Tumour Hypoxia and Hyperthermia

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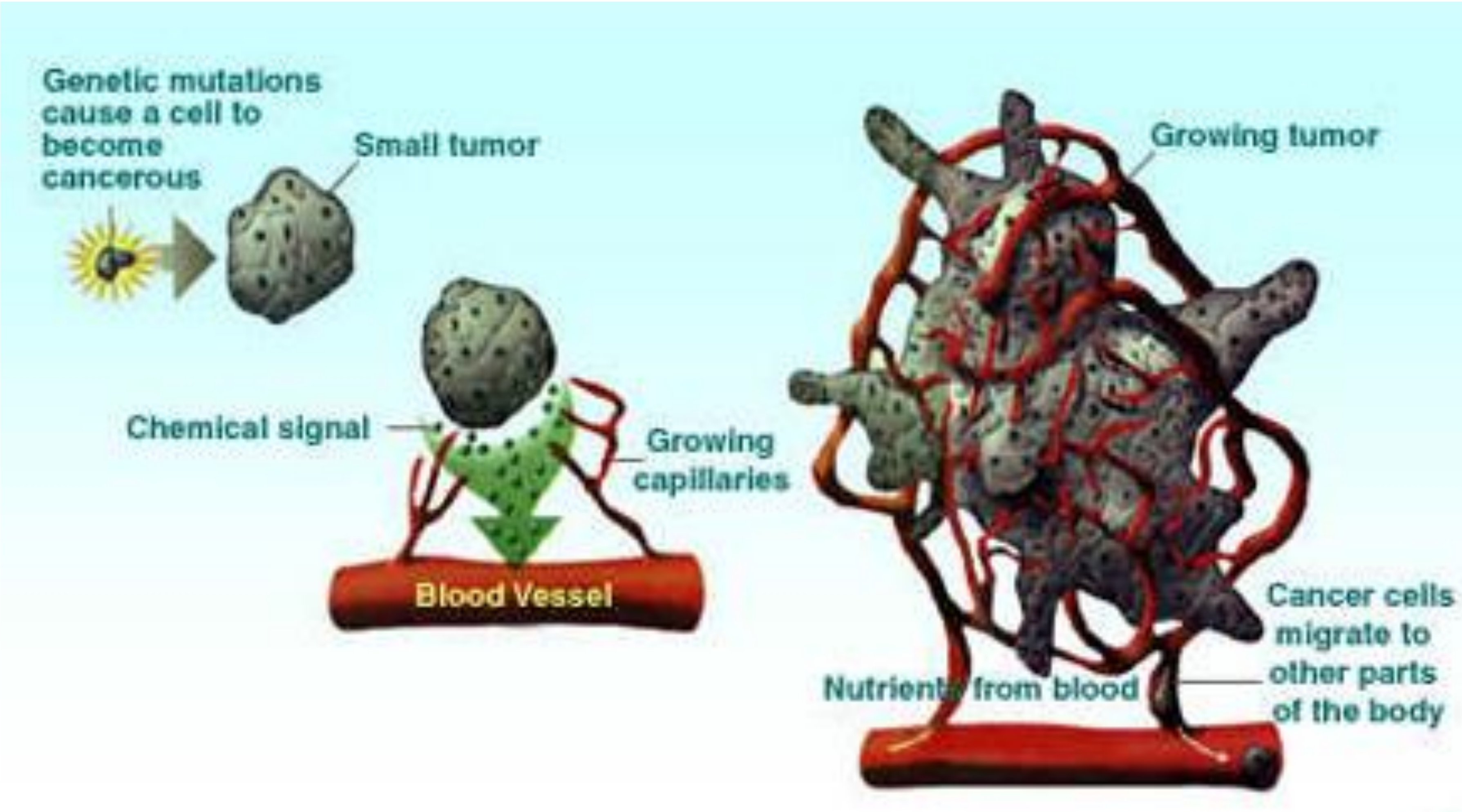
European Commission

Kræftens Bekæmpelse

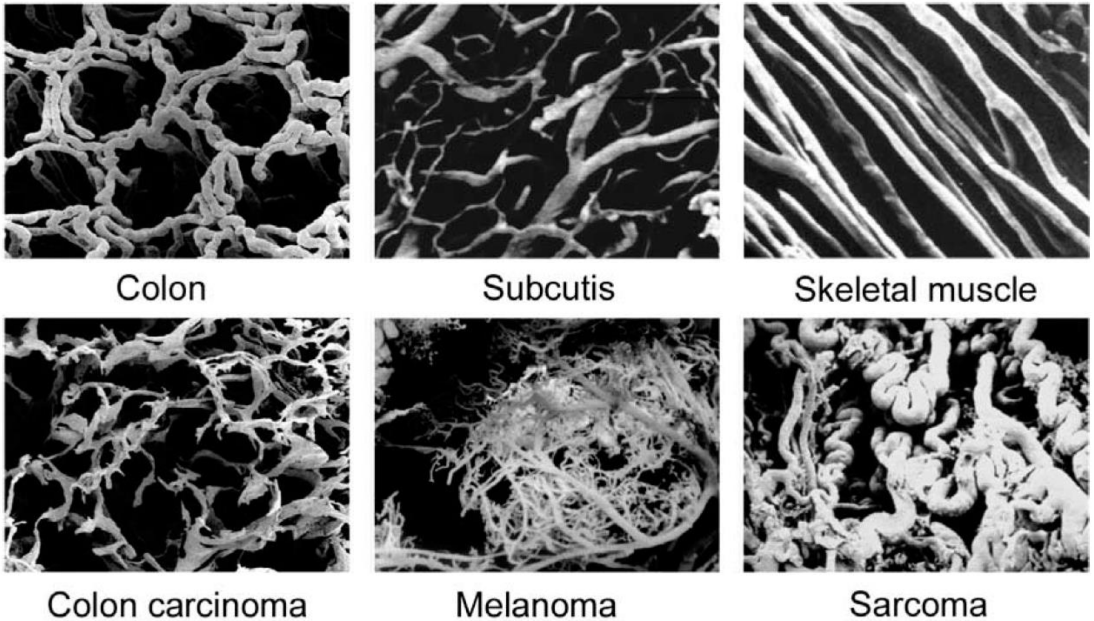
DANMARKS FRIE FORSKNINGSFOND

DANISH HEALTH AUTHORITY



Lertola (1998) Time Magazine 151 (20):40-46

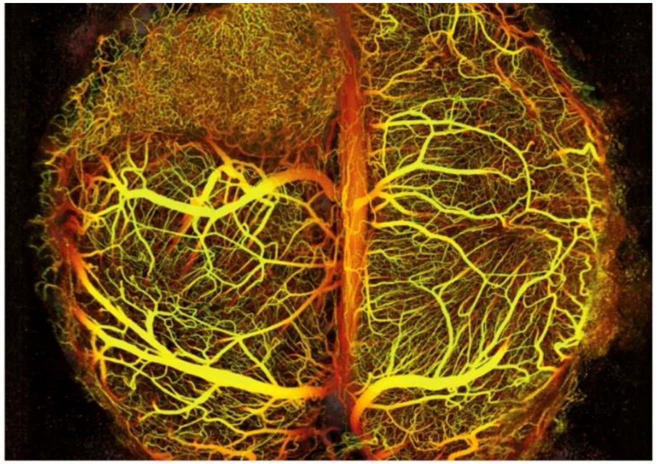
Tumour .v. Normal tissue vessels



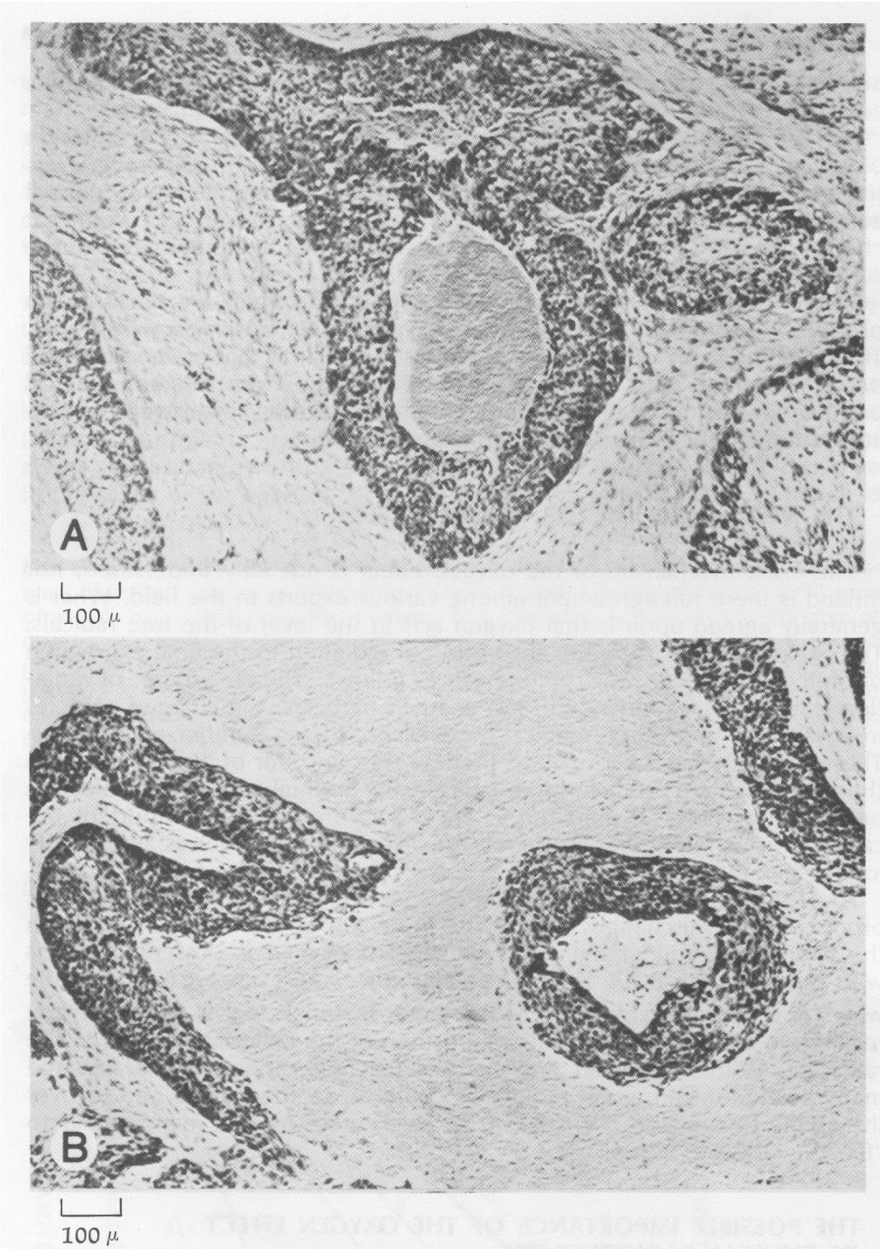
<u>Major Structural and Functional Abnormalities :</u>

- ***Abnormal vascular density***
- ***Contour irregularities***
- ***Loss of hierarchy***
- ***Lack of regulatory control mechanisms***
- ***Structural defects in vessel walls***
- ***Increased vascular permeability***
- ***Flow irregularities***
- ***Cellular aggregations/blockage***
- ***Increased haematocrit***

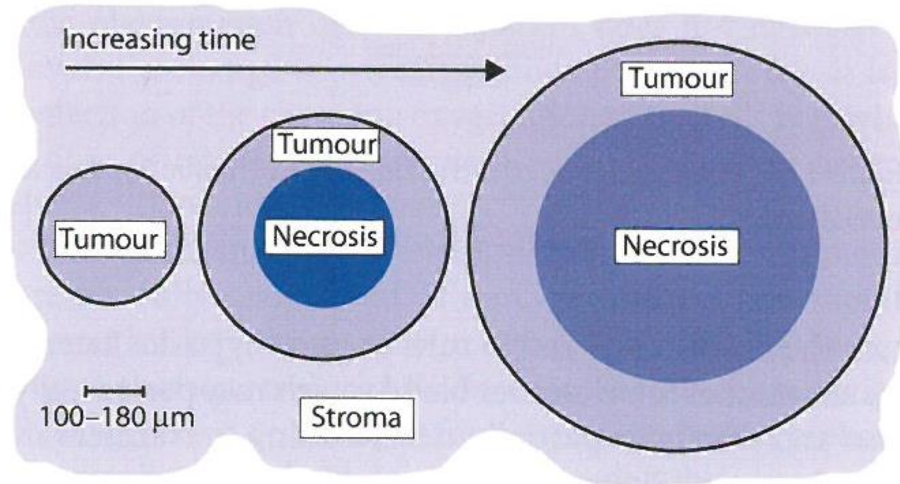
Horsman & Vaupel (2016) Frontiers in Oncology Research Topics



