

WHITE PAPER
Hyperthermia



Hyperthermia Treatment of the Liver

Celsius42's TCS – Tumor Cell Solution regional hyperthermia system in the treatment of hepatocellular cancer & liver metastases

Compiled by Martin Roesch on behalf of Celsius42 GmbH, Germany
with expert contributions by Dr. Hüseyin Sahinbas, Bochum, Germany

Given the methodological similarities of the treatment, this paper makes no distinction between the treatment of hepatocarcinoma and liver metastases.

Content

1. Physical principle of TCS – Tumor Cell Solution capacitive heating	4
2. Effect and evidence of deep-seated heating	5
2.1. Phantom measurements	6
2.2. In-vivo measurements	8
3. Hyperthermia liver treatments with TCS – Tumor Cell Solution	10
3.1. Treatment application	10
3.2. Standard protocols	12
3.3. Warnings and contraindications	14
3.4. Patient thermosensitivity and ways to expand limitations	14
4. Rationale of using hyperthermia in liver treatments	15
5. Trials including hyperthermia	15
5.1. Trial of Samsung Univ. Clinic in Seoul, Korea using the TCS – Tumor Cell Solution regional hyperthermia device	15
5.2. Other selected trials in the literature on liver cancer treatment that include a loco-regional hyperthermia option	16
6. Treatment strategies for liver cancer, including hyperthermia	18
6.1. Available treatment strategies	18
7. Conclusion	21
8. Case Reports	22
Case Report 1: Liver metastases, Patient A	22
Case Report 2: Liver metastases, Patient B	23
Case Report 3: Liver metastases, Patient C	24
Case Report 4: Liver metastases, Patient D	26
Creating an agar phantom	31

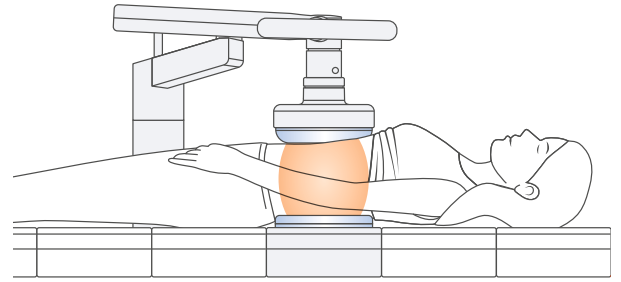
1. Physical principle of TCS – Tumor Cell Solution capacitive heating

Celsius42 TCS – Tumor Cell Solution is a device based on the functional principle of capacitive heating. It includes an upper and a lower electrode, which are protected by a grid and a water bolus containing deionized water. The electrodes can be adjusted to the patient's position. Care should be taken to ensure full contact with the patient's body since the liver is located on the right side partly below the plane of the abdomen. This gap needs to be filled (further details below).



The system operates at a frequency of 13.56 MHz with a power capacity of up to 600 W. For liver treatments, the largest electrode currently available is 250 mm in diameter. Due to thermotolerance limitations of patients, no more than 50% of the nominal maximum power capacity should be used for this application in the liver area with the 250 mm electrode.

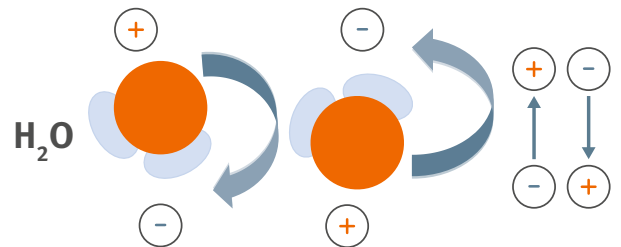
The patient's body – placed between these two electrodes – serves as a dielectric.



The Celsius42 TCS HF field between the upper and lower electrode changes its positive/negative charge 13.5 million times per second.

Ions within the dielectric (in each cell and in the matrix) react to the electromagnetic field by rotating according to its polarity → this creates heat.

Water molecules are electrically unbalanced: Since O-molecules bind electrons more strongly than H-molecules, the O-side of water is electrically negative and adjusts to the rapidly changing electrical field. The resulting friction with adjacent molecules generates warmth/ heat.



The device operating frequency of 13.56 MHz is capable of deeply penetrating the body. In contrast, micro-ovens that typically operate at 2.4 GHz can only penetrate a few millimeters. Frequencies in the 400 MHz range and above would only be suitable for surface heating.

Assuming a given constant rate of attenuation in water-dominant tissue structures (such as the human body), deep-seated penetration rises with decreasing frequency. As documented below with temperature measurements, the

Celsius42 TCS – Tumor Cell Solution devices designed for 13.56 MHz are perfectly capable of reaching sufficient temperature gradients. The value of 13.56 MHz was chosen deliberately because it is a freely available frequency which does not require Faraday cage shielding and thus helps reduce cost.

For more details regarding the technical concept of the Celsius42 TCS – Tumor Cell Solution device, please refer to our User Guide, Part I, “Regional Hyperthermia”.

Tissue differences

As shown in the graph below (Fig. 1), tissues vary in their response to electromagnetic fields. It should be noted that high and low are relative terms regarding the various tissues. For example, liver tissue is easier to warm up than regular muscle tissue.

Similarly, if malignant tissue (as it is claimed by some authors) had a higher rate of ionization (negative electrical charge), it would be more susceptible to capacitive heating. If that were to be so, it would have an additional selective effect.

Fig. 1:

2. Effect and evidence of deep-seated heating

Celsius42 TCS – Tumor Cell Solution devices are well suited to generate heat in deep-seated body locations. Some limitations exist for obese patients in the abdominal area since overly thick layers of fat tend to absorb the energy. Moreover, such layers increase the distance between electrodes, which further reduces the deep-seated impact. Temperature measurements should be performed to determine the effect in morbidly obese patients with considerable fat tissue.

The section below includes brief summaries for phantom measurements, which show the impact of the Celsius42 TCS - Tumor Cell Solution device without the variable and unstable effect of blood flow cooling.

In contrast to earlier years, we now recommend the use of higher energy inputs in order to achieve sufficient temperature gradients. In liver treatments, patients by and large can tolerate higher power input (see Section 3.2 for further details).

Biological effects of capacitive electromagnetic fields

Different tissue structures	Conductivity σ	Dielectricity ϵ_r
fat	low	high
muscle	moderately higher	moderately high
internal organs	comparatively higher	slightly less
Healthy vs. malignant tissue	healthy	malignant
σ - Conductivity ϵ - Dielectricity	↓	↓
	<i>in relation to each other</i>	

All measurements were performed using fiber-glass optics with small sensors attached to the tip (*Fig. 2*).



Diameters: 0.5 to 0.9 mm according to model

2.1. Phantom measurements

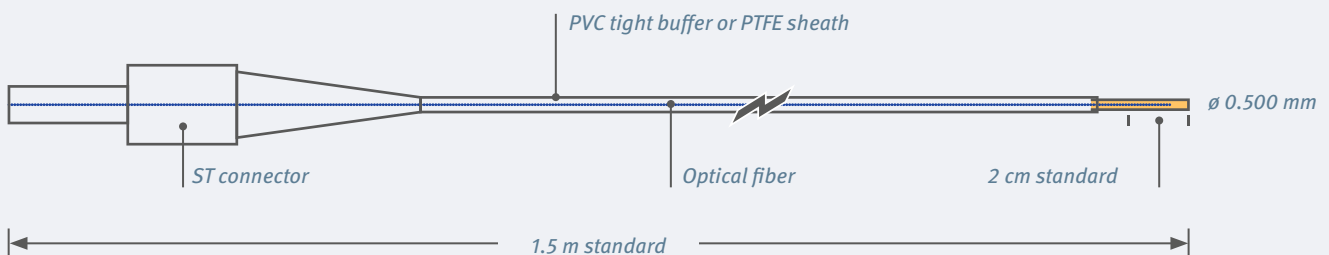
An agar gelatin corpus, created by heating water to just below the boiling point and containing 4% agar-agar and 0.9% NaCl serves as a muscle equivalent temperature model.



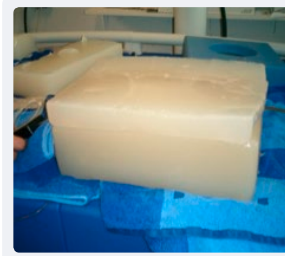
Invasive temperature sensors now can be inserted easily in all desired locations

The experiment shown below was designed to determine the predominantly horizontal effect of temperature distribution in the model. It provides evidence that a sufficient temperature has been generated in the phantom.

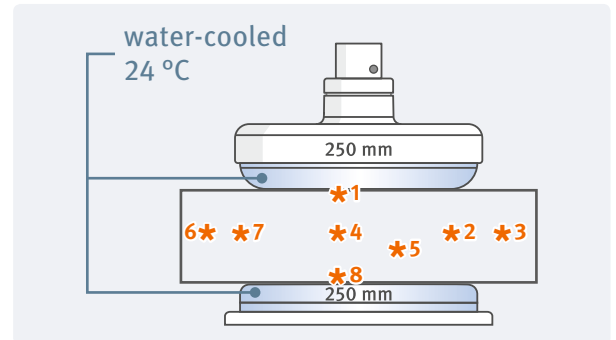
Fig. 2:



Total system accuracy is ± 0.3 °C



Muscle equivalence phantom: 1% agar and 0.9% NaCl



Results:

» Finding 1:

***1 and *8:** Relative temperature increase of 3.1/3.3 °C over 45 minutes (period 5-50); consistent cooling effect due to cooled water bolus at the surface

» Finding 2:

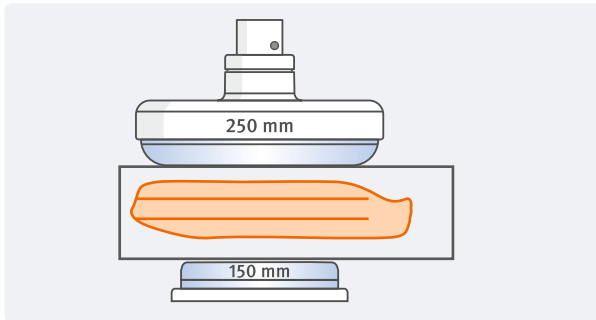
***4 and *5:** High, consistent temperature impact in the center and between electrodes with an increase of 9.3 °C and 8.5 °C

» Finding 3:

***3 and *6:** No temperature impact outside electrodes

***7 and *2:** Small increase of 2.0 and 1.6 °C at the electrode edge

In the next phase, a cow's liver was included in the phantom to measure the different tissue sensibilities. As expected, the power input created a much faster temperature rise in the liver tissue than in the muscle tissue. However, as in all the other cases, no cooling blood flow was present.



