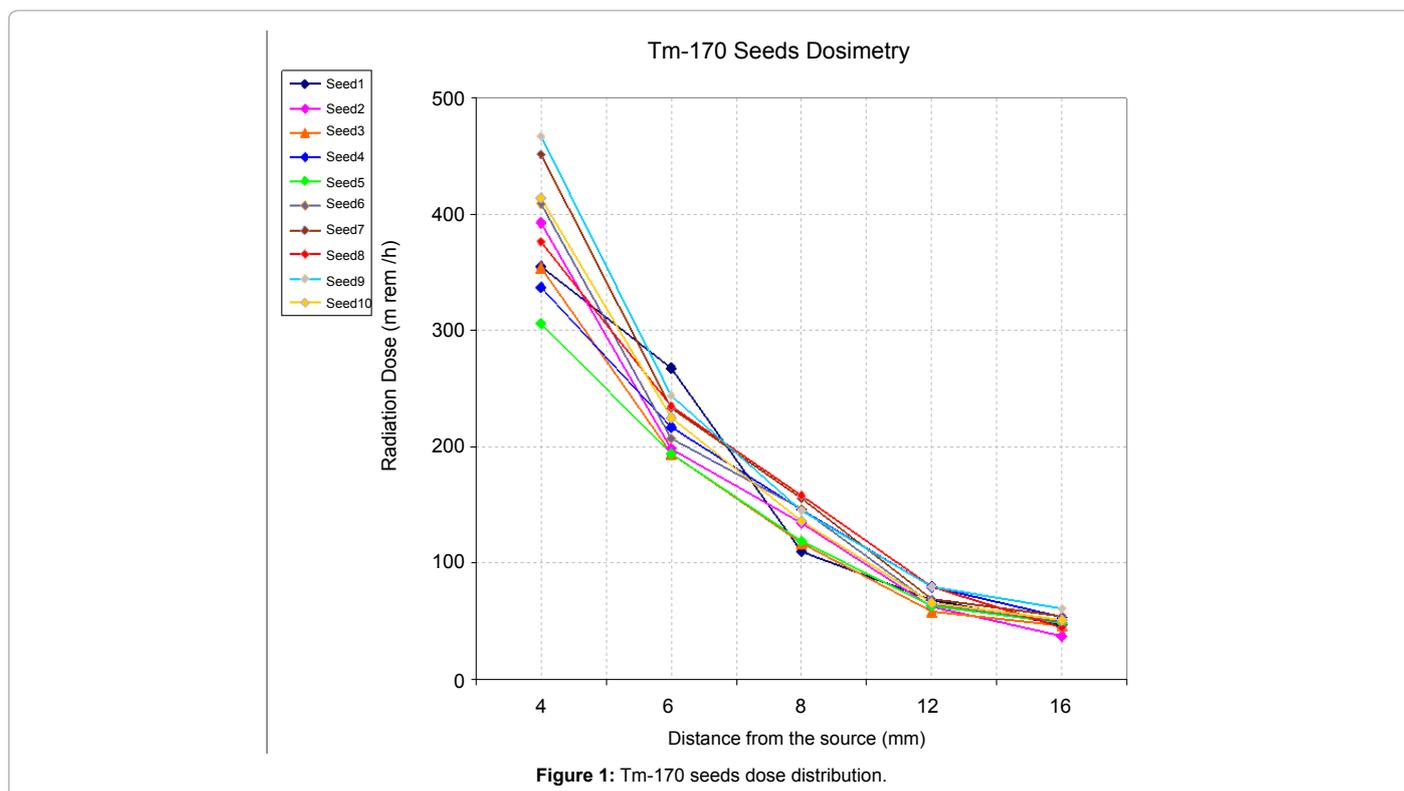


Figure 1: Tm-170 seeds dose distribution.

Therefore, Beta related dose is:

$$30 \text{ Sv/h} \times 645.5 \times 0.127 = 2459 \text{ Sv/h}$$

The total dose to the tumor on the various rats was calculated according to the total dwell time. It is in the range 30 Sv to 60 Sv due to photons and 2500 Sv to 5000 Sv due to beta at 2 mm from the source.

## Results and Discussion

### LDR brachytherapy

**Control group 1, no treatment:** CNS-1 tumors grow exponentially with time, as shown in Figure 3.

**Control group 2, implantation of dummy seeds into CNS-1 tumors:** Dummy seeds are empty titanium capsules as described in Materials & Methods. Those capsules have the same dimensions as the Tm-170 capsules and were also sent for neutron activation at the Soreq Nuclear Research Center.

