Associazione Italiana Radioterapia Oncologica

Simposio AIRO-AIRB

Ipossia, neoangiogenesi e radioresistenza: dalla ricerca di base agli studi clinici

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Key facts concerning Hypoxia

1.Hypoxia is by far the most explored, and most widely cited [10], biological phenomena in radiotherapy,

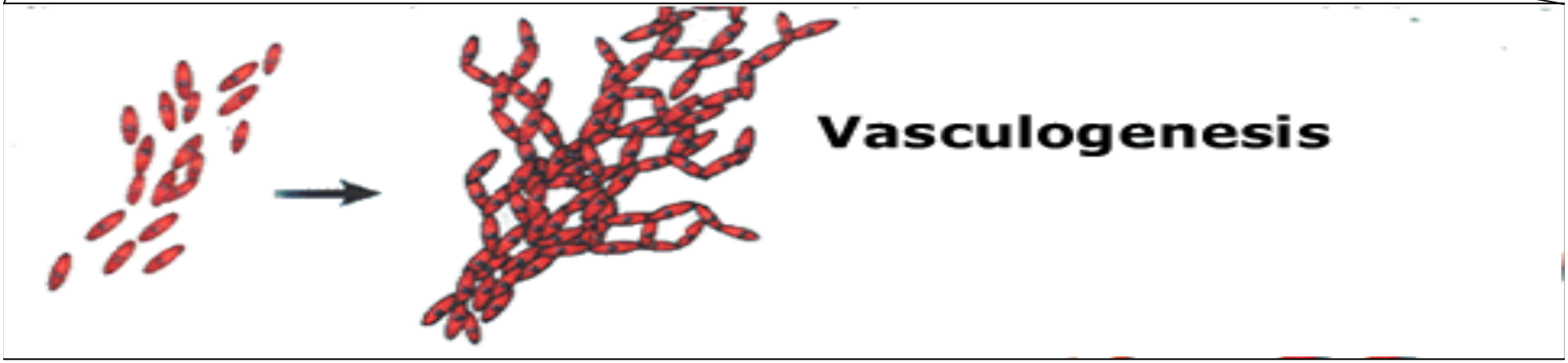
2.That hypoxia can cause clinical radioresistance has been known for more than a century, and since the pivotal work by Gray and colleagues have attempts to overcome it been explored in controlled clinical trials.

3. Literature strongly supports that there is a biological rationale and a valid treatment strategy, and when used it may result in improved loco-regional tumour control and consequently an improved survival probability.

4. However it has yet a limited impact on daily routine practice and it has been expressed: hypoxia is "adored and ignored".

Modern terminology of angiogenesis

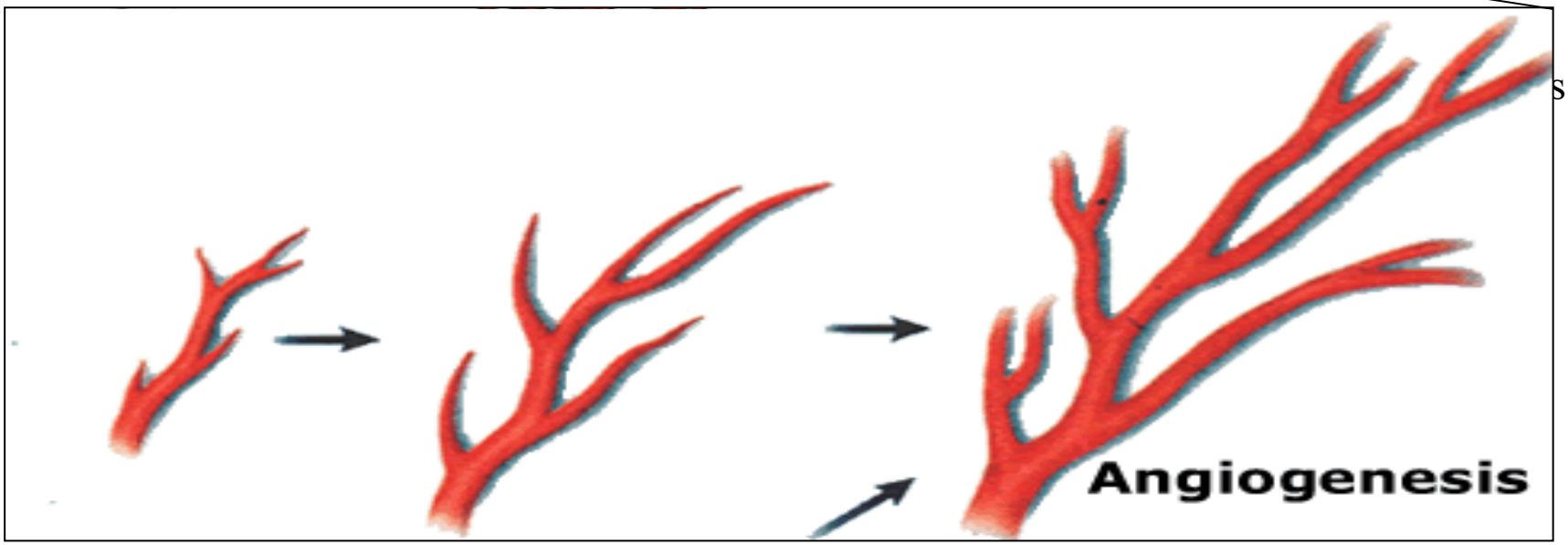
Vasculogenesis – Formation of vascular structures from circulating or tissue-resident endothelial stem cells(angioblasts), which proliferate into de novo endothelial cells. This form particularly relates to the embryonal development of the vascular system.