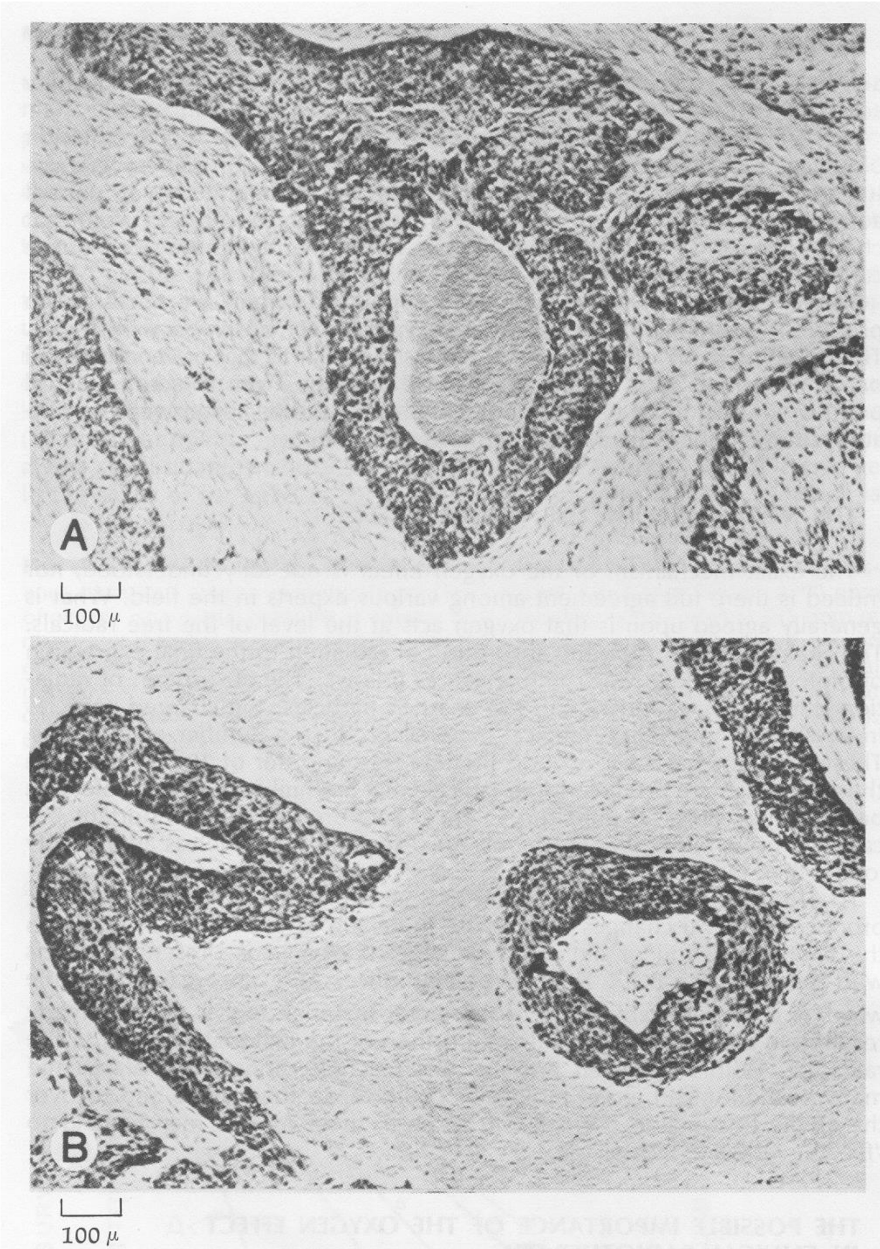
Vakoc et al. (2009) Nat. Med. 15:1219-1223.



Thomlinson & Gray (1955) Br. J. Cancer 9:539-549



Horsman et al. (2019). Basic Clinical Radiobiology, 5th edit., pp.188-205



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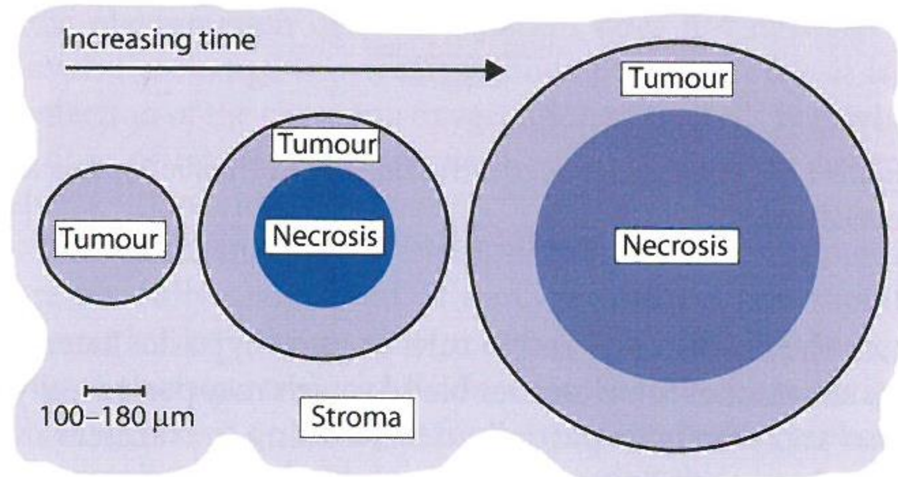
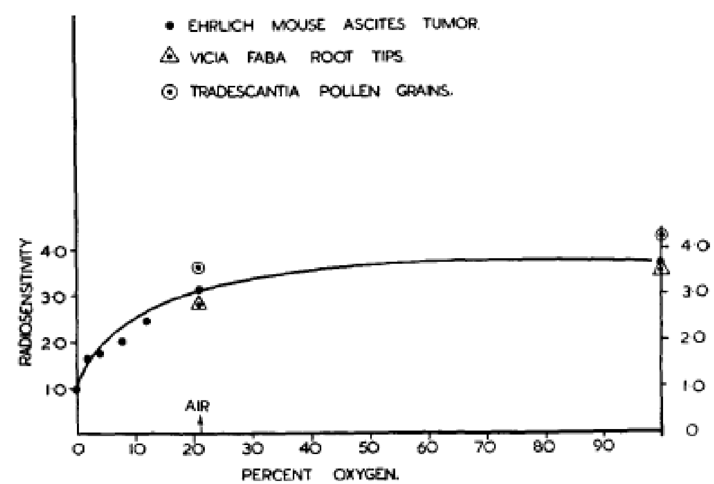
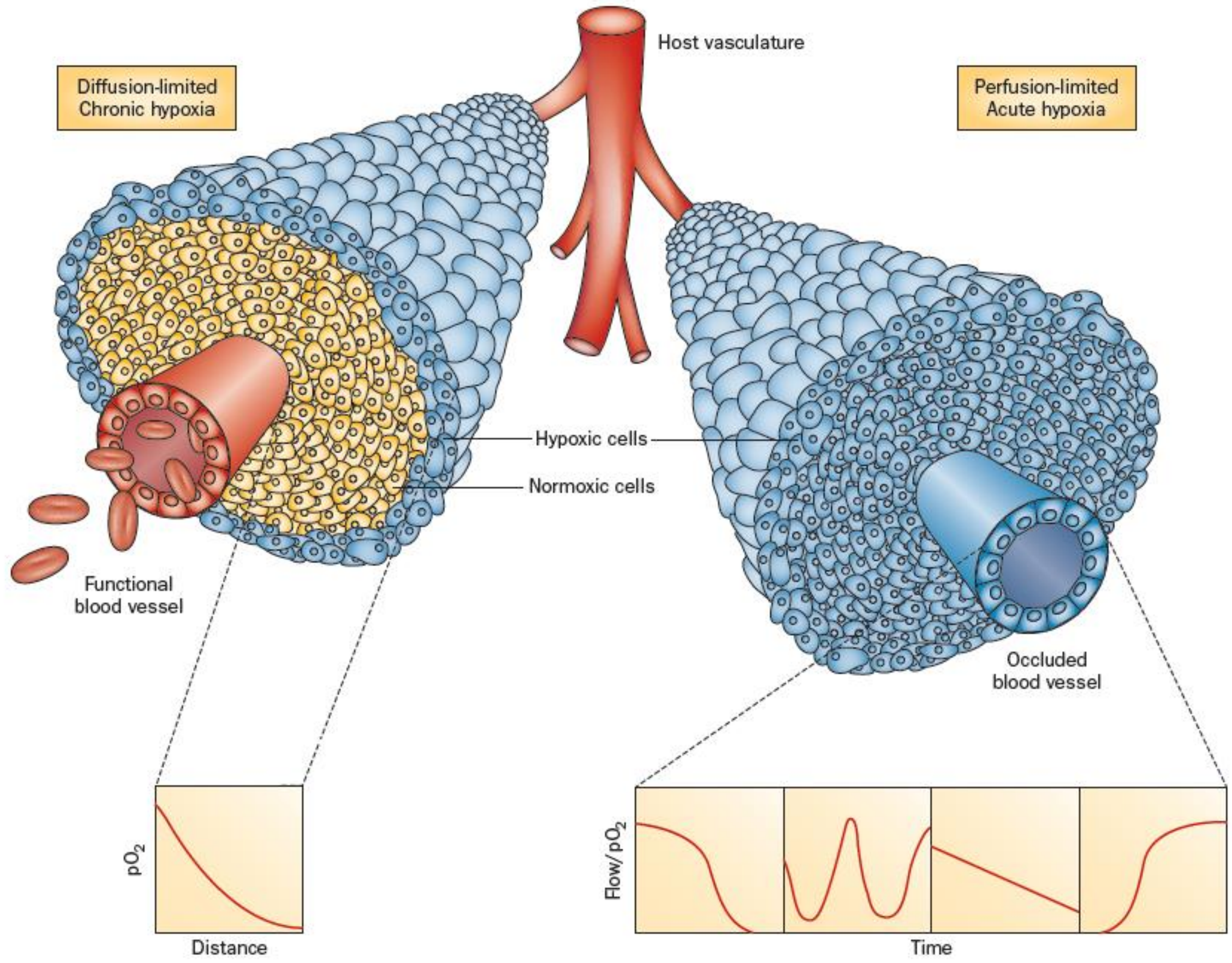


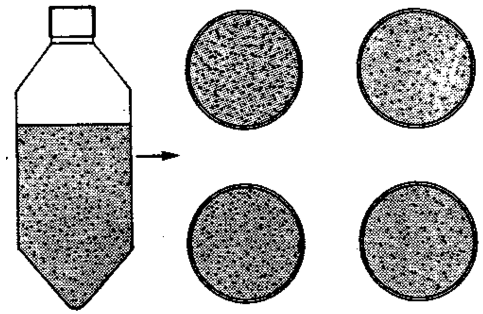
Horsman et al. (2019). Basic Clinical Radiobiology, 5th edit., pp.188-205