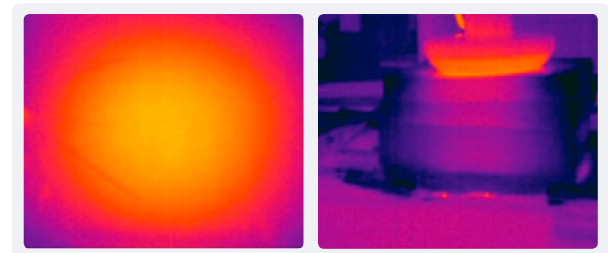
Results:

- » *In this configuration with a 250 mm upper electrode and a 150 mm lower electrode the maximum temperature impact occurs in the center of the treated area (with a temperature rise of 3.3 °C at only 150 Watt of power input and 9.4 °C at 300 Watt)*
- » *In this configuration with a 250 mm upper electrode and a 150 mm lower electrode, the maximum temperature impact occurs in the center of the treated area.*
- » *Liver tissue transfers the power impact much faster to a higher temperature than the surrounding muscle (temperature equivalent) tissue. (without blood (cooling) circulation)*

The infrared images shown below only illustrate the characteristics of heating. In preparation, the agar phantom was cut into slices. After heating, it was separated in the center, with immediate infrared images taken. In the image, a rather homogenous heating zone is clearly visible and measurable underneath the electrodes. Outside of the electrode's circular line, temperatures drop quickly.

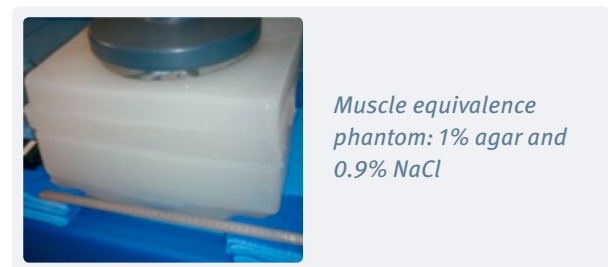
However, in an in-vivo model, larger blood flow dissemination would transfer heat into adjacent tissues.

As expected, the temperature impact near the upper electrode is slightly stronger than below. This effect accounts for about 20% and can be used in positioning the patient appropriately. However, liver treatments are best performed with patients in supine position.



SAT00224.SAT

SAT00238.SAT



Results:

- » *It is clearly evident that the temperature impact reaches well into the depth of the volume. Although a slightly larger temperature increase can be observed in the center of the electrodes (probably due to heat dissemination), the effect is also noticeable in the area between the two electrodes. In spite of the slightly weaker output of the bottom electrode, there is an obvious loco-regional impact in area and depth.*

2.2. In-vivo measurements

Naturally, temperature measurements taken in the liver would be the final proof, but invasive measurements always represent point measurements. The adjacent areas can easily be warmer or cooler if the vascularization structure is different.

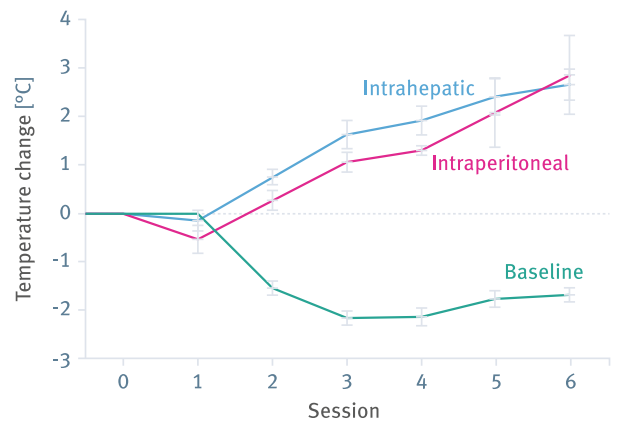
A Korean team from Samsung University conducted a study of in-vivo temperature measurements in the liver of three live pigs (40 kg).

Noh, M., Kim, H. Y., Park, H. C., Lee, S. H., Kim, Y. S., Hong, S. B., ... Han, Y. (2014). In vivo verification of regional hyperthermia in the liver. *Radiation Oncology Journal*, 32(4), 256–261. <https://doi.org/10.3857/roj.2014.32.4.256>

The fiberoptic sensors – as described above – were located in the liver and verified by CT scan (Fig. 3) after the measurements.

The study protocol was based on the recommendation given in Section 3.2 of this paper. While the baseline core temperature was decreased due to anesthesia of the pig, the intrahepatic and intraperitoneal temperatures showed a sufficient temperature gradient. The graph also includes a less effective first session with much less power. However, this pattern is needed in order to achieve sufficient heat tolerance in the later sessions. The desired temperature gradi-

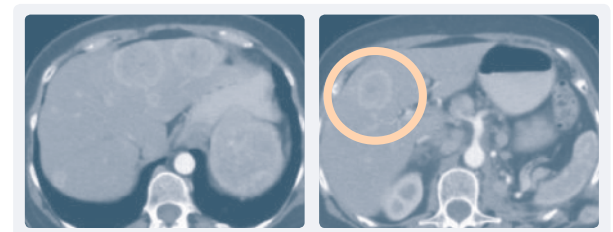
ent of 2 °C was achieved. The authors concluded that a mean increase of 2.67 °C in the liver and 2.87 °C in the peritoneal cavity was observed.



Radiat Oncol J. 2014 Dec; 32(4): 258

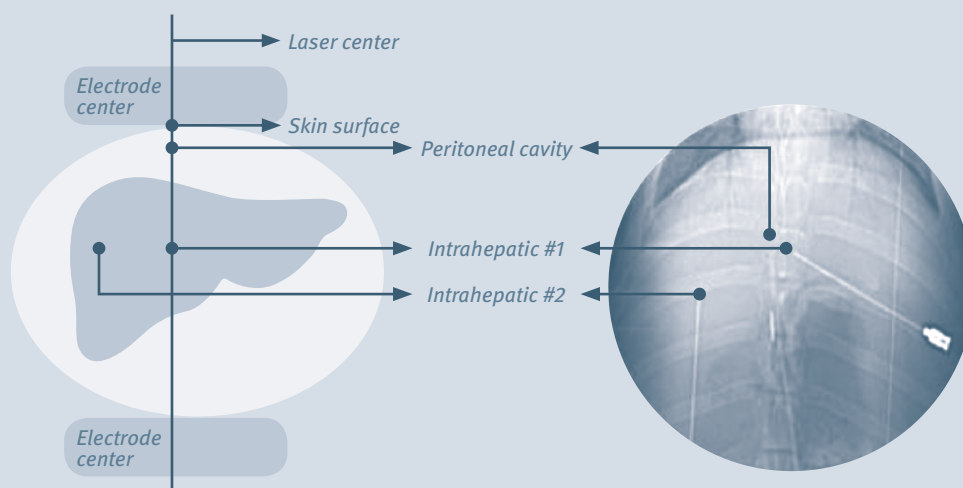
Measurements in humans

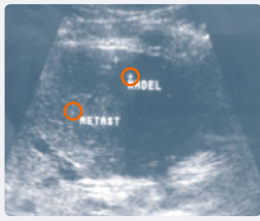
The section below refers to two measurements taken in a human patient. The CT images of this patient show liver metastases (primary tumor in the pancreatic head).



CT image of liver, Feb 28, 2013 S., M.

Fig. 3:



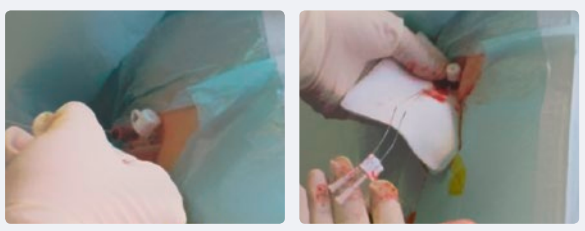


○ Needle

Ultrasound image,
Mar 19, 2013
S., M.

The two temperature sensors within the needles were placed under ultrasound guidance.

All treatments were performed by Dr. Sahinbas, Hyperthermia Center, Bochum, Germany.



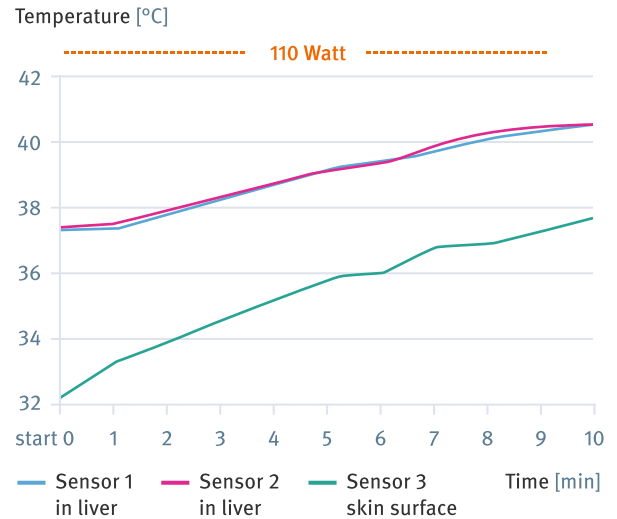
Temperature sensors, Mar 19, 2013 S., M.



