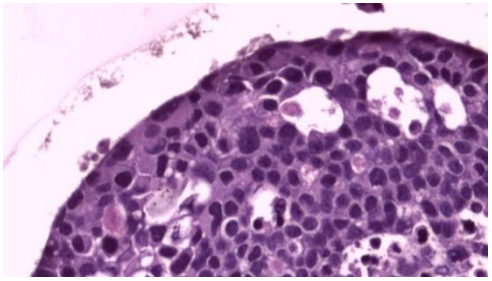
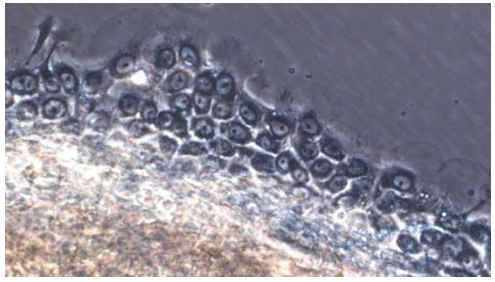
Differential effects of 42°C- hyperthermia on radiation response of breast cancer spheroids *vs.* normal human skin explants





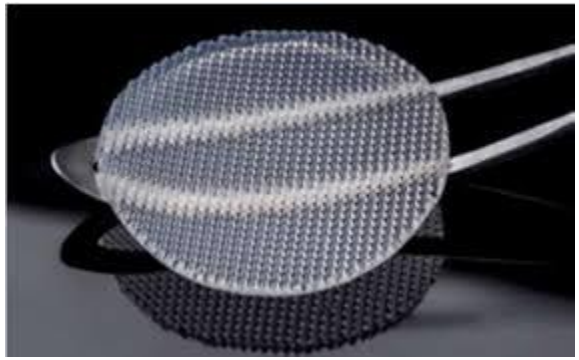
Thomsen AR[1,2], Aldrian C [1], Niedermann G[1,2], Grosu AL[1,2], Vaupel P[3], Lund PG[1,4]

[1]*University Medical Center Freiburg, Radiation Oncology Dept., Freiburg, Germany*
[2]*German Cancer Consortium (DKTK) - Partner Site Freiburg, Heidelberg, Germany*
[3]*University Medical Center Mainz, Radiation Oncology Dept., Mainz, Germany*
[4]*Ortenau-Klinikum Offenburg-Gengenbach, Radiation Oncology Dept., Offenburg, Germany*

Conflict of Interest

A.T. developed the presented 3D cell culture system
and works as a consultant for *abc biopply* (Solothurn, Switzerland), the current manufacturer of the system.

The remaining authors have no conflict of interest to declare.



3D CoSeedis™

abc biopply
applied biomedical concepts

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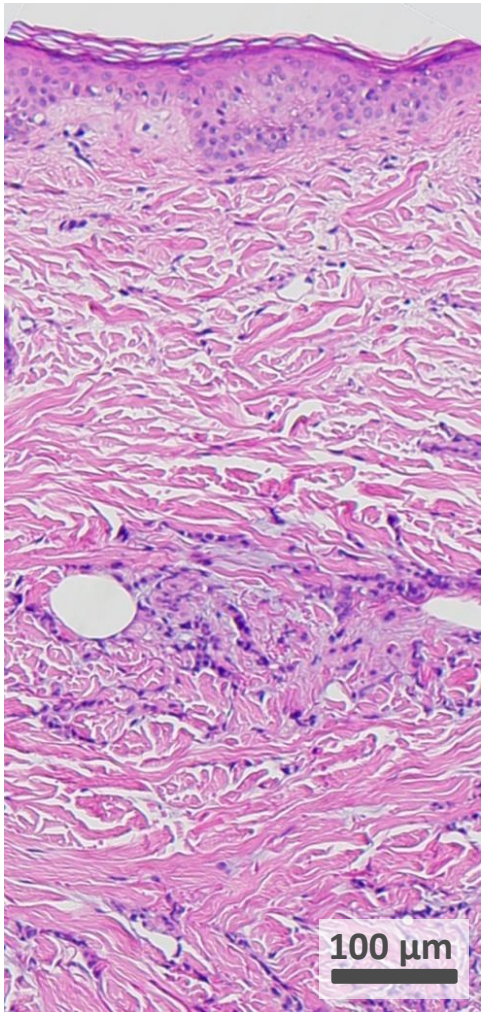
Background

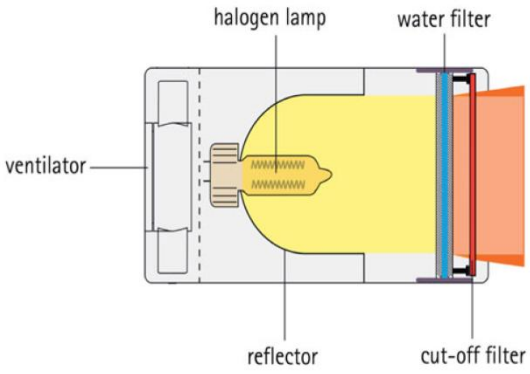
1. Recurrent breast cancer in pre-irradiated regions remains a challenging situation.

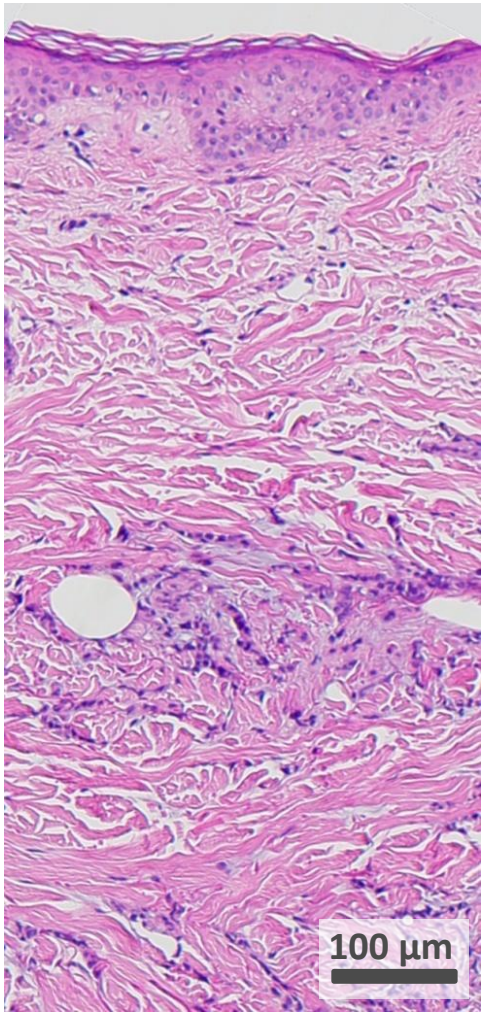


H&E staining
J.-O. Gebbers, Chur (CH)

Background

1. Recurrent breast cancer in pre-irradiated regions remains a challenging situation.
2. For radiosensitization of superficial tumors, loco-regional HT can be applied using water-filtered infrared radiation (wIRA)*.
3. In January 2018, we started treating patients with locally recurrent breast cancer using wIRA-HT, followed by hypofractionated re-irradiation (5 x 4 Gy; 1x/wk).





H&E staining
J.-O. Gebbers, Chur (CH)

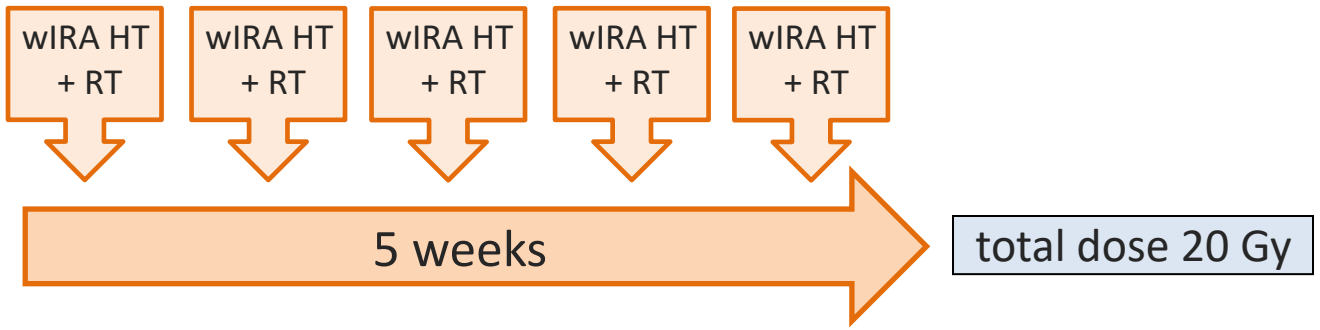
*Notter et al., IJH 2017

Hypofractionated hyperthermia + radiotherapy

Hyperthermia 1x/wk

within 5 min

Radiotherapy 4 Gy



Hypofractionated hyperthermia + radiotherapy

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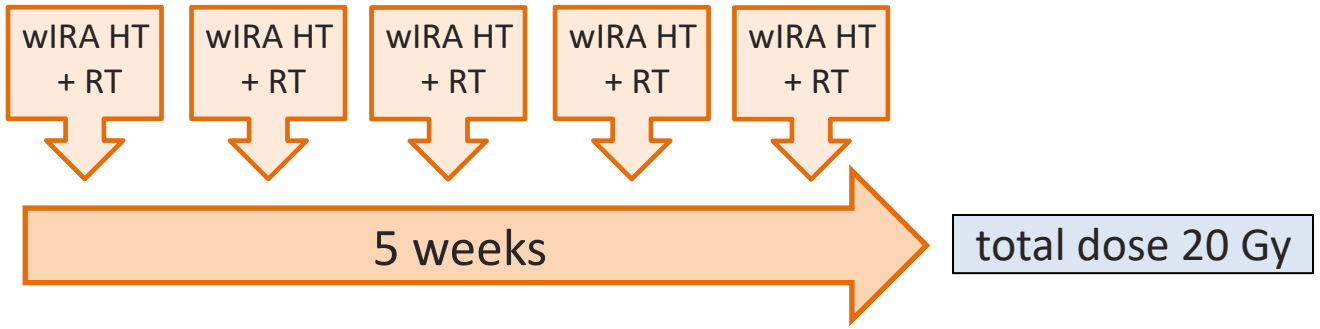
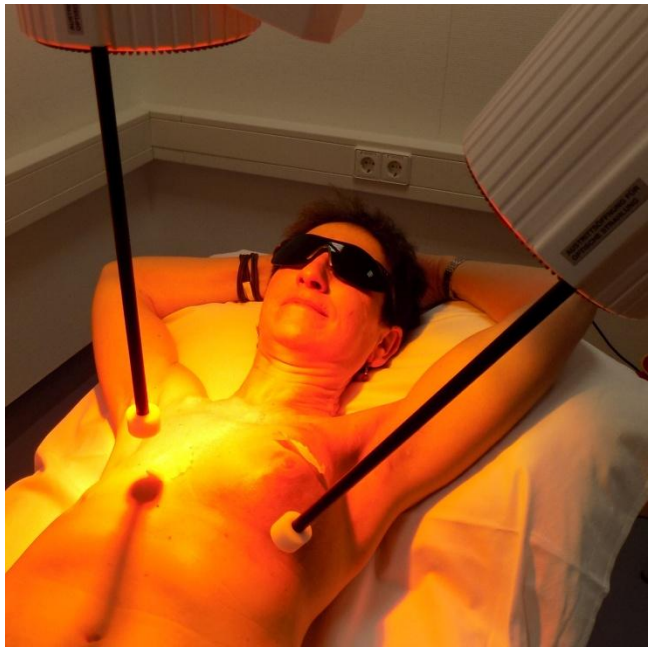
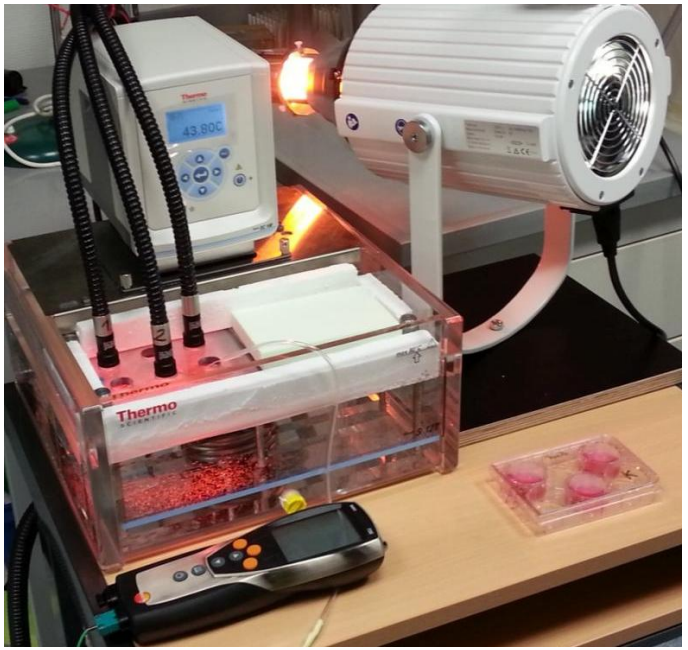
Radiotherapy 4 Gy