Gray et al. (1953) Br. J. Radiol. 312: 638-648

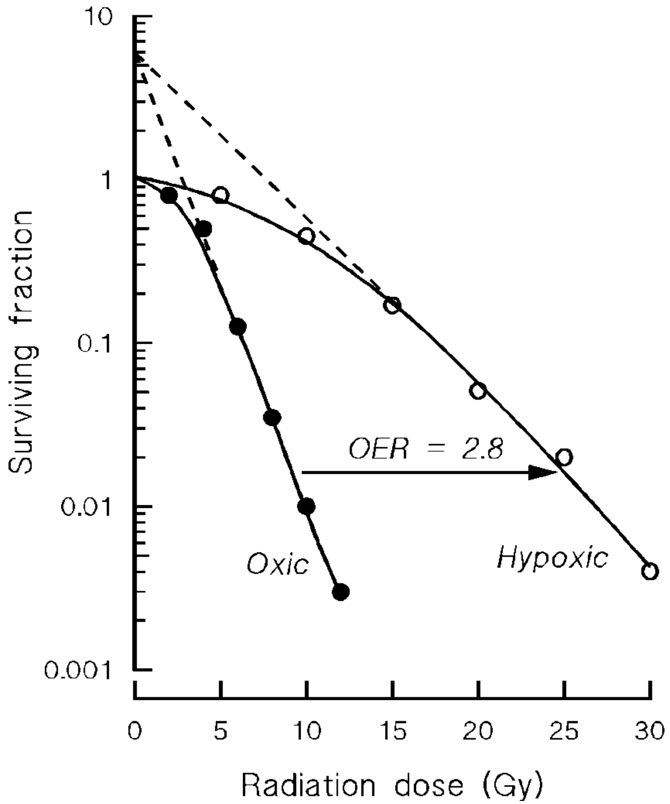


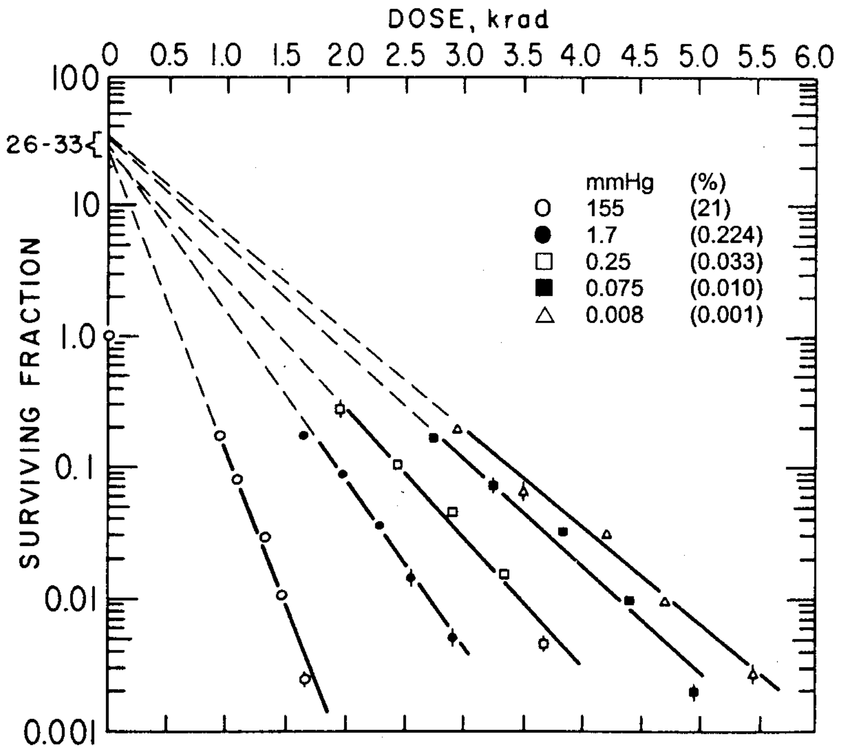
Horsman et al. (2012) Nat. Rev. Clin. Oncol. 9:674-687



Surviving fraction = $\frac{\text{No. colonies formed}}{\text{No. cells plated}}$

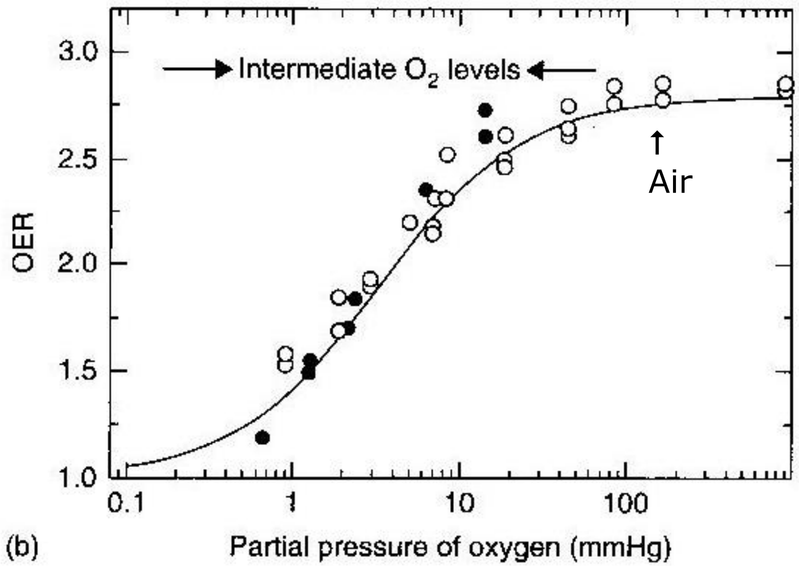
e.g., $\frac{\text{10 colonies}}{\text{100 cells}}$ = 0.1

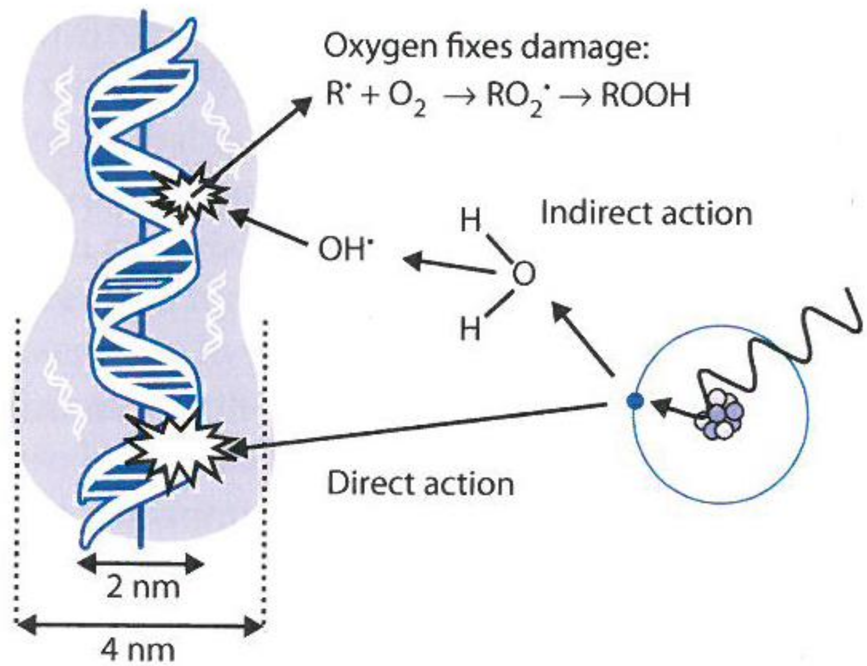




Horsman et al. (2019). Basic Clinical Radiobiology, 5th edit., pp.188-205

Elkind et al. (1965) Cellular Radiation Biology, pp.442-461





Horsman et al. (2019). Basic Clinical Radiobiology, 5th edit., pp.188-205

Reactions with the Target

1. Direct effect – Ionisations occur in the target

$$RH \longrightarrow RH^{+} + e^{-}$$
$$RH^{+} \longrightarrow R^{\cdot} + H^{+}$$

2. Indirect effect – Ionisations occur in water

$$H_2O \longrightarrow H_2O^{+} + e^{-}$$
$$H_2O^{+} \longrightarrow H^{+} + OH^{\cdot}$$
$$H_2O^{+} + H_2O \longrightarrow H_3O^{+} + OH^{\cdot}$$
$$e^{-} + H_2O \longrightarrow H_2O^{-} \longrightarrow H^{\cdot} + OH^{\cdot}$$
$$e^{-} + H_2O \longrightarrow e^{-}aq$$
$$e^{-} + H^{+} \longrightarrow H^{\cdot}$$

The most important product is believed to be the $OH^{\cdot}$

$$H_2O \longrightarrow H_2O^{+} + e^{-}$$
$$H_2O^{+} \longrightarrow H^{+} + OH^{\cdot}$$
$$RH + OH^{\cdot} \longrightarrow R^{\cdot} + H_2O$$

What happens to $R^{\bullet}$?

In absence of oxygen or in presence of –SH

$$R^{\cdot} + SH \longrightarrow RH + S^{\cdot}$$

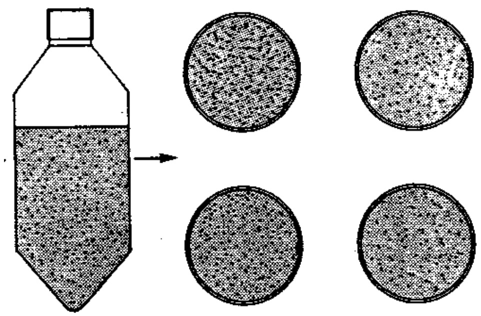
(Target restitution)

In presence of oxygen

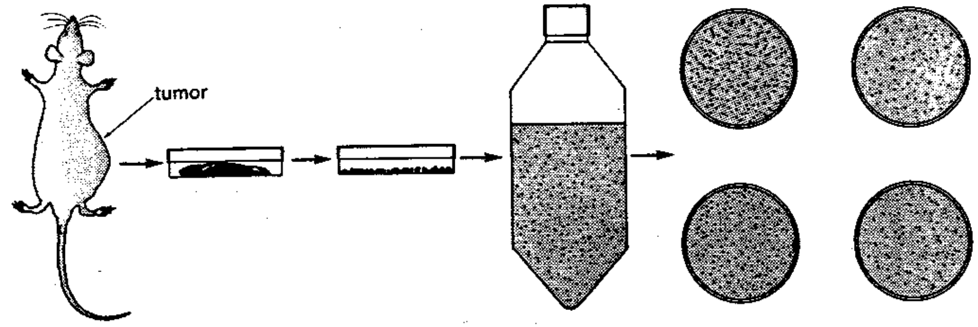
$$R^{\cdot} + O_2 \longrightarrow RO_2^{\cdot} \longrightarrow ROOH$$

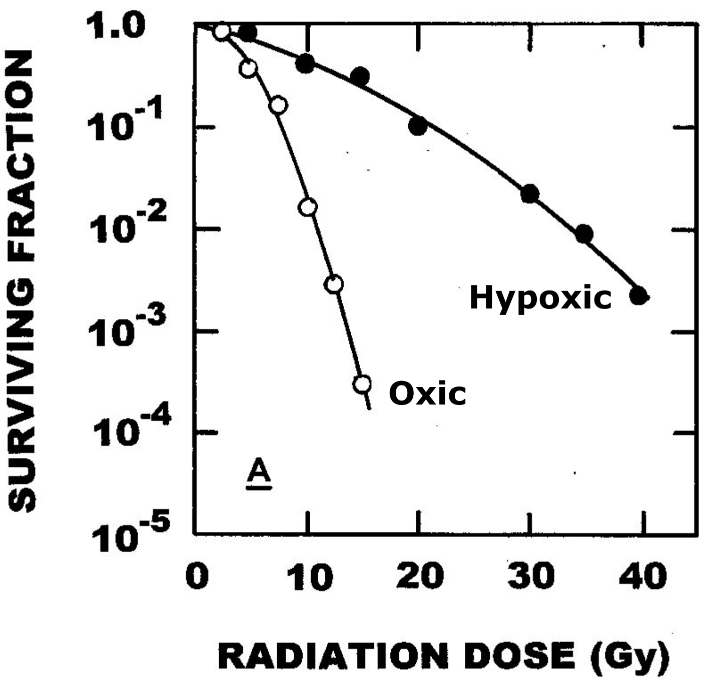
(Oxygen fixation hypothesis)