No radiation could be measured from these seeds; hence, no effect was expected on tumor growth. As expected the tumors kept growing exponentially, as did the tumors in control group 1. Tumor volume vs. time is shown in Figure 4.

**Control group 3, implantation of I-125 seeds:** Rats bearing tumors that continued growing, in spite of being irradiated, were sacrificed when tumors reached volumes above ~2800 mm<sup>3</sup>, or when the animal seemed to be ill and suffering.

In ten of twelve animals the tumors were solid tissue, as was seen after animal sacrifice and seed removal from tumor tissue. In one of twelve animals (that which was cured) the tumor disappeared and subcutaneous bloody fluids were found. After carefully removing those fluids with a syringe, no malignant tissue volume was observed where the tumor had been. The wounds healed within a few days. The rat which was fully cured was kept for further observation. Figure 5 shows the change in tumor volume vs. time. Results are summarized in Table 1.

**Experimental group, implantation of Tm-170 seeds:** Tm-170 seeds, of measured activity of ~0.5 mCi, prepared as described, were

implanted into twelve rats bearing CNS-1 tumors on their thighs. Not all rats were irradiated at the same time due to the limited number of seeds and the number of seeds implanted to each tumor (3-4). Tm-170 seeds were used when available, hence, their activity varied, which was taken into account in total dose calculation.

Rat survival of the Tm-170 LDR experiments is shown in Figure 6 and results are summarized in Table 2.

### HDR brachytherapy

**Control group 1, no treatment:** Untreated CNS-1 tumors grow exponentially with time as shown in Figure 7.

**HDR control group 2, dummy implantation:** In this group of animals, procedures were performed in every way similar to active-source HDR procedure: animal was anesthetized; a syringe (dummy source) was inserted into the tumor for a period of time, as in HDR treatment experiment, with tumor of the same size. Tumor growth is shown in Figure 8.

**HDR experimental group:** HDR treatments were performed in several fractions; the minimum number of fractions for each animal was three. For some rats four or even five fractions were needed for tumor disappearance and successful cure. Tumor growth of this group of rats is shown in Figure 9 and Results are summarized in Table 3.

### Summary of Statistical Analysis of Tm-170 Rat Experiment

For the statistical analysis the experimental groups are designated as follows, 12 rats per group:

1. Control group before LDR experiment, No treatment
2. Control group before LDR experiment, Dummy seeds
3. Control group before HDR experiment, No treatment
4. Control group before HDR experiment, Dummy source
5. Experimental group, LDR, Tm-170 seeds