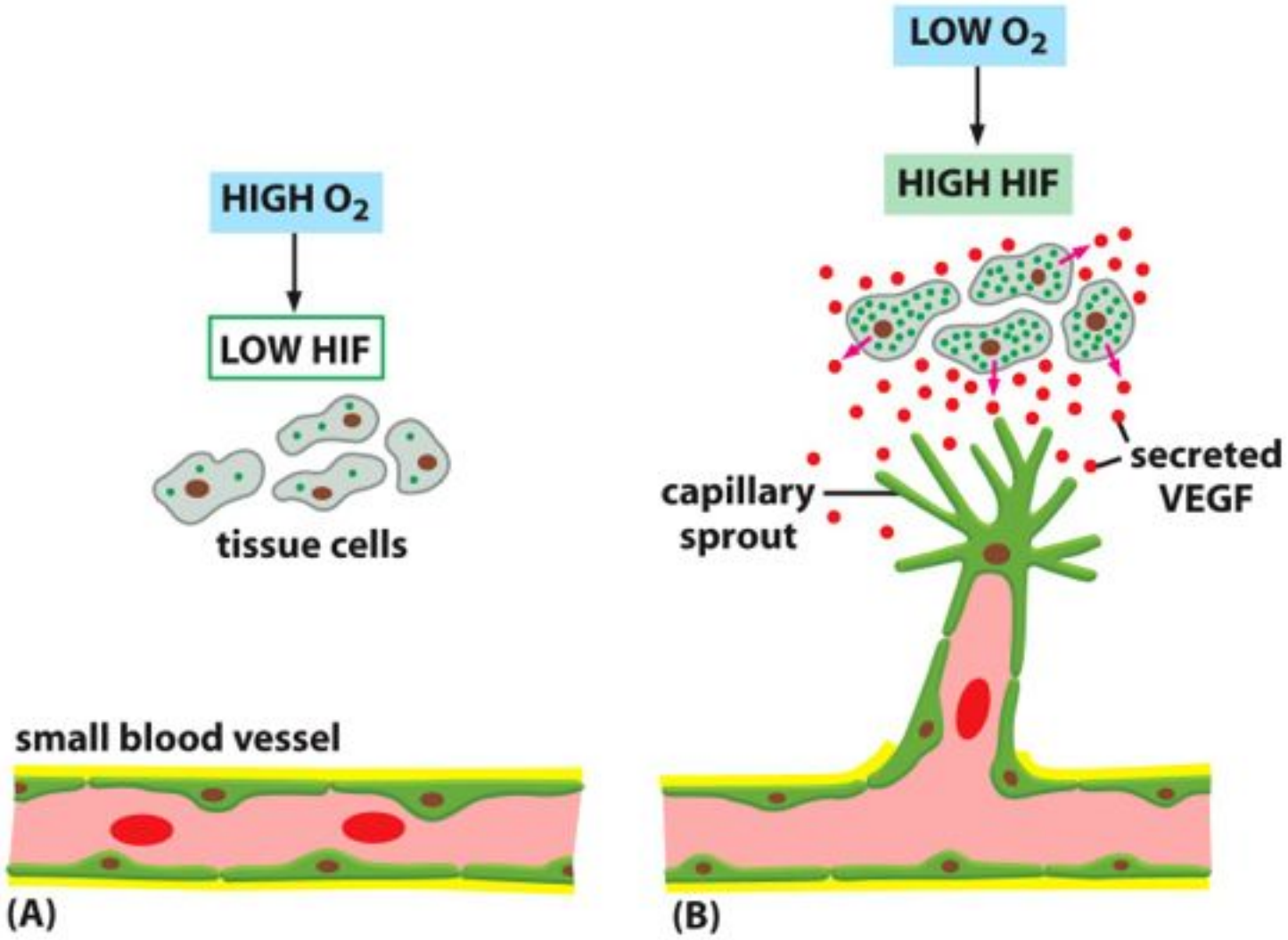
Modern terminology of angiogenesis

Vasculogenesis – Formation of vascular structures from circulating or tissue-resident endothelial stem cells(angioblasts), which proliferate into de novo endothelial cells. This form particularly relates to the embryonal development of the vascular system.

Angiogenesis – Formation of thin-walled endothelium-lined structures with /without muscular smooth muscle wall and pericytes (fibrocytes). This form plays an important role during the adult life span, also as "repair mechanism" of damaged tissues.



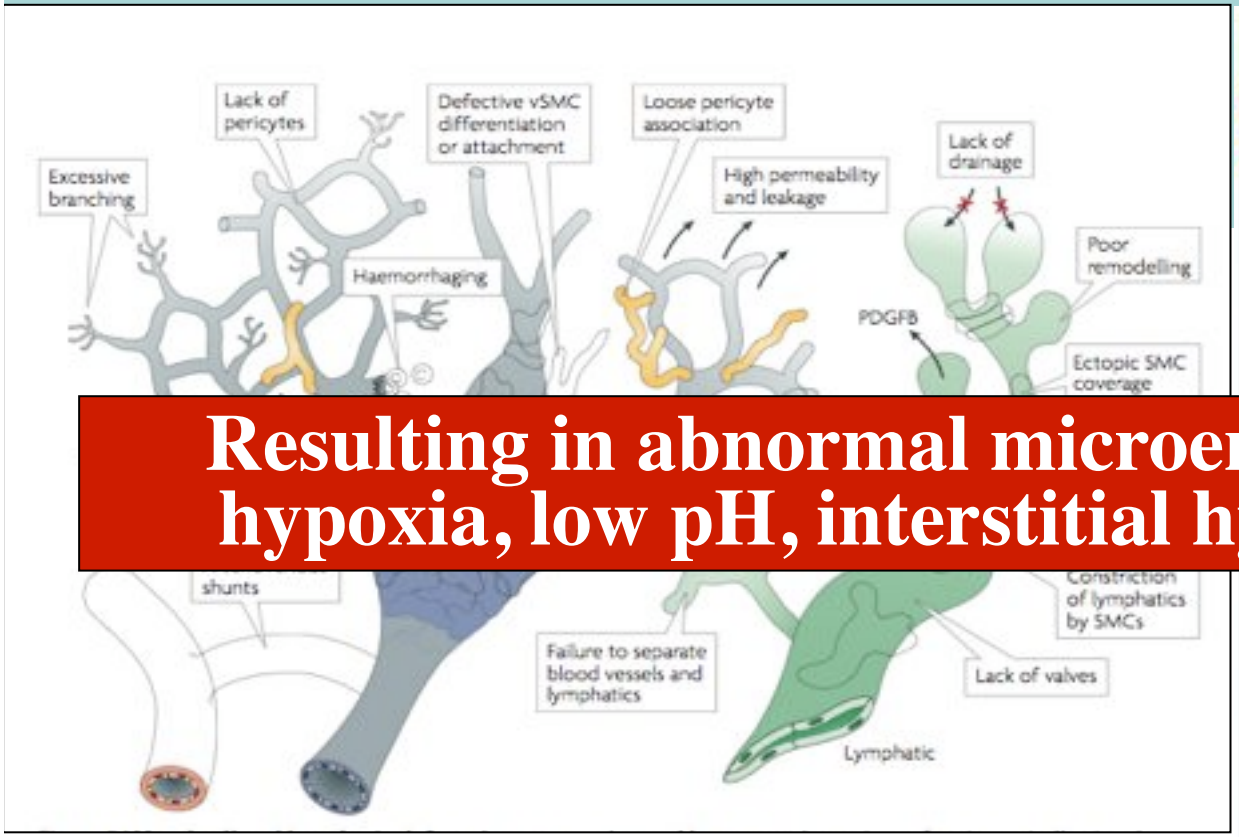
Sprouting towards chemotactic gradient: VEGF