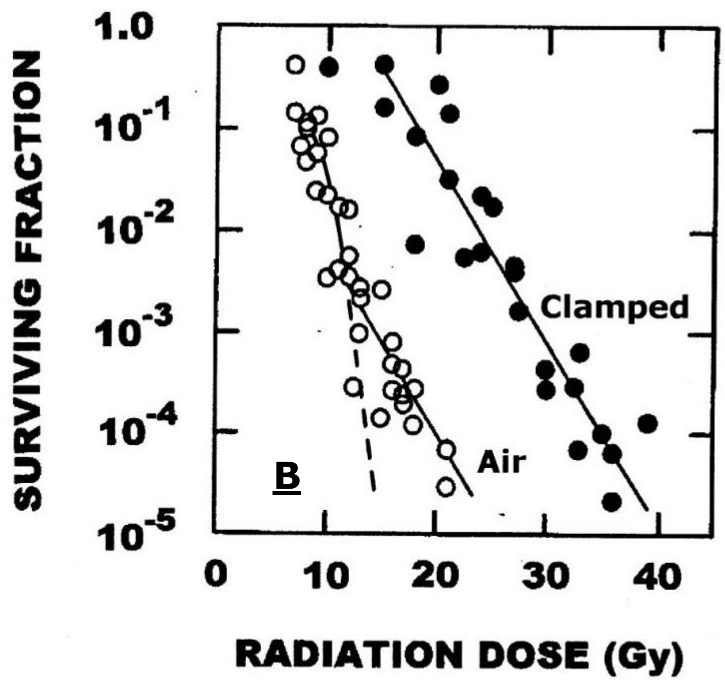
In Vitro



In Vivo/In Vitro

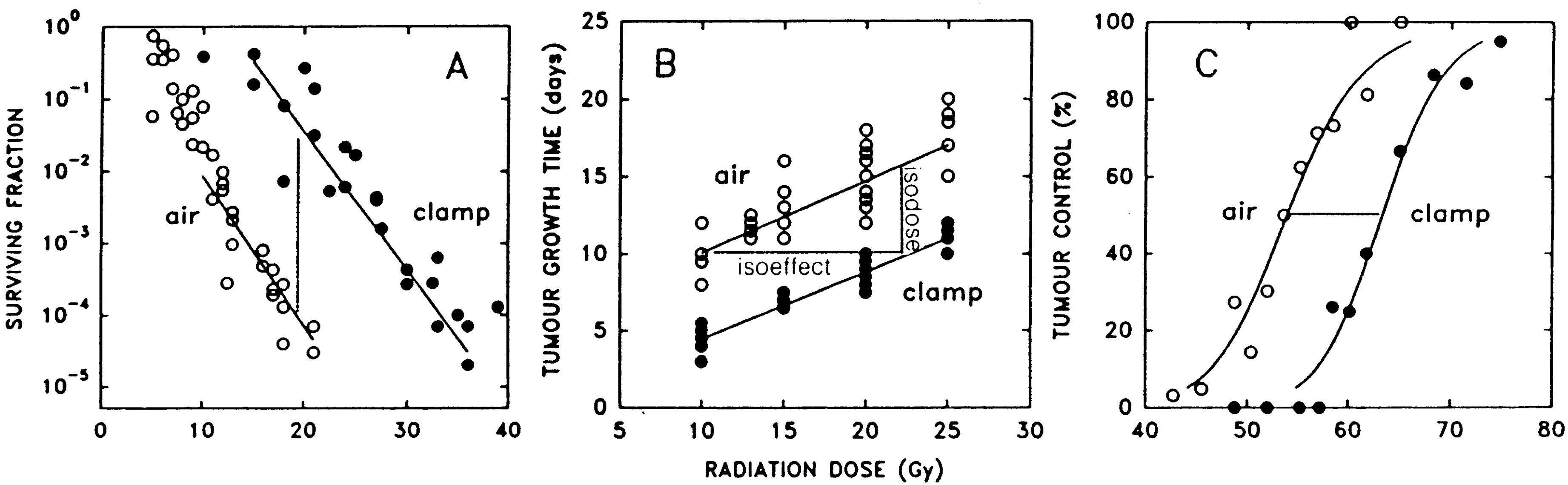






Classical assays for assessing hypoxia

A) Clamped survival **B) Clamped growth delay** **C) Clamped tumour control**

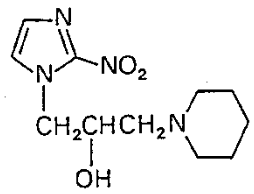


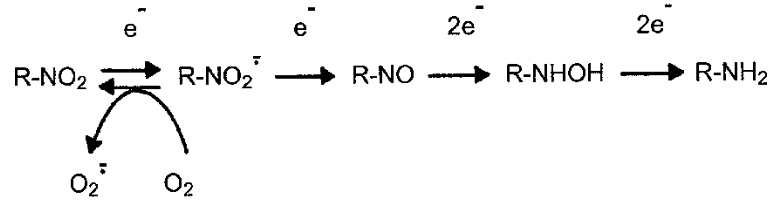
Grau (unpublished observations)

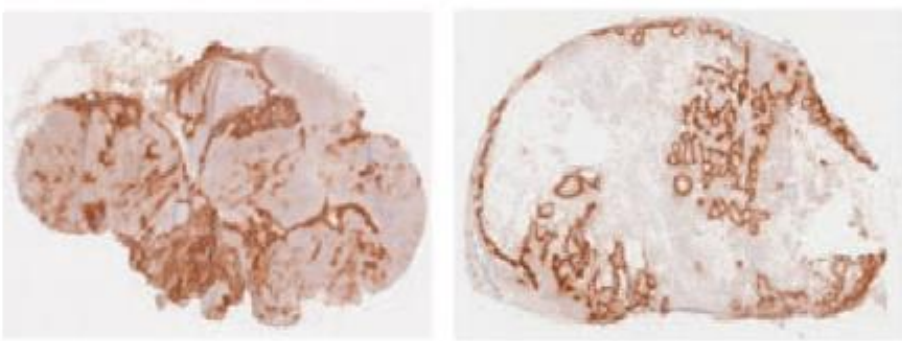
Measuring hypoxia in human tumours

- **Vascular based methods**
  - Biopsy/immunohistochemistry
    - Intercapillary distance
    - Vascular density
    - Cell to nearest vessel distance
    - HbO_2 saturation
  - Imaging approaches
    - HbO_2 saturation (NIRS/BOLD)
    - PET (^{15}Oxygen labelled water)
    - CT perfusion
    - DCE-MRI
- **Direct Oxygen measurements**
  - Electrodes (Glass/Eppendorf)
  - EPR
- **Endogenous markers**
  - Individual genes/proteins
    - Biopsy studies
    - Serum/Plasma
  - Gene signatures
- **Exogenous markers**
  - Biopsy/immunohistochemistry
    - Nitroimidazole markers
  - Imaging approaches
    - PET (nitroimidazoles)
    - CuATSM
    - MRI (nitroimidazoles)
    - SPECT (nitroimidazoles /technetium)
- **Surrogate markers**
  - Metabolism
    - Biopsy/bioluminescence (Lactate/ATP/glucose)
    - Imaging (FDG/MRS)
  - DNA damage
    - Comet assay
    - H2AX phosphorylation
  - Interstitial fluid pressure
    - Probes
    - DCE-MRI

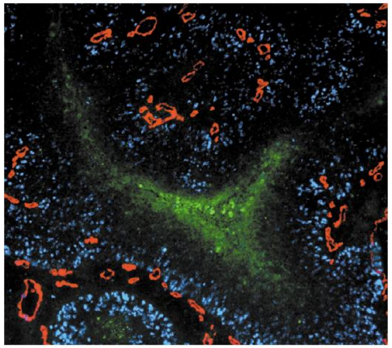
Pimonidazole Binding





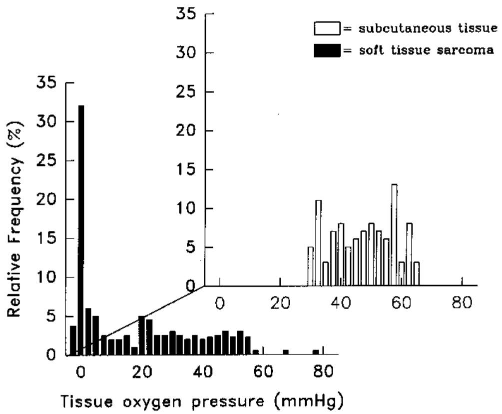


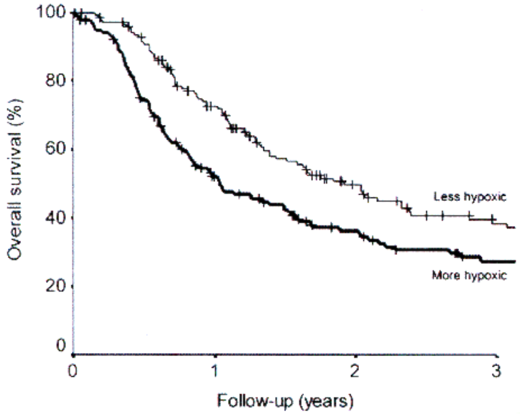
Busk et al. (2013) Acta Oncol. 52:1300-7



Kaanders et al. (2002) Lancet Oncol 3:728-37

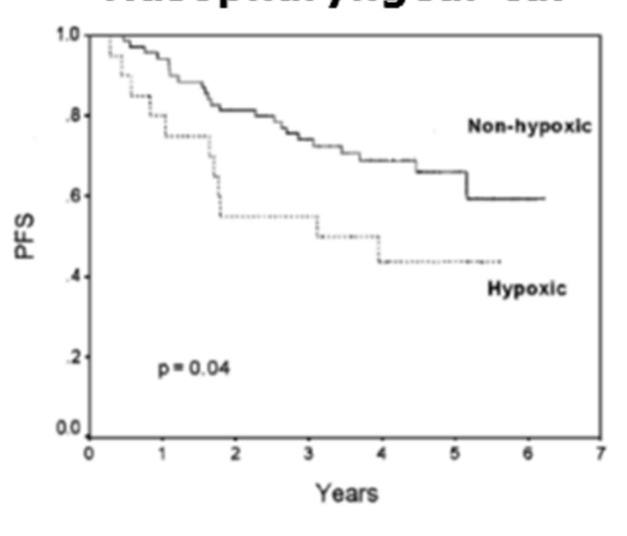
Eppendorf Oxygen electrode



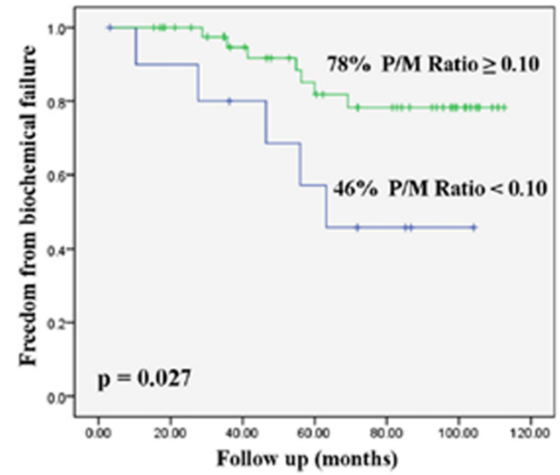


Nordsmark et al. (2005) R&O 77:18-24

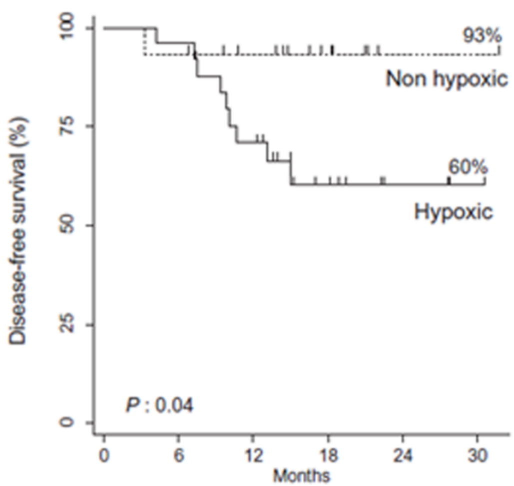
(A) Exogenous markers: Nasopharyngeal ca.



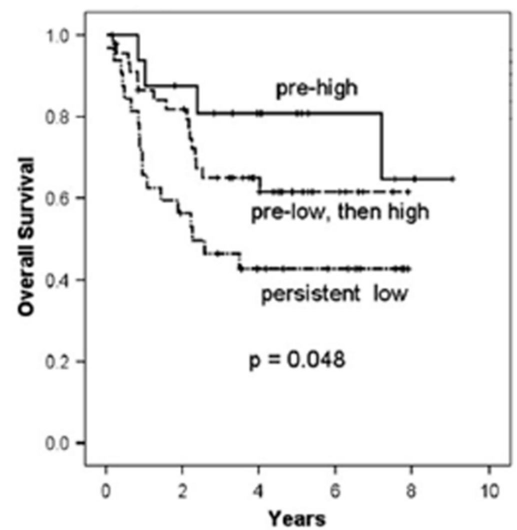
(B) Eppendorf pO2: Prostate



(C) [18F]-FAZA PET: Head & Neck SCC



(D) DCE-MRI: Cervix ca.