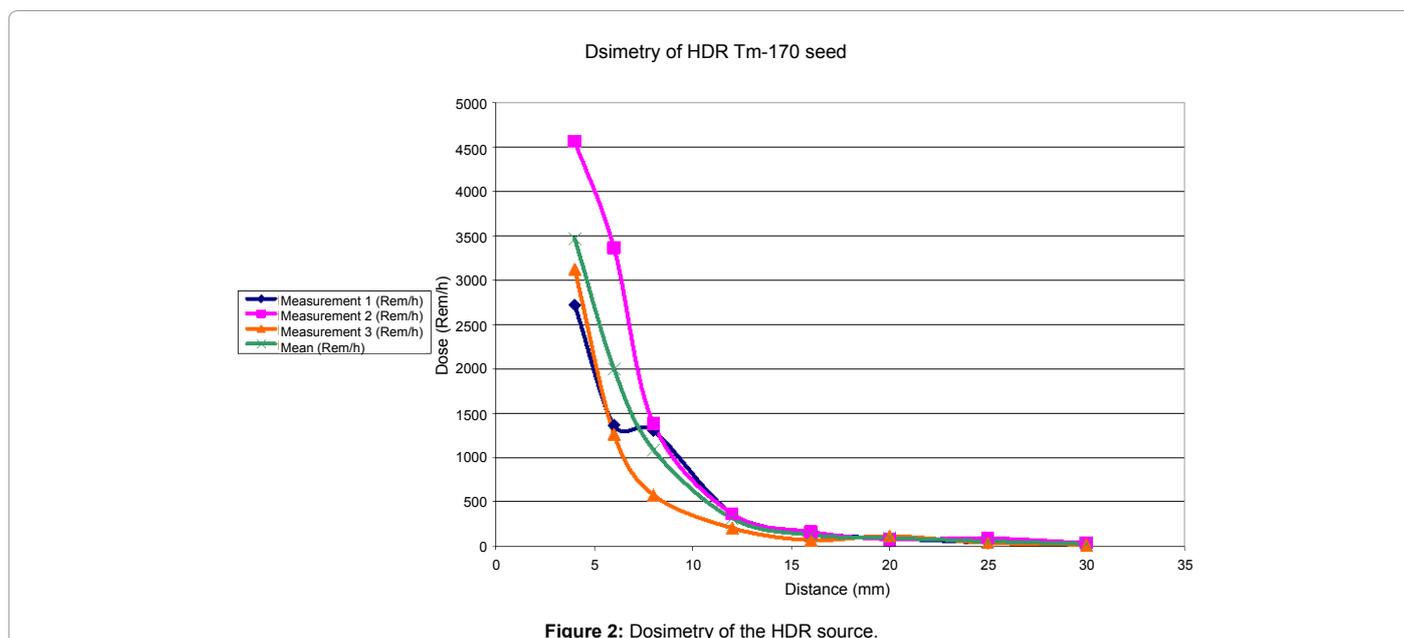


Figure 2: Dosimetry of the HDR source.

### LDR Control group 1 - No Treatment - Volume vs. Time

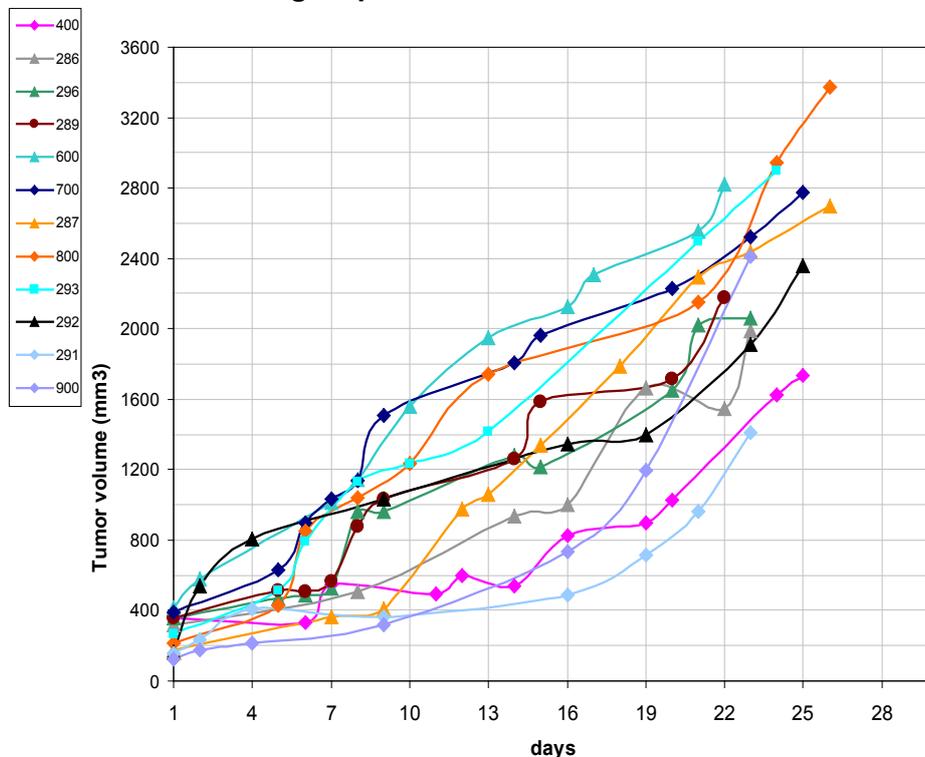


Figure 3: LDR Control group 1, no treatment: tumor volume vs. time.