clinical protocol



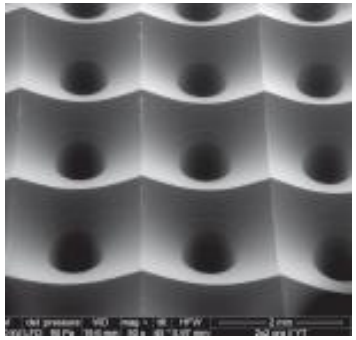
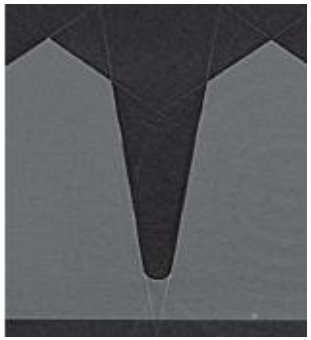
protocol *in vitro*

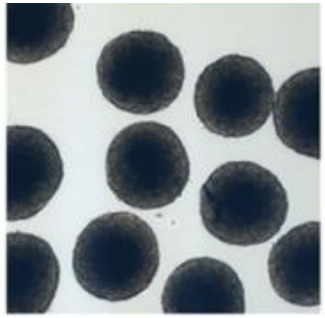


T_{max}= 43° C at surface, 45-60 min

T= 42° C, 60 min

(A) Cancer model: 3D breast cancer spheroids



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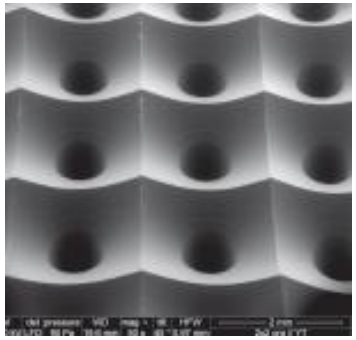

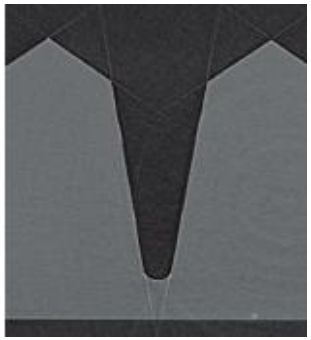

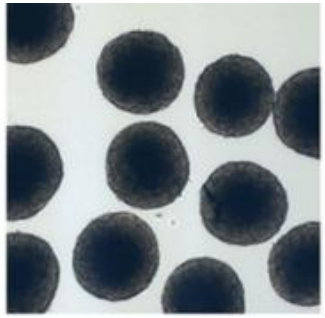

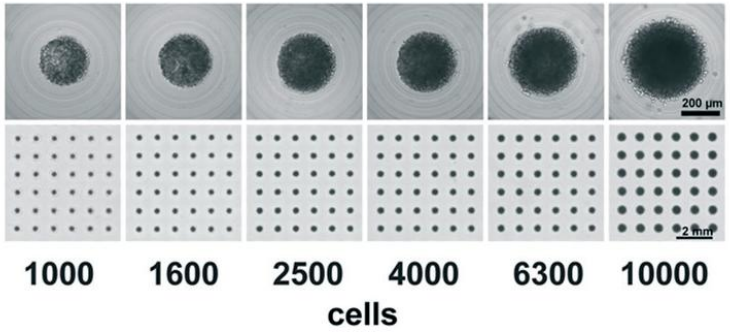

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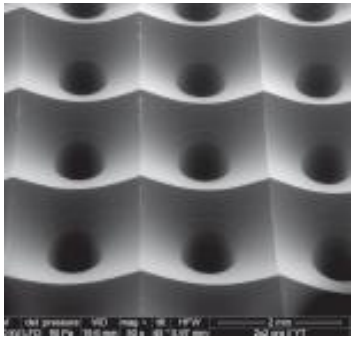

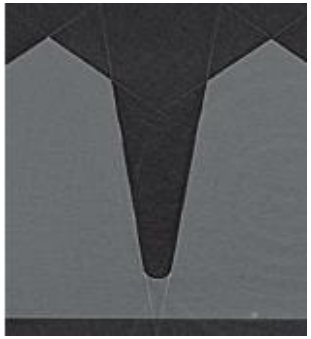

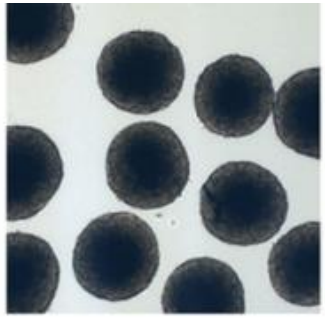

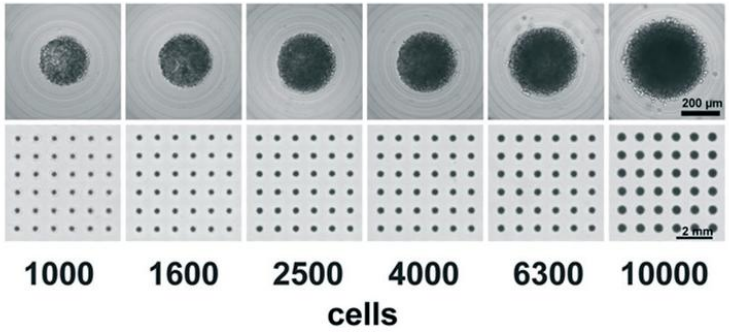

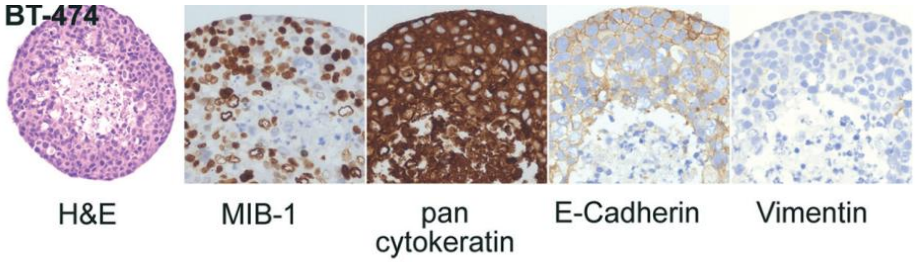

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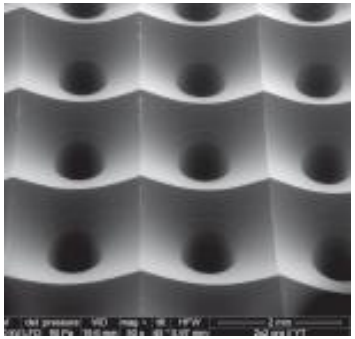
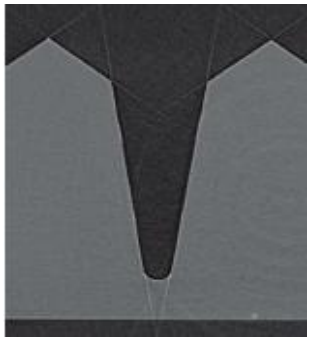
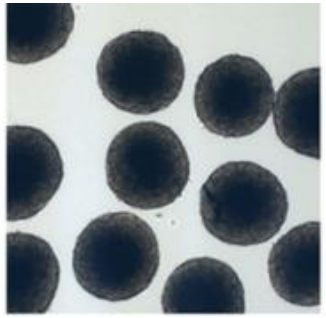
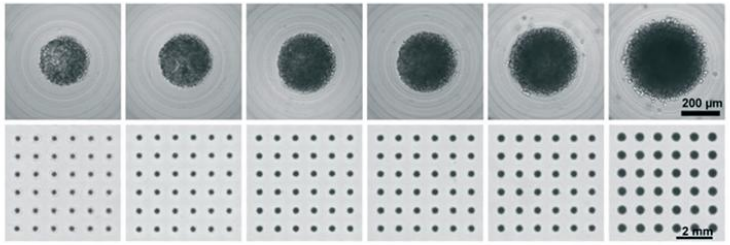
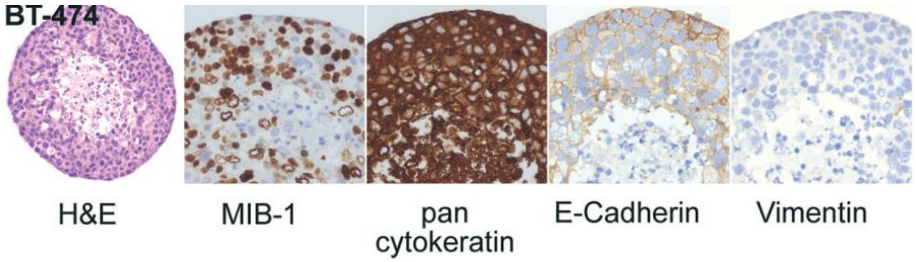
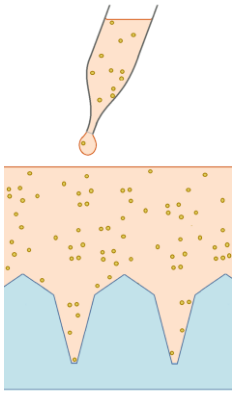