Siemann & Horsman (2015) Pharmacol. Ther. 153:107-124

Hypoxia Gene Expression Classifier

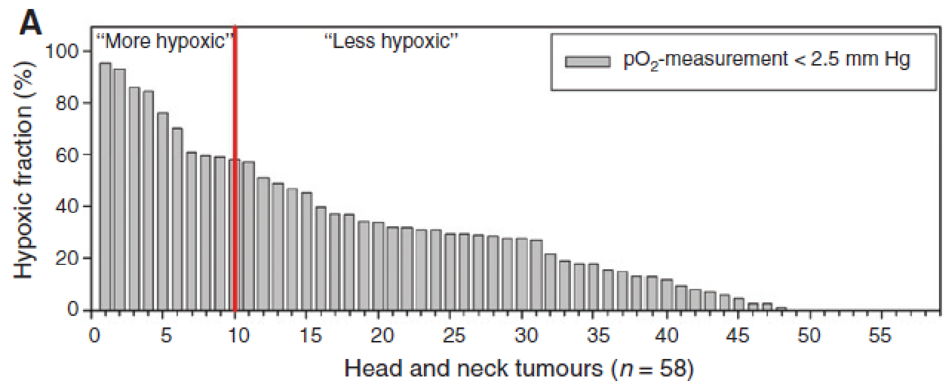
In vitro & in vivo

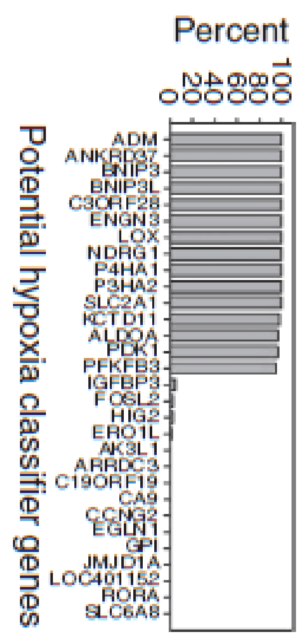
Table 1. Hypoxia-responsive genes

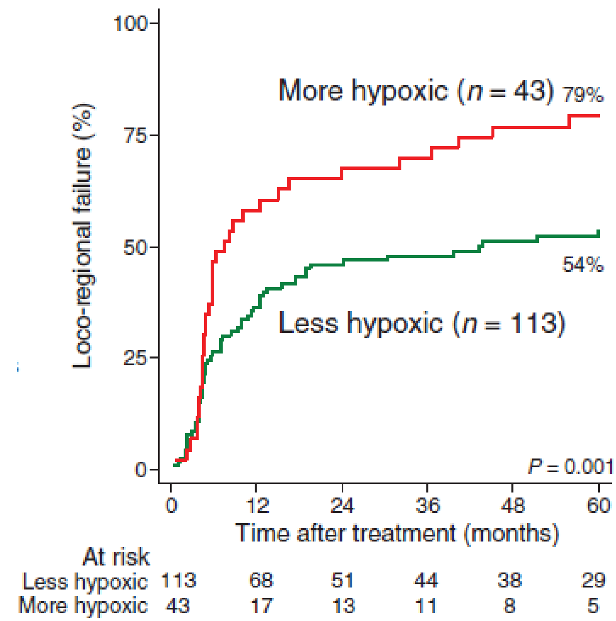
In vitro derived genes	Included in hypoxia classifier	Function
ADM	ADM	Stress response
AK3L1		Nucleotide metabolism
ALDOA	ALDOA	Glucose metabolism
ANKRD37	ANKRD37	Protein–protein interactions
ARRDC3		Cell surface metabolism
BNIP3	BNIP3	Apoptosis
BNIP3L	BNIP3L	Apoptosis
C3orf28	C3orf28	Unknown
C18orf19		Unknown
CCNG2		Cell cycle regulation
EGLN1		Regulation of HIF-1 activity
EGLN3	EGLN3	Regulation of HIF-1 activity
ERO1L		Oxidoreductase
FOSL2		Cell proliferation
GPI		Glucose metabolism
HIG2		Stress response
IGFBP3		Cell proliferation
JMJD1A		Histone demethylase
KCTD11	KCTD11	Apoptosis
LOC401152		Unknown
LOX	LOX	Extracellular-matrix metabolism
NDRG1	NDRG1	Stress response
P4HA1	P4HA1	Extracellular-matrix metabolism
P4HA2	P4HA2	Extracellular-matrix metabolism
PDK1	PDK1	Energy metabolism
PFKFB3	PFKFB3	Glucose metabolism
RORA		Unknown
SLC2A1	SLC2A1	Glucose metabolism
SLC6A8		Glucose metabolism
CA9[a]		pH regulation

[a]Gene based on previous studies.

Clinical







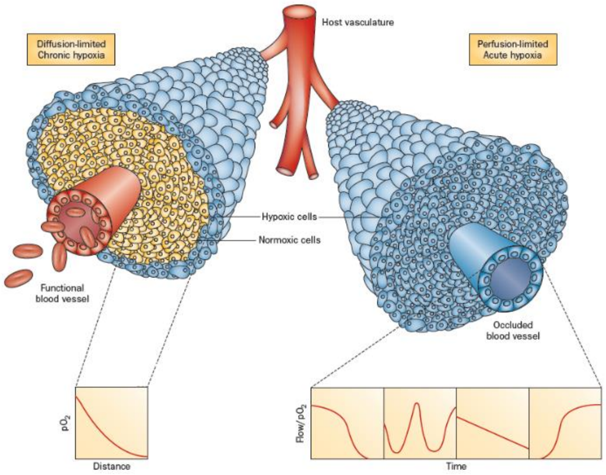
Toustrup et al. (2011) Cancer Res. 71:5923-5931

Hypoxic Gene Signatures

Author and publication date	Number of gene symbols
Koong *et al.*, 2000	10
Denko *et al.*, 2003	80
Jogi *et al.*, 2004	107
Ning *et al.*, 2004	104
Manalo *et al.*, 2005	107
Wang *et al.*, 2005	56
Detwiller *et al.*, 2005	27
Bosco *et al.*, 2006	177
Mense *et al.*, 2006	111
Aprelikova *et al.*, 2006	236
Chi *et al.*, 2006	111
Elvidge *et al.*, 2006	181
Peters *et al.*, 2006	159
Seigneuric *et al.*, 2007 (early 0% O_2)	71
Seigneuric *et al.* 2007, (early 2% O_2)	34
Seigneuric *et al.*, 2007 (common genes between early 0% and 2% O_2 signatures)	14
Winter *et al.*, 2007	**99**
Shi *et al.*, 2007	32
Sung *et al.*, 2007	90
Beyer *et al.*, 2008	159
Van Malenstein *et al.*, 2010*	7 (3*)
Benita *et al.*, 2009*	81 (57*)
Fardin *et al.*, 2009	8
Hu *et al.*, 2009	**13**
Fardin *et al.*, 2010	35
Sorensen *et al.*, 2010	27
Buffa *et al.*, 2010	**51**
Ghorbel *et al.*, 2010	166
Toustrup *et al.*, 2011	15
Ghazoui *et al.*, 2011	**70**
Starmans *et al.*, 2012	759
Eustace *et al.*, 2013	**26**

Harris et al. (2017) Clin. Oncol. 27:547-560

Hypoxic Cells: Impact on Therapy

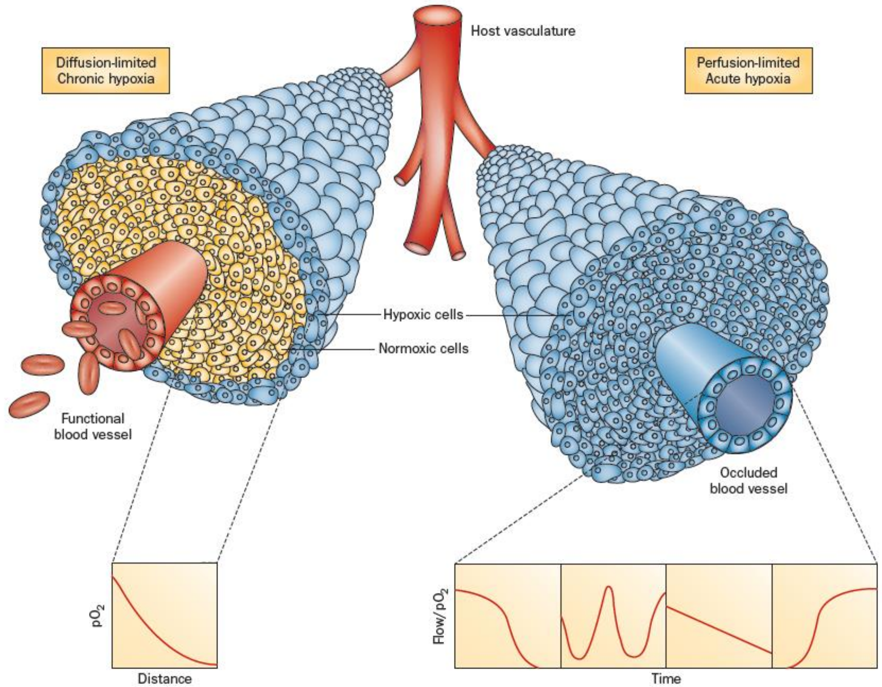


Resistant

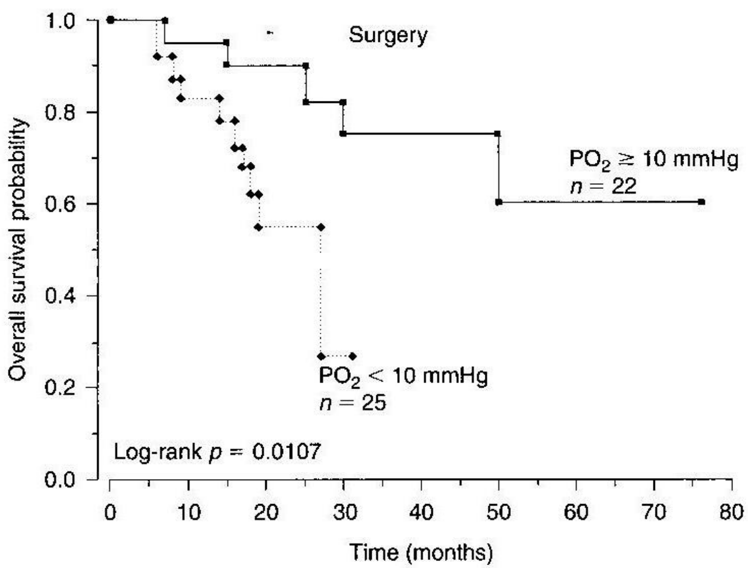
- Radiation
- Doxorubicin
- Actinomycin D
- Bleomycin
- Vincristine
- Methotrexate (?)
- 5-flurouracil (?)
- Cisplatin (?)
- Streptonigrin
- Procarbazine

Sensitive

- Hyperthermia
- Etoposide
- BCNU/CCNU (?)
- Alkylating agents (?)
- Mitomycin C
- E09
- PR-104
- TH-302
- Tirapazamine
- Banoxantrone

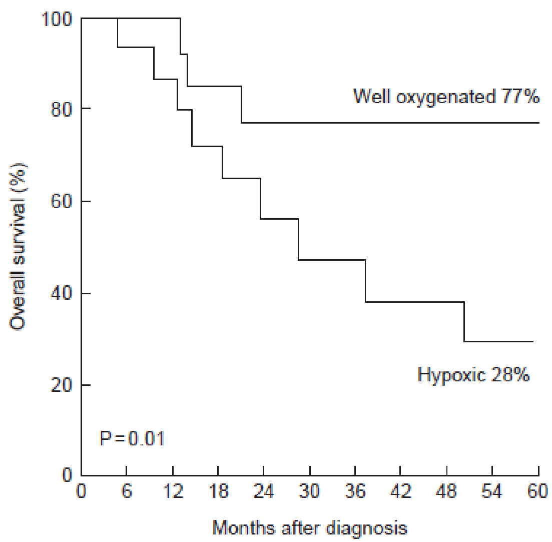


Cervix Carcinomas



Hoeckel et al. (1996)
Cancer Res. 56:4509-15

Soft Tissue Sarcomas



Nordsmark et al. (2001)
Br. J. Cancer 84:1070-75

Approaches for dealing with hypoxia

- **Increasing oxygen delivery**
  - High oxygen content gas breathing (e.g., HBO, carbogen)
  - Altering haemoglobin (e.g., transfusion, EPO, 2,3-DPG, PFE)
  - Reducing fluctuations in flow (e.g., nicotinamide, pentoxifylline)
  - Decreasing oxygen consumption (e.g., metformin, phenformin, atovaquone)
  - Increasing blood flow (e.g., hyperthermia)
- **Radiosensitizing hypoxic cells**
  - Nitroaromatic sensitizers (e.g., Misonidazole, Nimorazole, Doranidazole)
  - Hyperthermia
- **Preferentially killing hypoxic cells**
  - Hyperthermia
  - Bioreductive drugs (e.g., Tirapzamine, AQ4N, PR-104, TH-302)
- **Vascular targeting therapies**
  - Angiogenesis inhibitors (e.g., avastin, DC101, TK inhibitors)
  - Vascular disruptive agents (e.g., CA4P, OXi4503, DMXAA, hyperthermia)
- **Radiation**
  - Dose painting
  - High LET radiation

