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Accelerating Scientific Discovery

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Tumor regression by means of nanotherapy with and without magnetic drug targeting

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Introduction

Experimental research into the treatment of primary and secondary liver tumors shows promising alternatives. Magnetic drug targeting is another possibility.

One modification of this method is coupling solely of iron oxides (Fe_3O_4) to cytostatics.

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Introduction

Due the physiological capacity of the liver for phagocytosis,
the iron is sluiced into the liver,
and undergoes phagocytosis by the terminal vascular macrophages and is thereby absorbed.

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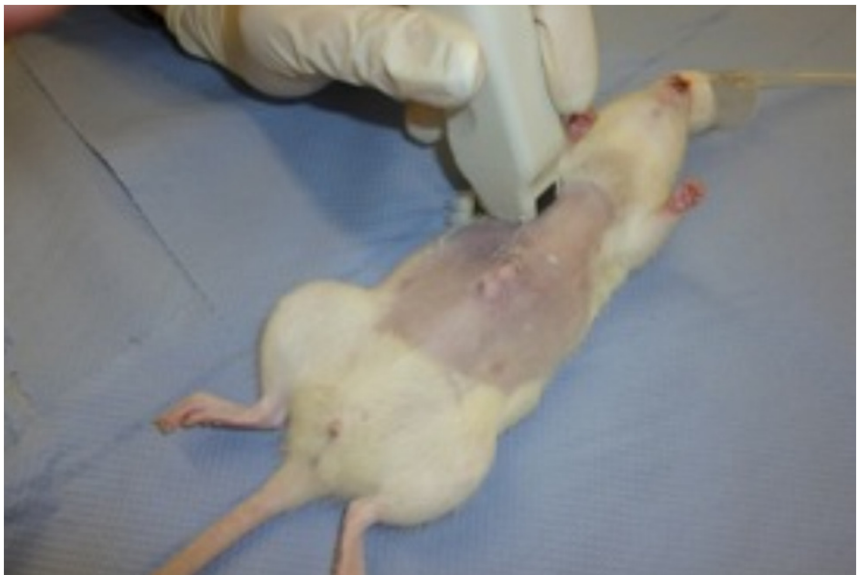
Animal studies

adult Wag/Rij rats
n=72
transfected with rhabdomyosarcoma R_1H
in their right gastrocnemius muscle
and in the liver



treated with Mitoxantrone
(Fe_3O_4)
with and without a magnet.

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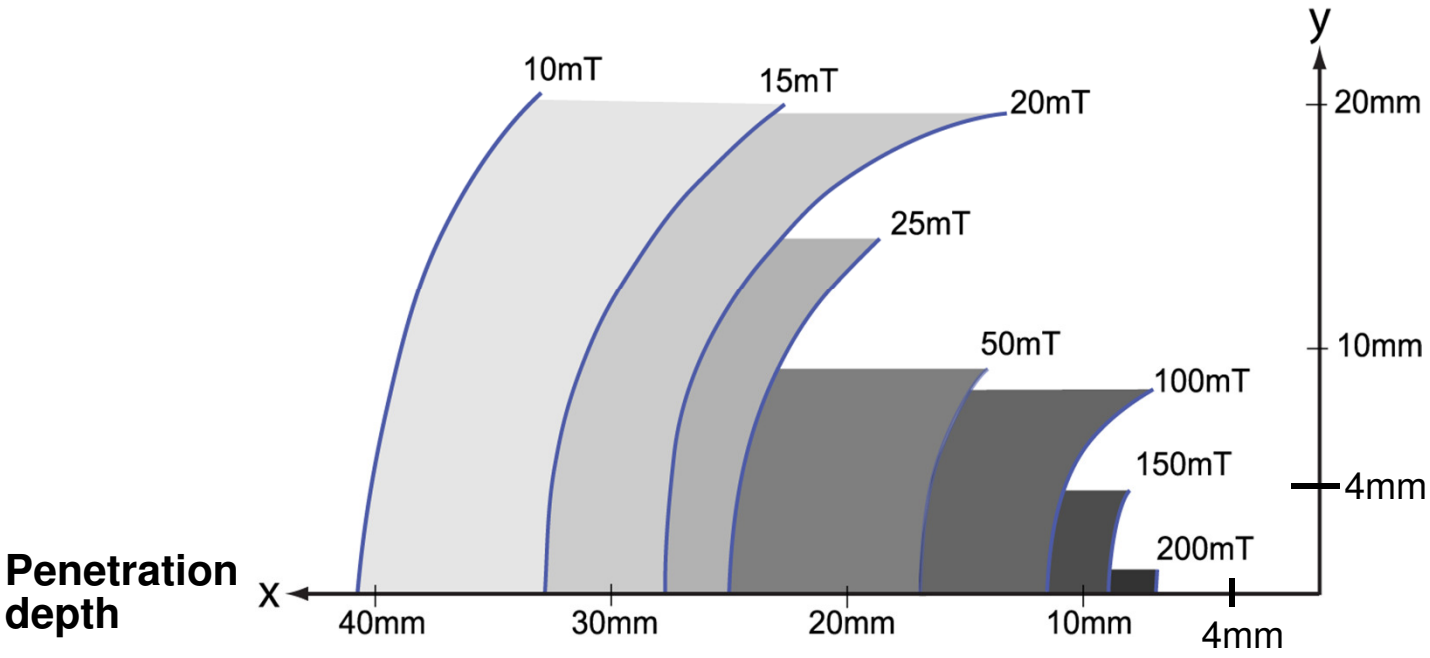
Methods