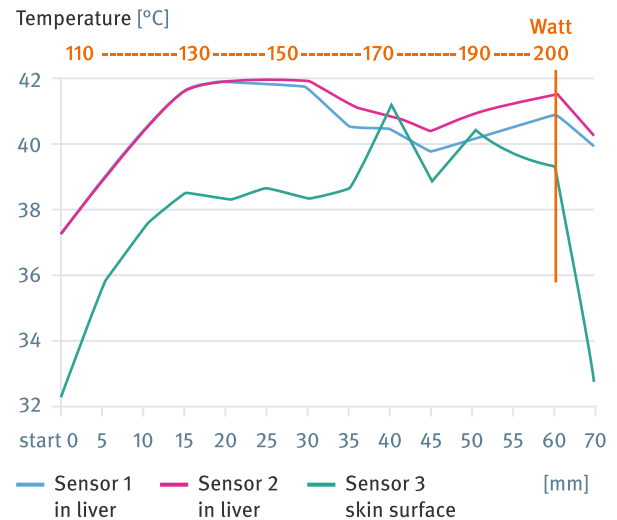
Two sensors were placed in the liver and one on the skin surface; water cooling was set to 8 °C.

Treatment duration: 60 min from 110 to 200 Watt, 590 kJoule.

Results:



Excerpt of first 10 minutes (at 110 W)



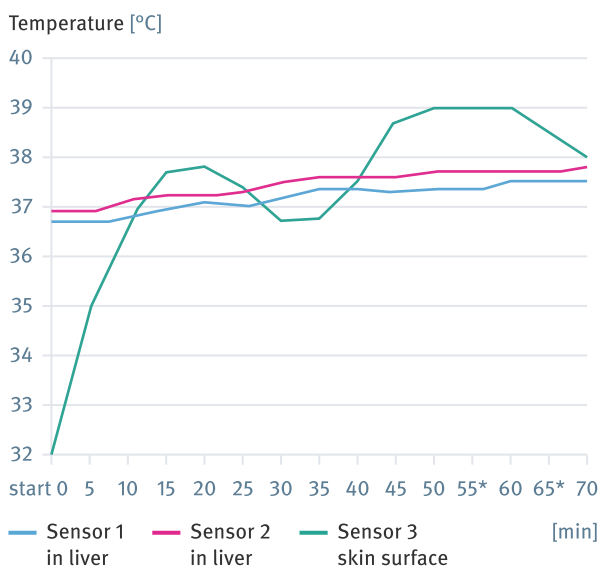
Full 60 minutes (20 W increase every 10 min)

- » For the first 10 minutes, the temperature rise in the liver was 3.2 and 3.3 °C, to 40.6 °C.
- » Accordingly, this represents a SAR (specific absorption rate) of much more than the required increase of 1 °C per 5 min as defined in the matching criteria by the European Society of Hyperthermia in Oncology (ESHO).

The overall peak temperature reached 42.0 °C; the plateau remained above 40 °C for 50 minutes.

It should be noted that in the first previous session with the same patient two days earlier, which used the same protocol, only a very moderate temperature rise of 0.8 and 0.9 °C was measured in the liver, with a peak temperature of only 37.8 °C. Toward the end of the session, the patient asked to interrupt the treatment because it exceeded his heat tolerance.

We still cannot quite interpret this low measurement. In the next session, the same protocol reached temperatures of 42 °C. The location of the sensors may be more influential than initially assumed.



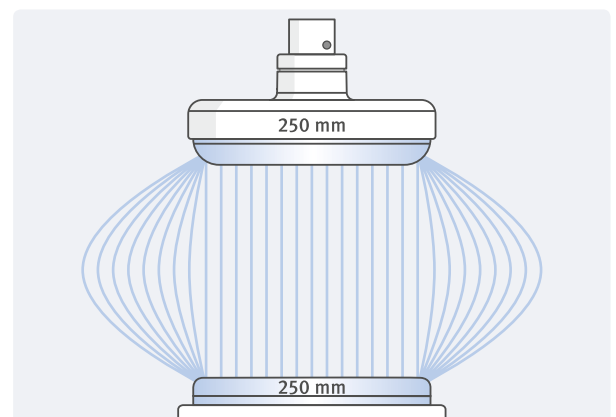
Power applied: starting at 110 W for 20 minutes, then increasing by 20 W every 10 min.

3. Hyperthermia liver treatments with TCS – Tumor Cell Solution

Liver treatments are usually well tolerated and seem to be quite beneficial. However, temperature measurements for quality control are not easy and would require an invasive procedure. Based on past experience and several measured temperature gradients, we can provide recommendations for administering TCS sessions for liver patients as summarized below. For general advice on the application of Celsius42 TCS devices, please refer to the User Guide, Part I, which is available from Celsius42.

3.1. Treatment application

For liver treatments it is recommended to mount 250-mm electrodes on the upper and lower side. Make sure to position the electrodes in proper alignment. Check that the center of the arm electrode is precisely positioned over the bottom electrode (see User Manual and User Guide, Part I). In this configuration, the energy field is symmetric as shown below.



Carefully ensure that the upper electrode is in full contact with the patient's body. Since the liver is located on the right side of the electrode system, the upper electrode tends to shift to the side of the body and can easily lose full contact. This would not be beneficial since part of the energy would not be absorbed through the patient's body and the electrode area that is in full contact would have a higher energy concentration. Air is a much better insulator than water-dominated tissue.

It is helpful to connect rounded and uneven body areas to the electrode with the help of water cushions. The image on the right below illustrates the principle, although a smaller water cushion of the type shown on the left is more suitable for liver treatment.



Liver treatment in cachectic patients requires additional water cushions to smoothly adapt the upper electrode to the body contours.

- » 3. Seal the bag with a conventional sealing device, starting with a straight line to leave just a 1 cm opening. Squeeze out all air bubbles and seal the bag completely. The bag should contain as few air bubbles as possible. Seal the bag again in a horizontal line to create additional thickness.
- » 4. That's it... The bag may need to be replaced after 10-20 treatment uses.



Example of a commercially available sealing device (approximate cost: <50 €)

Excursion: How to create a water cushion

- » 1. It is best to use a polyester or polyurethane plastic bag. These are available in different sizes and are already sealed on three sides. We recommend a thickness of approx. 100-200 μ .
- » 2. Fill of the bag with deionized water (not regular tap water!). Tap water usually contains approx. 600-900 μ Siemens, while deionized water only contains approx. 3 to 15 μ Siemens.

3.2. Standard protocols

A discussion of therapy strategies can be found in Section 4 below. Our recommendations in this context only pertain to the power structures to be applied. Based on our growing experience, we have increased power levels over the last years in order to maximize impact. We have observed the effects of doing so in single case outcomes, which in turn has motivated the treating physicians to aim for high power input and reach higher temperatures.

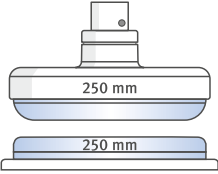
It is imperative to use so-called “step-up heating” with two dimensions of

- » a) an increase within each session and
- » b) an increase from session to session.

This allows for safely observing how well the patient is able to tolerate the sessions, while generating a much higher thermotolerance as an additional benefit.

Protocol for liver treatments recommended by Dr. Hüseyin Sahinbas.

Please note: To increase patient tolerance, cool the water bolus down to 10-12 °C

			Session 1	Session 2	Session 3	Session 4	Session 5+	Session 8+
Cooling		[°C]	20-22	18-20	16-18	12-14	8-10	8-10
Duration of fraction		[min]	60	60	60	60	60	60
LEVEL 1	Duration	[min]	20	20	20	20	20	20
	Power	[W]	40	50	60	80	80	100
LEVEL 2	Duration	[min]	10	10	10	10	10	10
	Power	[W]	60	70	80	100	110	120
LEVEL 3	Duration	[min]	10	10	10	10	10	10
	Power	[W]	70	85	100	130	140	150
LEVEL 4	Duration	[min]	10	10	10	10	10	10
	Power	[W]	90	100	120	160	160	180
LEVEL 5	Duration	[min]	10	10	10	10	10	10
	Power	[W]	100	>120	max. tolerable power 150	max. tolerable power 180	max. tolerable power 200	max. tolerable power 200
Cumulative applied energy in kJoule		[kJ]	240	~285	~340	~400-425	~450-500 min. >400	~500 min. >400

› Level 4 needs to be adjusted to patient; fraction 5+ at level 5 still experimental; fraction 8+ still experimental, needs to be observed and evaluated individually.

Our experience over the years has guided us to the power settings recommended here. It has further evolved since the early days of therapy with the Celsius42 TCS devices. However, we want to emphasize that these recommendations provide only rough guidance. Patients show different temperature tolerance patterns at different times and in response to different combination therapies. The therapy protocol may well need to be adapted to the individual session.

The cooling temperature of the electrodes must be set accordingly for high energy inputs. For example, at 150 watts and higher, it seems optimal to set the cooling temperature to 16-12 °C, and with power outputs over 180 watts a cooling in the range of 10-8 °C is advisable. Patients must be closely observed when using higher power settings. An additional water cushion should be placed between the electrode and the patient whenever a better overall contact can be established in this way. Always wipe off any humidity in the latter course of the treatment! Continually check a patient's well-being to avoid eventual negative side effects such as skin burns or pain.