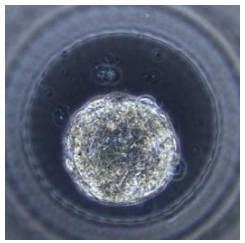








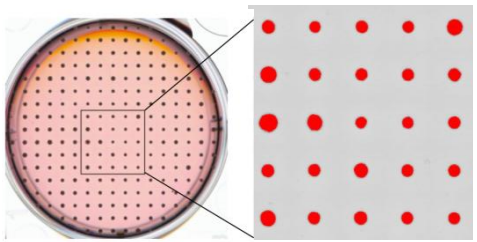
Seeding



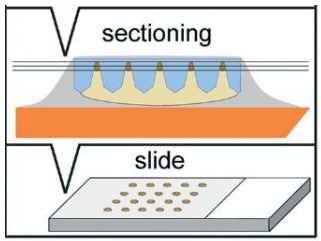
Spheroid formation

Treatment

up to 12 weeks



Volume readout



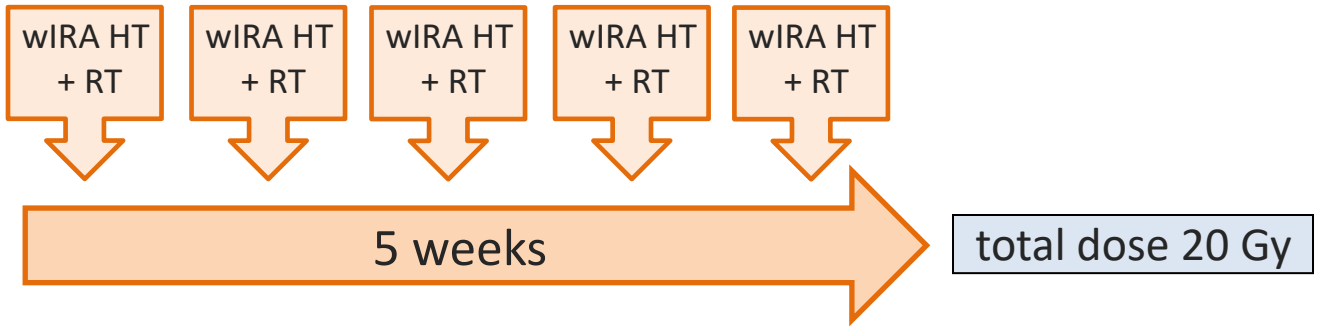
Analysis

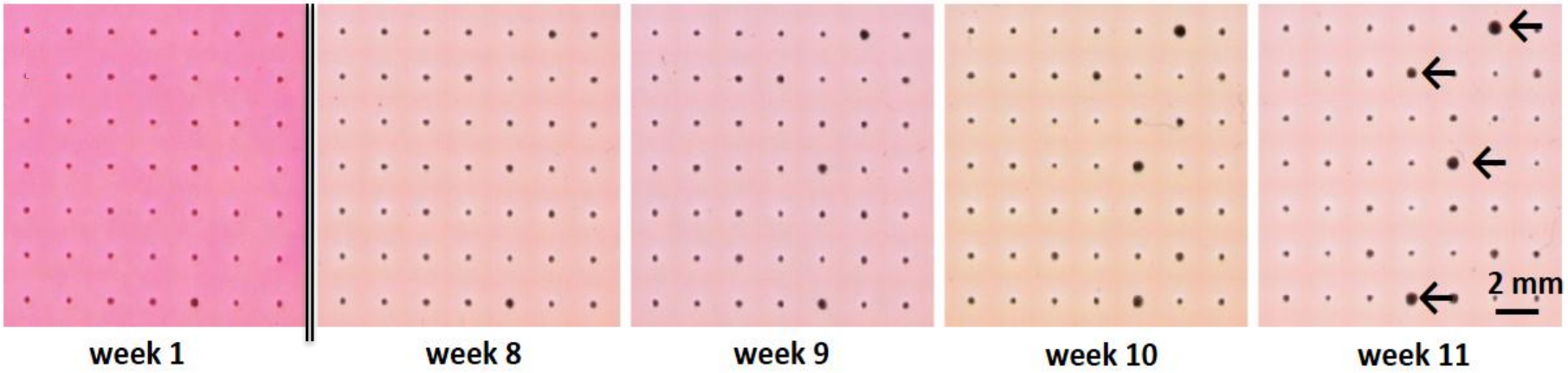
Spheroid growth during and after hypofractionated treatment

Hyperthermia 1x/wk

within 5 min

Radiotherapy 4 Gy





Spheroid growth during and after hypofractionated treatment

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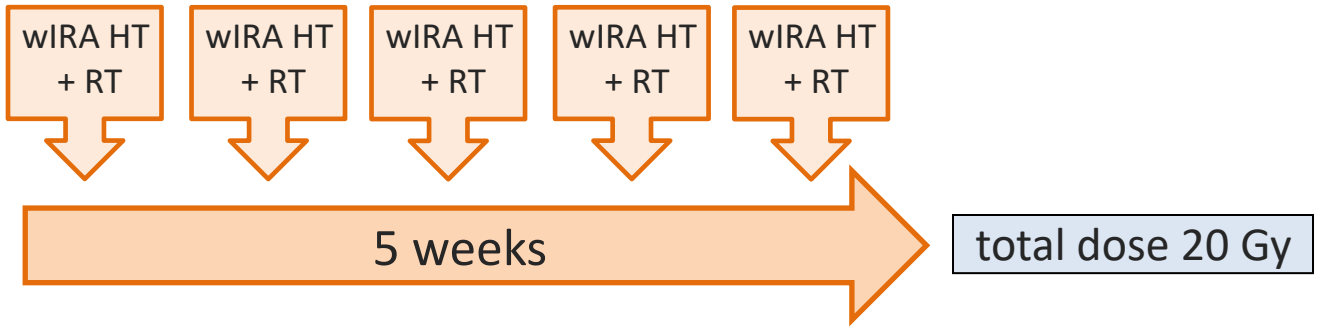
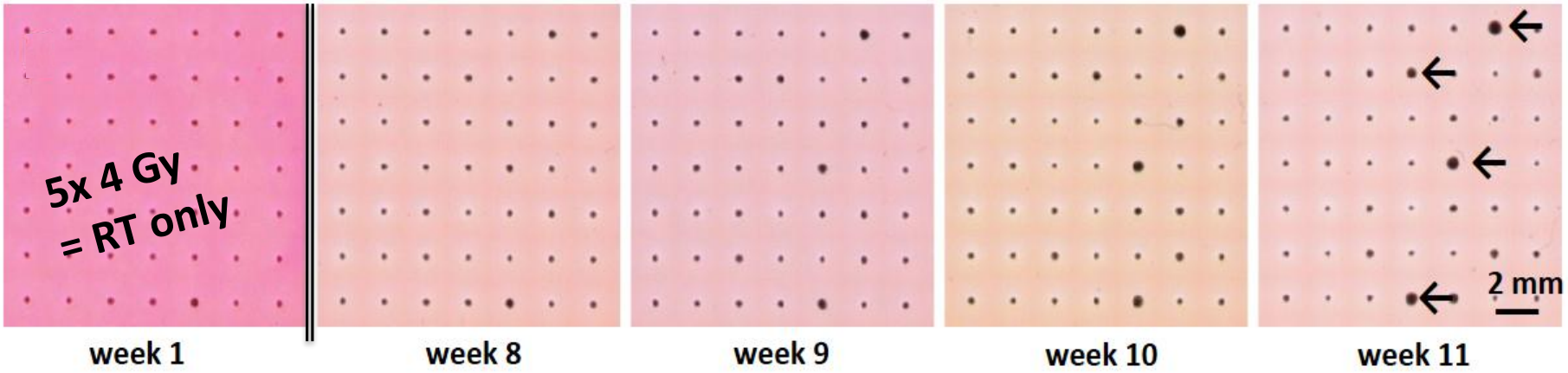
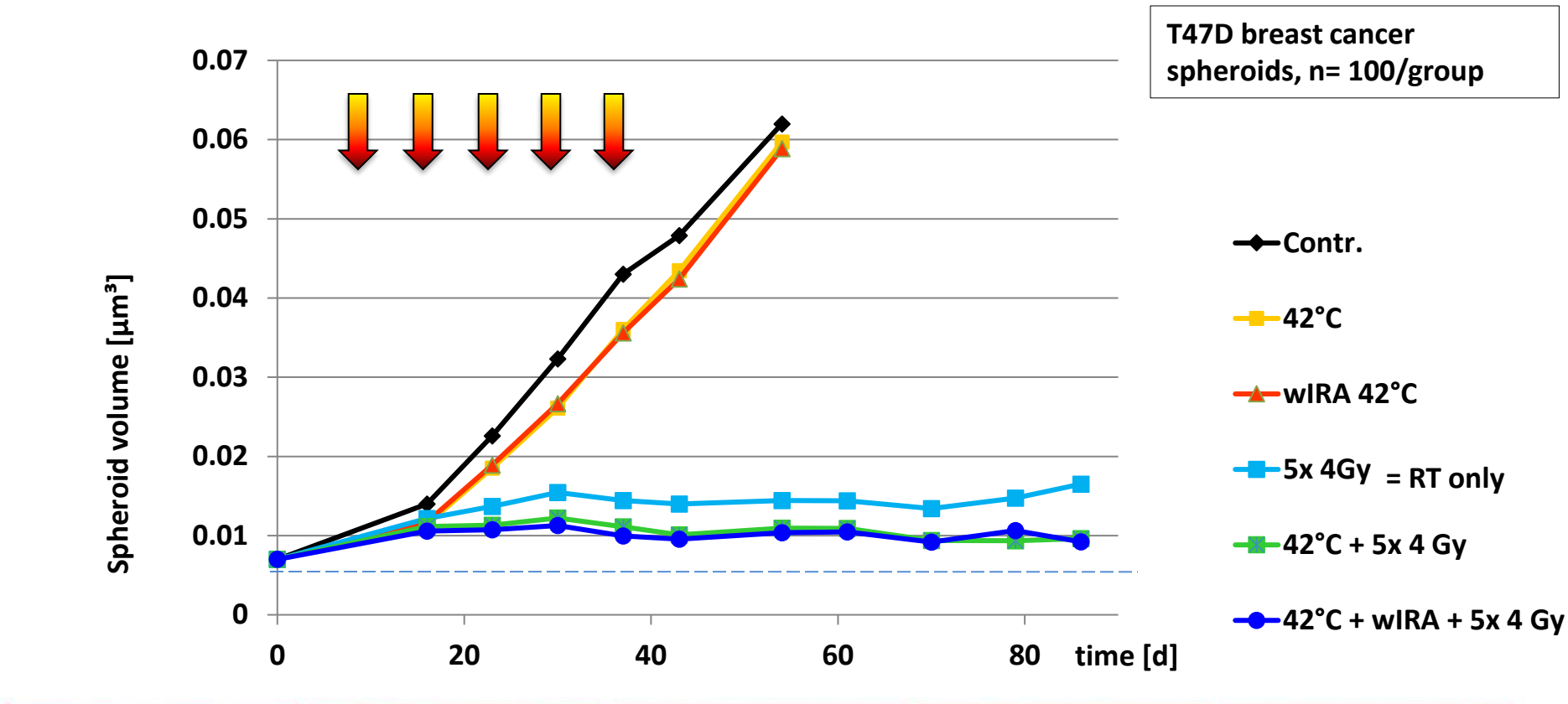
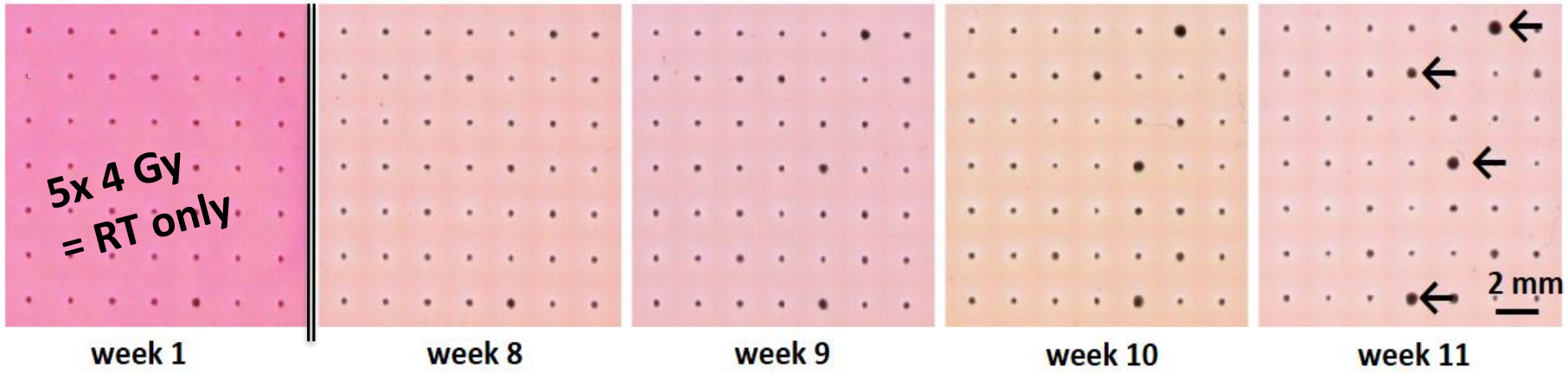