Mitoxantrone-iron oxide with and without an extracorporal 0.6 tesla magnet and uncoupled mitoxantrone were measured in plasma and tumor tissue for different doses administration.

The unit of the force flux density B in the SI system [International System of Units] is the tesla. 1 tesla = 1 Vs M^{-02}. The tesla unit was named after Nicola Tesla [1846 - 1943], a Serbo-American inventor and researcher in the field of electromagnetism.



In all our experiments we used a 0.6 tesla permanent magnet

The magnet was used for tumors outside the liver!

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Principle of magnetic drug targeting

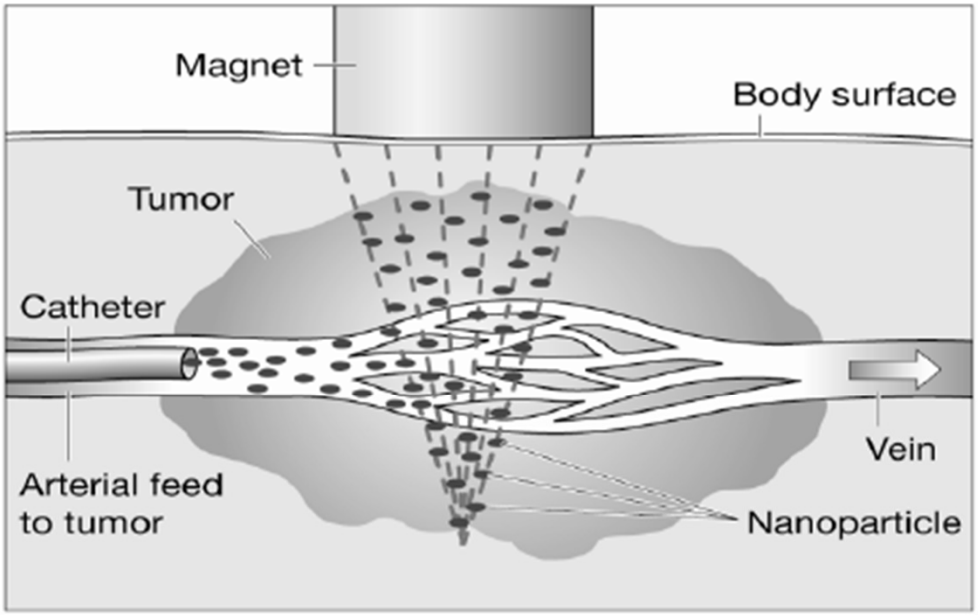
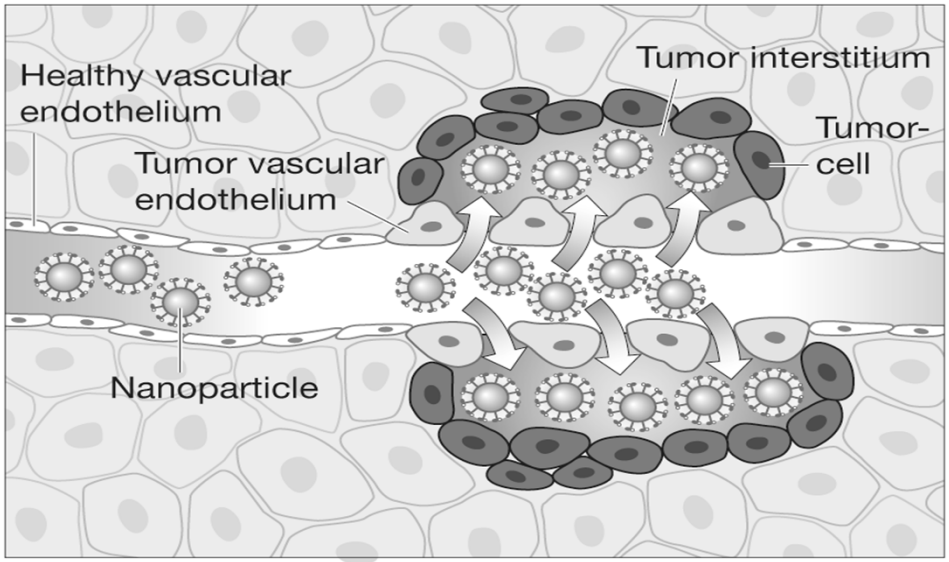
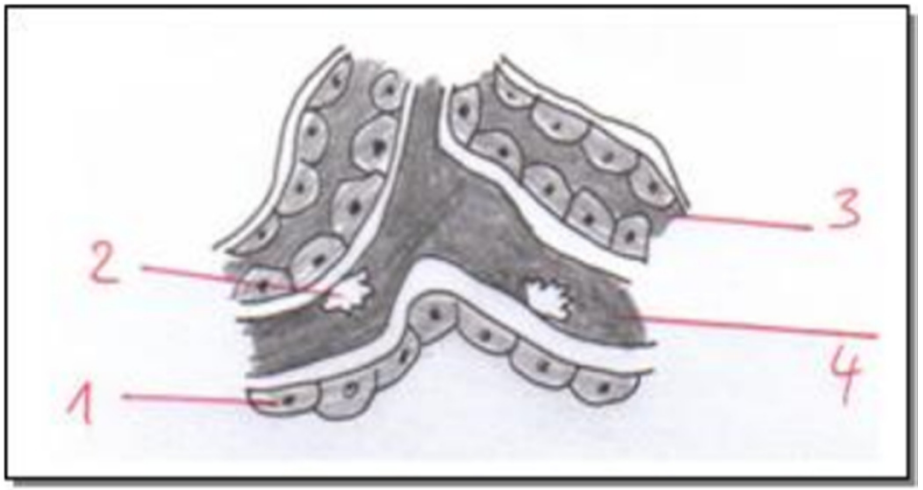