Of course, no protocol should be followed blindly. Patients need to be observed and questioned about any treatment side effects they may have experienced before the respective next session.

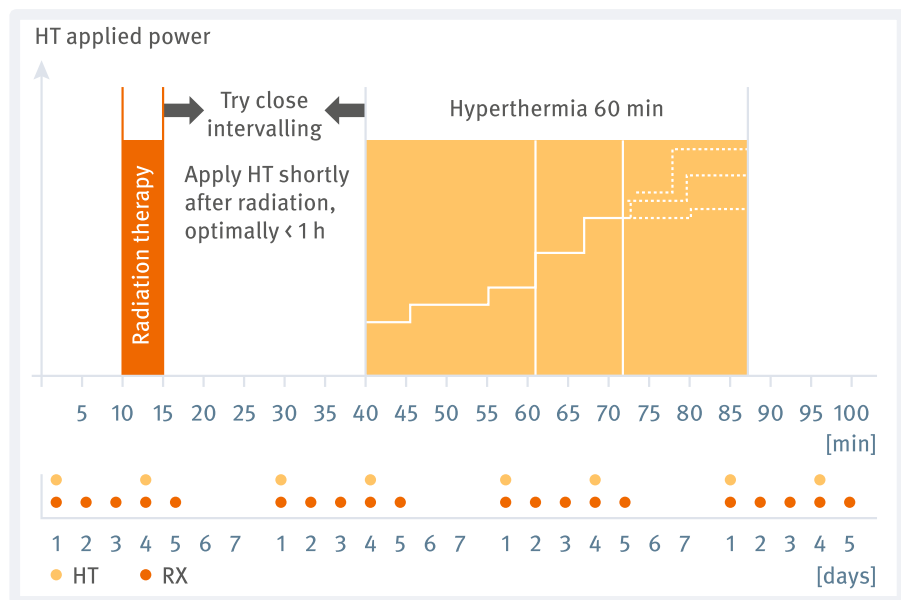
The next section briefly covers our recommendations regarding the combination of

- » a) radiation and
- » b) chemotherapy

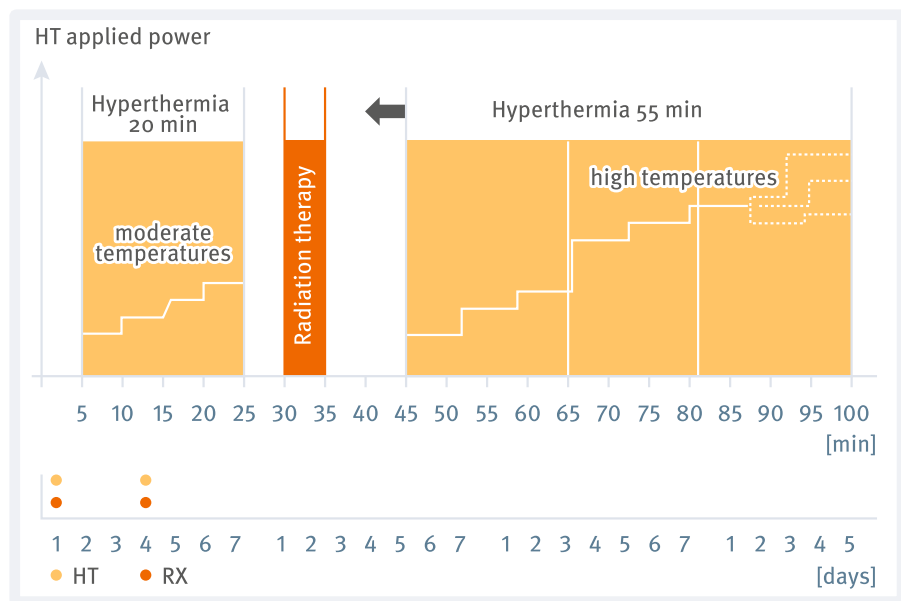
However, not much detail is known about the latter.

Regarding point a: If treatment is combined with radiation, we generally recommend the following therapy regimen. 48 hours should pass between hyperthermia sessions to allow for the dissolution of heat shock proteins. Otherwise, the subsequent session will be less effective since the purpose of HSPs is to protect cells against stress such as heat. Section 4 includes further details on therapy strategies.

Recommended regimen and therapy regimen protocol (here in combination with radiation)



Optimal treatment regimen (useful in case of hypo-fractionated radiation):



Regarding point b: Treatment is combined with chemotherapy. Unfortunately very little is known about the ideal scheduling of cytostatic drug infusions and the timing and fractions of hyperthermia sessions.

I. Scheduling

Preliminary research and application experience suggests the following:

- » * *Doxorubicin and cisplatin probably best during or in close proximity to hyperthermia*
- » * *Gemcitabine, oxaliplatin, 5-FU probably best 24 h after infusion*¹

II. Recommended temperatures

- » *Moderate HT in the range of up to 40.5 °C may be preferable*²

¹ Satoko Adachi et al: Effects of hyperthermia combined with gemcitabine on apoptotic cell death in cultured human pancreatic cancer cell lines, *Int.J.Hyperthermia* 2009 25(3):210-219

² Joan M.C. Bull et al: Fever range whole-body thermal therapy combined with cisplatin, gemcitabine and daily interferon-alpha: A description of a phase I-II protocol, *Int J.Hyperthermia* 2008: 24(8):649-662

3.3. Warnings and contraindications

The section below summarizes a few warnings and precautions. For a general list, please refer to the User Guide, Part I.

- » *pacemaker in the ROI*
- » *insulin pumps in the ROI*
- » *newly operated patients / fresh scars in ROI (risk of grade II / III burns)*

- » *new thrombosis in abdominal area and lungs*
- » *large pleural effusion (breathing restriction)*
- » *epileptics with EM sensitivity (risk of provoking a seizure)*
- » *partial loss of temperature sensation (check stroke patients)*
- » *delicate cardiac status (be cautious with delicate cardiac status)*
- » *be sure to remove all metal objects, belts etc. in the ROI*

3.4. Patient thermosensitivity and ways to expand limitations

Limitations to more aggressive power inputs can be observed in some patients who obviously are more sensitive to heat and have lower thermotolerance. There are many ways to overcome such obstacles. Please refer to Chapters 4 and 5 in the User Guide Part I, Regional Hyperthermia for further details about the technical concept of the Celsius42 TCS – Tumor Cell Solution device.

4. Rationale of using hyperthermia in liver treatments

HCC and liver metastases are almost never treated exclusively with hyperthermia. Hyperthermia is usually combined with various other therapies. For a general discussion of hyperthermia, contributions on the following topics are available on the Celsius42 website:

- » *a. Augmenting the effects of radiotherapy*
- » *b. Augmenting the effects of chemotherapy*
- » *c. Immunological stimulation*
- » *d. Effects of hyperthermia on DNA and cell behavior*

5. Trials including hyperthermia

5.1. Trial of Samsung Univ. Clinic in Seoul, Korea using the TCS – Tumor Cell Solution regional hyperthermia device

Researchers from Samsung University conducted a prospective phase II trial on 69 patients with hepatocellular carcinoma and a portal vein tumor thrombosis in order to investigate the efficacy and safety of a combined treatment: transarterial chemoembolization (TACE) followed by radiation therapy plus - as a further therapy option - subsequent regional hyperthermia. These combined therapies are referred to as CERT.*)

*)

Yu, J. Il, Park, H. C., Oh, D., Noh, J. M., Jung, S. H., Kim, H. Y., ... Yoo, B. C. (2016). Combination treatment of trans-arterial chemo-embolisation, radiotherapy and hyperthermia (CERT) for hepatocellular carcinoma with portal vein tumour thrombosis: Interim analysis of prospective phase II trial. *International Journal of Hyperthermia*, 6736(March), 1–8. <https://doi.org/10.3109/02656736.2016.1144895>

Yu, J. Il, Park, H. C., Jung, S. H., Choi, C., Shin, S. W., Cho, S. K., ... Paik, S. W. (2017). Combination treatment with transarterial chemoembolization, radiotherapy, and hyperthermia (CERT) for hepatocellular carcinoma with portal vein tumor thrombosis: Final results of a prospective phase II trial. *Oncotarget*, 8(32), 52651–52664. <https://doi.org/10.18632/oncotarget.17072>

The criterion for evaluating efficacy was the objective response rate (ORR), which was evaluated 3 months after completion of CERT. The overall ORR in all 69 patients was 43.5% (30/69) and the ORR in the radiotherapy target region was 69.6% (48/69). Liver function was not significantly affected by CERT.

The 2-year survival data of all patients was as follows:

- » Overall survival 62.9%
- » Local progression-free survival 47.6%
- » Progression-free survival 14.3%

Toxicity related to the combined treatment was manageable. However, as the authors report, pain intolerance to hyperthermia sessions was observed as the main obstacle. This highlights the eminent importance of managing a patient's thermotolerance as long-term experience has demonstrated potential in this respect (see User Guide, Part I, Chapter 5). This potential was not adequately leveraged in the trials hyperthermia treatments. Although the demanding power output suggestions should be followed in order to truly achieve the desired temperature gradients, the various mechanisms for reducing heat sensation (correct lateral positioning, wipe off sweat etc.) and patient comfort (manual adjustments, personal presence etc.) also have to be observed and applied.