### LDR Control group 2 - Dummy seeds - Volume vs. Time

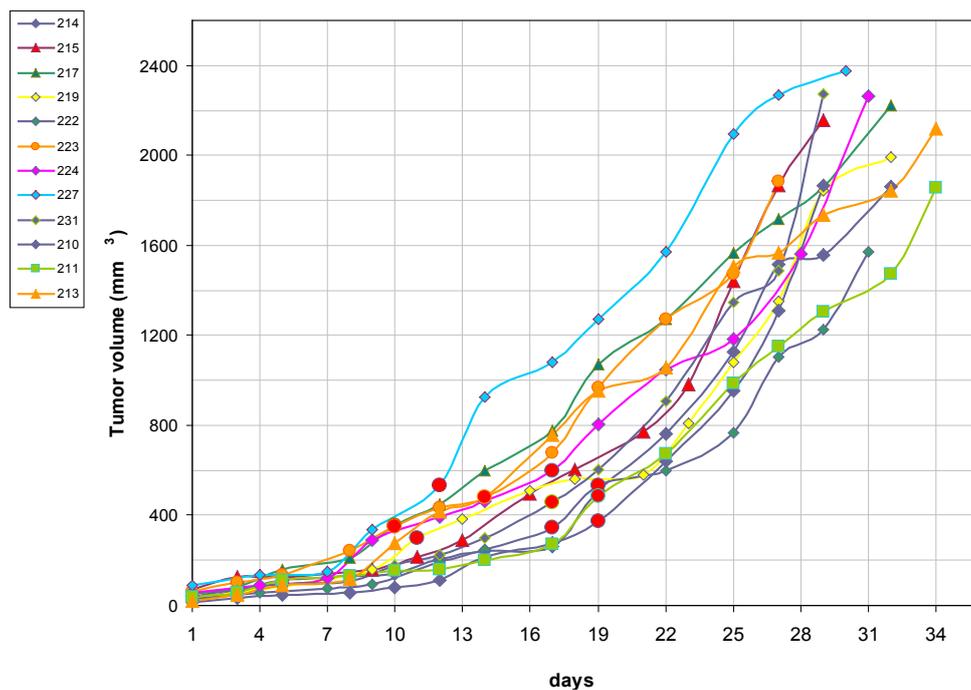


Figure 4: LDR Control group 2: tumor volume vs. time, tumors implanted with dummy seeds. Red dots indicate implantation date.

### LDR trials - I-125 group

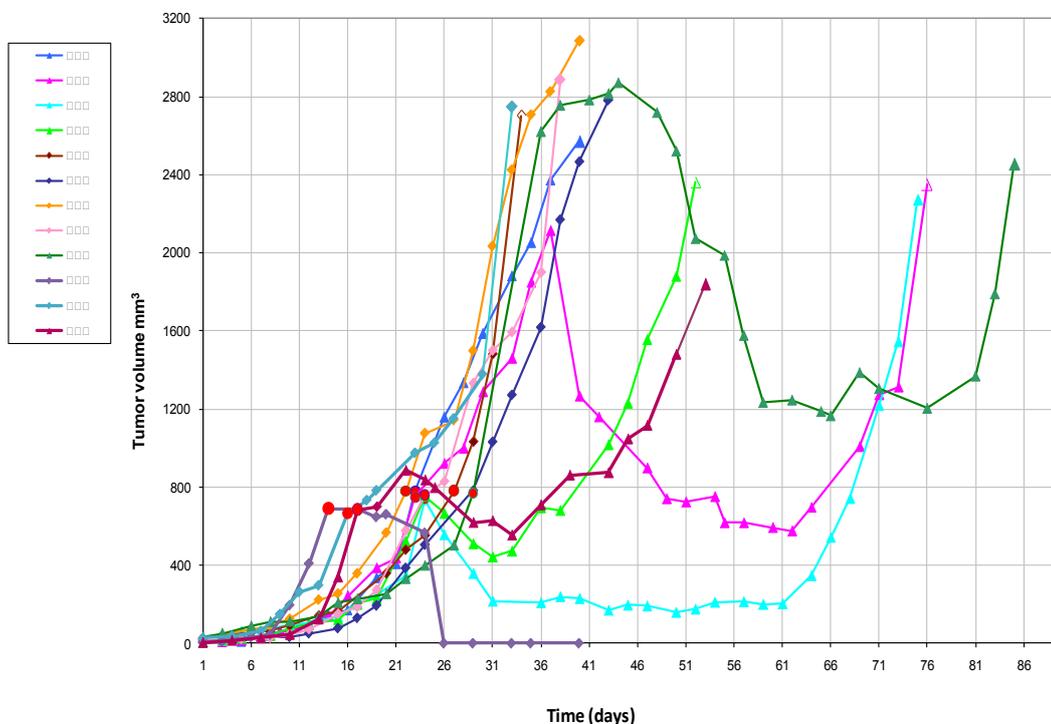


Figure 5: LDR Control group: tumors implanted with I-125 seeds growth as a function of time.

	Number	Percentage
Fully cured	1	8.3
Tumor growth delay	5	41.7
Not cured	6	50
Total	12	100.0

Table 1: Summary of I-125 group results.

6. Experimental group, HDR, Tm-170 source
7. Experimental (control) group, LDR, I-125, for comparison with Tm-170.

#### Tumor volume

A Summary of tumor volume measurements ( $\text{mm}^3$ ) is given in the Endnote section.