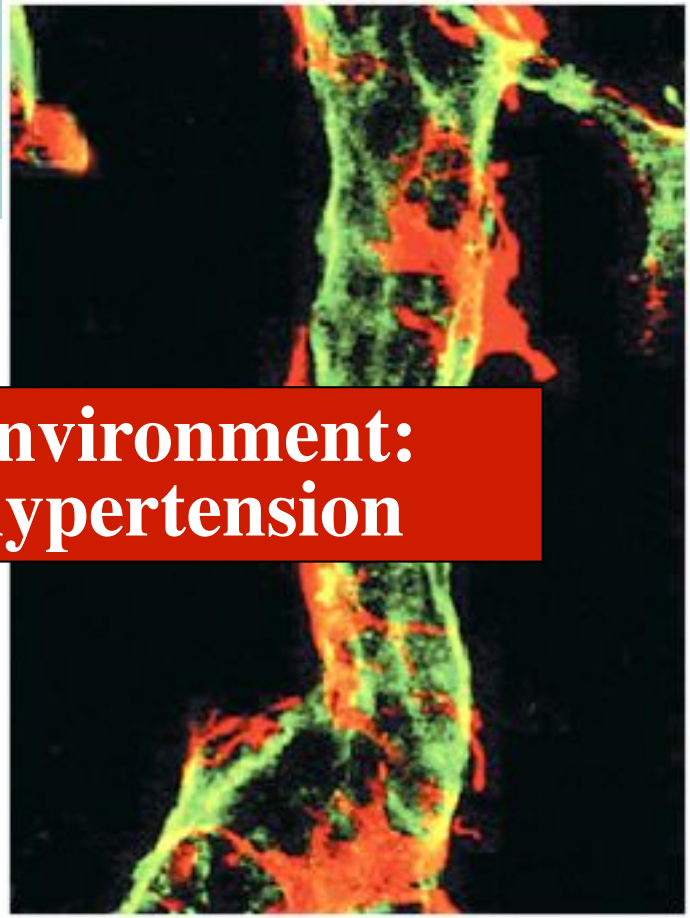
Neoplastic vessels are morphologically and functionally deficient

- Highly irregular and tortuous
- Dependent on cell survival factors (VEGF)
- Hyperpermeable
  - deficient pericyte coverage
  - absence of a basement membrane
  - deficient intercellular junctions
  - presence of cellular lacunae
  - vascular mimicry

Chaotic organization of tumor-associated vasculature



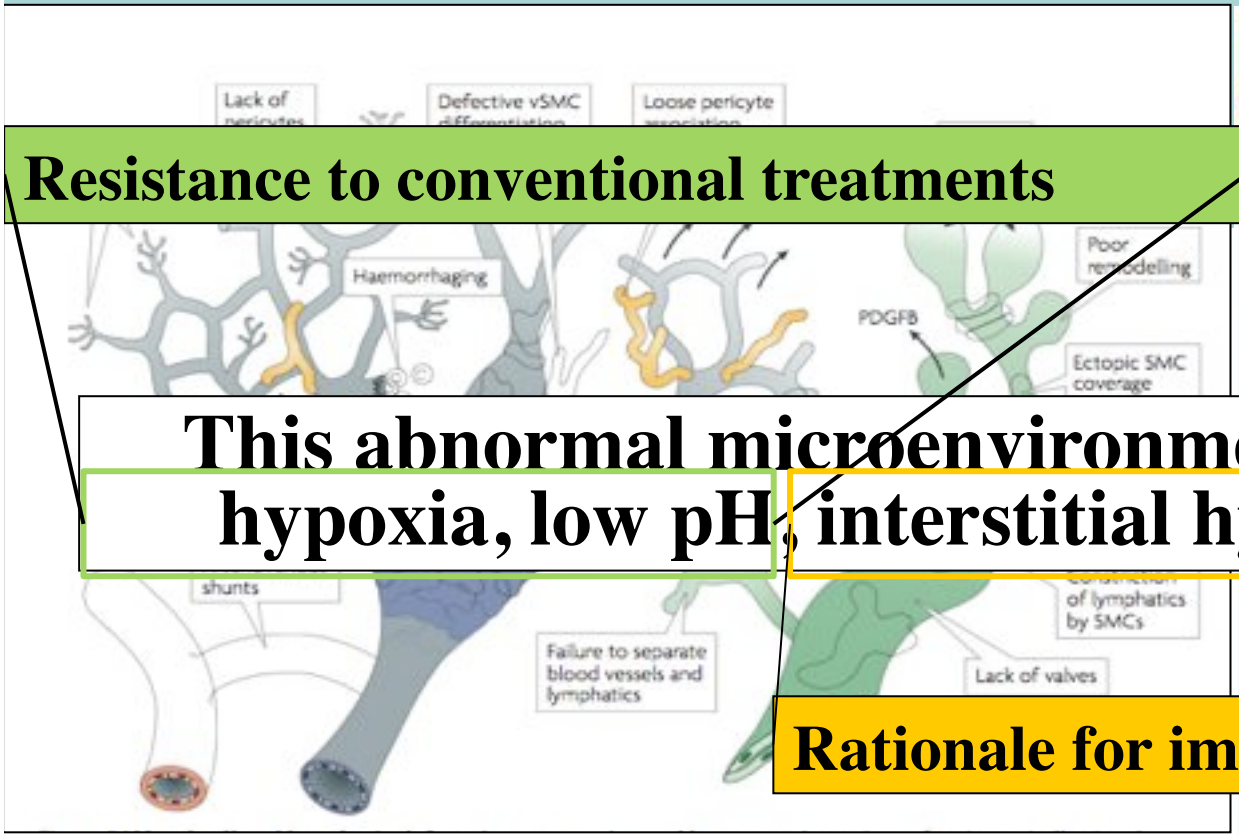
Resulting in abnormal microenvironment: hypoxia, low pH, interstitial hypertension



Examples of vascular defects

Tumor vessel is only partially overlaid by pericytes and SMC

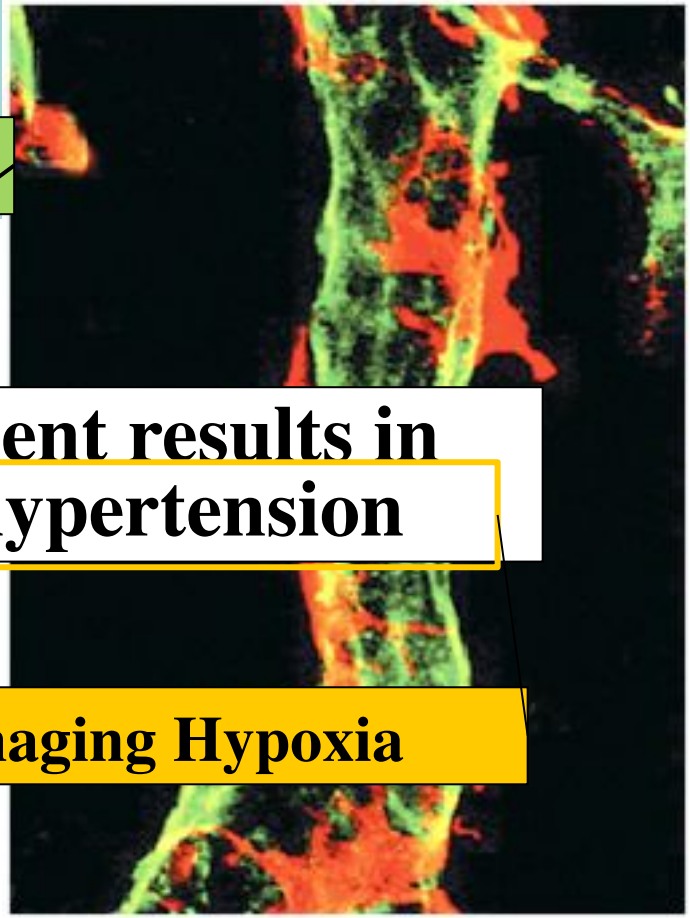
Chaotic organization of tumor-associated vasculature