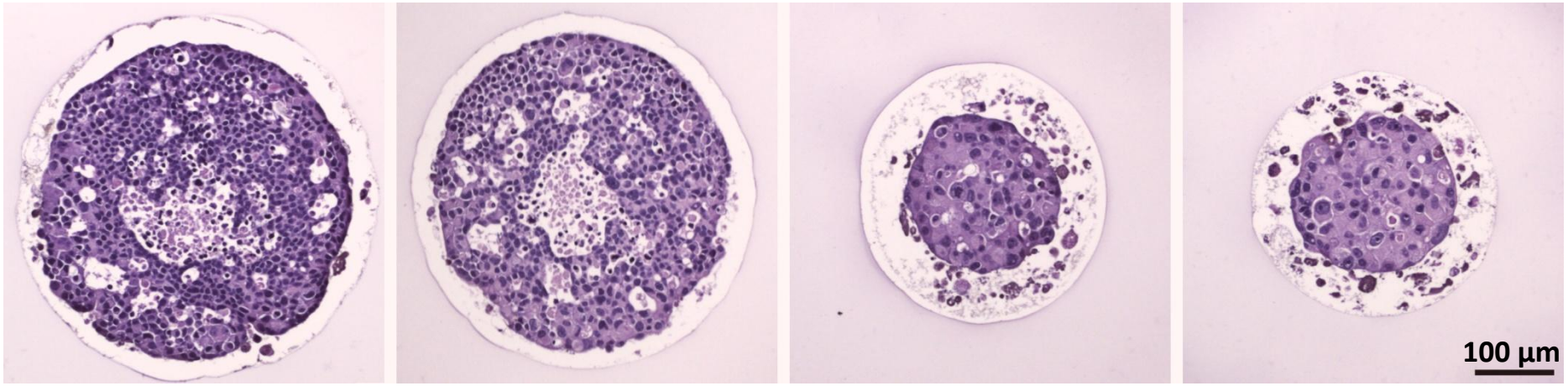


Spheroid growth during and after hypofractionated treatment





Analysis d 42 Paraffin sections, H&E staining

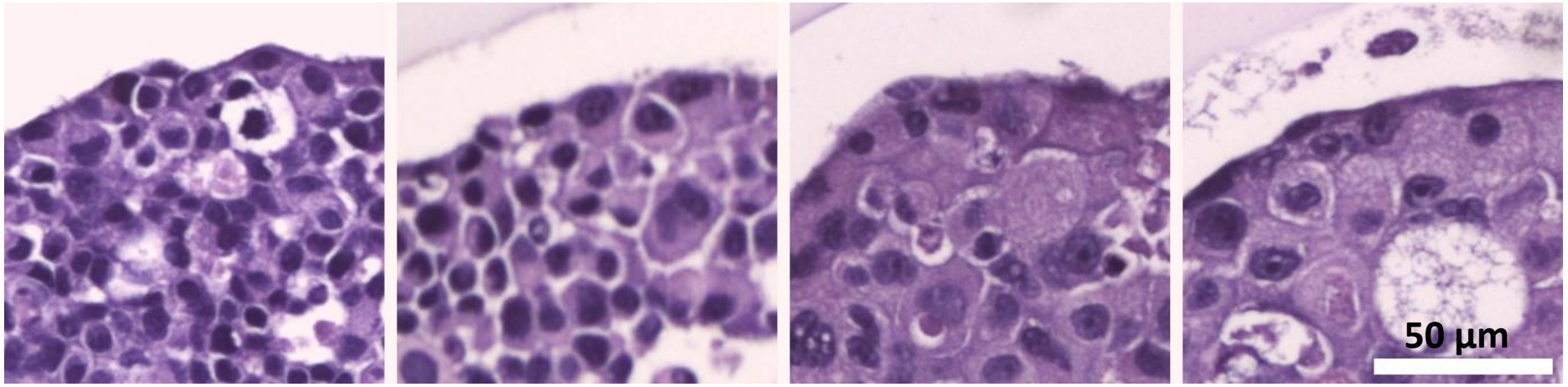


5x 0 Gy
37°C

5x 0 Gy
42°C

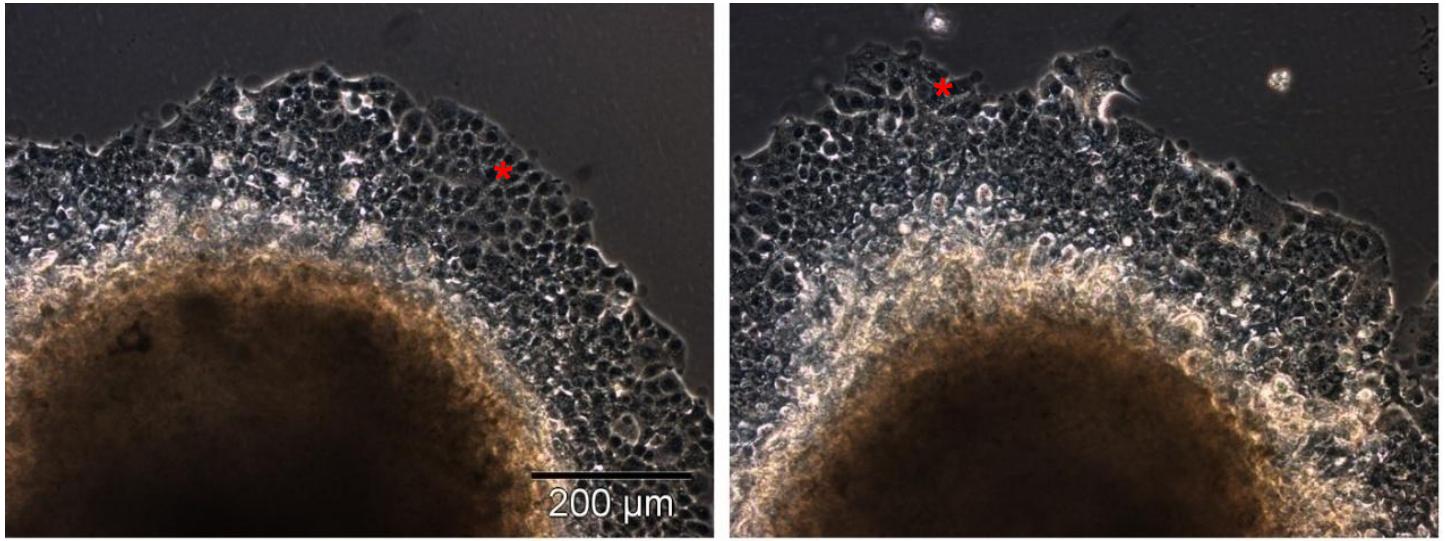
5x 4 Gy
37°C

5x 4 Gy
42°C



Functional test on cell survival

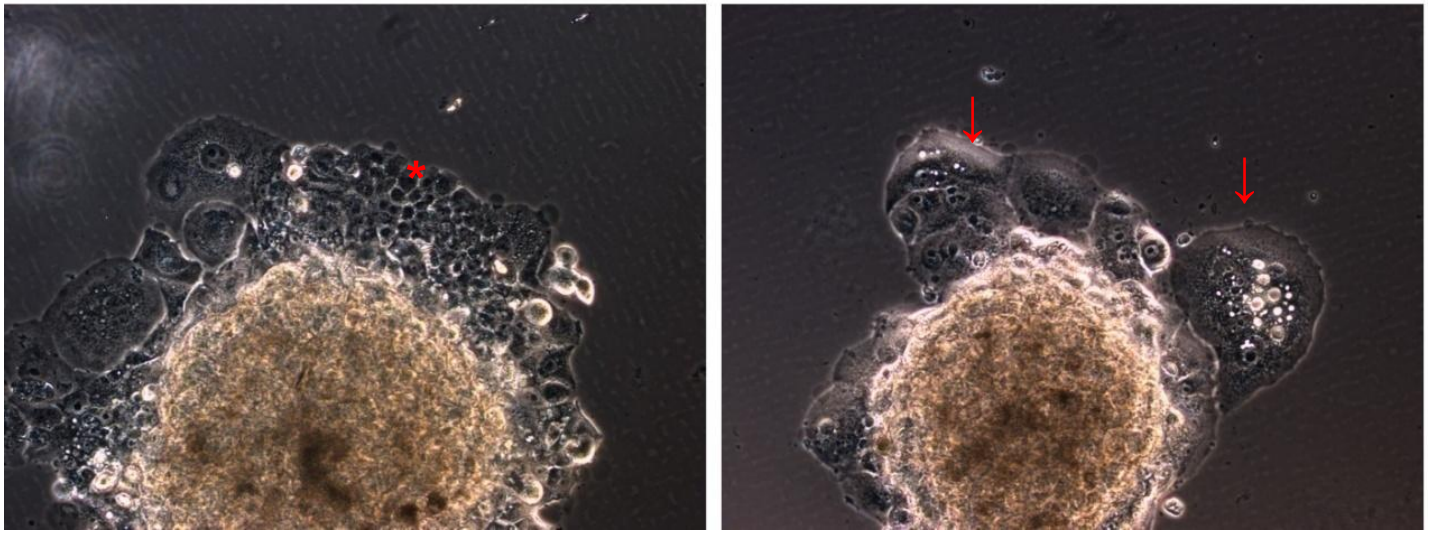
5x 0 Gy



37°C

42°C

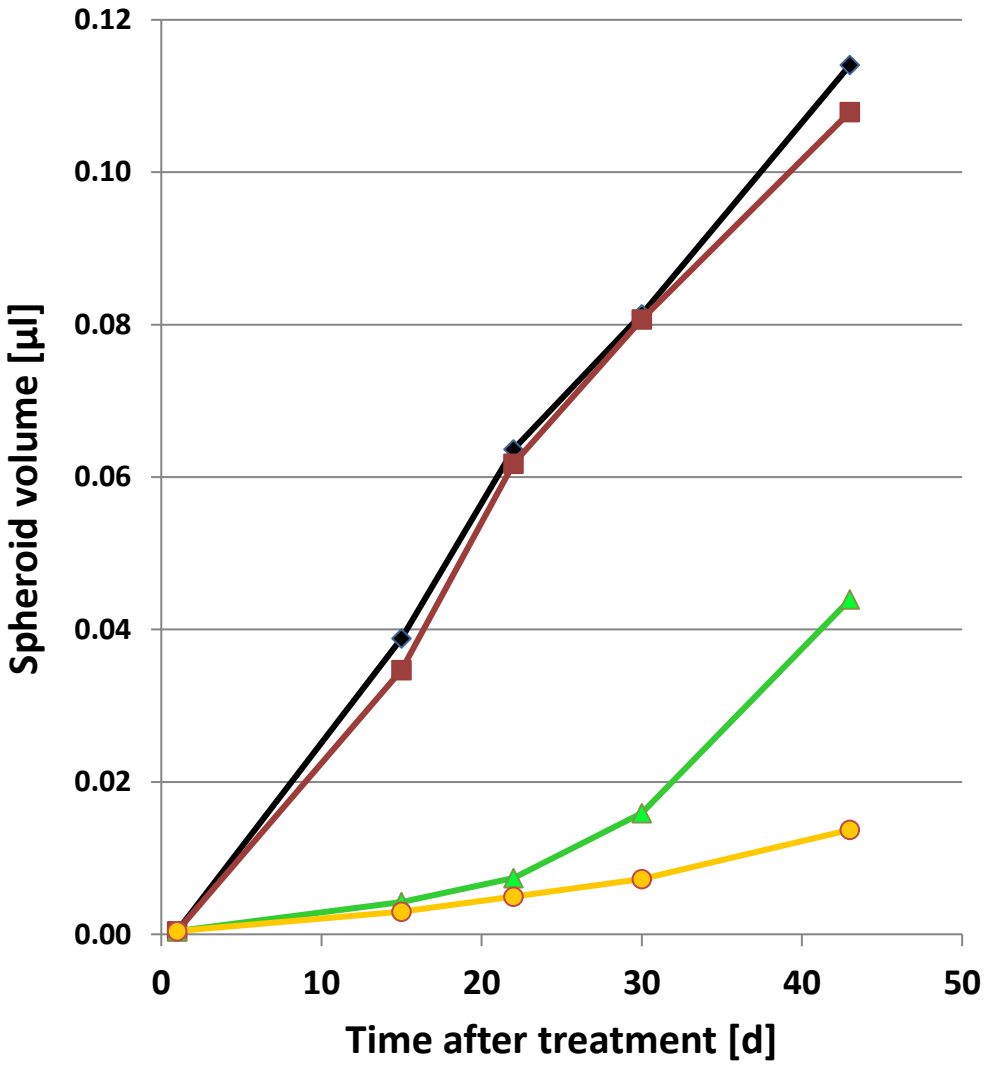