Fig. 2: Magnet and magnetic field in the tumor.

Iron particles will be concentrated under the magnet

Reticuloendothelial system (RES)





Extravasation of the nanoparticles
in the tumor tissue because of
pathological endothelium
and absorbation
in the reticuloendothelial system (RES)

1 = liver cells
2 = Kupffer star cells
3 = bile flow
4 = sinusoidal epithelial cells

Study design and treatment plan

The animals were divided into three groups:
Group I comprised animals which received only mitoxantrone in a dosage of 1 mg/kg BW.
Group II comprised animals which received mitoxantrone coupled with magnetic nanoparticles. The dosage was 1 mg/kg BW.
Group III as the control group received sodium chloride, 1 mg/kg BW.

Group	Administration	Drug	Number
I	i.v.	Mitoxantrone	6♀ : 6♂
II	i.v.	Fe_3O_4 + Mitoxantrone	6♀ : 6♂
III	i.v.	NaCl	6♀ : 6♂
		with and without magnet	∑ 72

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A batch of 2 x 36 rats divided into groups each of 6 female and 6 male rats formed the basis of the study. 14 days before the start of therapy R_1H tumor cells were injected into the right-hand liver lobe and in the musculus gastrocnemius.

Day -3:	Blood sample with blood count, iron and ferritin assay.
Day 0:	Administration of substances and tumor ultrasound.
Day 1:	Blood count, iron and ferritin assay.
Day 5:	Blood count, iron and ferritin assay.
Day 14:	Blood count, iron and ferritin assay.
Day 14:	At the end of the study: Tumor detection by ultrasound.

Autopsy of the animals and measurement of tumor size.
Samples taken for pathohistological examination.

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Drug

The active substance is mitoxantrone hydrochloride. Detachment of the iron oxide from mitoxantrone takes place during the half-life of mitoxantrone of approximately 30 minutes.

Parameter	Specifications
Product No.	05-66-302 S55009
Product Name	nanomag^{R}-CLD-Mitoxantrone
Product Description	magnetic dextran composite particles, cross-linked
Surface	immobilized mitoxantrone
Size (mean)	300 nm
Polydispersity index	< 0.2
Particle content	14 mg/ml
Iron oxide content	10.5 mg/ml
Mitoxantrone content	0.6 mg/ml
Quantity	33 ml
Shape	cluster-typed
Density	2.5 g/cm^3
Magnetization	43 emu/g particles (H = 1,000 Oe)
Saturation Magnetization	> 67 emu/g particles (H = 1,000 Oe)
Stable in	aqueous buffers pH >4
Not stable in	organic solvents, acidic solutions pH < 4
Product form	suspension in 0.01M PBS (pH = 7.4) filled in 50 ml Macoflex Empty Bag in PVC
Particles per ml	4.0*10E11
Particles per mg	2.8*10E10
Sterility Status	non-sterile
Additional remarks	Storage at 2-8° for 6 months, do not freeze

Mitoxantrone is obtained from micromod Partikeltechnologie GmbH, Rostock, Germany.