5.2. Other selected trials in the literature on liver cancer treatment that include a loco-regional hyperthermia option

Maeta M et al: A case-matched control study of intrahepatoarterial chemotherapy in combination with or without regional hyperthermia for treatment of primary and metastatic hepatic tumors. *Int J Hyperthermia* 1994; 10 (1):51-58

(n =64 patients)

Treatment arm A:

- » *intraarterial chemotherapy (5 protocols) plus hyperthermia*

Treatment arm B:

- » *intraarterial chemotherapy only (5 protocols)*

Hyperthermia arm with 32 patients; total of 228 sessions for 60-70 min each; temperature target 41-43 °C for at least 30 min.

Results:

HCC:

- » *Arm A: PR 2 /8, NC 6 /8, PD 0 /8*
- » *Arm B: PR 1 /8, NC 5 /8, PD 2 /8*

Liver metastases:

- » *Arm A: PR 10/24, NC 8/24, PD 6/24*
- » *Arm B: PR 8/24, NC 6/24, PD 10/24*

Sugiyama A. et al: Hepatic arterial infusion chemotherapy combined with hyperthermia for metastatic liver tumors of colorectal cancer. *Semin Oncol* 1997; 24 (2 Suppl 6): S6-135-8

(n =17 patients)

Treatment arm A:

- » *isolated chemo liver infusion (5-FU, in part doxorubicin + mitomycin C)*

Treatment arm B:

- » *isolated chemo liver infusion plus hyperthermia*

Hyperthermia arm with 9 patients; total of 10-31 sessions for 45 min. or less;

Results:

- » *2-year survival arm A: 12% (2 /8)*
- » *2-year survival arm B: 35% (4 /9)*

Kurpeshhey O. et al: Immediate results of loco-regional hyperthermia and chemotherapy for liver metastases of colorectal cancer. Presentation at ESHO conference 2010 Rotterdam, May 22, 2010

(n =30 patients)

Results:

- » *CR 2/30, PR 8/30, SD 19/ 30 and PD 1/30*
- » *Combination treatment of chemotherapy plus HT “was found to be tolerated well by patients without producing marked general toxic effects.”*

Mayrhauser et al: published results on liver cell lines. Their findings show a correlation between temperature and thermal cell death; however, their heat application was much higher than what could be achieved in non-invasive in-vivo heating. It is interesting to note that their sensitivity to heat induced apoptosis decreased based on the grade of fibrosis in the liver.

Mayrhauser U, Stiegler P, Stadlbauer V, Koestenbauer S, Leber B, Konrad K, Iberer F, Portugaller RH, Tscheliessnigg K **Effect of hyperthermia on liver cell lines:** important findings for thermal therapy in hepatocellular carcinoma. *Anticancer Res.* 2011 May;31(5):1583-8.

Further:

Hager ED et al: Deep hyperthermia with radiofrequencies in patients with liver metastases from colorectal cancer. *Anticancer Research* 1999; 19 (4C):3403-8

(n =80 patients)

Kim BS et al: Phase II trial for combined external radiotherapy and hyperthermia for unresectable hepatoma. *Cancer Chemother Pharmacol* 1992; 31 (Suppl):S119-27

(n =30 patients)

Moffat FL et al: Effect of radiofrequency hyperthermia and chemotherapy on primary and secondary hepatic malignancies when used with metronidazole. *Surgery* 1983; 94 (4): 536-42

(n =102 patients)

Moffat FL et al: Further experience with regional radiofrequency hyperthermia and cytotoxic chemotherapy for unresectable hepatic neoplasia. *Cancer* 1985; 55 (6): 1291-5

(n =178patients)

Nagata Y, Hiraoka M, Nishimura Y et al: Clinical results of radiofrequency hyperthermia for malignant liver tumors. *Int J Radiat Oncol Biol Phys* (1997) 38:359-365

Nagata Y, Hiraoka M, Akuta K et al: Radiofrequency thermotherapy for malignant liver tumors, *Cancer* 65(8) 1990: 1730-1736

Alexander HR, Libutti SK, Pingpank JF et al: Hyperthermic isolated hepatic perfusion using melphatan for patients with ocular melanoma metastatic to liver. *Clin Cancer Res* (2003) 9: 6343-6349

Kasianenko IV, Osinsky SP, Pivnyuk VM et al: Thermochemotherapy for liver metastases in patients with mammary carcinoma and gastrointestinal tumors. *Oncol UKR* (2000) 2:34-36

Yamamoto K, Tanaka Y: Radiofrequency capacitive hyperthermia for unresectable hepatic cancers. *J Gastroenterol* 1997; 32:361-366

Seong J, Lee HS, Han KH et al: Combined Treatment of Radiotherapy and Hyperthermia for Unresectable Hepatocellular Carcinoma. *Yonsei Med J* 35(3) (1994):252-259

Ohguri T, Imada H, Yahara K et al: Effect of 8-MHz radiofrequency-capacitive regional hyperthermia with strong superficial cooling for unresectable or recurrent colorectal cancer. *Int J Hyperthermia* 2004; 20(5):465-475

Nishimura Y, Hiraoka M, Abe M: Thermoradiotherapy of locally advanced colorectal cancer. In: Matsuda T (ed) *Cancer Treatment by Hyperthermia, Radiation and Drugs*. Taylor Francis, London, 1993. 278-289

Nishimura Y, Hiraoka M, Akuta K et al: Hyperthermia combined with radiation therapy for primarily unresectable and recurrent colorectal cancer. *Int. J Rad Onc Biol* (1992) 23(4):759-768

Berdov BA, Menteshashvili GZ: Thermoradiotherapy of patients with locally advanced carcinoma of the rectum. *Int J Hyperthermia* (1990) 6(5):881-890

Jeon T, Yang H, Lee C et al: Electro-hyperthermia up-regulates tumour suppressor Septin 4 to induce apoptotic cell death in hepatocellular carcinoma. *International Journal of Hyperthermia* (2016) 32(6) 648-656 DOI: 10.1080/02656736.2016.1186290

6. Treatment strategies for liver cancer, including hyperthermia

Liver metastases are a common occurrence in colorectal cancer, gastrointestinal cancer, lung and breast cancer, and esophageal cancer. Hepatocellular cancers are comparatively rare, at least in Western countries. Since there is a large incidence rate of liver metastases, a variety of treatment options and strategies has been developed.

The primary strategy relies on surgery, whether open or minimally invasive (RFA, LITT etc.), with the recent addition of non-invasive HIFU. In cases where the tumor is already too large or has too many lesions, transarterial Chemoembolisation (TACE) is usually the next consideration, followed ultimately by systemic chemotherapy.

6.1. Available treatment strategies

Hyperthermia is typically considered as an option when surgery or ablation is no longer feasible. It rarely serves as a single strategy; the evidence we can rely upon only indicate benefits of hyperthermia in combination with other treatment options. The section below discusses two scenarios and an innovative third approach.

Scenario I

» *CERT protocol: initial chemoembolization, followed by radiation and hyperthermia*

Scenario II

- » *Liver chemoembolization or conventional systemic chemotherapy including hyperthermia*

Scenario III

- » *Innovative immunological concept: combination of low-dose chemo, radiation and hyperthermia*

Regarding Scenario I**Initial chemoembolization (TACE) plus subsequent radiation including hyperthermia**

This is the strategy employed by the Korean research group (see Section 5.1). Our suggestion would be to administer a hyperthermia session followed by the TACE infusion, ideally in tight sequential order and to use hyperthermia in the same pattern as the Korean trial, twice a week, with the hyperthermia session following as soon as possible. We recommend following the suggested power output of the two-dimensional step-up heating pattern. The active management of the patient's thermotolerance as discussed in the User Guide Part I is an important concern.

As an alternative, the hyperthermia session can be applied shortly after radiation (closely following, ideally within 30 minutes). There should be a break of at least one day before the next hyperthermia session to allow for denaturation of heat shock proteins within the cells.

Regarding Scenario II**Liver chemoembolization (TACE) or conventional systemic chemotherapy including hyperthermia**

Institutions typically have different approaches and preferences for cytostatic drugs. Of course, this also depends on the patient and whether or not a first-line therapy has already taken place. Hyperthermia may be included in all phases. The benefit/side effect ratio is quite in favor of including this therapy option. Common cytostatic drugs include 5-FU, cisplatin, oxaliplatin, doxorubicin, mitomycin C, sorafenib, gemcitabine and others.

Hyperthermia in this context focuses on moderate temperature gradients (39-41 °C, see power recommendations in Section 3.2). A typical treatment cycle could be five to six weeks with hyperthermia not more than every other day (3x/week) or twice a week. Allow for 48 hours between sessions. A treatment series can consist of 15 to 30 single sessions.

A few years ago a protocol was under discussion for a trial involving hyperthermia. Although the trial unfortunately never happened, the protocol is available from celsius42 GmbH.

Regarding Scenario III Combination of low-dose chemo, radiation plus hyperthermia

This scenario requires the availability of radiation equipment (Linac) and the willingness of the treating physicians to employ this rarely applied option. Promising results have been reported on the basis of various cases using this protocol (Brockmann, Sahinbas).

This is especially true for a scenario with advanced multifold carcinogenic liver penetration where full systemic chemotherapy is no longer feasible.

A variation of Scenario III would be to stimulate the adaptive immune response. In that case, radiation would serve the purpose of damaging cancer cells to cause necrotic cell death. The APC cells and dendritic cells would take up the tumorous cell material and travel to the closest lymph nodes to prime specific T-cells. Hyperthermia would support this lymphatic transport and stimulate the process with temperature gradients. Radiation would be hypofractionated (as suggested by Prof. Park in the Korea trial, which used 3 Gy/session) and ideally have a break of several days between sessions. This is an unusual pattern, but the rationale would be not to interfere with this immunological process by jeopardizing the agents in a radiation session the next day.

