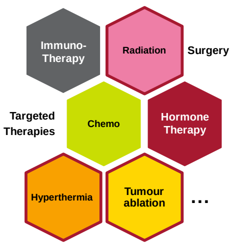
Therapeutic ultrasound and radiation therapy dose relationships

S. C. Brüningk

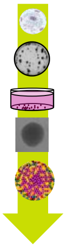
Making the discMaking the discoveries that defeat cancer

Motivation - Multimodality therapies



- Modern cancer treatments are multimodality
- Biological effects may vary significantly between modalities
- Scope for optimizing treatment dosing and scheduling
- Personalized treatments
- Quantification and modelling of biological effects induced

Analysis and quantification of biological effects



At cell level – Cellular targets, cell cycle sensitivity, micro-environmental influence

Cell survival modelling – isoeffective treatments

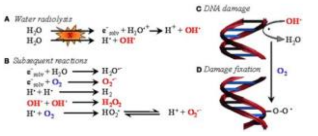
Importance of dynamic cell death (Monolayer and 3D cultures)

Systems biology simulations – modelling dynamic response

Biological effects of radiation

Radiation

- **Stress**: Ionizing radiation (Dose)
- **Biological target**: DNA – Double strand breaks
- **Microenvironment**: Oxygen effect
- **Cell cycle:** Increased resistance in G2,S
- **Cell death**: Mitotic catastrophe, Senescence, Apoptosis, Necrosis



Jordan et al., 2012, Frontiers in Pharmacology (3)

Biological effects of hyperthermia

Hyperthermia

- **Stress**: Elevation of temperature above physiological range (thermal dose)
- **Biological target**: Multiple cellular components, functional and structural proteins
- **Microenvironment**: pH dependence
- **Cell cycle:** Increased resistance in G1
- **Cell death**: Necrosis/Apoptosis/Mitotic cell death

Thermal dose concept: Calculate the time at 43´C to achieve equivalent cell survival

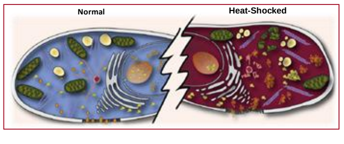
$$t_{43} = t \cdot R^{T-43^{\circ}\mathrm{C}} \quad \text{with} \quad R = \begin{cases} 0.25 & T < 43^{\circ}\mathrm{C} \\ 0.5 & \geq 43^{\circ}\mathrm{C} \end{cases}$$

- **T** Temperature
- **t** Duration
- **t_{43}** Thermal dose

Biological effects of hyperthermia

Hyperthermia

- **Stress**: Elevation of temperature above physiological range (thermal dose)
- **Biological target**: Multiple cellular components, functional and structural proteins
- **Microenvironment**: pH dependence
- **Cell cycle:** Increased resistance in G1
- **Cell death**: Necrosis/Apoptosis/Mitotic cell death

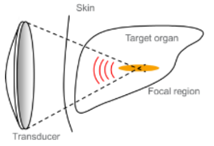


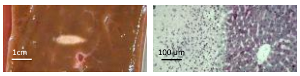
Richter et al., 2010, Molecular Cell (40)

Biological effects of focused ultrasound - thermal

Tumour Ablation

- **Stress**: Elevation of temperature to boiling point
- **Biological target**: -
- **Microenvironment**: -
- **Cell death**: Coagulation necrosis
- **Cell cycle:** -

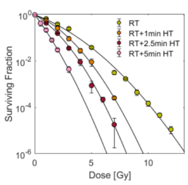




Heat-induced radio-sensitization

Hyperthermia | **Radiation** | **Tumour ablation**

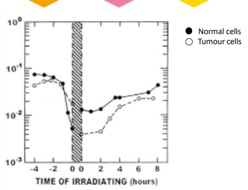
- **Synergistic effects** of hyperthermia/tumor ablation and radiation
- Mechanism of action: **inhibition of DNA repair**, different cellular targets, difference in cell cycle sensitivity



Heat-induced radio-sensitization

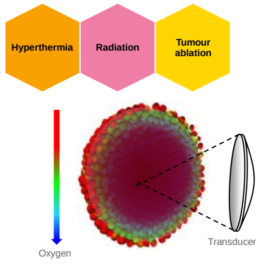
Hyperthermia | **Radiation** | **Tumour ablation**