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Results of the tumor measurement

	Volume in [mm³]
Group I	24,83 mean
ID01	14,40
ID02	†
ID03	†
ID07	25,00
ID08	2,17
ID09	31,82
ID19	†
ID20	44,80
ID21	23,04
ID28	30,97
ID29	12,85
ID30	18,90

	Volume in [mm³]
Group II	15,12 mean
ID04	7,68
ID05	13,44
ID06	†
ID12	12,60
ID14	16,65
ID15	22,05
ID22	17,57
ID23	9,90
ID24	†
ID31	†
ID32	17,78
ID33	18,46

	Volume in [mm³]
Group III	18,83 mean
ID10	17,55
ID11	15,60
ID13	†
ID16	Reference
ID17	19,60
ID18	23,25
ID25	†
ID26	†
ID27	18,18
ID34	†
ID35	†
ID36	†

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The tumor volume showed the lowest growth in **Group II**, which was treated with mitoxantrone-coupled iron oxides.

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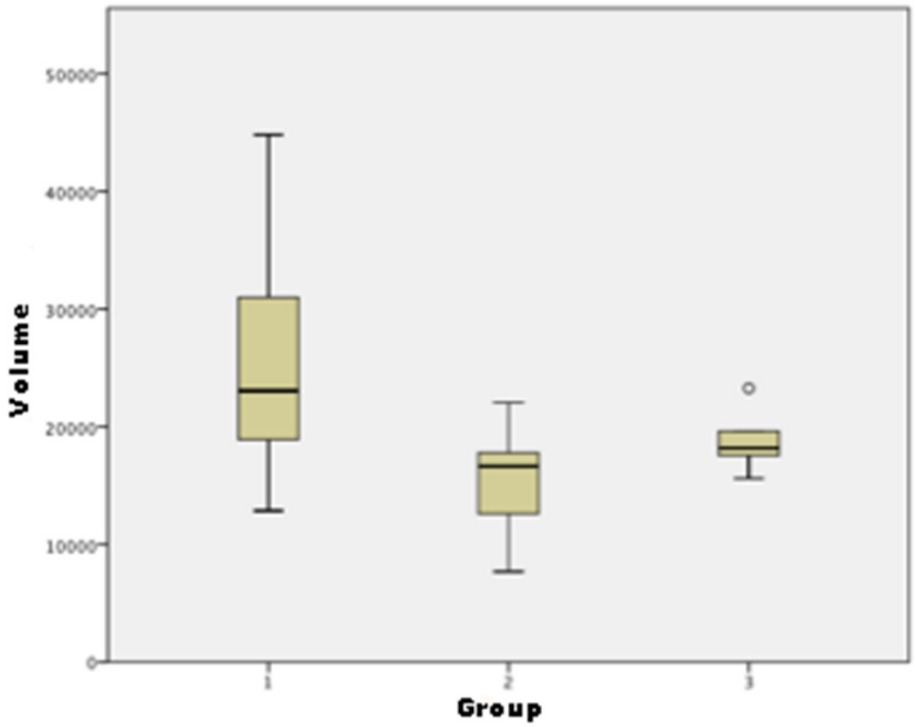
Box plot diagram of volume [mm³]



Each of the three diagrams comprises the study animals of its group. The thick black line represents the average animal (called median or 50th percentile). The brown column shows the animals within the 25th and 75th percentile. The horizontal lines represent the minimum and maximum respectively. In Group III, the maximum lies more than 1.5 times away from the 75th percentile, and is therefore identified as an outlier by the circle. Significant difference between group I and group II.