Criteria of success:

- » * *number and size of metastases reduced by a minimum of 50%*
- » * *tumor markers – if elevated prior to treatment – reduced as well*

Control:

- » *A CT scan is advisable prior to treatment (no more than 2 weeks). Another control CT should be performed 4-6 weeks after the end of radiation.*

7. Conclusion

Our experiences and the results achieved by a large variety of our Celsius42 TCS - Tumor Cell Solution clients have taught us that liver treatments, including loco-regional hyperthermia, clearly seem to be rewarding and successful in their prospective outcome. This is all the more remarkable given the fact that treatment strategies and regimens, the administered cytostatic drugs and even the hyperthermia application itself varied considerably across institutions. On several occasions, single liver cases have been presented at conferences and were confirmed by others in informal conversations. We therefore encourage everyone to incorporate hyperthermia in the treatment of liver cancer.

Institutes that have ready access to radiation should consider establishing a CERT protocol (chemoembolization (TACE) plus radiation with hyperthermia) administered very shortly after radiation (with at least 1 day between HT sessions)

Part of our observations included favorable feedback from patients, who reported an increase in life quality. In our opinion, QoL aspects should be considered in future trials.

Celsius42 GmbH in Germany will be pleased to answer any further questions you may have.

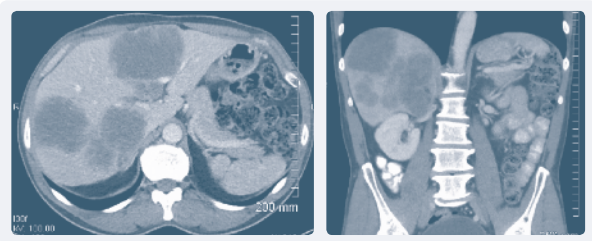
8. Case Reports

Case Report 1: Liver metastases, Patient A

(Treatment provided by Dr. Sahinbas, NRW, Germany)

The following case report pertains to a 52-year-old patient with a primary colon tumor who had developed extensive metastases in the liver. The initial treatment consisted of five cycles of the FolFox regimen over 3 months; this chemotherapy was subsequently discontinued. At the time of treatment, the liver metastases were the primary therapeutic target.

The CT image shown below was generated prior to starting the hyperthermia treatment.



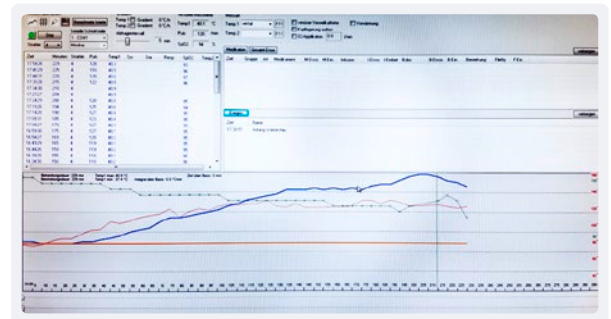
In the coronal view (shown on the right), also of November 2015, the tumor lesions are even more extensive.

As is evident in these CT images, surgical removal (either with open surgery or with RFA or LITT) was no longer an option, given the tumor size of the individual fields, with the larger metastases showing expansions of 8-12 cm, and the scope of the spread. The patient suffered from anemia, had lost 5-6 kg of body weight, and showed additional fatigue symptoms. At the beginning of the treatment, he showed an increased CA 125 level: 1630 (Dec. 9, 2015) and CEA: 379.

This patient was treated as follows:

Following a chemosensitivity test, he received chemotherapy with 90 mg oxaliplatin in two-weekly intervals from Dec. 2015 to Feb. 2016 as well as oral capecitabine daily for 2 weeks, followed by a 1-week break. A supplementary oncological infusion therapy with supportive agents (high-dose vitamin C, vitamin B-complex, and curcumin as well as selenium, procaine bases and artemisinins etc.) was administered in a changing rhythm and with varying compositions.

The thermal therapy consisted of active hyperthermia with mistletoe preparations as well as whole-body hyperthermia and regional deep-seated hyperthermia with focus on the liver. The whole-body hyperthermia was administered three times, in each case 24 hours after the administration of oxaliplatin. The peak core body temperatures in the sessions reached 40.9 °C, 40.2 °C, and 40.6 °C, respectively.



Moderately extended fever-like whole-body hyperthermia

The patient underwent 10 sessions of regional hyperthermia with power settings up to 500 kJ. This involved several invasive intratumoral temperature measurements for quality assurance of the desired temperature gradients. Fiber-optic temperature probes were placed into one of the large tumor lesions under ultrasound control. The intratumoral temperature measurements ranged from 39.5 to 43 °C.

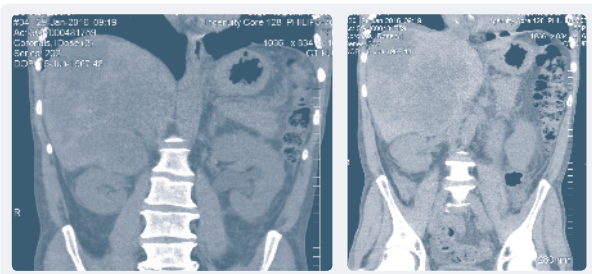


Invasive intratumoral temperature measurement as a quality assurance measure

Clinical outcome:

The first evident effect was a decline in the fatigue symptoms that are typically observed under this tumor burden together with anemia and the given therapy regimen. The patient's vitality and drive as well as his anemia improved during the further course. The tumor markers were also developing positively and showed significant declines.

Major changes were evident in the follow-up examination some two and a half months later:

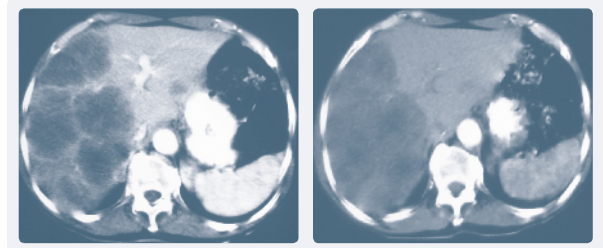


Although the imaging still showed necrotic tumor tissue, it could be presumed based on the tumor markers that this tissue was no longer highly metabolically active.

Case Report 2: Liver metastases, Patient B

(Treatment provided by Dr. Pistofides, Athens, Greece, and Dr. Sahinbas, Bochum, Germany)

This case involved a 72-year-old male patient with a primary colon cancer. The treatment had been preceded by a sigmoidectomy and concomitant mistletoe therapy. The CT images of the liver shown below were taken 5 months and 9 months later, at the time when the patient presented himself in the hyperthermia center.

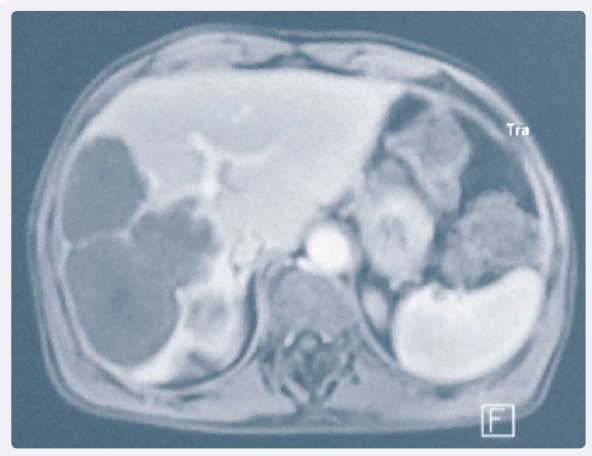


Tomography images taken prior to TACE/hyperthermia therapy in September 2010

At this point, the therapy began with a TACE (superselective transarterial chemoembolization) The following chemotherapy drugs were administered: 10 mg mitomycin C, 40 mg cisplatin, 100 mg irinotecan and 8 ml lipiodol.

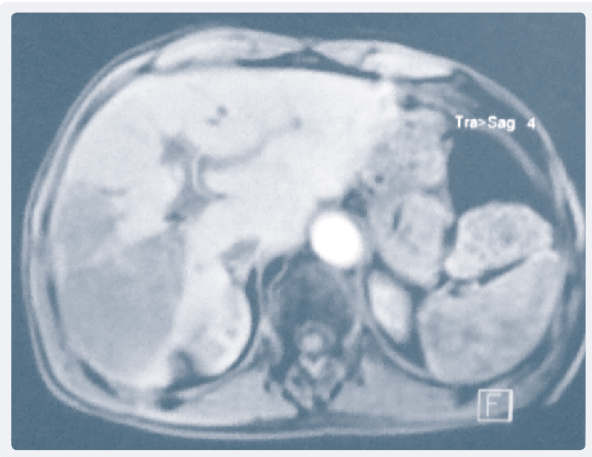
At the same time, the patient received local deep-seated hyperthermia with the Celsius42 device as part of the embolization therapy. These treatments were accompanied by infusion therapies with “biologicals”. Following the primary TACE therapy, the patient continued biweekly individual sessions with local deep-seated hyperthermia and the accompanying infusions. This involved a total of ten individual sessions with breaks of 4-6 weeks in between. No further systemic chemotherapies were applied.

The MRI images taken 6 months later as part of an interim evaluation demonstrate that the former tumor lesions had become necrotic, as evident by the hypodense tissue quality.



MRI 2-2011

In the next follow-up five months later, another MRI showed a significant reduction of the liver metastases.