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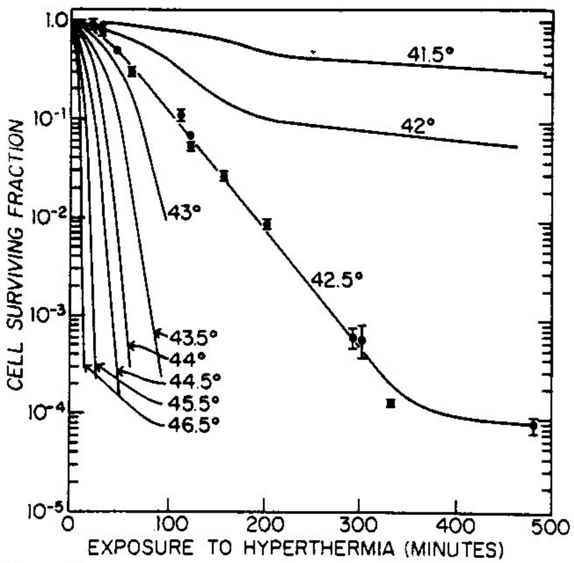
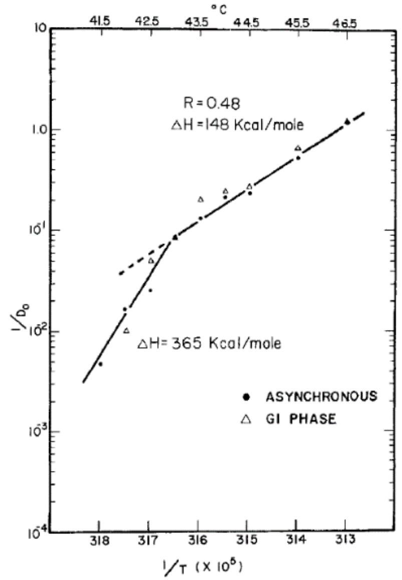
  - Dose painting
  - High LET radiation

Cell killing by heat

Fever range: ***37-42°C***
Hyperthermia: ***40-45°C***
Thermal ablation: ***>45°C***

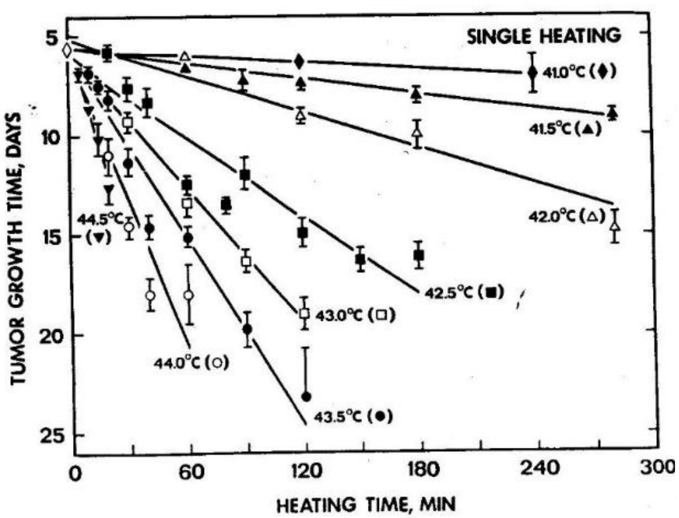
In Vitro

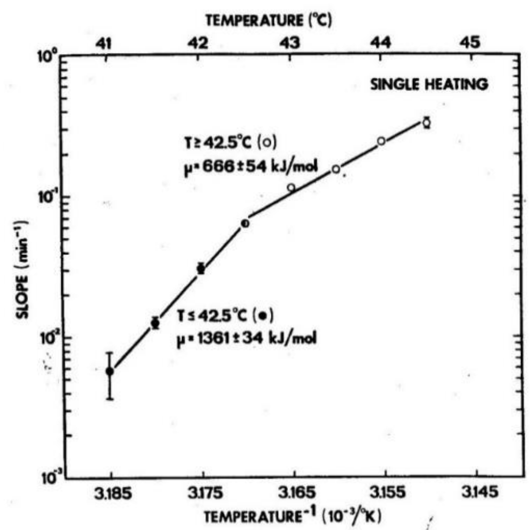




Dewey et al., Radiol (1977) 123:463-474

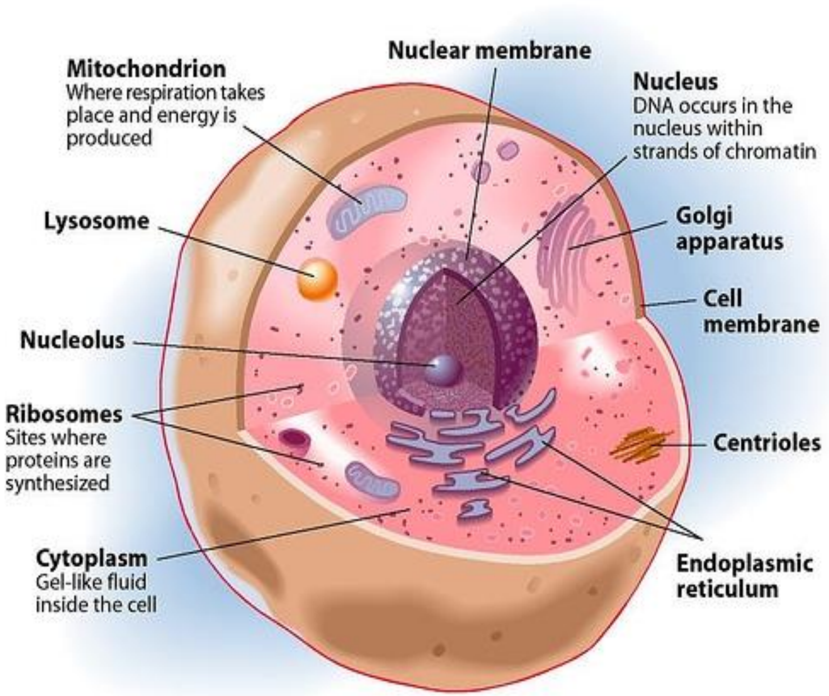
In Vivo





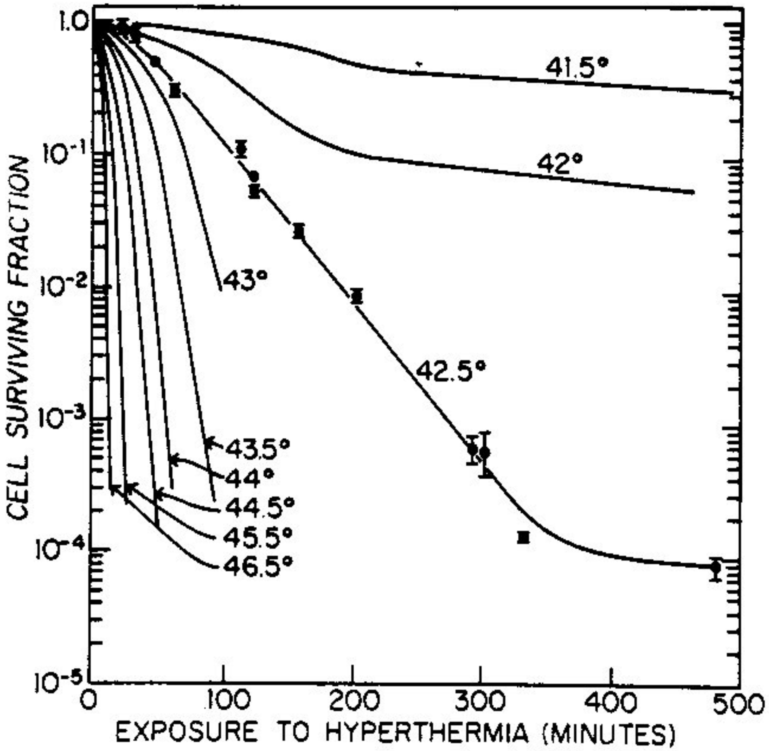
Lindegaard & Overgaard (1987) Int. J. Hyperthermia 3:79-81

Targets for heat



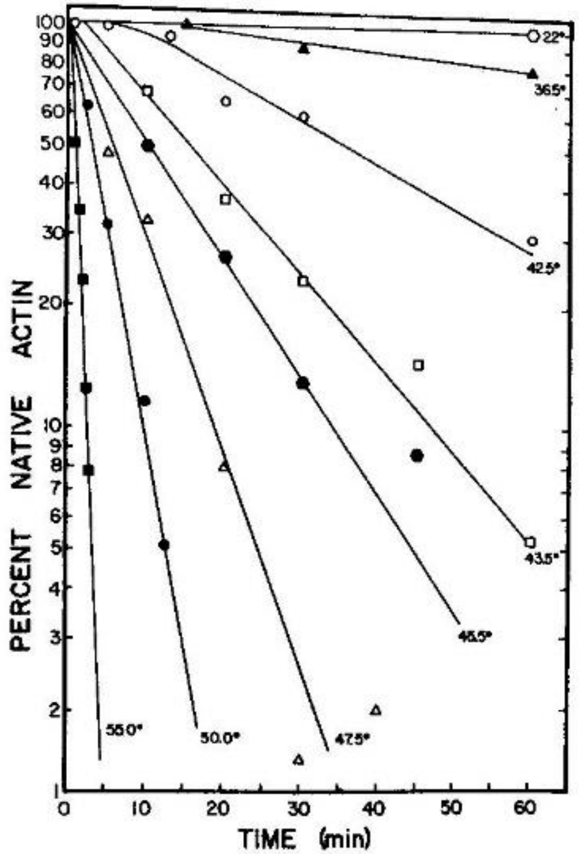
- Membranes
  - lipids
  - proteins
- Cytoskeleton
  - microfilaments
  - microtubules
- Cytosol
  - mitochondria
  - lysosomes
  - respiration/glycolosis
  - protein synthesis
- Nucleus
  - DNA replication
  - RNA synthesis
  - chromosomal damage