Resistance to conventional treatments

This abnormal microenvironment results in hypoxia, low pH, interstitial hypertension

Rationale for imaging Hypoxia



Examples of vascular defects

Tumor vessel is only partially overlaid by pericytes and SMC

Tumor Hypoxia

- There are two types of hypoxia
  - Transient Hypoxia
    - Intermittent in nature
    - Can be quite severe
  - Permanent Hypoxia
    - Unrelieved hypoxia
    - Severe to the point of causing cell death

Tumor Hypoxia

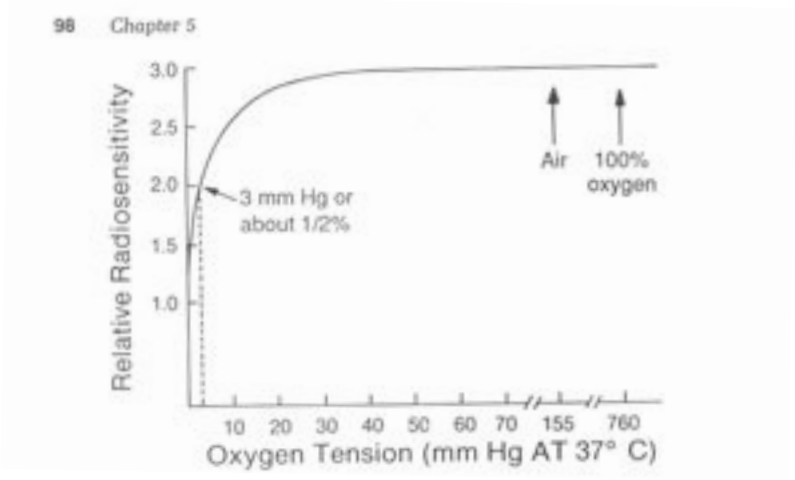
- Intermittent Hypoxia
  - Caused by vascular spasm
  - Spasm usually at the arteriole level
  - Due to lack or neurologic control of vessels
  - May be mediated by vasopressors secreted by the tumor
  - Increases radiation resistance
  - Increase resistance to some drugs

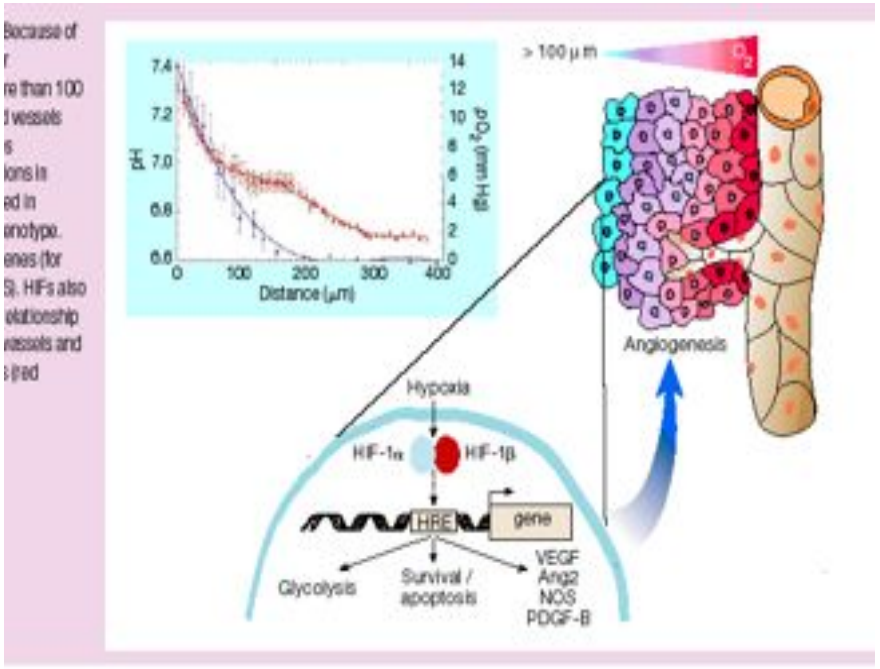
Tumor Hypoxia

- Permanent Hypoxia
  - Occurs when tumor growth outstrips vascular supply
  - Hypoxic cells are physically displaced from vessels.
  - Tumor pressure on surrounding tissues may further impede blood supply.
  - Increases radiation resistance
  - Increase resistance to some drugs

Tumor Hypoxia

- Permanent Hypoxia and radiation resistance
  - Must be relatively profound.
    - O_2 tension below 3mmHg
    - Present during main phase of repair





Oxygen diffusion distance varies with metabolism but beyond 100 microns hypoxia is probably profound.

Consequences of pH changes induced by hypoxia

To potentiate survival in hypoxic conditions, tumour cells adapt by increasing glycolysis causing external acidosis via secretion of lactic acid and protons to preserve intracellular pH.

Alkaline pHi :-

- **Inhibits activity of endonucleases, acid sphingomyelinase, and caspases.**
- **Inhibits apoptosis.**

Acidic pHe :-

- **Activates extracellular proteases (MMP-2 and 9) allowing degradation of ECM and basement membrane.**
- **Increases cell migration, invasion and metastasis.**

Clinical implications of hypoxia

Radio-genomics: Perfusion Surrogate Markers in MRI

ORIGINAL RESEARCH

R. Jain
L. Poisson
J. Narang
L. Scarpace
M.L. Rosenblum
S. Rempel
T. Mikkelsen

Correlation of Perfusion Parameters with Genes Related to Angiogenesis Regulation in Glioblastoma: A Feasibility Study

BACKGROUND AND PURPOSE: Integration of imaging and genomic data is critical for a better understanding of gliomas, particularly considering the increasing focus on the use of imaging biomarkers for patient survival and treatment response. The purpose of this study was to correlate CBV and PS measured by using PCT with the genes regulating angiogenesis in GBM.

MATERIALS AND METHODS: Eighteen patients with WHO grade IV gliomas underwent pretreatment PCT and measurement of CBV and PS values from enhancing tumor. Tumor specimens were analyzed by TCGA by using Human Gene Expression Microarrays and were interrogated for correlation between CBV and PS estimates across the genome. We used the GO biologic process pathways for angiogenesis regulation to select genes of interest.