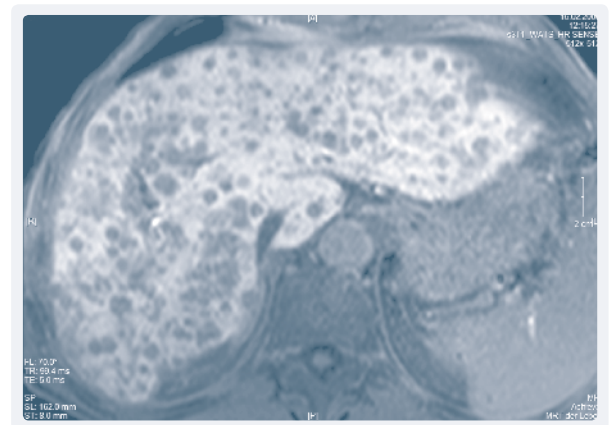
MRI 7-2011

Case Report 3: Liver metastases, Patient C

(Treatment provided by Dr. Sahinbas, NRW, Germany)

This case involved a 68-year-old male patient with liver metastases, with a diffusely metastatic adenocarcinoma in the pancreatic head, initially G3. The liver metastases had been first diagnosed in March 2007. The patient presented with the disease in the advanced stages: History of neoadjuvant therapy, history of SIRT therapy in July + August 2008; history of simultaneous chemosensitization radiotherapy of the abdominal lymph nodes from Feb. 6 to March 17, 2009. Since February 2008, fatigue syndrome with weight loss and increasing deterioration of physical performance.

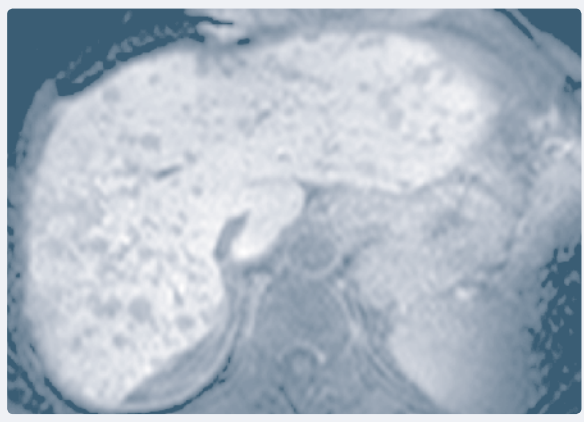
A diffuse progression (liver metastasis) had developed under ongoing chemotherapy since February 2009. Onset of liver impairment since April 2009 with tumor anemia.



The T1-weighted MRI image of Feb. 2009 shows the liver essentially covered with large-volume lesions.

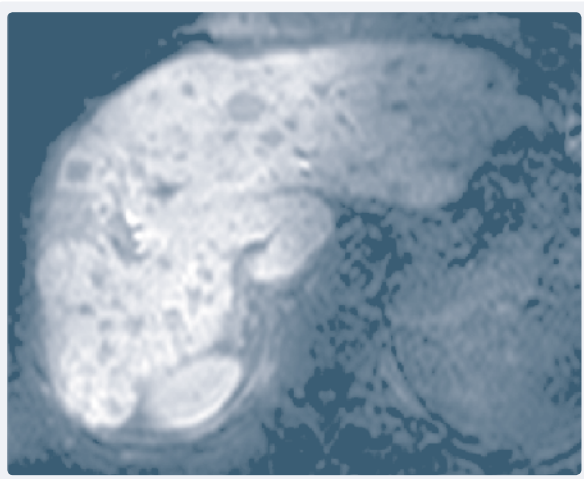
After the completion of the regional deep-seated hyperthermia with focus on the liver from May 6 to July 21, 2009 (total of 20 individual sessions) and the accompanying support therapy with “biologicals”, the follow-up MRI

examinations of the abdomen performed on July 11, 2009 showed another tumor regression. This alone is remarkable in the given situation.



MRI examination of the abdomen/liver of July 11, 2009

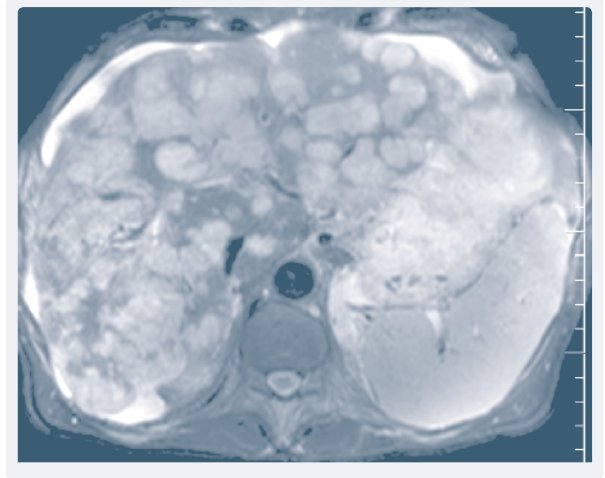
For further treatment, the chemosensitization hyperthermia was continued until September 2009. The imaging diagnostics of October 2009 showed another regression of the liver metastases, and the laboratory values indicated declining tumor markers as well. The patient also reported an improvement of his fatigue syndrome and physical condition.



MRT T1-weighted image of October 20, 2009

The patient no longer received any parallel hyperthermia in the time from October 2009 to February 2010. However, the chemotherapy was continued on its own. The next imaging fol-

low-up in April 2010 unfortunately showed another significant tumor progression.



MRI T2-weighted image of the liver of April 2010

The patient subsequently no longer received parallel hyperthermia, both for financial reasons and due to transport problems in a rural area (he was living on his own with no relatives close by). It is noticeable that this change in the ongoing therapy (discontinuing hyperthermia) brought the regression to a halt. The author is aware that this cannot be taken as proof of hyperthermia efficacy, but it is worth noting.

Case Report 4: Liver metastases, Patient D

(Treatment provided by Dr. Kalden, Berlin, Germany)

This is the case of a patient (born in 1968) who has undergone an amazingly long therapy course for treatment. The local tumor development in her liver and other body regions was repeatedly addressed successfully with local applications of hyperthermia in combination with other therapy options. This course of illness, which was originally considered a palliative case, has involved an unusually long treatment with over 300 hyperthermia sessions over the course of ten years.

Starting point ...

...was an invasive ductal breast carcinoma on the right, first diagnosed in 9/02 with poorly differentiated histology; grade III; hormone status: ER 90% pos, PR 90% pos; HER 2 neu: +++; in primary staging: pT2 pN0 (0/10) M0 R0

2002

September 2002

Diagnosis: Initial cancer diagnosis in the right breast (see above)

Immediate surgery: BCT right side and LNE on the right axillary

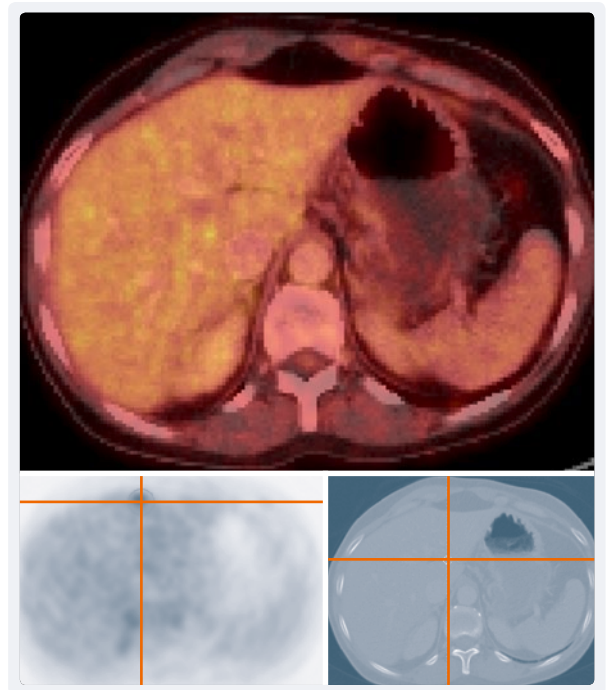
November 2002

First-line therapy: Chemotherapy (6 x FEC) and radiation, followed by Zoladex treatment until July 2004

2007

June 2007

Recurrence and development of further liver metastases (and others): Recurrence (liver, bone, lymph node metastases), Documentation: PET CT of Jul 10, 2007, Therapy suggestion of a university hospital: palliative chemotherapy with a projected survival time of approx. 6 months (which the patient therefore declined)



PET CT liver images

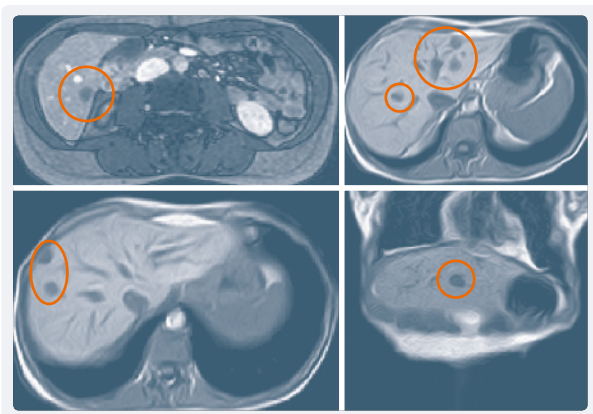
July 2007

Tam plus Zoladex - mistletoe HD + local hyperthermia bisphosphonates

November 2007

PD: Liver, tumor marker increase

In spite of this therapeutic approach, further tumor growth occurred.