- **Synergistic effects** of hyperthermia/tumor ablation and radiation
- Mechanism of action: **inhibition of DNA repair**, different cellular targets, difference in cell cycle sensitivity
- Treatment **sequence** and **scheduling** impacts radio-sensitization potential and may act differently on normal and tumor cells



Horsman et al., 2007, Clinical Oncology (19)

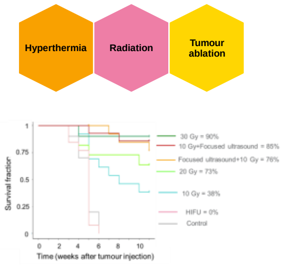
Heat-induced radio-sensitization



- **Synergistic effects** of hyperthermia/tumor ablation and radiation
- Mechanism of action: **inhibition of DNA repair**, different cellular targets, difference in cell cycle sensitivity
- Treatment **sequence** and **scheduling** impacts radio-sensitization potential and may act differently on normal and tumor cells
- **Heating applied locally** to the tumor may sensitize resistant tumors (or sub regions) to radiotherapy

Image: G. Mirams *et al*., PLoS Comput. Biol. 9 (3), 2013

Heat-induced radio-sensitization

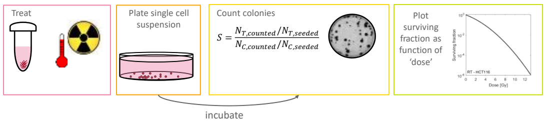


- **Synergistic effects** of hyperthermia/tumor ablation and radiation
- Mechanism of action: **inhibition of DNA repair**, different cellular targets, difference in cell cycle sensitivity
- Treatment **sequence** and **scheduling** impacts radio-sensitization potential and may act differently on normal and tumor cells
- **Heating applied locally** to the tumor may sensitize resistant tumors (or sub regions) to radiotherapy

PhD thesis M. Costa, 2017, The Institute of Cancer Research

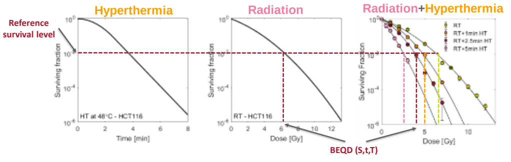
Quantifying heat-induced radio-sensitization: Evaluating cell survival

- Gold standard: Clonogenic assay



- Account for **reproductive capability** of isolated cells only
- **No** information on **dynamic processes**
- **Easy to control** micro-environmental conditions (hypoxia/normoxia/pH)
- Use cell survival data to **calculate biological dose weighting** for hyperthermia treatments

Quantifying heat-induced radio-sensitization: Evaluating cell survival



- Shape of the survival curve may be treatment dependent
- Calculate **biological equivalent dose** (BEQD) to express hyperthermia treatments in terms of radiation dose based on **iso-effective** treatments

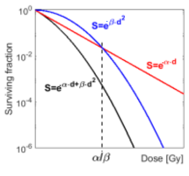
$$\text{TER}(S,t,T) = \frac{D(S,0,37C)}{BEQD(S,t,T)}$$ thermal enhancement ratio (TER)

- Parameterize the **dependence** of BEQD **on thermal dose**

Quantifying heat-induced radio-sensitization: Evaluating cell survival

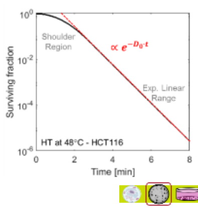
- **Linear-quadratic model (radiation)**

$$-\log(S) = \alpha d + \beta d^2$$



- **Arrhenius model (hyperthermia)**

$$-\ln(S) = D_0 t \quad \text{with} \quad \frac{1}{D_0} = k = Ae^{-\frac{E_a}{k_B T}}$$

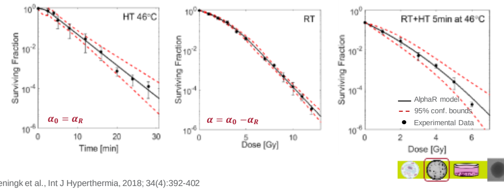


Evaluating cell survival: Radiation + Hyperthermia

- AlphaR model: Action and counter-action of damage and repair

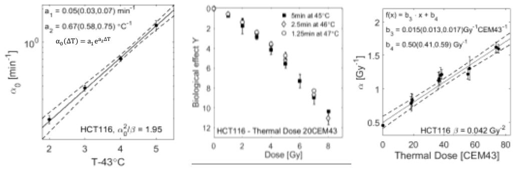
$$-\ln(S) = \begin{cases} (\alpha_0 - \alpha_R)d + \beta d^2 & Y \le Y_0 \\ \alpha_0 d + c & Y > Y_0 \end{cases}$$