RESULTS: We observed expression levels for 92 angiogenesis-associated genes (332 probes), 19 of which had significant correlation with PS and 9 of which had significant correlation with CBV ($P < .05$). Proangiogenic genes such as *TNFRSF1A* (PS = 0.53, $P = .024$), *HIF1A* (PS = 0.62, $P = .0065$), *KDR* (CBV = 0.60, $P = .0084$; PS = 0.59, $P = .0097$), *TIE1* (CBV = 0.54, $P = .022$; PS = 0.49, $P = .039$), and *TIE2/TEK* (CBV = 0.58, $P = .012$) showed a significant positive correlation; whereas antiangiogenic genes such as *VASH2* (PS = −0.72, $P = .00011$) showed a significant inverse correlation.

CONCLUSIONS: Our findings are provocative, with some of the proangiogenic genes showing a positive correlation and some of the antiangiogenic genes showing an inverse correlation with tumor perfusion parameters, suggesting a molecular basis for these imaging biomarkers; however, this should be confirmed in a larger patient population.

Jain, R. et al. Am J Neuroradiology Published March 15, 2012 as 10.3174/ajnr.A2956.

Pro-angiogenic Genes

- **KDR VEGFR-2**

  (CBV r=0.60, p=0.0084; PS 0.59, p=0.0097)

- **HIF 1a (Hypoxia inducible factor 1-alpha) (CBV 0.29, p=0.29; PS r=0.66, p=0.008)**

- **TNFRSF-1A (Tumor necrosis factor receptor superfamily, member 1A)**

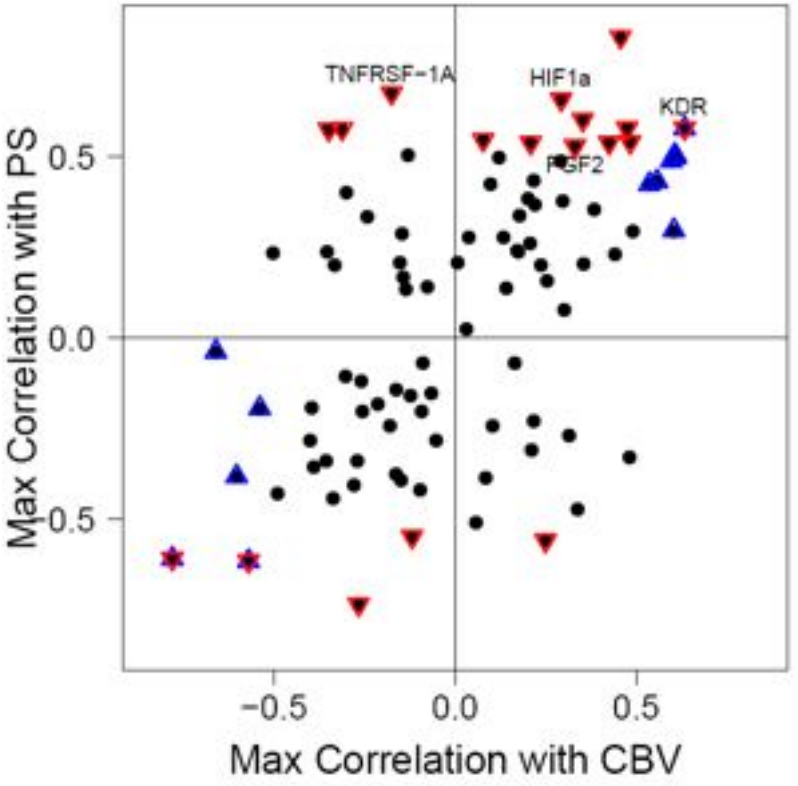
  (CBV 0.23, p=0.3673; PS r=0.53, p=0.0239)

- **TIE1**

  (CBV r=0.54, *P* = 0.0217; PS r=0.49, *P* = 0.0389)

- **TIE2/TEK**

  (CBV r=0.58, *P* = 0.0119; PS 0.46, *P* = 0.0550)



Significant Correlation with CBV

Significant Correlation with PS

Significant correlation with both CBV and PS

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Anti-angiogenic Genes

- **VASH 2 Vasohibin 2**

  (CBV correlation co-efficient -0.35, *P* = 0.1568, PS r= -0.71, *P* = 0.0011)

- **CX3CR1**

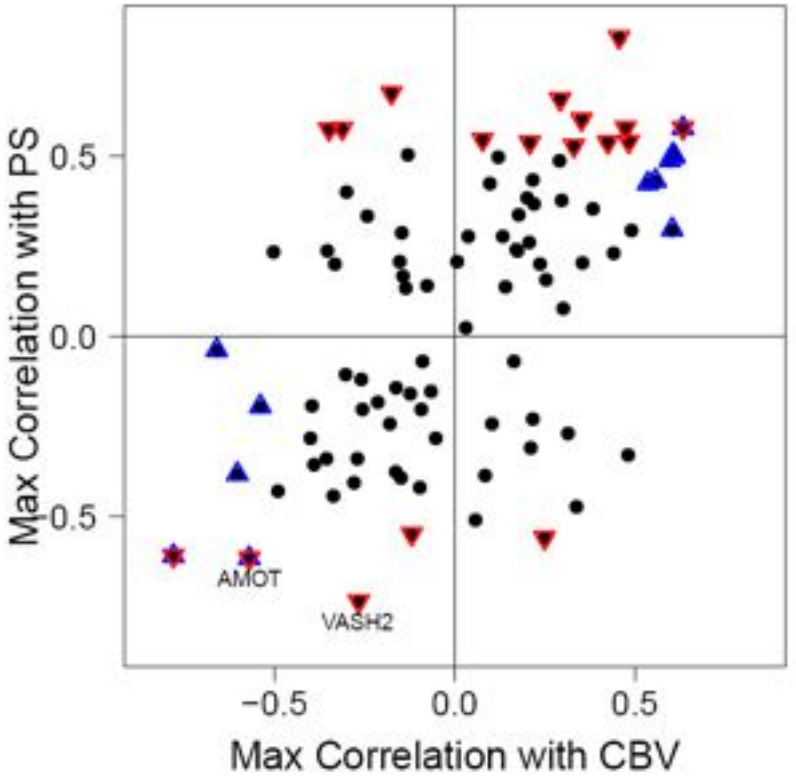
  (CBV r= -0.66, *P* = 0.0028; PS -0.49, *P* = 0.0375)

- **WNT5A**

  (CBV r= -0.10, *P* = 0.6833; PS -0.52, *P* = 0.0284)

- **C3**

  (CBV r=-0.63, *P* = 0.0051; PS -0.41, *P* = 0.0953)



Jain, R. et al. Am J Neuroradiology Published March 15, 2012 as 10.3174/ajnr.A2956.