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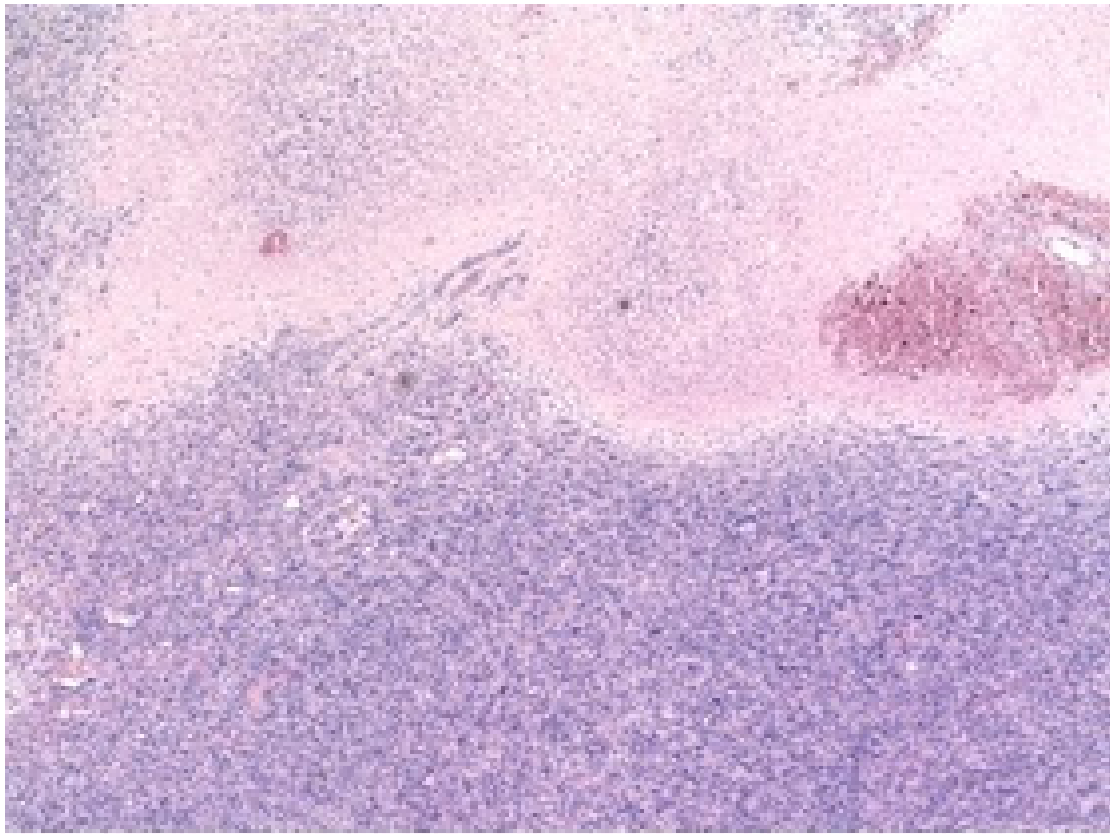

Group II, necrosis with erythrocyte extravasates.
In this tissue section the area of necrotic tissue makes up over 20 %

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Results I
outside the liver

Mitoxantrone iron-oxide concentration in plasma was significantly ($p<0.05$) lower
when a magnet was placed over the tumor area
and as low as uncoupled mitoxantrone.

Mitoxantrone coupled iron-oxide concentration in tumor tissue was always significantly higher
with magnetic drug targeting
when compared to uncoupled mitoxantrone.

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Results II

Mitoxantrone coupled iron oxide (Fe_3O_4) reduces the tumor volume to a greater extend than administration solely of mitoxantrone.

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Results III
inside the liver

Inside the liver and spleen the highest concentrations of coupled mitoxantrone iron oxide could be measured without a magnet because of adherence to RES.

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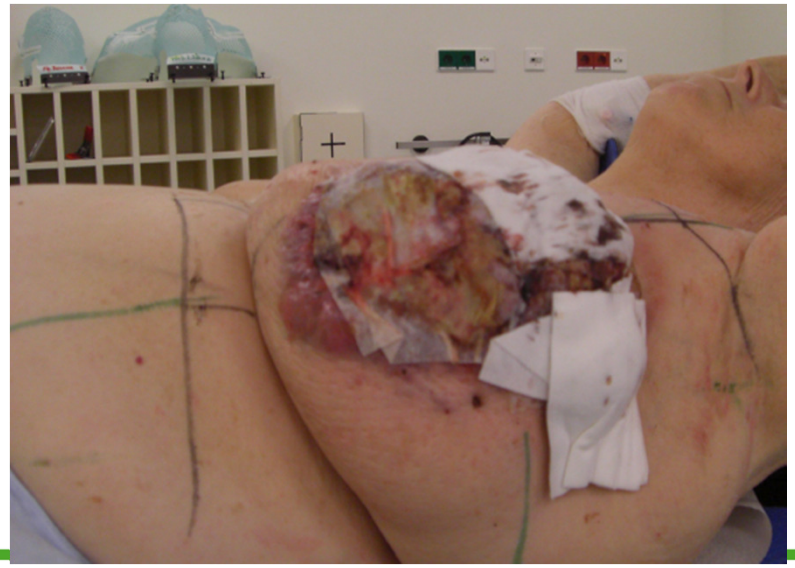
The case - individual therapeutic trial

70-year old female patient
with metastasized mammary carcinoma on the left;
initial diagnosis May 2008.
Diagnostics: Computer tomography (CT) demonstrated an exulcerated tumorous mass approximately 10 cm in diameter in the region of the left breast (volume: 500 ml)