5x 4 Gy



Small proliferating tumor cells (*)

Non-proliferating giant cells (↓).

Response of breast cancer spheroids: Impact of treatment sequence and time interval



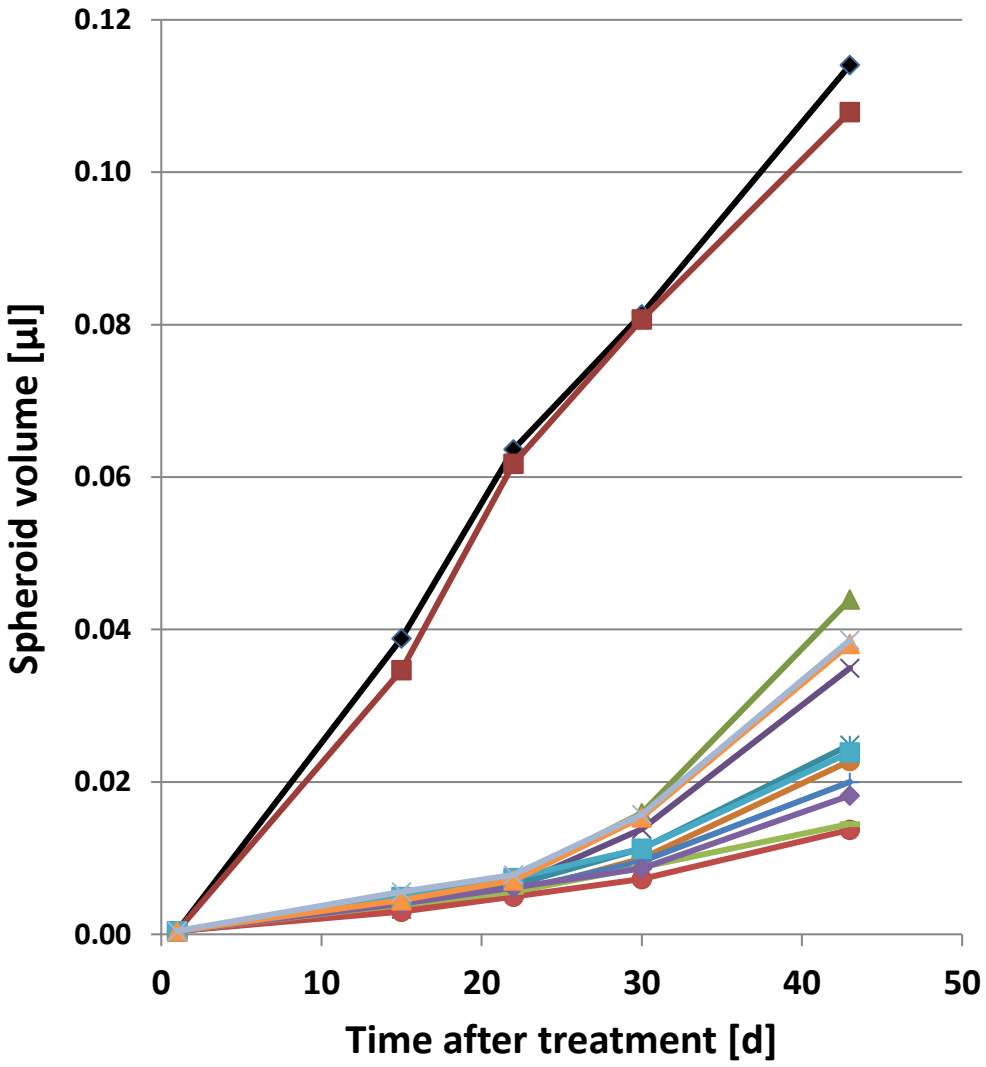
Control
HT only
RT only
0

T47D breast cancer spheroids
n= 100 per treatment condition

RT: 7.5 Gy
HT: 42°C/1h

simultaneous HT + RT

Response of breast cancer spheroids: Impact of treatment sequence and time interval