Correlation of Perfusion Parameters with Genes Related to Angiogenesis Regulation in GBM

- **CBV and PS estimates in GBMs can correlate positively with pro-angiogenic genes**
- **and inversely with anti-angiogenic genes.**
- **The results of this preliminary analysis can help establish a genomic/molecular basis for these commonly used imaging biomarkers and potentially add to our knowledge of their immuno-histological bases.**

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Angiogenic targets for therapeutic intervention

Radiation-Induced Vascular damage

Early endothelial effects

- Apoptosis
- Activation: increased expression of cell adhesion molecules and cytokine secretion
- Recruitment of inflammatory cells
- Pro-coagulant and pro-thrombotic phenotype
- Increased permeability
- ROS production

Late endothelial effects

- Microvessel collapse: rupture and dilatation of capillaries
- Thickening of the basal membrane Thrombosis
- Chronic pro-inflammatory phenotype
- Chronic production of ROS
- Senescence

Effects of irradiated endothelium on surrounding normal tissues

- Ischemia
- Necrosis
- Tissue fibrosis

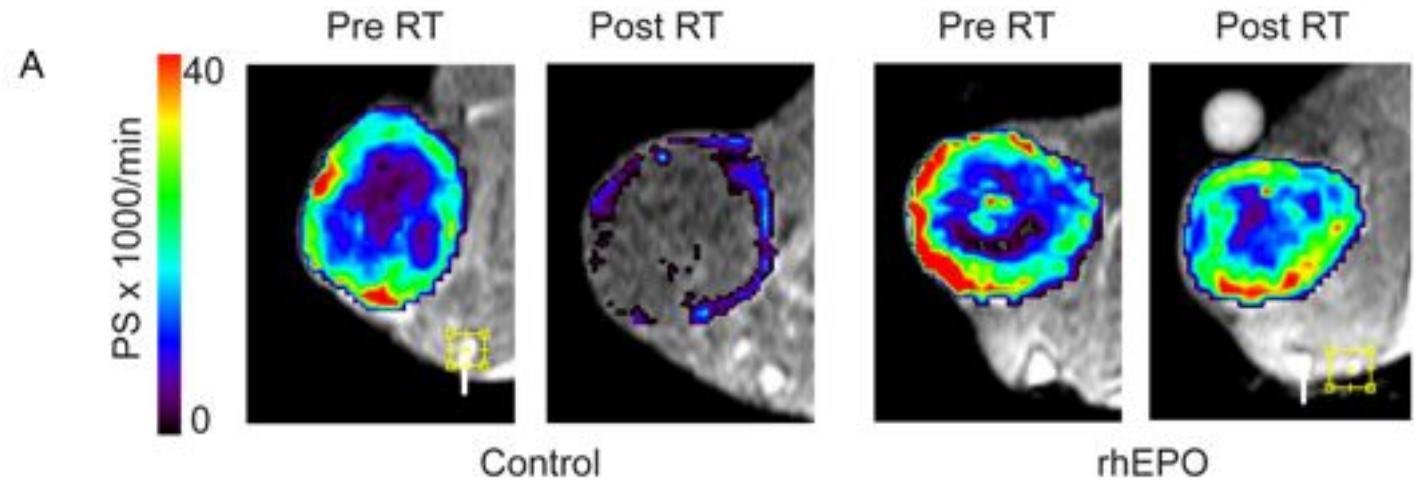
Figure Legend:

Proposed Mechanism of Involvement of NF-ϰB in Radiation-Induced Vascular DiseaseNF-ϰB = nuclear factor-kappa B.

Isabelle Corre et al *Int. J. Mol. Sci. 2013, 14, 22678-22696*

Effects of RT on neovascular permeability measured with DCE-MRI

rhEPO prevents radiotherapy-induced reduction in neovascular permeability

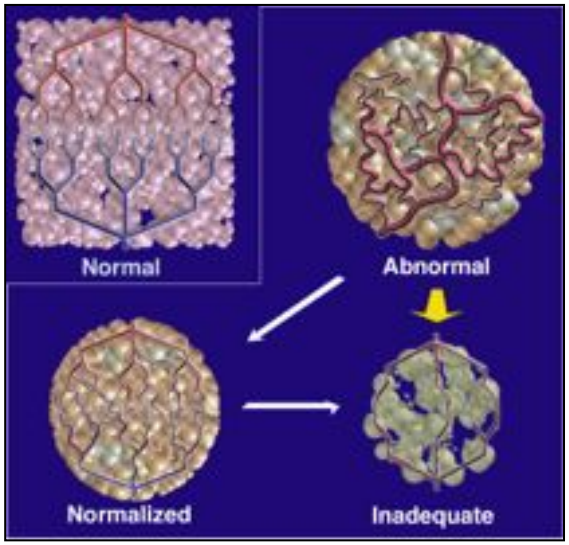


Ceelen et al. Br J Cancer 2007

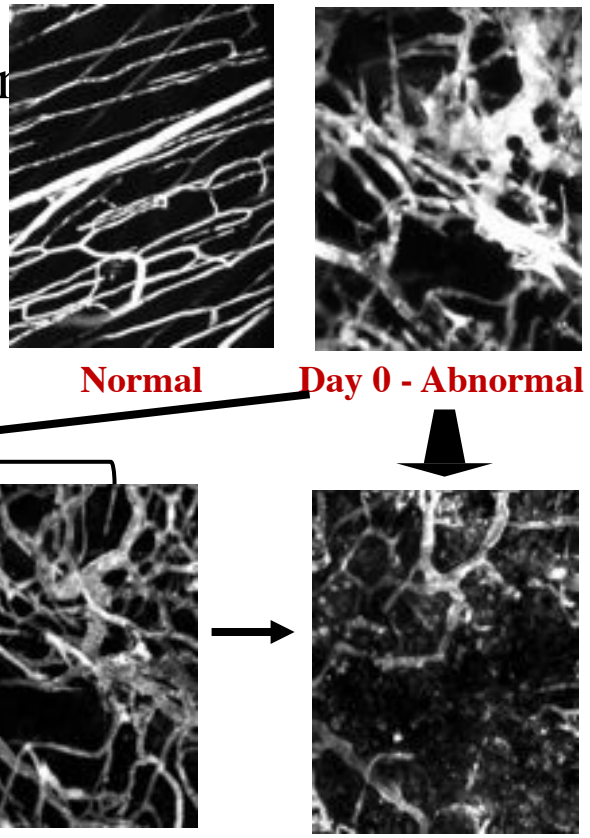
The concept of vessel **normalisation** by anti-angiogenic therapy

- Return to 'normal' phenotype by vascular *pruning*
- Results in more efficient drug delivery by lowering IFP and restoring microvessel function → paradoxical **synergism** of anti-angiogenesis agents and cytostatic drugs
- Results in more efficient RT by enhanced oxyger