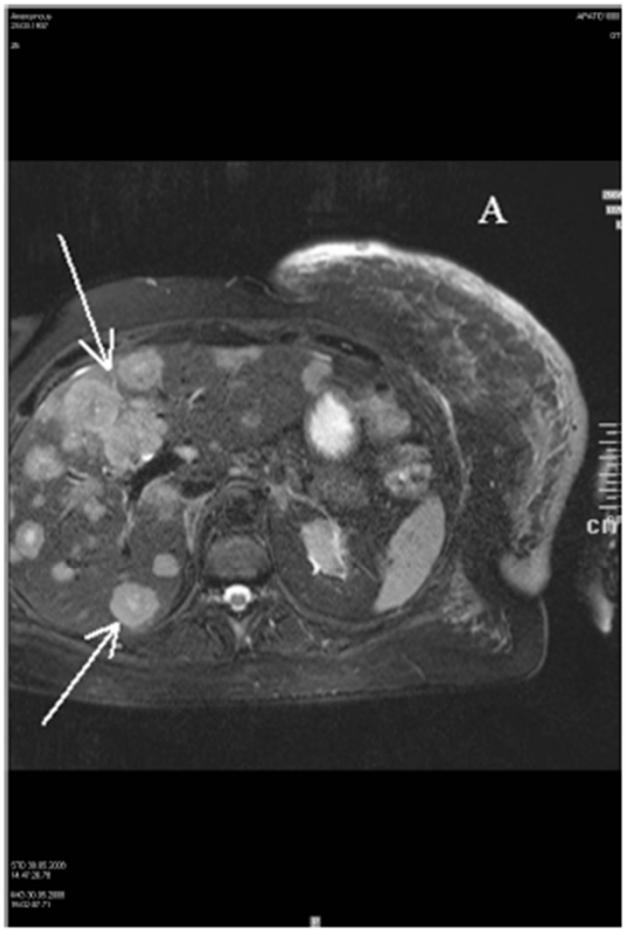


Figure 1 30 May 2008: metastasis of liver treatment. Arrows marks metastasis and lower liver. Small node has a size of 14.9 cm^3

MR demonstrates a massive tumor involvement of the liver

Therapy

Mitoxantrone-iron oxide [Fe_3O_4] corresponds to 32 ml fluids and 20 mg/m² body surface mitoxantrone.
Therapy started for four days.
After the end of each therapy a peripheral blood sample was taken.

Normal dosis in systemic treatment is 14 mg/m² body surface.

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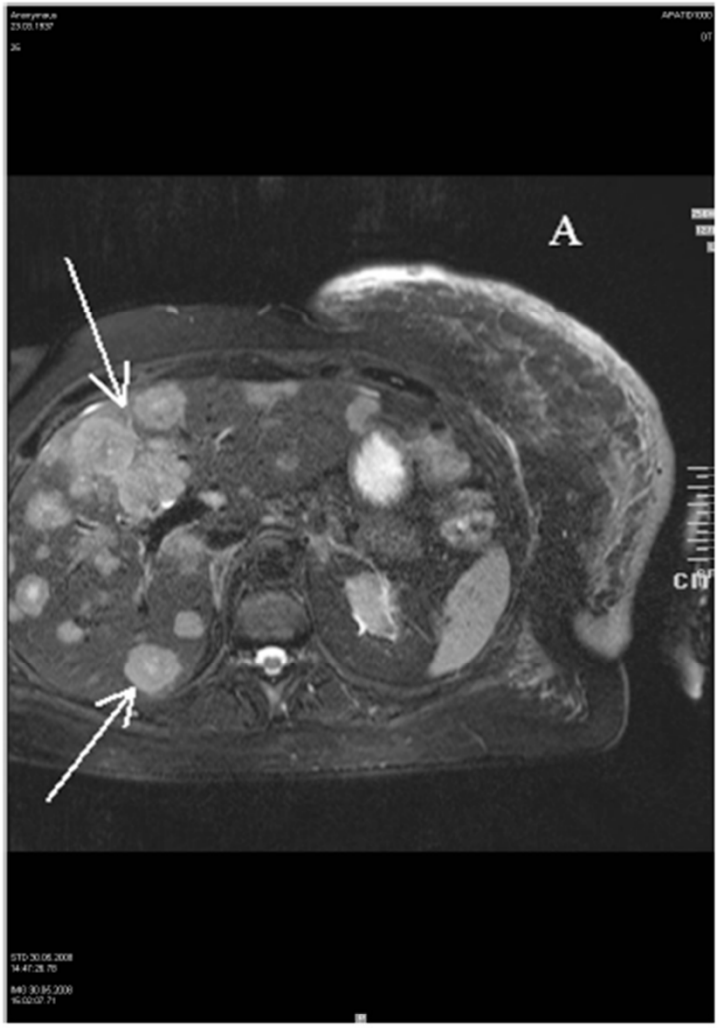
Serum levels of iron oxide and ferritin prior, during and after treatment