Control
HT only
RT only
-6
-4
-2
-0,7
0
0,3
1
1
2
6

T47D breast cancer spheroids
n= 100 per treatment condition

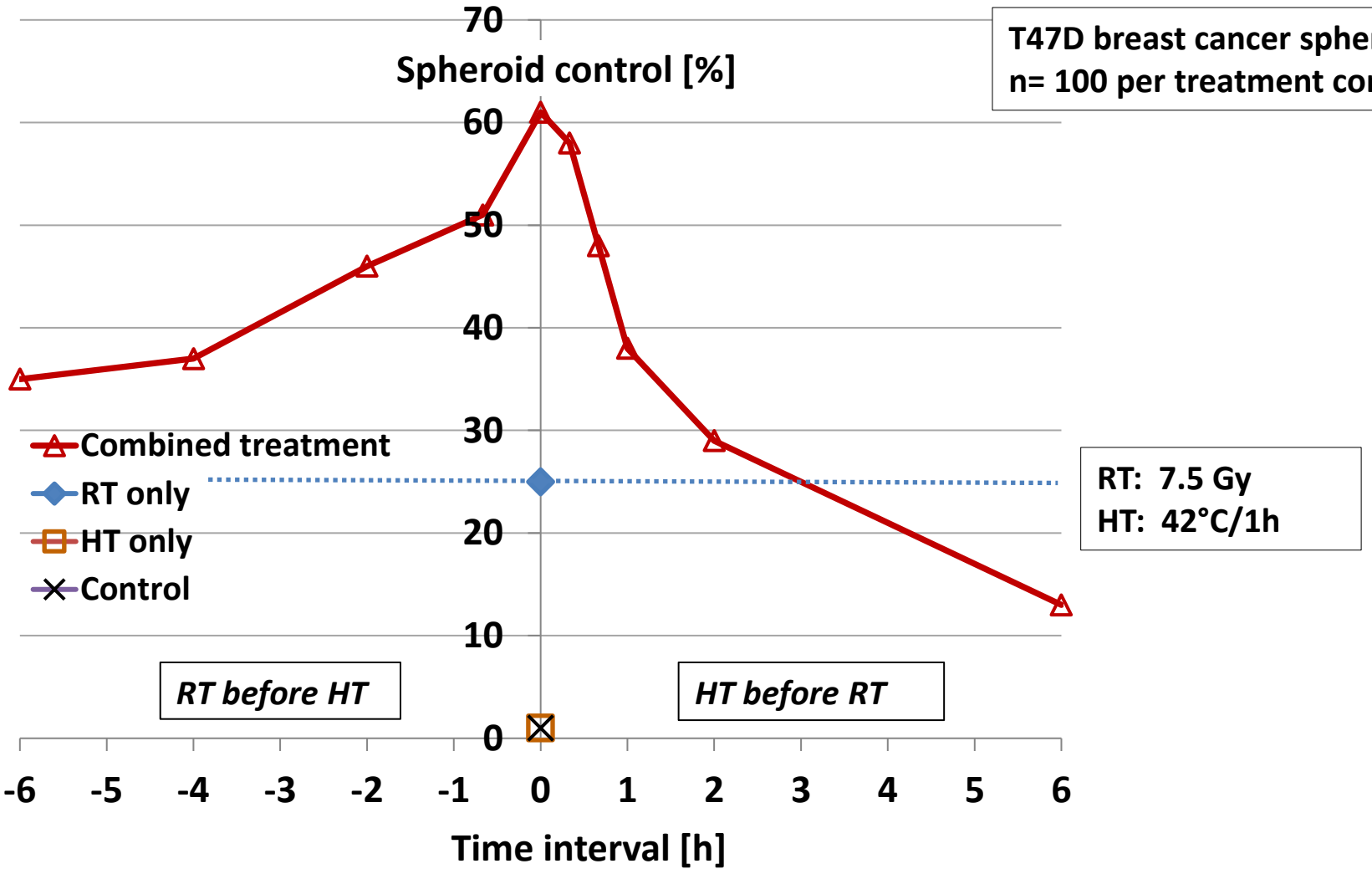
RT: 7.5 Gy
HT: 42°C/1h

HT before RT

simultaneous HT + RT

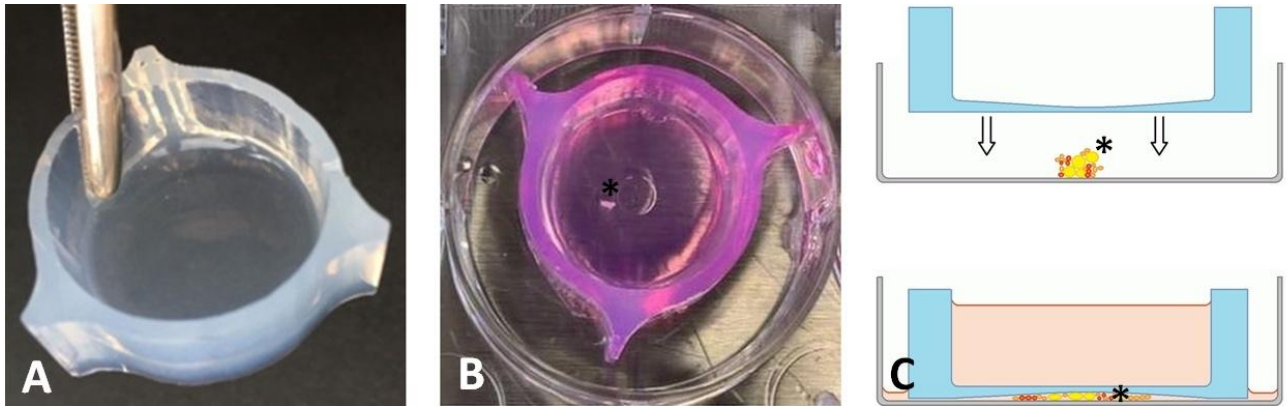
RT before HT

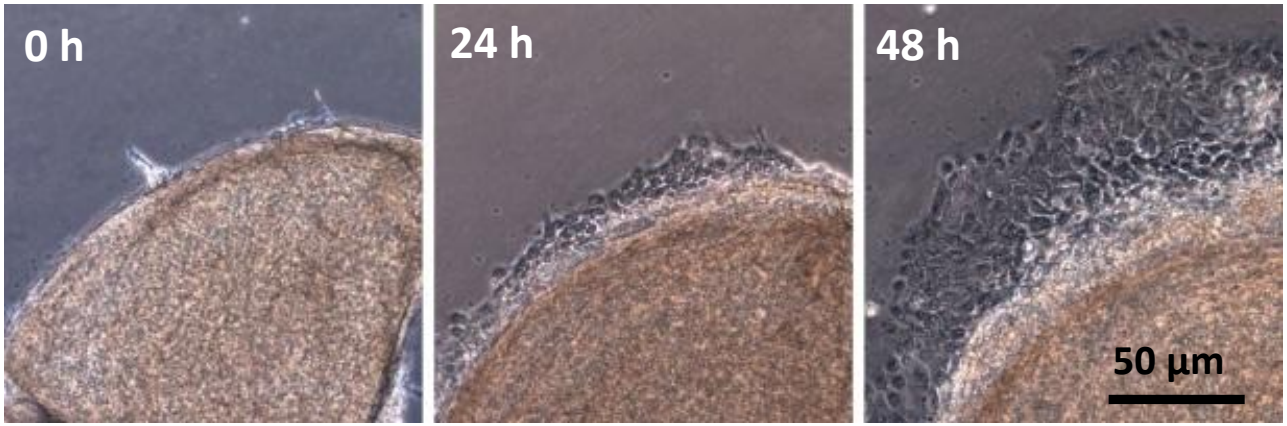
Response of breast cancer spheroids: Impact of treatment sequence and time interval



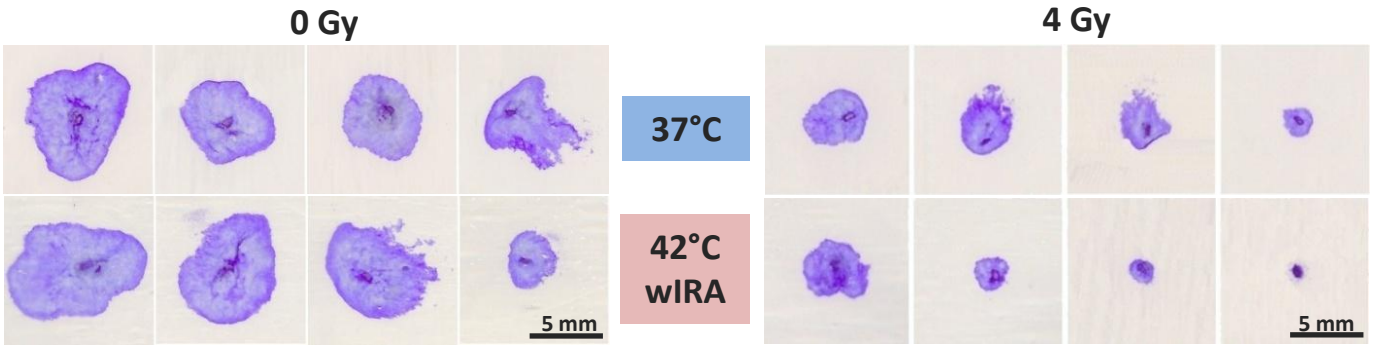
(B) Normal tissue model: Primary keratinocytes

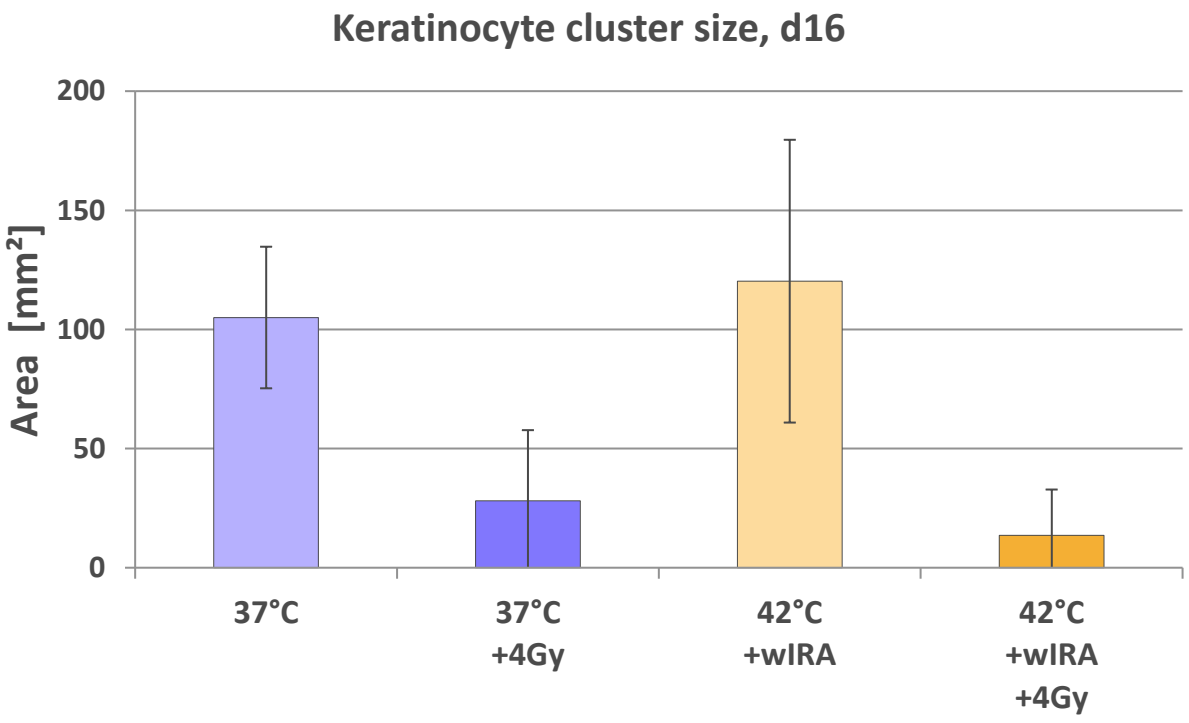
Skin mini explant cultures





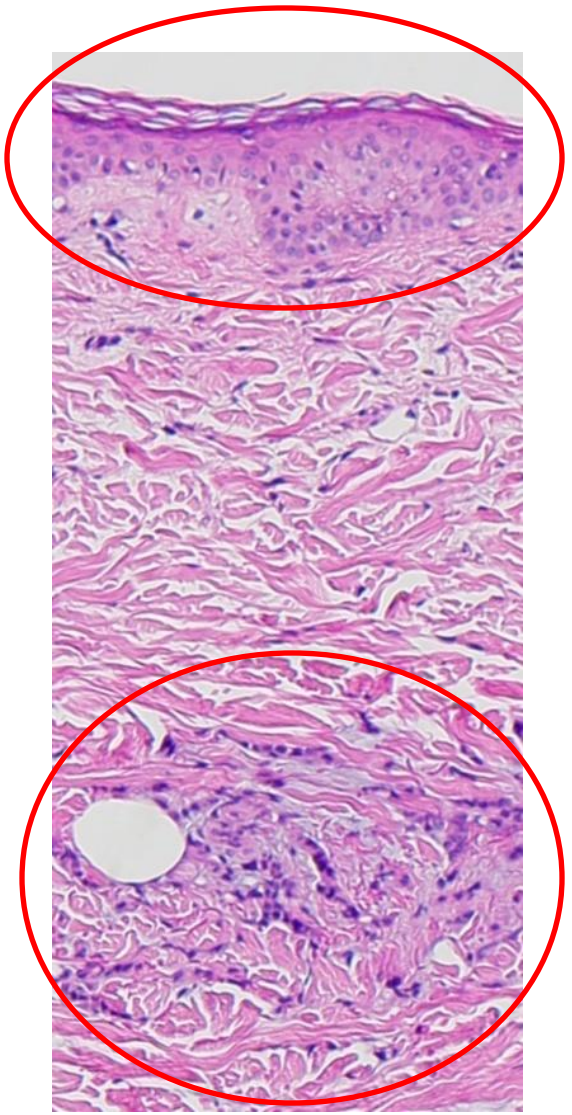
wIRA hyperthermia has no significant impact on primary keratinocyte outgrowth *ex vivo*