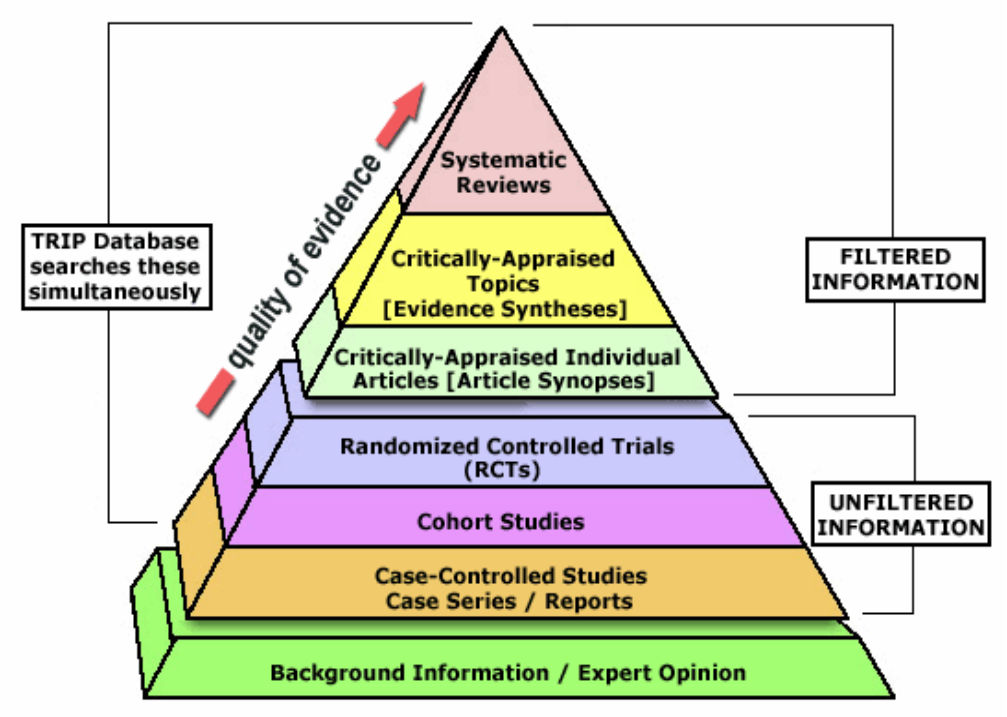
Normalization Hypothesis



Tong et al. (2003)
Jain, Nature Medicine (2001)



Hypoxia Modifiers, quality of evidence and strength of recommendations



Qualità degli studi primari e revisioni sistematiche: rating del livello delle evidenze

Ia	Metaanalisi o review sistematiche basate su più studi di livelli **Ib**
Ib	Trial diagnostici or studi di esito di buona qualità
II	Trial diagnostici or studi di esito di media qualità, numero insufficiente di pazienti, o altri trials (case-control, altri designi)
III	Studi descrittivi, case report ed altri studi
IV	Indicazioni di comitati, opinioni di esperti, e così di seguito (reviews non sistematiche etc.)

Rating della forza delle evidenze a supporto delle raccomandazioni nelle linee guida

A	Supportati da almeno due studi di livello Ib o da una review di livelli Ia ("E' stato dimostrato")
B	Supportate da almeno due studi indipendenti di livello II o estrapolazioni da studi di livelli I ("E' plausibile")
C	Non supportati da adeguati studi di livello I o II ("indicazioni")
D	Indicazioni di esperti ("non ci sono prove")

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Meta-analysis of hypoxia in HNSCC

Hypoxic modification of radiotherapy in squamous cell carcinoma of the head and neck – A systematic review and meta-analysis

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ABSTRACT

Background: The importance of tumour hypoxia for the outcome of radiotherapy has been under investigation for decades. Numerous clinical trials modifying the hypoxic radioresistance in squamous cell carcinoma of the head and neck (HNSCC) have been conducted, but most have been inconclusive, partly due to a small number of patients in the individual trial. The present meta-analysis was, therefore, performed utilising the results from all clinical trials addressing the specific question of hypoxic modification in HNSCC undergoing curative intended primary radiotherapy alone. *Methods:* A systematic review of published and unpublished data identified 4805 patients with HNSCC treated in 32 randomized clinical trials, applying, normobaric oxygen or carbogen breathing (5 trials); hyperbaric oxygen (HBO) (9 trials); hypoxic radiosensitizers (17 trials) and HBO and radiosensitizer (1 trial). The trials were analysed with regard to the following endpoints: loco-regional control (32 trials), disease specific survival (30 trials), overall survival (29 trials), distant metastases (12 trials) and complications to radiotherapy (23 trials). *Results:* Overall hypoxic modification of radiotherapy in head and neck cancer did result in a significant improved therapeutic benefit. This was most dominantly observed when using the direct endpoint of loco-regional

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Inclusion criteria

1. Radiation treatment with curative intended
2. Radiotherapy alone with randomization to a hypoxic modifier which should be known only to influence hypoxic radioresistance
3. No other cytotoxic effect.

Exclusion criteria

1. Chemoradiotherapy
2. Chemotherapy treatment with hypoxic activity (e.g. mitomycin C)
3. Patients with metastatic disease included since the analysis was focused on the effect of curatively intended radiotherapy.

Hypoxic modifiers used

The hypoxic modification in the trials were :
1. Oxygen breathing under normobaric or hyperbaric pressure
2. Nitroimidazoles.

The few studies with haemoglobin modification by either transfusion or the use of EPO are not included because there have been some uncertainty about their interpretation, and especially the EPO-related studies are not available in sufficient detail, but are currently under intense scrutinisation.

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Table 1
Randomized clinical trials with hypoxic modification of radiotherapy in HNSCC.