$\alpha_0(t,T)$ Damage
$\alpha_R(t,T)$ Damage repair
$\beta(t,T)$ Damage to repair mechanisms
Y_0 Threshold to repair mechanism
c Integration constant



Brueningk et al., Int J Hyperthermia, 2018; 34(4):392-402

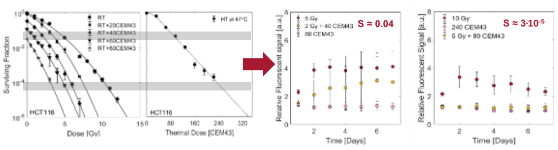
Evaluating cell survival: Radiation + Hyperthermia



- Hyperthermia: Exponential temperature dependence of α_0 and β
- Thermal dose is a valid predictor of heat-induced radio-sensitization
- Linear increase of α with thermal dose, β ≈ constant

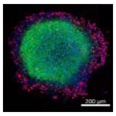
Brueningk et al., Int J Hyperthermia, 2018; 34(4):392-402

Isoeffective treatments – 2D growth



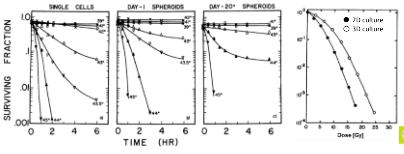
- **Same BEQD** delivered by radiation, hyperthermia or a combination thereof
- Clonogenic survival does **not account for** the influence of the cell **death mechanisms** induced
- More sophisticated models are required to capture this process
- Ideally this model accounts for a **more physiological micro-environment**

Analysing spheroid response



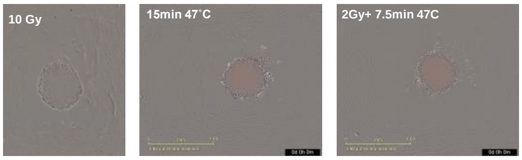
Proliferating
Quiescent/ Hypoxic
DAPI

- Physiological **micro-environment**
- Extracellular **matrix**
- **Layered structure**: Proliferating-quiescent-necrotic core
- "**Contact effect**": increased treatment resistance of cells in 3D
  - Hyperthermia: Build up of thermo-tolerance



[1] Sutherland et al. , 1988, Science (240)
[2] Durand et al., 1978, Radiation Research (75)

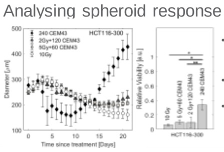
Analysing spheroid response



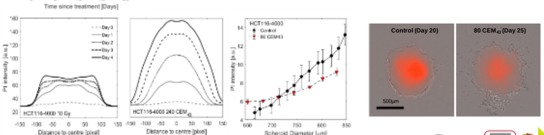
Time-lapse images on an Incucyte S3, **PI fluorescence** overlaid on phase contrast images of HCT116 tumor spheroids over a time course of 21 days post treatment.

- **Radiation:** Shrinkage from outside inwards from the proliferating zone
- **Hyperthermia:** Cell death and detachment independent of proliferation status
- **Combination:** Mixed response

Analysing spheroid response



- Despite isoeffective (thermal) doses delivered spheroid growth and cell viability differ significantly
- Difference in the cell death dynamics
- In spheroids hyperthermia leads to re-oxygenation/supply of more inner cells with nutrients



Modelling dynamic response - overview

- Simulate the **dynamic** biological response to multimodality therapies at a cellular level. By simulating a **large number of interacting cells**, insight into emergent tissue level phenomena can be achieved.
- **Cellular automaton** model simulates **individual cells** on a fixed sized grid (one voxel = one cell) with **probability driven responses** to treatments delivered on a macroscopic scale.