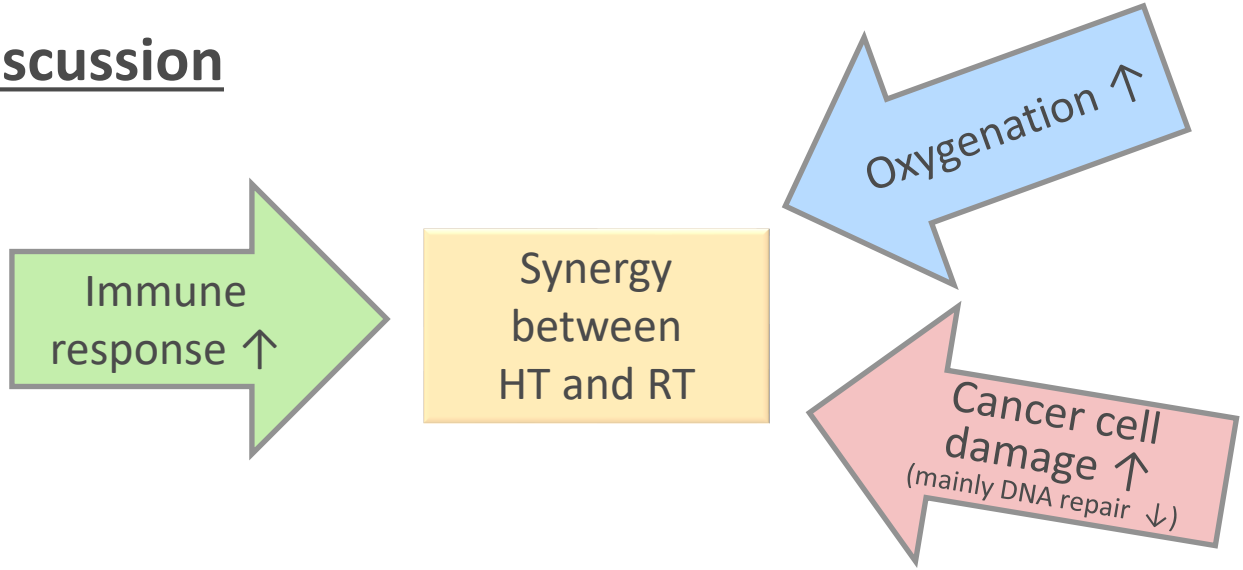


Summary

1. Hyperthermia (42°C, 1 hour) radiosensitizes breast cancer spheroids both as single dose and as hypofractionated treatment.
2. Synergy was inversely correlated with time interval between HT and γ-irradiation.
3. When time interval was < 1 h, sequence only had minor impact on control of 3D breast cancer cell cultures.
4. Function of normal epidermal keratinocytes was not significantly impaired by addition of HT to γ-irradiation.



Discussion



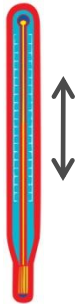
- In contrast to the clinical setting, where increased blood flow and oxygenation are discussed as key mechanisms for radiosensitization and for improved immune response, our model reduces HT treatment to thermal mechanisms, as non-thermal effects of wIRA could not be observed.
- In the cancer model, synergistic effects of RT + HT were inversely correlated with time interval. This confirms earlier observations in the experimental and clinical setting [Overgaard J 1982, van Leeuwen 2018), but is opposed to data published recently (Kroesen M 2019).

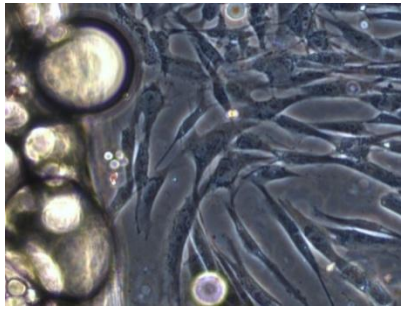
Thank You!

Funding:
This work was supported by the Dr. med. h.c. Erwin Braun Foundation, Basel.

Outlook

- Additional experiments are underway to further approximate the conditions present *in vivo*
- Focus on hyperthermia effects on other relevant normal tissue cell types (fibroblasts, mesenchymal stem cells)





Extra
slide 1a

INTERNATIONAL JOURNAL OF HYPERTHERMIA
https://doi.org/10.1080/02656736.2018.1469169

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Biophysical and photobiological basics of water-filtered infrared-A hyperthermia of superficial tumors

Peter Vaupel[a], Helmut Piazena[b], Werner Müller[c] and Markus Notter[d]

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ORIGINAL ARTICLE

OPEN ACCESS

Hypofractionated re-irradiation of large-sized recurrent breast cancer with thermography-controlled, contact-free water-filtered infra-red-A hyperthermia: a retrospective study of 73 patients

Markus Notter[a]*, Helmut Piazena[b] and Peter Vaupel[c]

Lab on a Chip

PAPER

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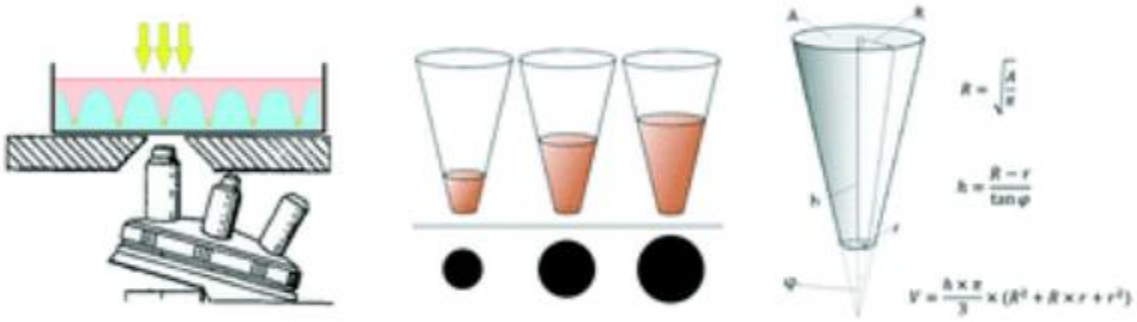
Cite this: *Lab Chip*, 2018, 18, 179

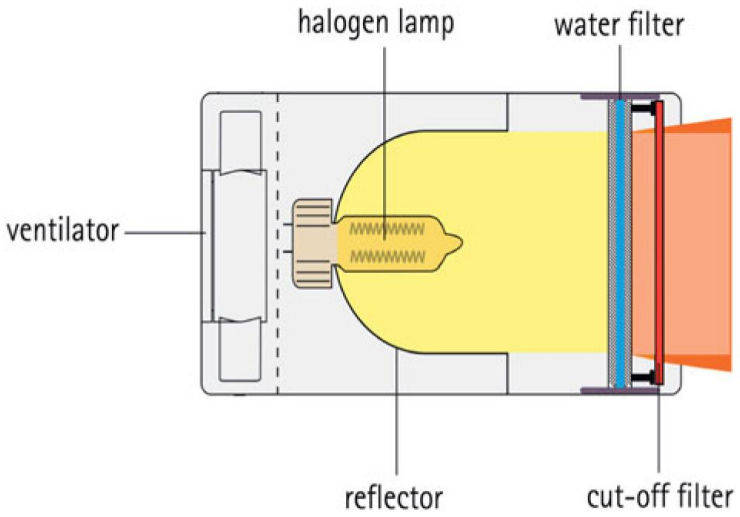
A deep conical agarose microwell array for adhesion independent three-dimensional cell culture and dynamic volume measurement†

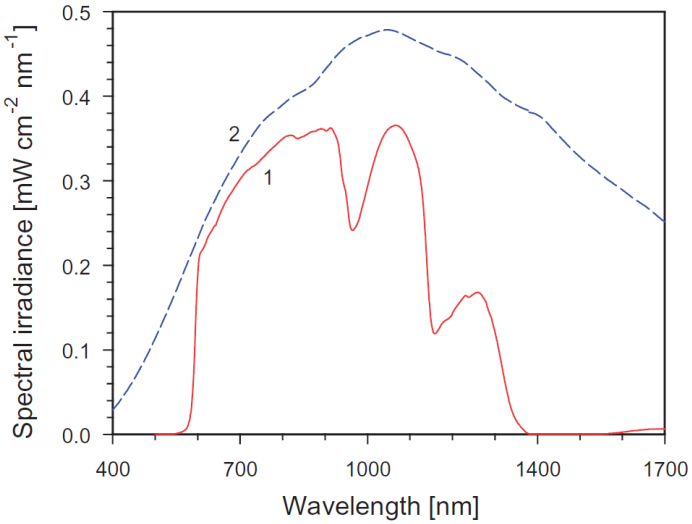
Andreas R. Thomsen, *[abc] Christine Aldrian,[abc] Peter Bronsert, [bcde] Yi Thomann,[f] Norbert Nanko,[ac] Nicolas Melin,[g] Gerta Rücker, [ch] Marie Follo, [ci] Anca L. Grosu, [abc] Gabriele Niedermann, [abc] Paul G. Layer,[j] Anja Heselich [k] and Per G. Lund *[ac]