Cell survival



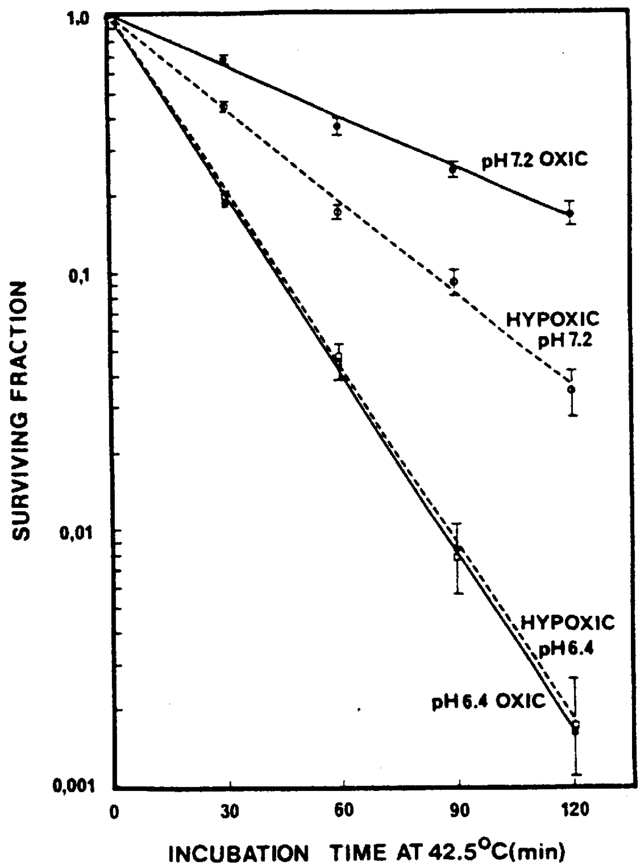
Dewey et al. (1977)
Radiol. 123:463-474

Protein inactivation



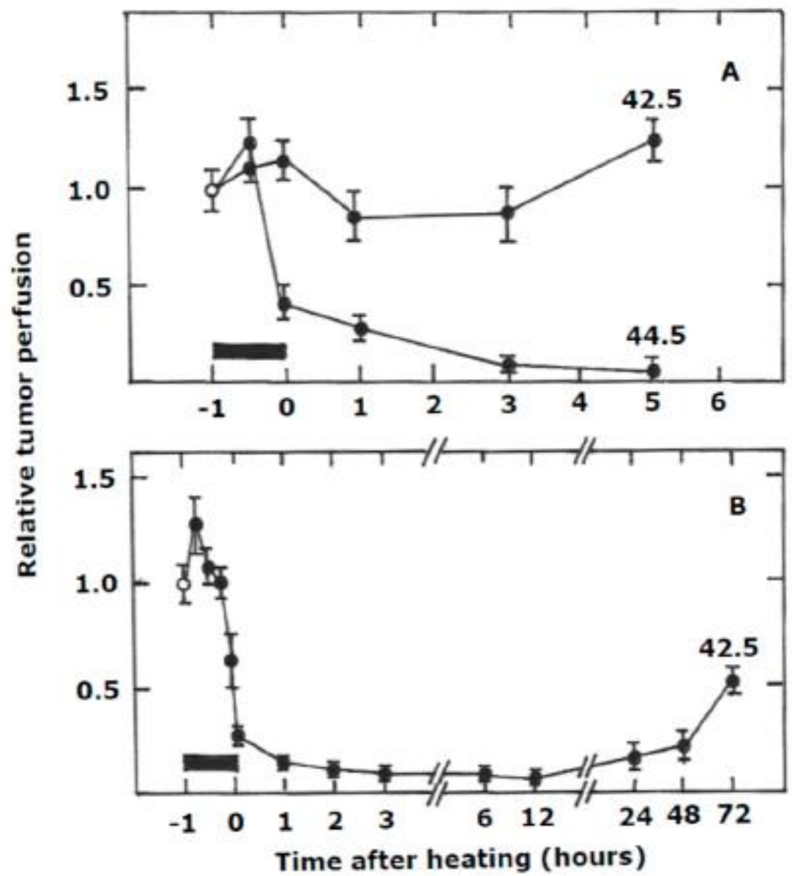
Heacock et al. (1982)
JNCI 61:73-75

Hypoxia and pH



Overgaard & Bichel (1977) Radiol. 123:511-514

Vascular damage

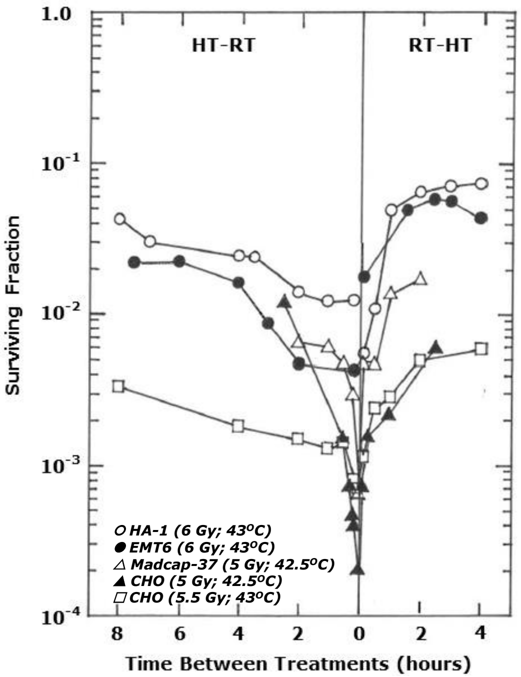


Elming et al. (2019) Cancers

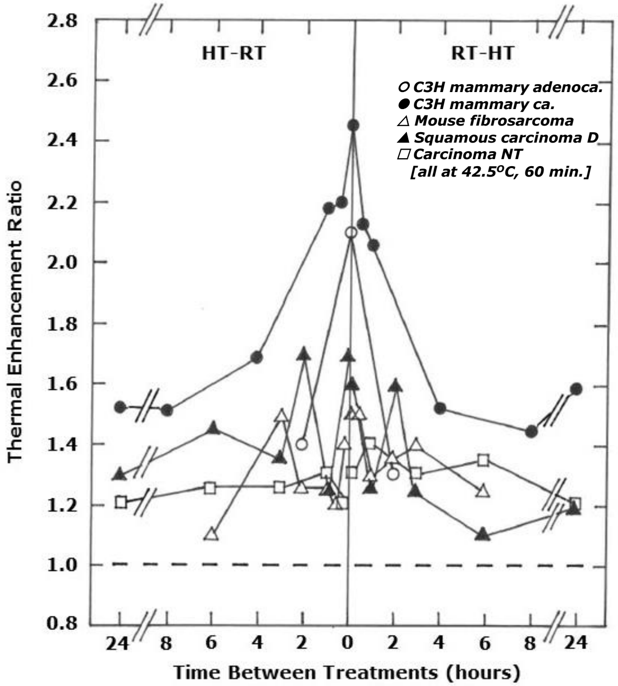
Combining radiation and hyperthermia

Influence of sequence and interval

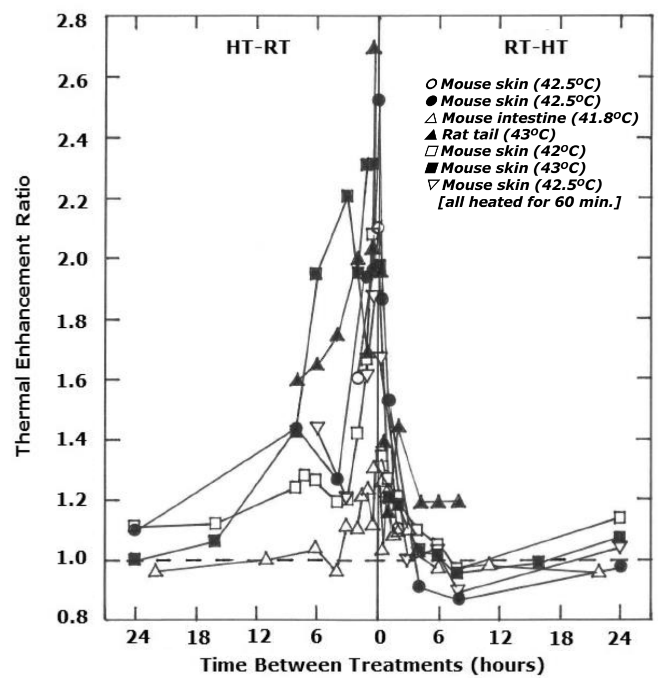
In vitro cell lines



In vivo tumors

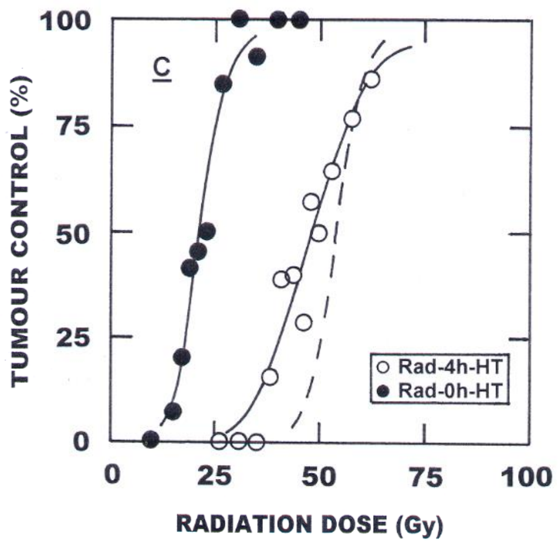


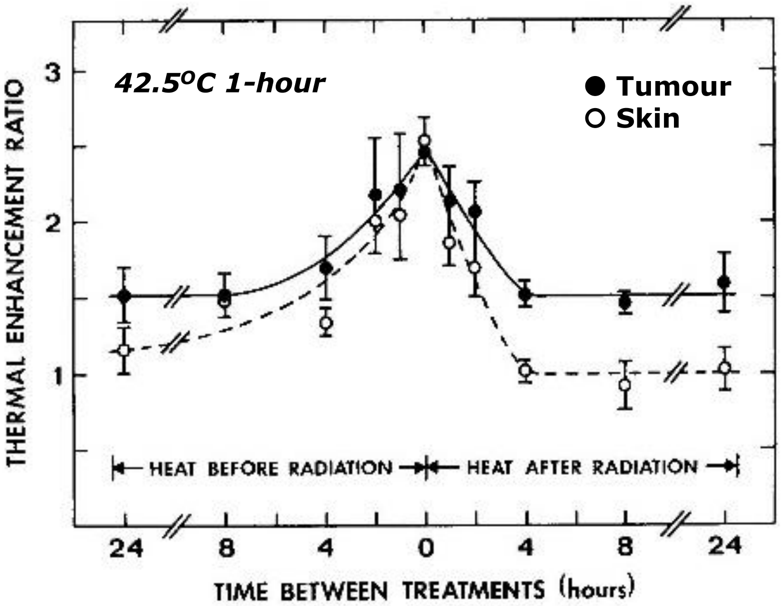
In vivo normal tissues

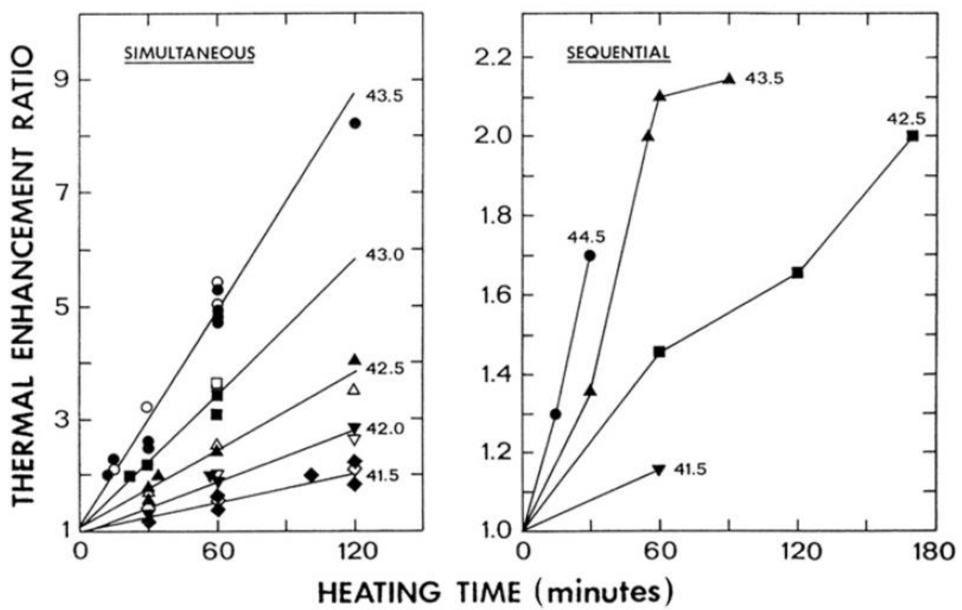


Sinha et al. (submitted to Cancer)