#### Chi-square test

For the Chi-square calculation we added the partial cured group once to full-cured and compared it with failure, or we compared full cured to the rest (adding partial to the failure). Then we compared the first control group to the experimental groups in those three combinations as well as comparing the experimental groups with each other.

A table of chi-square values is shown in the Endnote section.

**Remarks:** The Chi-square values for the whole data, whether in 3 groups (success, partial, failure) or in 2 groups (failure vs. success+partial, or success vs. failure+partial) are very high and the p values are therefore very low.

There is no fit between the experimental groups 5 and 6 and the control group 1. There is some fit with experimental group 7, i.e. using I-125 seeds instead of Tm-170. That is due to the fact that the tumors started re-growing after shrinking in the first few weeks.

The experimental groups 5 and 6 fit each other quite well. They have some resemblance with the I-125 group.

When we take full success vs. other results again there is a big difference between the experimental groups 5 and 6, and the control group, and some similarity between the I-125 group and the control. The fit between the experimental groups 5, 6 is smaller, and between those and group 7 is better.

When we compare failure to any success, full or partial, groups 5 and 6 do not fit the control but group 7 – I-125 fit it quite well. That is in this case the I-125 experiment results are similar to the control.

The Tm-170 experimental groups 5 and 6 fit perfectly,  $p=1.000$ .

#### Kolmogorov-Smirnov test

Kolmogorov-Smirnov test of the distribution of the tumor volume at the end of the experiment in each group.

Group	Normal distribution	Poisson distribution
1	yes	no
2	yes	no
3	yes	yes
4	yes	no
5	no	no
6	no	no
7	no	no

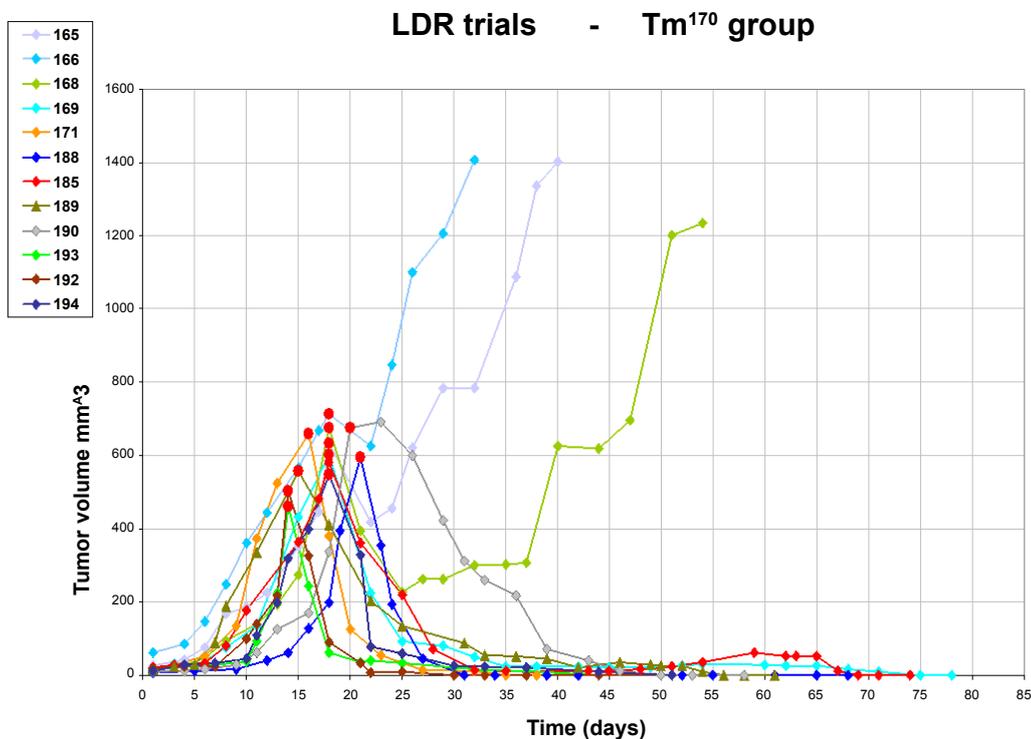


Figure 6: LDR Experimental group, tumors implanted with Tm-170 seed volume vs. time.

	Number	Percentage
Fully cured	9	75
Tumor growth delay	1	8.3
Not cured	2	16.7
Total	12	100

Table 2: Summary of Tm-170 group results.

**Remarks:** There is no good explanation for the positive result of Poisson distribution of group 3.

It seems reasonable that the end tumor volume of the control groups has a normal distribution.

### T-test (F test)

Results are shown in a table in the Appendix.