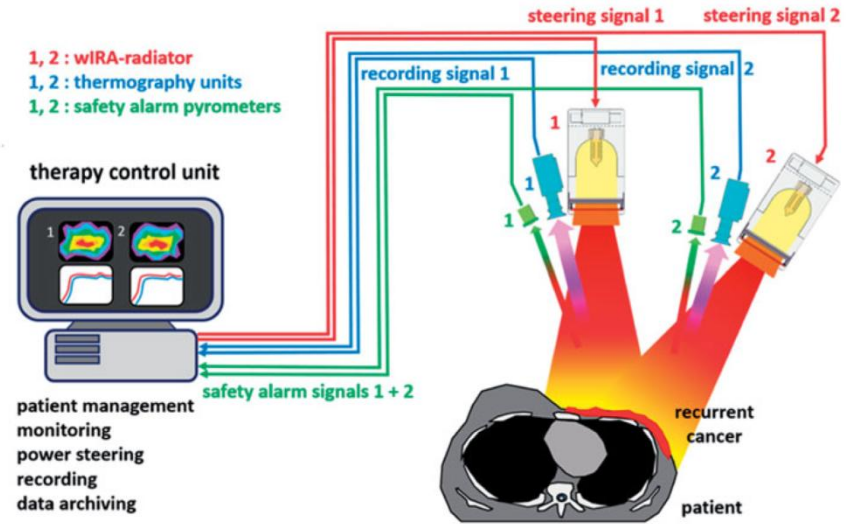
Extra slide 2

Water-filtered Infrared A (wIRA) Hyperthermia





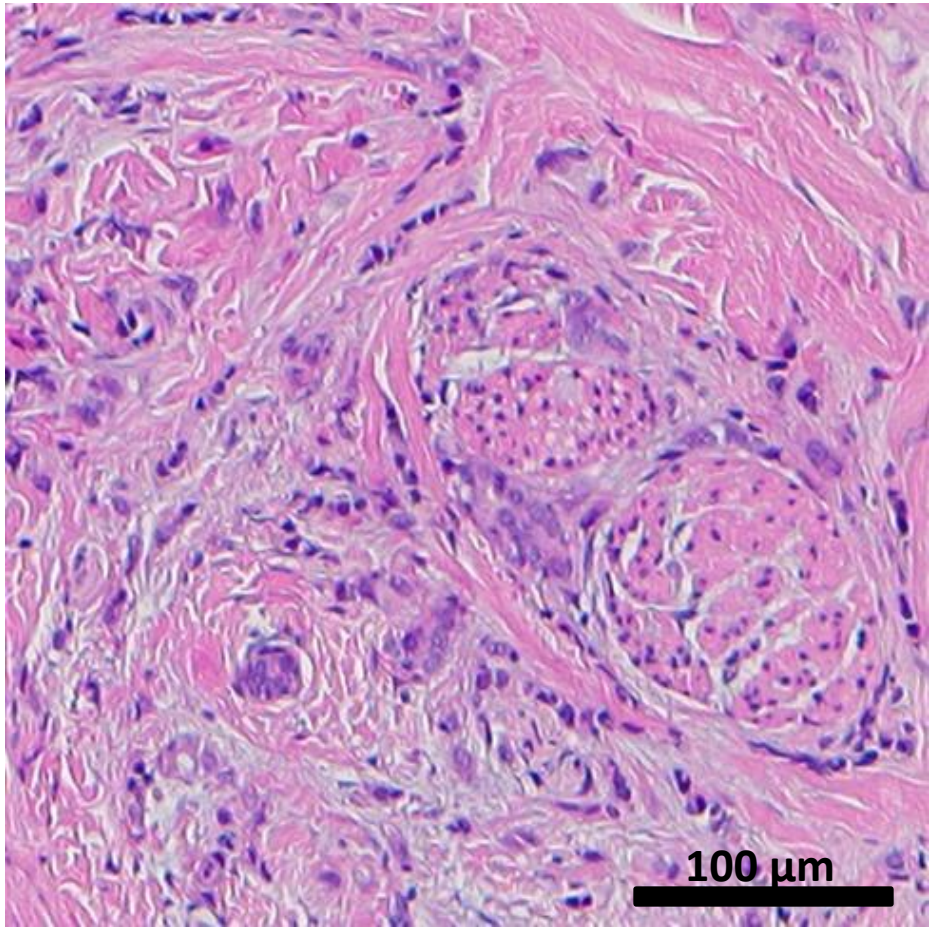
TWH1500, Hydrosun®, Germany



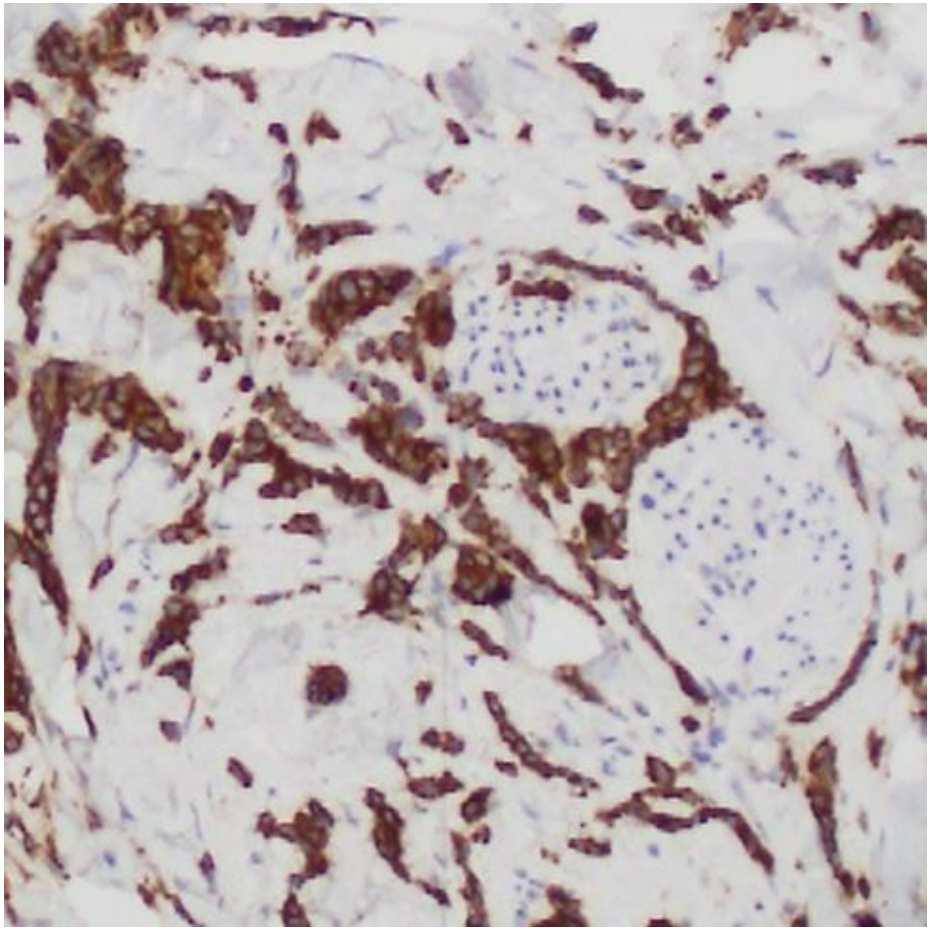
Vaupel et al., IJH 2018

Extra
slide 3

Histology



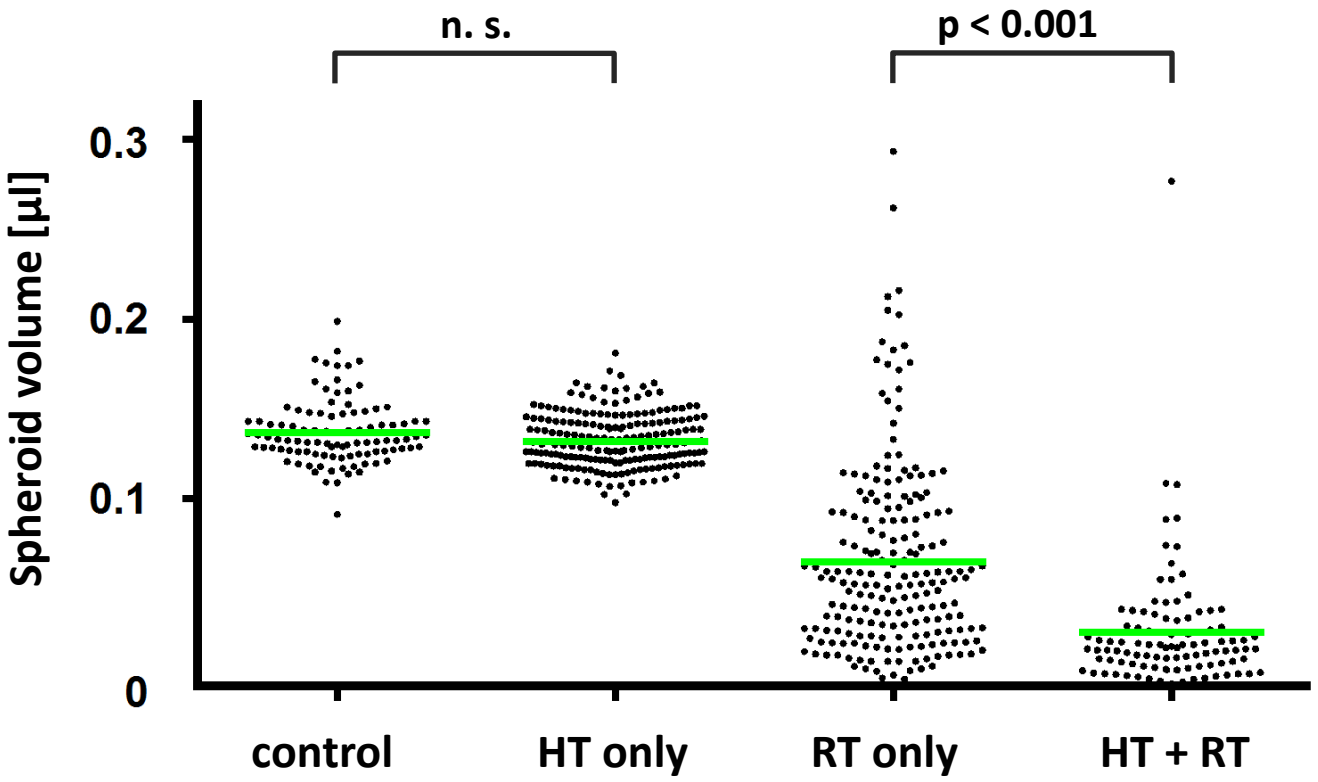
H&E



Pan Cytokeratin

Extra slide 4

Hyperthermia sensitizes breast cancer spheroids to γ-irradiation, but has no impact on spheroid growth itself



T47D spheroids,
initial volume 0.007 µl

d43

Scatter plot with median

RT: 7.5 Gy
HT: 42°C, 1h

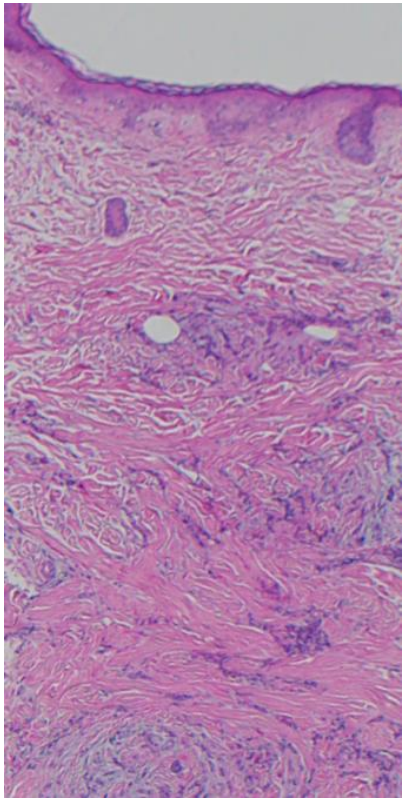
Clinical Case

Extra slide 5

57 yo. Patient

2003	First diagnosis breast cancer, left side pT1c pN1 (1/8) cM0 ER+, PR+, HER-2/neu: neg.
2003	breast-conserving surgery + postoperative radiotherapy
2011-15	Multiple surgeries for local recurrences
2015	Bilateral mastectomy
2015	Local recurrence left chest wall, Re-RT
2017	Local recurrence left chest wall, hypofract. Re-RT (20 Gy) + wIRA HT
2018	Re-Recurrence, hypofract. Re-Re-RT (20 Gy) + wIRA Hyperthermia

Cumulative dose on left chest wall:
> 120 Gy



Histology prior to Re-Re-RT + HT

H&E staining
Prof. J.-O. Gebbers, Chur (CH)

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