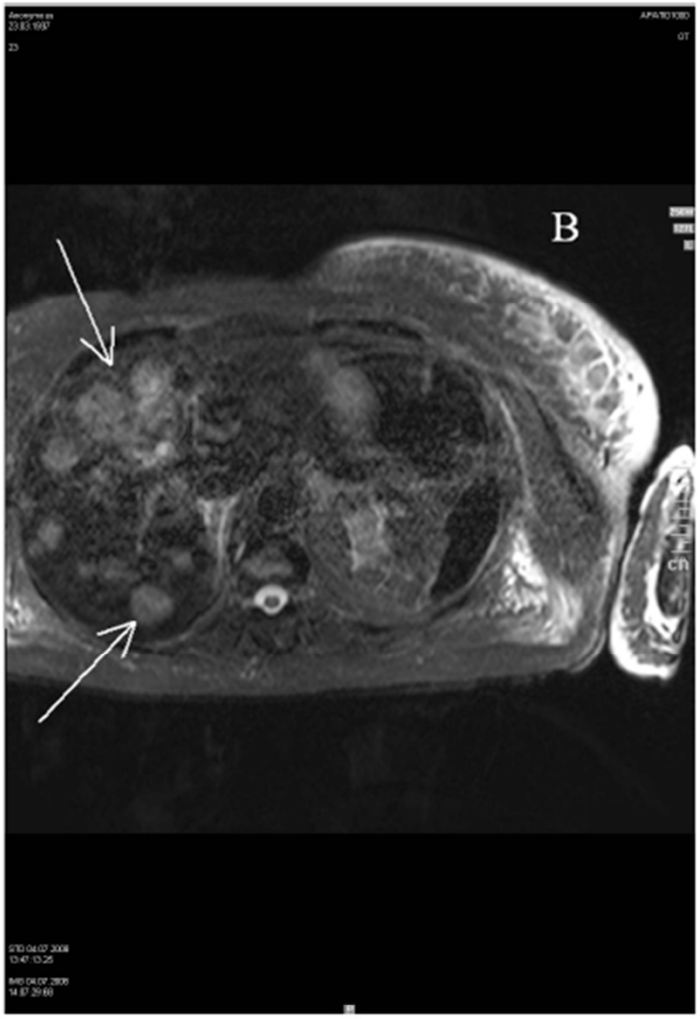
Parameter		Day -2	Day 0	Day 1
Iron	µg/dl	131	44	46
Ferritin	ng/ml	185,7	305,1	366,8

Parameter		Day 2	Day 3	Day 4	After 2 weeks
Iron	µg/dl	96	117	151	119
Ferritin	ng/ml	535,4	729,5	1011,0	1985,0

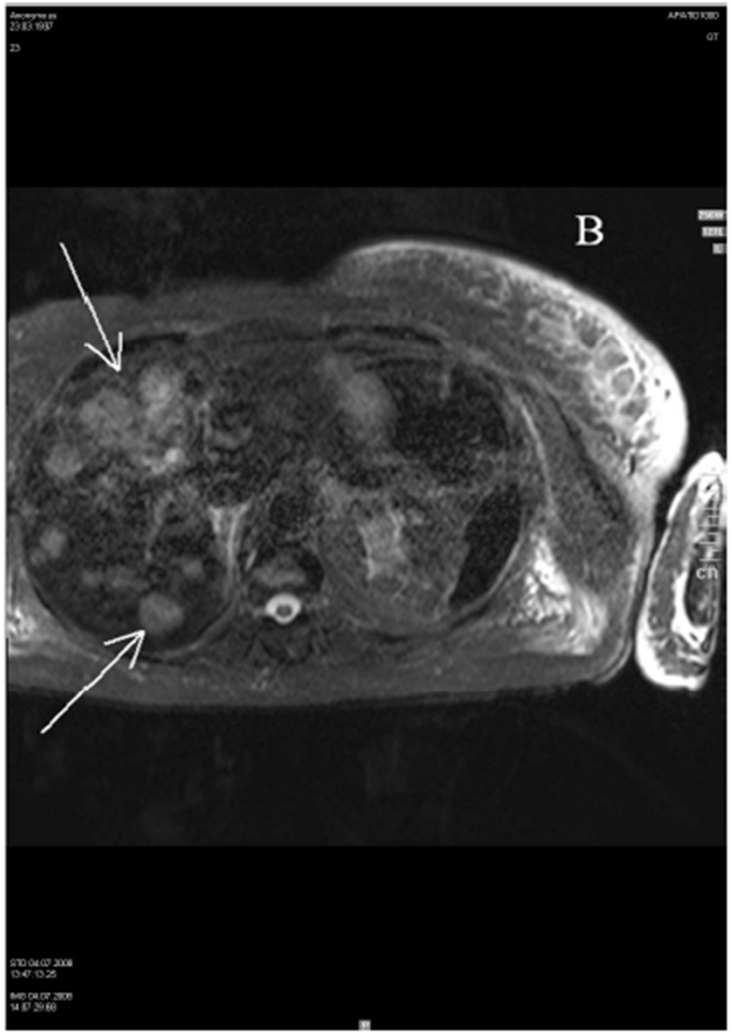
Normal values Iron: 33 – 193 µg/dl
Ferritin: 34 – 310 µg/ml