Radiation + Hyperthermia

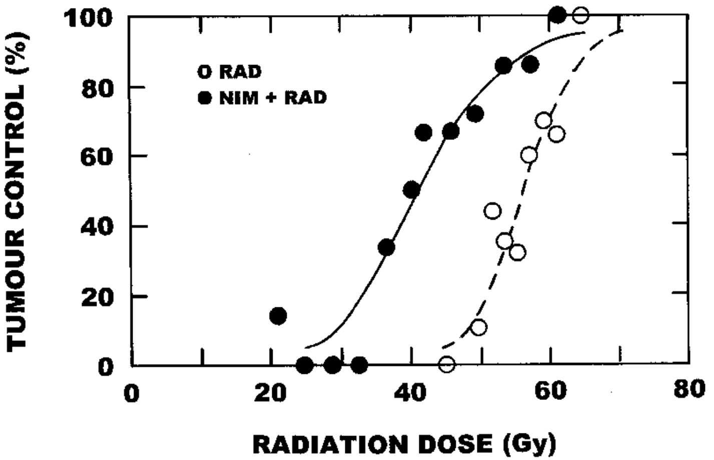


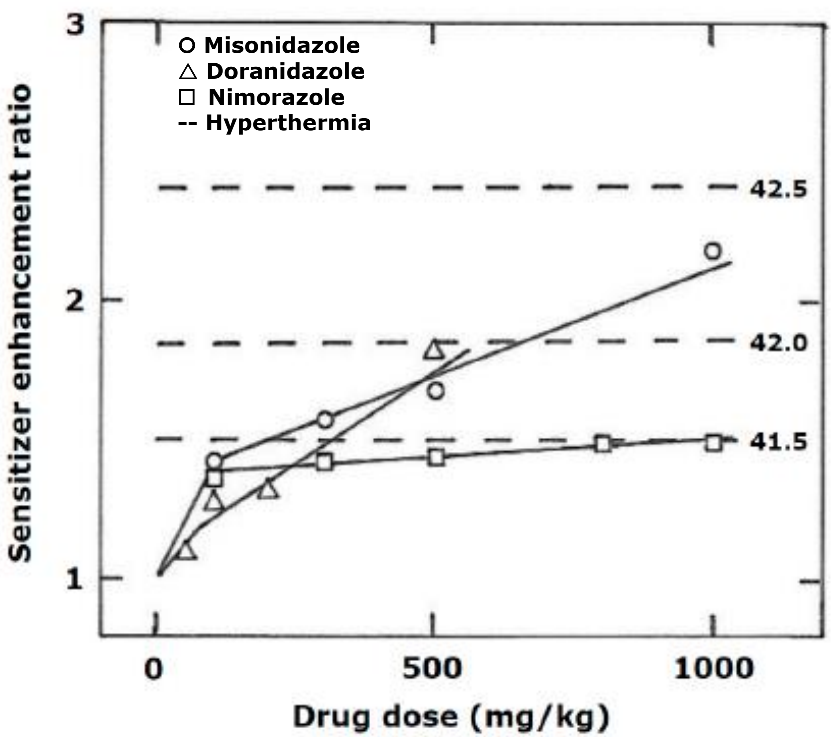




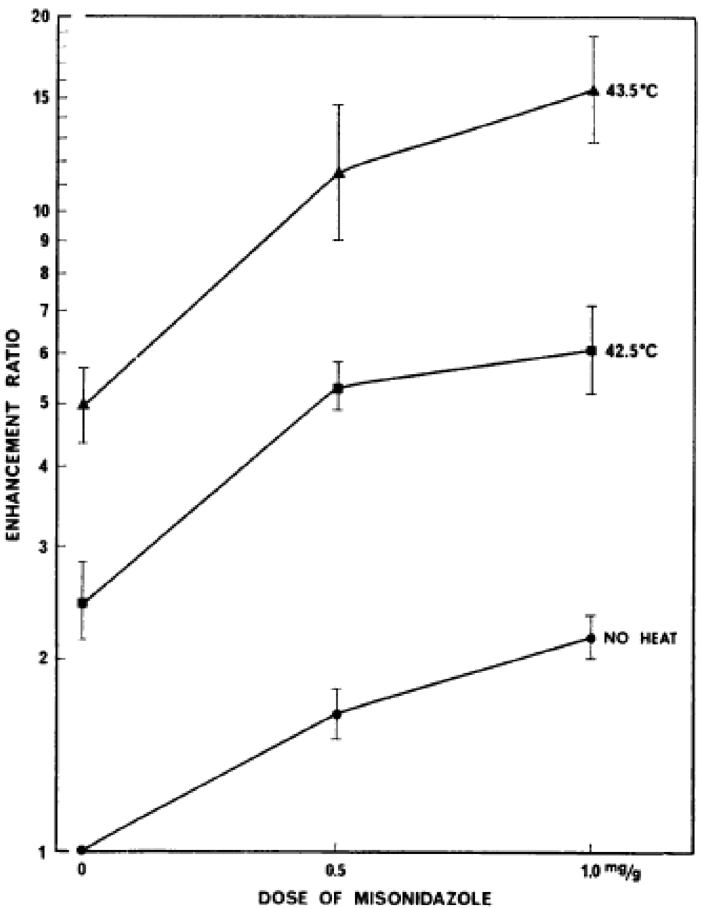
Horsman & Overgaard (2007) Clin. Oncol. 19:418-426

Radiosensitization by Hyperthermia



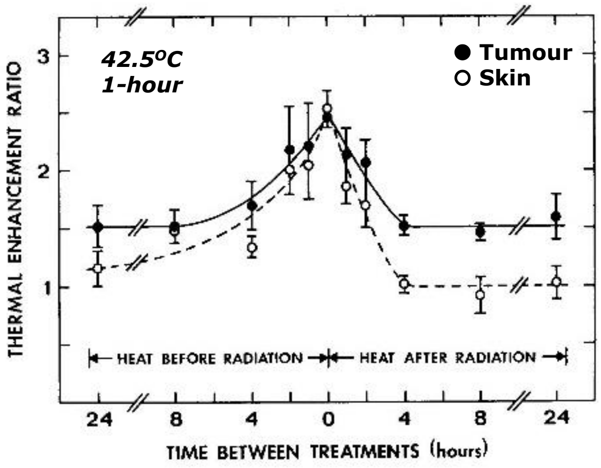


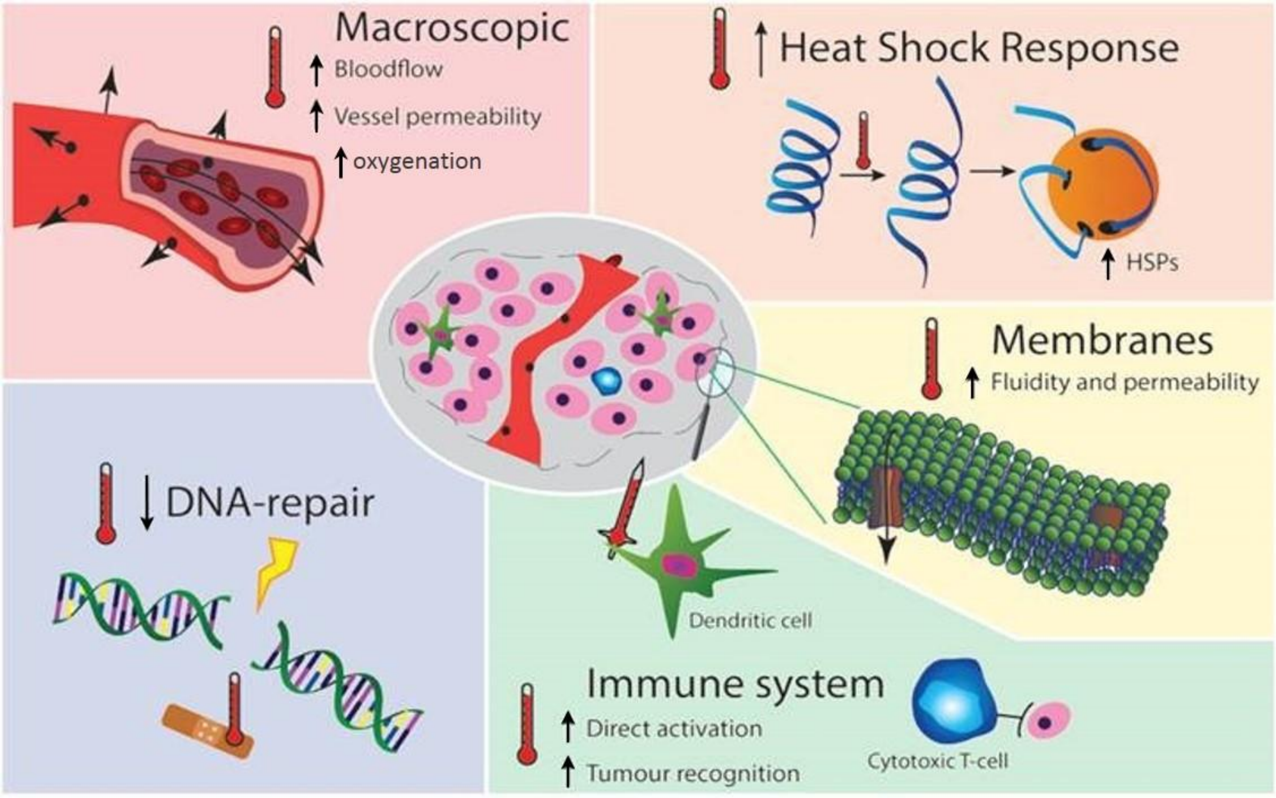
Elming et al. (2019) Cancers



Overgaard (1980) Br. J. Cancer 41:10-21

Mechanism for the interaction





Van den Tempel et al. (2016) Int. J. Hyperthermia 32:446-454

Hyperboost project

(Hyperthermia boosting the effect of Radiotherapy)

European Union Horizon-2020 Marie Sklodowska-Curie Actions Innovative Training Networks (H2020-MSCA-ITN-2020)

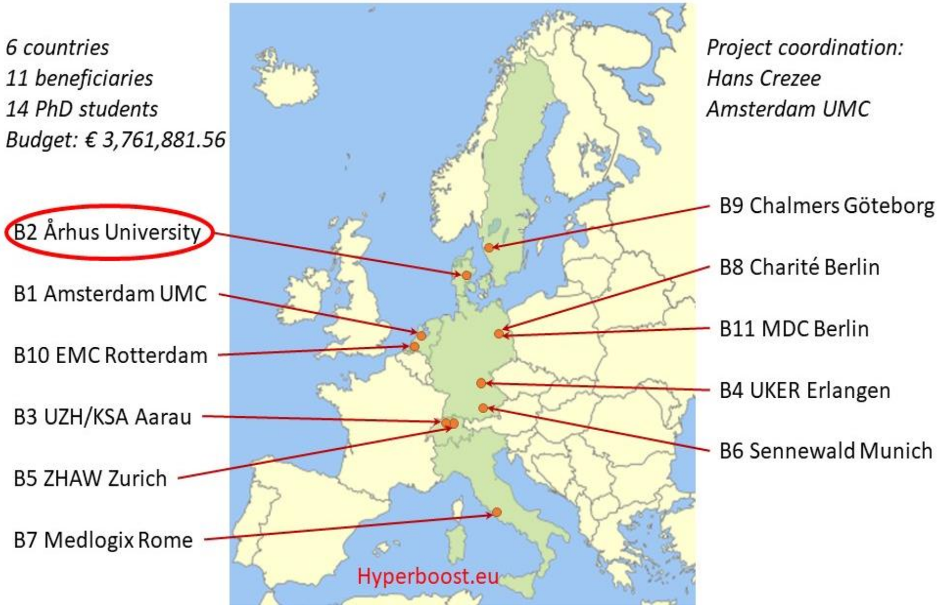
[Two PhD students]

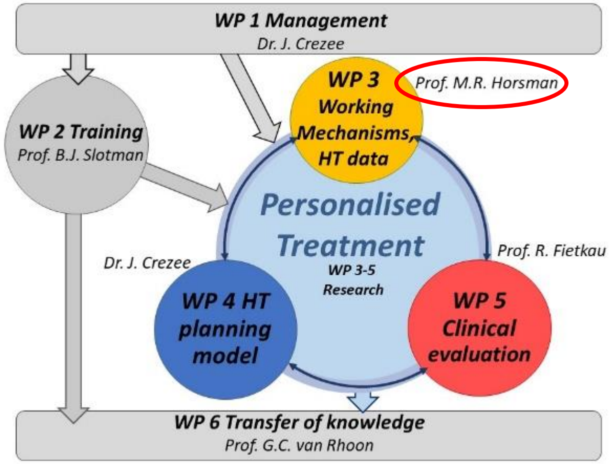
Funding from EU for Hyperboost

	Institute	Country	No.	Person months	Amount (€)
B1	AMC	Netherlands	2	72	531,240
B2	AU	Denmark	2	72	595,044
B3	KSA	Switzerland	1	36	281,277
B4	UKER	Germany	2	72	505,577
B5	ZHAW	Switzerland	1	36	281,277
B6	SMT	Germany	1	36	252,788
B7	ALBA	Italy	1	36	261,500
B8	CUB	Germany	1	36	252,788
B9	CUT	Sweden	1	36	281,983
B10	EMC	Netherlands	1	36	265,620
B11	MDC	Germany	1	36	252,788
Total			**14**	**504**	**3,761,882**

€ 595,044 = 4,433,078 kr.

Additional support: Danish Cancer Society

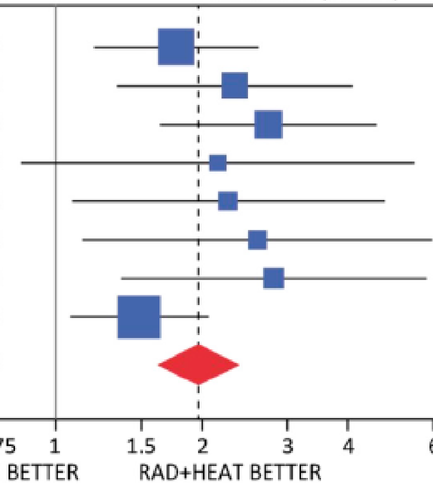




Meta-analysis of randomised clinical trials of radiation (RAD) ± hyperthermia (HEAT)

Tumor site	Trials	All pt.	RAD+HEAT Failure	RAD+HEAT All	RAD+HEAT Without failure	RAD ALONE Failure	RAD ALONE All	RAD ALONE Without failure	Endpoint: CR/loco-regional control Odds ratio (95% CI)
BREAST	6	419	86	229	62 %	98	190	48 %	1.77 (1.20 - 2.62)
HEAD & NECK	4	207	45	104	57 %	66	103	36 %	2.34 (1.34 - 4.09)
UTERINE CERVIX	4	249	41	125	67 %	71	124	43 %	2.74 (1.64 - 4.60)
LUNG	1	80	11	40	73 %	18	40	55 %	2.16 (0.85 - 5.48)
RECTUM	2	258	104	128	19 %	118	130	9 %	2.27 (1.08 - 4.76)
BLADDER	1	101	14	52	73 %	24	49	51 %	2.61 (1.14 - 5.98)
MELANOMA	1	128	28	63	56 %	45	65	31 %	2.81 (1.36 - 5.80)
MIXED	4	610	177	306	42 %	204	304	33 %	1.49 (1.07 - 2.07)
OVERALL	**23**	**2052**	**506**	**1047**	**52 %**	**644**	**1005**	**36 %**	**1.97 (1.63 - 2.37)**

I-squared = 0.0%, p = 0.48



Elming et al. (2019) Cancers

Questions?