Spheroid/Monolayer growth – 2D/3D Lattice

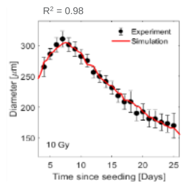
Oxygenation – Diffusion model, central necrosis

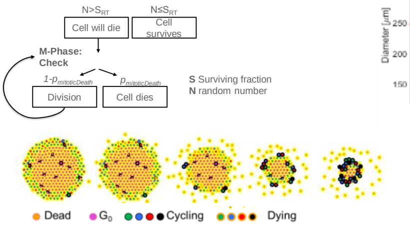
Cell survival – Alpha R model (weighting for oxygenation, cell cycle stage)

Dynamic cell death – Probability driven response cascade

Dynamic response: Radiation

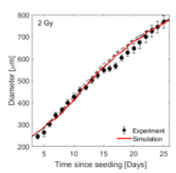
- **Mitotic catastrophe**: Death upon division
- **Initial delay**: Less likely to die

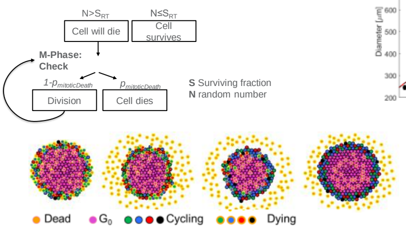




Dynamic response: Radiation

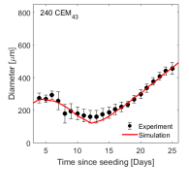
- **Mitotic catastrophe**: Death upon division
- **Initial delay**: Less likely to die

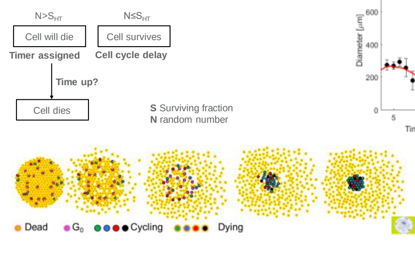




Dynamic response: Hyperthermia

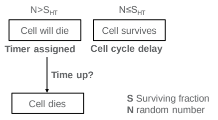
- Build up of **thermo-tolerance** in 3D cultured cells
- Cell death on average within 4 days – **independent of cell proliferation**



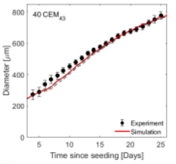


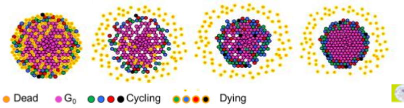
Dynamic response: Hyperthermia

- Build up of **thermo-tolerance** in 3D cultured cells
- Cell death on average within 4 days – **independent of cell proliferation**



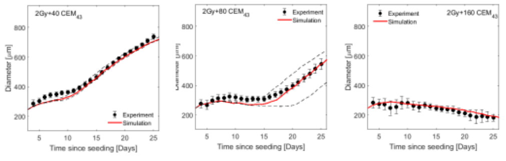
S Surviving fraction
N random number





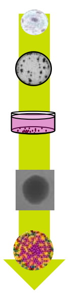
Combination treatments

- Overall surviving fraction $\mathbf{S=S_{HT} \cdot S_{HTRT}}$
- Treat the proportion $\mathbf{1\text{-}S_{HTRT}}$ as undergoing **radiation induced death**
- From the remaining cells assign heated-induced cell death to a subpopulation $\mathbf{1\text{-}S_{HT}}$



Summary

- The biological effects induced **at cellular level by radiation and hyperthermia differ** significantly.
- **Clonogenic cell survival is currently the gold standard assay** to quantify treatment efficacy and synergism between radiation and hyperthermia.
- Clonogenic assays do not account for **differences in dynamic cell death.**
- **3D tumour spheroids** provide a much more physiological cellular microenvironment than 2D cultures.
- **Biological equivalent dose** levels calculated from **clonogenic survival** data was a **poor predictor** of spheroid growth response.
- **More advanced biological models** are needed that account for micro-environmental effects and differences in cell death dynamics to be applied to predict in vivo response.



Prof. Dr. Gail ter Haar
Prof. Dr. Uwe Oelfke
Dr. Ian Rivens
Dr. Simeon Nill

Dr. Peter Ziegenhein
Dr. Marcia Costa
Dr. Carol Box
Dr. John Civale
Richard Symonds-Tayler
Dr. Petros Mouratidis

Prof. Mark Chaplain
Dr. Gibin Powathil

Thank you!

ICR The Institute of Cancer Research

Funded by CANCER RESEARCH UK

The ROYAL MARSDEN NHS Foundation Trust