MRI liver images

December 2007

The following changes were made in response:

Arimidex plus Zoladex – intensification of local hyperthermia, now three HT sessions every third week, with a peak output of up to 150 W, liver-activating hyperthermia (= fever therapy) based on bacterial lysates, pseudomonas/ streptococcus pyogenes; low-dose epirubicin bisphosphonates

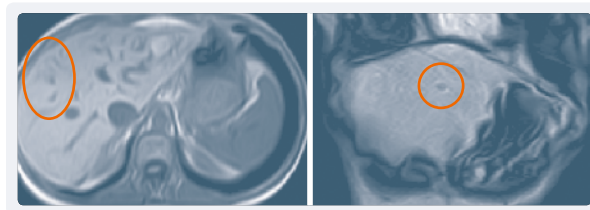
2008

January 2008

Patient responds to therapy. Drop in tumor markers CEA-CA15/3; additional administration of Herceptin

April 2008

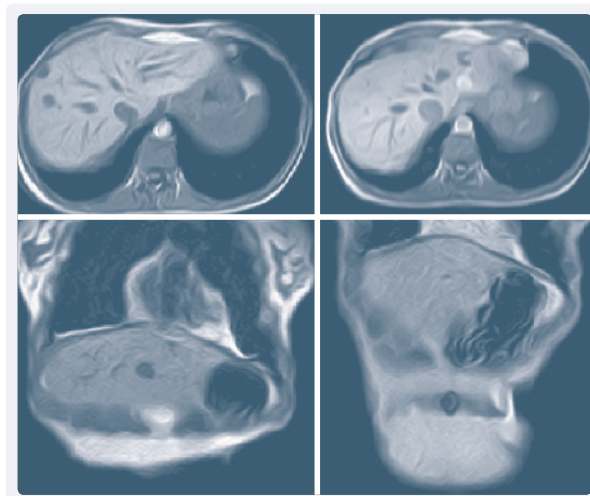
MRI follow-up **Liver PR** further reduction of tumor markers.



MRI liver images

July 2008

Repeated MRI follow-up **Complete remission in the liver**, a true therapeutic success!



Images of 11/2017 (left)
MRT comparison

Images of 4/2018 (right)

October 2008

PET CT follow-up: PR also in bones and lymph nodes; confirmed CR in the liver. Larger intervals for active and passive hyperthermia; sessions now every six weeks instead of every three weeks.

2009

June 2009

Repeated MRI follow-up: liver still in remission, but distinct progression in the bones; stable tumor markers

2010

September 2010

MRI: massive tumor growth in the bones; liver still in remission (with continued loco-regional hyperthermia of the liver (every six weeks for three days with peak output up to 150 W), Tumor markers are increasing, radiation therapy of the spine (thoracic vertebrae 5-8) in combination with regional hyperthermia; Aromasin

December 2010

PET CT: **new liver metastasis** is now visible; vital bone metastases in the pelvis. Consequence: Intensification of fever therapy and hyperthermia treatment (now focusing on liver and pelvis/lumbar spine)

2011

March 2011

Three months later: drop in tumor markers

May 2011

MRI: thoracic spine unchanged, again **remission of liver metastasis**, SD in the pelvic bones

December 2011

PET CT: compared to 12/10, distinct PD in the pelvic bones; **no sign of liver metastasis**. Tumor markers constant

2012

January 2012

MRI of the pelvis: no change compared to May 2011; meaning overall stable condition with unchanged tumor markers.

April 2012

SD

September 2012

SD

December 2012

Brain metastasis in the area of the right cerebellopontine angle, CyberKnife radiation (Charité hospital, Berlin), switch of systemic therapy to XGeva, chemical immune modulator corrected: instead of low-dose epirubicin now low-dose cyclophosphamide in conjunction with active and passive hyperthermia

2013

June 2013

Tumor markers are unchanged. MRI of the skull: SD - therapy is continued

2014

January 2014

Increase of tumor markers

February 2014

Re-staging: Brain metastasis PD, bones partly PD, liver normal

April 2014

TAM replaced with Faslodex, XGeva again replaced with bisphosphonates, intensification of hyperthermia plus Endoxan (IPT and fever)

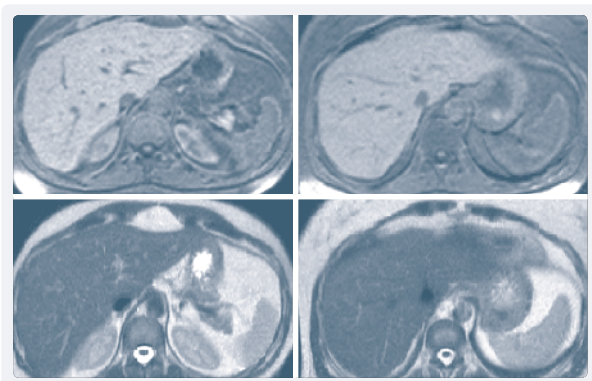
September 2014

Another increase of tumor markers: PD brain metastasis/metastases, Stereotactic radiation of the skull

2015

May 2015

PD in bones, liver in full remission under local hyperthermia



MRI liver images

2016

April 2016

High-dose radiation treatment (CyberKnife) of both pedicles in thoracic vertebra 7, radiation of the right lower pelvic ring incl. the right acetabulum, femoral head, femoral neck, prox. femoral stem

December 2016

PD (tumor markers/ supraclavicular lymph node package on the left!/increasing pain in the right leg

2017

February 2017

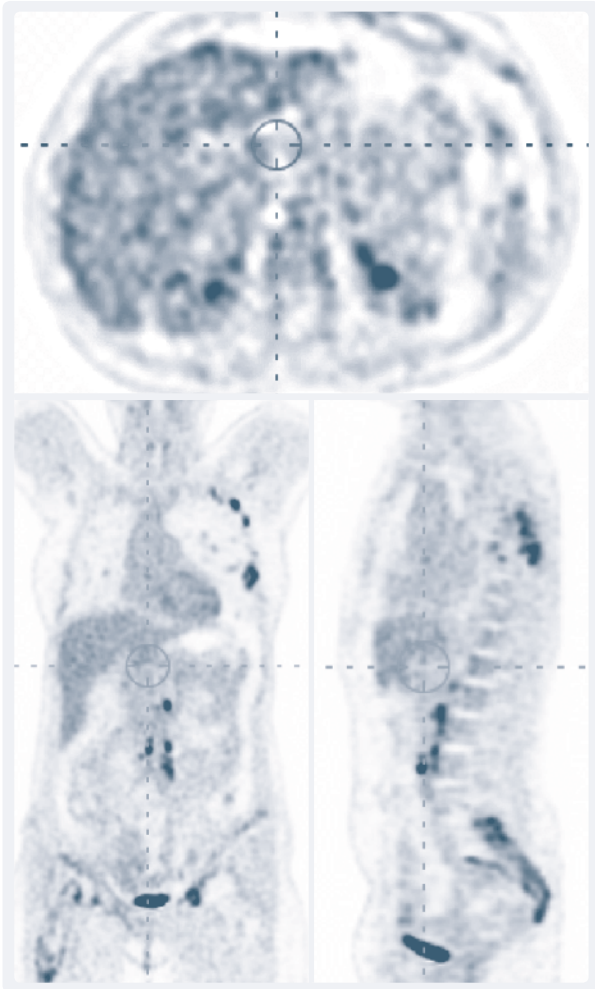
PD in bone: Therapy switch: metronomic therapy with capecitabine, IPT with FAFU. Continued Faslodex and Herceptin

March 2017

In addition, radiation therapy of the sacral bone and left iliac wing (total focal dose 30Gy)

October 2017

Another progression of the bone metastases, intra-spinal growth in thoracic vertebra 7, lung metastases; surgery: dorsal decompression of thoracic vertebra 7



Images: PET-CT (only PET images)

December 2017

Oncological therapy switched to Navelbine

2018**February 2018**

Drop in tumor markers

May 2018

Further progression of lung metastases; liver is stable, therapy: MMC/FAFU as IPT, which results in clinical stabilization, significant drop of tumor markers

November 2018

PD of pulmonary and lymph node metastasis; **liver continues to be in full remission.** Chemotherapy switched to IPT Abraxane

Summary assessment:

This clinical course is amazing given the fact that the patient's situation was considered palliative with a life expectancy of a few months in the summer of 2007. The recurrent metastases were treated locally, with successful to satisfactory results. The patient is looking back on over 10 years of treatment – now 11 years! Her quality of life has been excellent for many years (KPS 100%, increasing restriction due to sacral plexus damage since May 2017. KPS otherwise 90% with the exception of the perioperative period October 2017).

As of today, her quality of life can be considered relatively good. She suffers most from the consequences of the plexus damage and feels weaker overall, KPS 70%

Appendix

Creating an agar phantom

...for temperature measurements (to serve as a muscle tissue equivalent)

Phantoms may also include liver tissue from animal sources (see image below)



- » *Let the liquid cool down for about 3 min and then pour it in a plastic mold to set (usually in slices about 25-30 x 25-30 cm and about 5-10 cm in height)*
- » *To speed up the process, store the mold in the refrigerator or otherwise at room temperature. The phantom should be at room temperature for the experiment. Remove it from the mold prior to use.*

We sometimes add a mesh while the agar solution is still hot to help stabilize the shape. Temperature sensors can easily be placed in the phantom, which has the consistency of a firm pudding.

Agar can only be used for a few days until it decays.

Recipe:

- » *Use a liquid with physiological salt content (or for very large quantities, use deionized water and add 0.9% NaCl (physiological saline level))*
- » *4% agar powder*
- » *Precisely measure the salt quantity and add it to the deionized water.*
- » *Warm up, then add agar (40 mg of agar powder per 1,000 ml of water).*
- » *Heat up to almost boiling (95 °C) to produce a consistent liquid without flocculation.*

Technical Cancer Treatment

Celsius42 GmbH
Hermann-Hollerith-Str. 11
D-52249 Eschweiler
T. +49 (0) 24 03-78 29 230
F. +49 (0) 24 03-78 29 249
info@celsius42.de

www.celsius42.de