**Remarks:** In every comparison of two groups (experiment and control), when the p value (or 2 tail sigma) is smaller than 0.05 the t value is (much) larger than the critical value and the experiment is successful (compare to control). It means that the difference between the means of the two groups is large.

When  $p > 0.005$  the variances of the two groups are assumed to be equal. When  $p < 0.05$  the variances assumed unequal.

### Mann-Whitney test

(Independent Samples, Mann-Whitney U Test).

**Null hypothesis:** The distribution of Tumor Volume is the same across control and experimental groups.

**Summary of Test:** Significance level=0.05.

Groups	Asymptotic Significance	Decision
1-5	0.000	Null hypothesis rejected
1-6	0.000	"
1-7	0.932	Null hypothesis accepted
2-5	0.000	Null hypothesis rejected
2-6	0.000	"
2-7	0.006	"
3-5	0.000	"
3-6	0.000	"
3-7	0.001	"
4-5	0.000	"
4-6	0.000	"
4-7	0.010	"

**Remarks:** In general, the control groups 1-4 have a different distribution than the experimental groups 5-7. In case of comparing group 1 with group 7 i.e., I-125 seeds treatment, after tumor regrowth, the volume distributions had some similarity.

### Discussion

In the LDR treatment we were able to compare the use of Tm-170 seeds to the widely used (in human patients) I-125 seeds. Tm-170 superiority is demonstrated.

In the HDR experiment the only radiation source used for human patients' treatment is Ir-192, it was not available to us. Using Tm-170 for HDR at activity of about 2.5 Ci was done in a simple university laboratory without heavy shielding and remote control system. The personnel were present at the treatment location with a little lead shielding around the source and basic radiation safety measures. Preparation of the Tm-170

### Control 1 group - volume vs. time

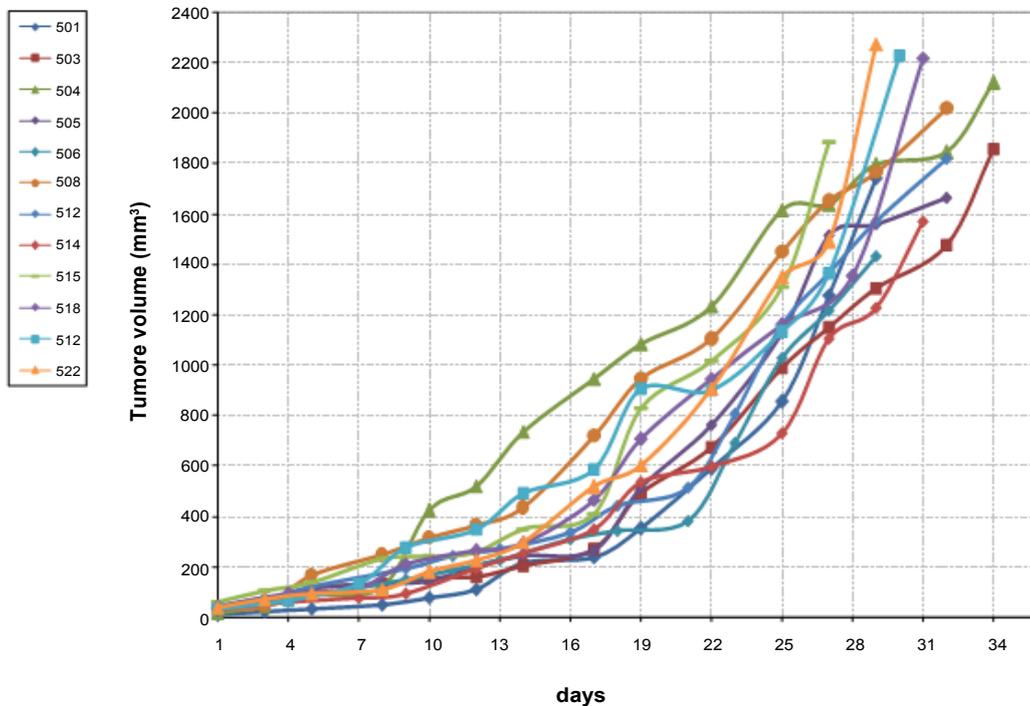


Figure 7: HDR Control group 1 tumor volume vs. time, untreated animals.