May 2008:
MR demonstrates
the liver full of metastases



July 2008:
MR demonstrates
a partial remission



September 2008:
MR demonstrates
stable disease

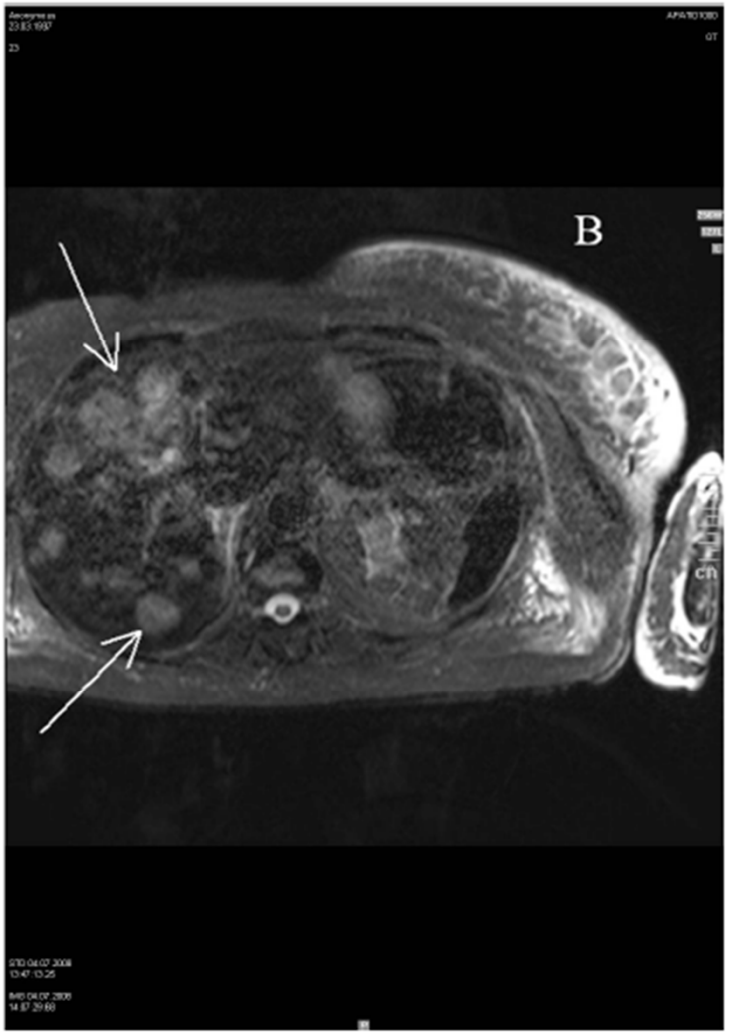


Figure 4: 29 January 2009: Visible reduction of all metastasis in the liver. Small liver metastasis in the right lobe decreased in volume by 75 % to 3 cm^3 in comparison to image Figure 1.

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Doses

Study doses	20 mg/m² body surface
Normal doses in systemic therapy	14 mg/m² body surface

The concentration of ferrofluids was 10.5 mg/ml, which corresponds to 6.4 mg iron/ml solution and 0.6 mg mitoxantrone/ml solution.

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Therapy results

- Tumor shrinkage in the liver was measured between 37,5 – 75% in size 3 months after the end of therapy
  ► partial remission

- At this time blood values of the liver had been partially normalised.
  Hence, the anaesthesist was able to gain narcosis for breast ablation.

- 2 weeks later the patient was discharged from hospital

- The patient survived about 2 years without clinical problems

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Discussion I

By means of the RES fluid iron has a large adherence to the liver with high concentration of mitoxantrone in the target organ and low concentration in the blood.

In the end, the success can be explained by the fact that in the liver iron undergoes phagocytosis by the Kupffer star cells.

Hence, we were able to perform a nearly local therapy by systemical approach.

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Discussion II

This new technique is a therapeutic option for primary and secondary tumors of the liver to reduce the tumor mass with higher cytostatic concentrations and less adverse effects for the patient, especially if the patient is unable to get a systemic standard therapy.

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