### Control 2 group - volume vs. time

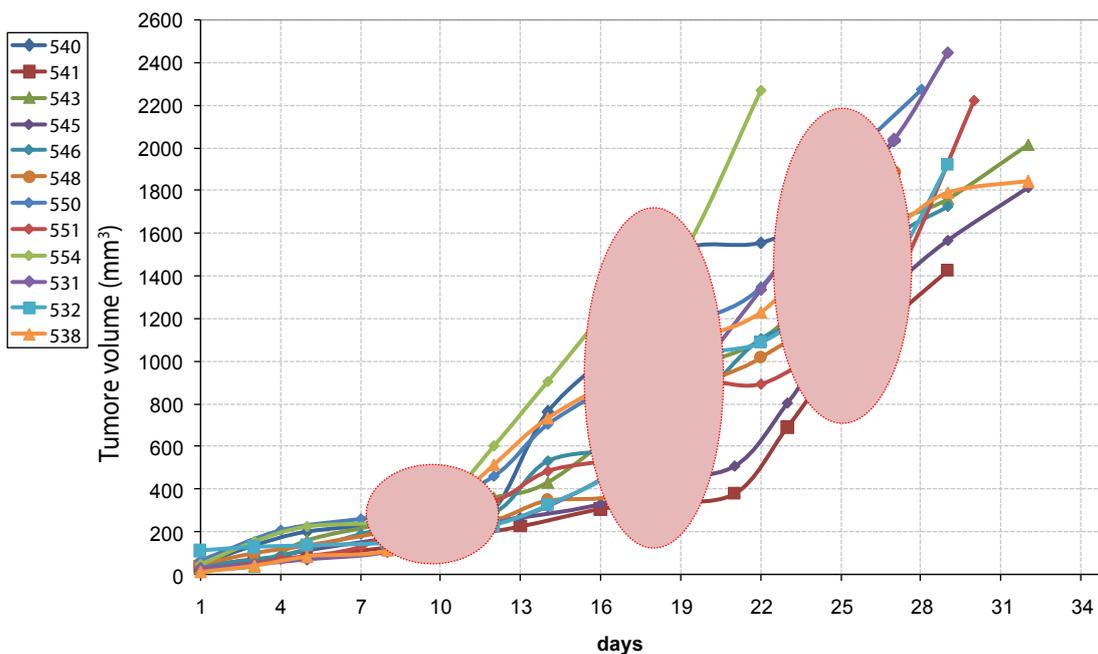


Figure 8: HDR Control group 2 tumor volume vs. time, dummy HDR procedure, clear red spheres indicate days of dummy HDR implantation.

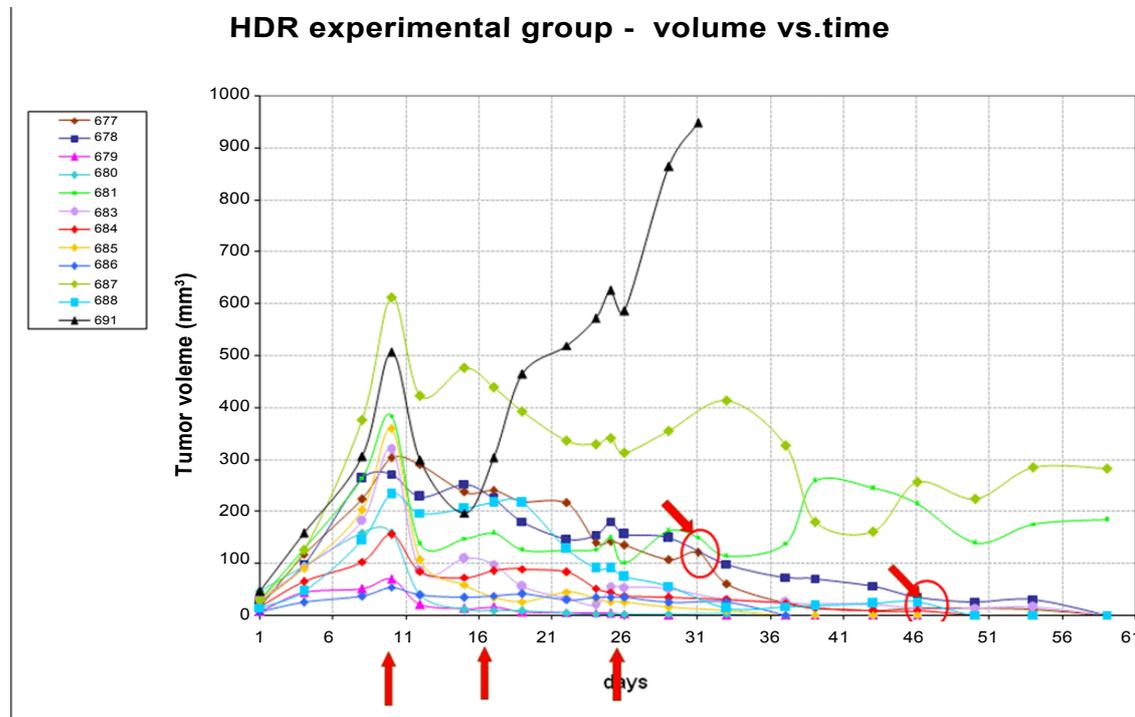


Figure 9: HDR Experimental group; arrows indicate days of HDR treatments.

	Number	Percentage
Fully cured	9	75
Tumor growth delay	2	16.7
Not cured	1	8.3
Total	12	100.0

Table 3: Summary of HDR group results.

HDR source required special means (stainless steel welding in a hot laboratory), this service was given to us by the Negev Nuclear Research Center in Israel.

The LDR seeds were made in a simple university laboratory.

We let the tumor grow for about 10 days to reach the volume of about 500 mm<sup>3</sup>, then we implanted the seeds or the source. The drop in tumor volume in the HDR experiment was immediate in most rats. In the LDR experiments the volume dropped slower. That means that there is a dose rate effect. The HDR source dwelling time in the tumor was 10 min, in the LDR experiments, the seeds stayed in the tumor for days as shown in the relevant figures.

LDR seeds implantation spacing was such that we get beta radiation from the various seeds overlaps, that is along three lines 6 mm apart, perpendicular to the tumor main axis. In the HDR experiments, the source moved along the central tumor axis. Therefore, in the first treatment the beta rays did not reach the outer region of the tumor. In the following treatments (second a third), the tumor shrank and the beta radiation reached the tumor edge. It should be mentioned that the dose due to beta, in the closest few mm to the source, is several hundred times higher than that of the photons.

Therefore, it can be concluded that cancer cells death was induced mainly due to the beta radiation, around the source, which caused a

severe damage to surrounding tumor cells' DNA, leading to cell death around the source, and to tumor shrinking. Interesting to note, that in LDR treatments cell death probably occurred via apoptotic pathways, due to lack of inflammation. However, in HDR treatments, probably due to severe tissue damage, cell death occurred via necrosis, since inflammatory tissue was observed around the source axis after the treatments.

When comparing the use of Tm-170 to that of I-125 for LDR, I-125 has the advantage of shorter half-life, but the dose delivered by Tm-170 of the same activity is much higher. The results obtained in the present research show it very clearly, that Tm-170 is much more efficient than I-125.

The exposure of the tumor to the Tm-170 source (dwell time), in the HDR treatment, was 3 times 10 min, with one-week interval between the treatments. This is a good demonstration of the Tm-170 source efficiency.

It is important to note that the total dose delivered to the tumor in both methods, HDR and LDR, were about the same. Still, in HDR treatments the survival percent was higher. We assume that HDR treatment higher success was caused by necrotic death in tumor cells, which leaves smaller chances for tumor recovery, due to inflammatory process taking place in radiation damage area.

Uncured tumors received much higher dose than those cured because they were irradiated for longer times. Yet, despite the higher radiation dose, small percent of the animals (25% in LDR and 8% in HDR) did not respond to the treatment, which may be explained by the individual response of each organism to cancer treatment, and, unfortunately, in some cases – unresponsiveness. Nevertheless, in LDR treatment failure percentage was much higher in I-125 compared to the Tm-170 group (92% and 25%, respectively), indicating Tm-170 better

compatibility to overcome tumor cells resistance against radiation treatment.

## Conclusions

The experiments results show that Tm-170 can be used as a simple and efficient radiation source for brachytherapy. With some optimization, the success percent can reach much higher values than presented here.

Using Tm-170 for HDR at activity of about 2.5 Ci can be done in a simple university laboratory or hospital operation room, without heavy shielding and remote control system. The personnel are present at the treatment location with a little lead shielding around the source and basic radiation safety measures. These facts demonstrate the simplicity of using Tm-170 for brachytherapy. Brachytherapy treatment can therefore be provided in any small hospital in the country, not just in major health centers.

The drop in tumor volume in the HDR experiment was immediate in most rats. In the LDR experiments the volume dropped slower. That means that there is a dose rate effect. The dose due to beta, in few mm radius from the source was several hundred times higher than that of the photons, therefore, it can be concluded that the cancer cells death was induced mainly by the beta emission, around the source. To avoid creating a hot spot in big tumor treatment, more than one source should be applied simultaneously.

## Competing Interest

There are no competing interests in this work, financial or other.

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