References	Trial acronym	Year	No. pts	fx[a]	RT schedule	Hypoxic modification	Endpoint[b]					Obs. time
[21]	van den Brenk	1968	30	HH	7.75 Gy x4vs7.25 Gy x4 with HBO	HBO 4 atm	L	D	S			2 + years
[22]	Evans 1	1970	40	LL	60 Gy/30 fx	Normobaric 02	L	D	S			2 + years
[23]	Tobin	1971	17	LL	60 Gy/30 fx	HBO 3 atm	L	D	S			2–3 years
[24]	Chang	1973	51	HHL	6 Gy x6+ HBO vs 6 Gy x7 or 60 Gy/30 fx	HBO 3 atm	L	D	S	M	C	5 years
[25]	Shigamats u	1973	31	HH	60–79 Gy/10 fx vs. 40–50 Gy/8–10 fx + HBO	HBO	L	D	S			2 + years
[26]	Evans 2	1975	44	LL	60 Gy/30 fx	Normobaric 02	L	D	S	M	C	2 + years
[27]	MRC 1 trial	1977	276	HH	35–45 Gy x10	HBO 3 atm	L	D	S	M	c	4 + years
[26]	MRC 3, trial	1979	24	HL	45–50/15 el 48.5–55/20 air vs. 40–45/10 HBO	HBO	L	D	S		c	5 years
[29]	RTOG 70-02	1979	254	LL	60–70 Gy/30 fx	Carbogen	L	D	S	M	c	2 + years
[30]	Sause	1979	44	HL	48 Gy/12 fx + HBO vs. 62 Gy/25 fx	HBO 3 aim	L	D	S		c	2 + years
[31]	Giaux	1962	56	II	50 Gy/16 fx	MISO	L	D	S			34 months
[32]	Sealy 1	1962	97	HH	36 Gy/6 fx/17 days	MISO	L					>1 year
[33]	B run in	1963	101	LL	72 Gy/36 fx	MISO	L	D	S			2 years
[34]	MRC 10 fx	1964	162	HH	40–45 Gy/10 fx	MISO	L	D	S		c	3 + years
[34]	MRC 20 fx	1964	89	LL	50–57 Gy/20 fx	MISO	L	D	S			3 + years
[35]	Panis	1964	52	MM	Split-course 1.1 Gy x6 daily/ 5 days – 4 weeks split-repeat	MISO	L	D	S		c	2 + years
[36,37]	EORTC 22S111	1966	330	MM	1.6 Gy x3/10 days – 3 weeks split + same to total of 67–72 Gy	MISO	L	D	S		c	5 + years
[38,39]	MRC 2, trial	1966	103	HL	64 Gy/30 fx vs. 41–44 Gy/10 fx + HBO	HBO 3 aim	L	D	S	M	c	4 + years
[40]	Sealy 2	1966	124	HL	63 Gy/30 fx (air); 36 Gy/6 fx (HBO)	HBO/MISO	L	D	S	M	c	1–2-year
[41,42]	IAEA study	1967	36	LL	70 Gy/35 fx	On ids zo e	L	D	S		c	2 + years
[43,44]	RTOG 79-15	1967	297	LL	66–74/33–37 fx	MISO	L	D	S	M	c	2 + years
[45]	Galecki	1969	35	LL	70 Gy/35 fx vs. 66 Gy/30 fx vs. 80.5 Gyx 70 fx	Metronidazole	L	D	S		c	3 + years
[46]	Dahanca 2	1969	622	LL	68–72/34–36 fx eller 61/22/9.5 weeks	MISO	L	D	S	M	c	5 + years
[47]	RTOG 79-04	1969	40	HH	4 Gy 11–13 fx	MISO	L	D	S		c	2 + years
[48]	RTOG 8S-27	1995	504	LL	66–74 Gy/33–37 fx	Etanidazole	L	D	S	M	c	5 + years
[49]	Huilgol	1996	18	LL	54 Gy/45 fx/22 days	AK-2123	L	D	S			2 + years
[50]	European trial	1997	374	LL	66–74 Gy/33–37 fx	Etanidazole	L	D	S		c	5 + years
[51,52]	Dahanca 5	1998	414	LL	66–68/33–34	Nirnorazole	L	D	S	M		5 years
[53]	Haffty	1999	48	HH	12.65 Gy x2 vs. 11.50 Gy x2 + HBO	HB04 atm	L	D		M	c	5 + years
[54]	Mendenhall	2005	101	MM	76 Gy/1.2 Gy fx BID	02 Carbogen	L	D	s	M		5 + years
[55]	Ullal	2006	46	LL	60 Gy/30 fx	AK-2123	L					3 + months
[56]	ARCON	2010	345	LL	64–68 Gy/32–34 fx accelerated fx	Nicotinamide	L	D	s			2 years

[a] H: Hypofract; L: conventional tract; M: hyperfract (multiple fx/day).
[b] L: Loco-regional failure; D: disease specific death; S: overall death; M: distant metastasis; C: complications.

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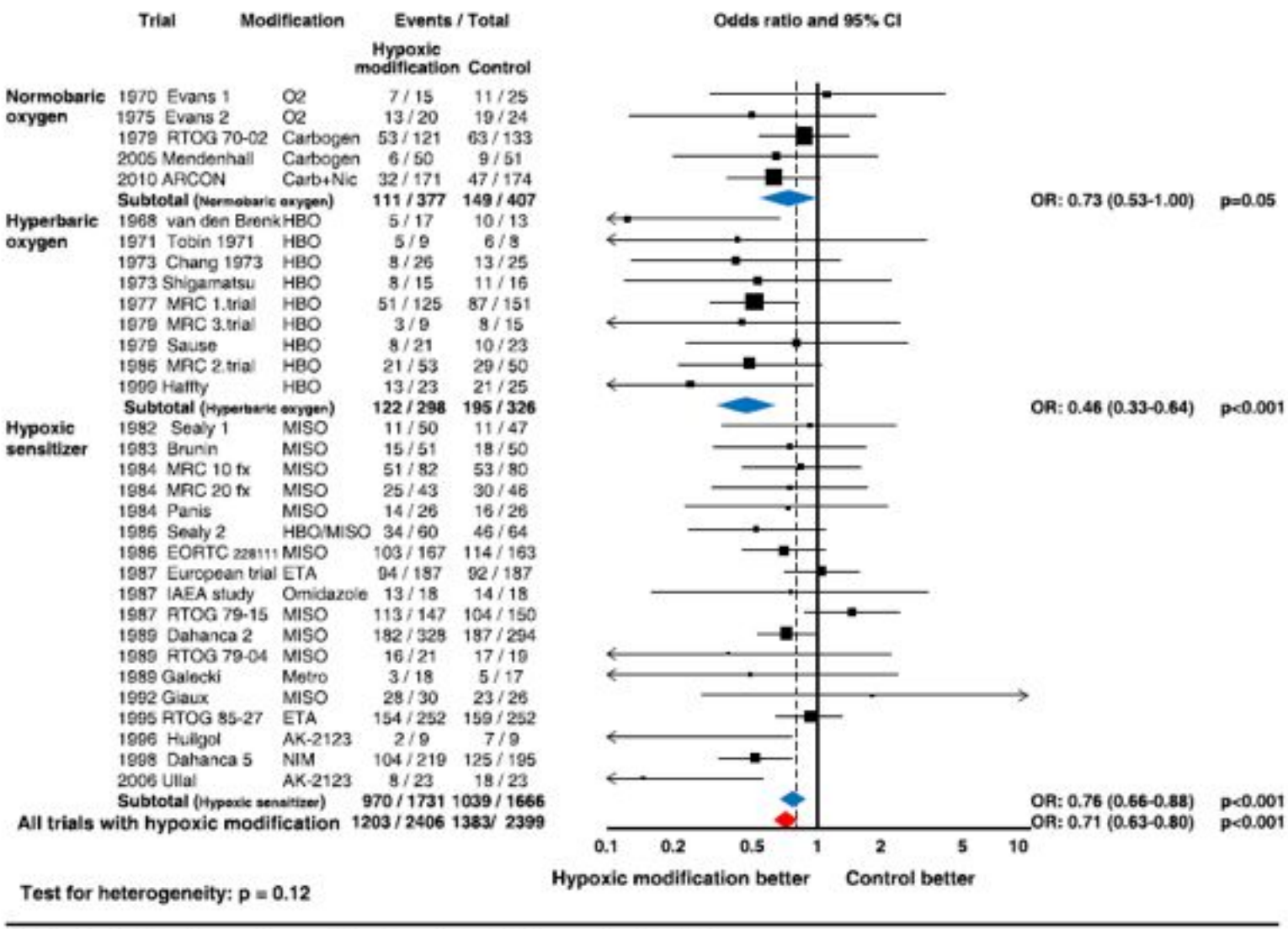
Endpoint: Loco-regional failure



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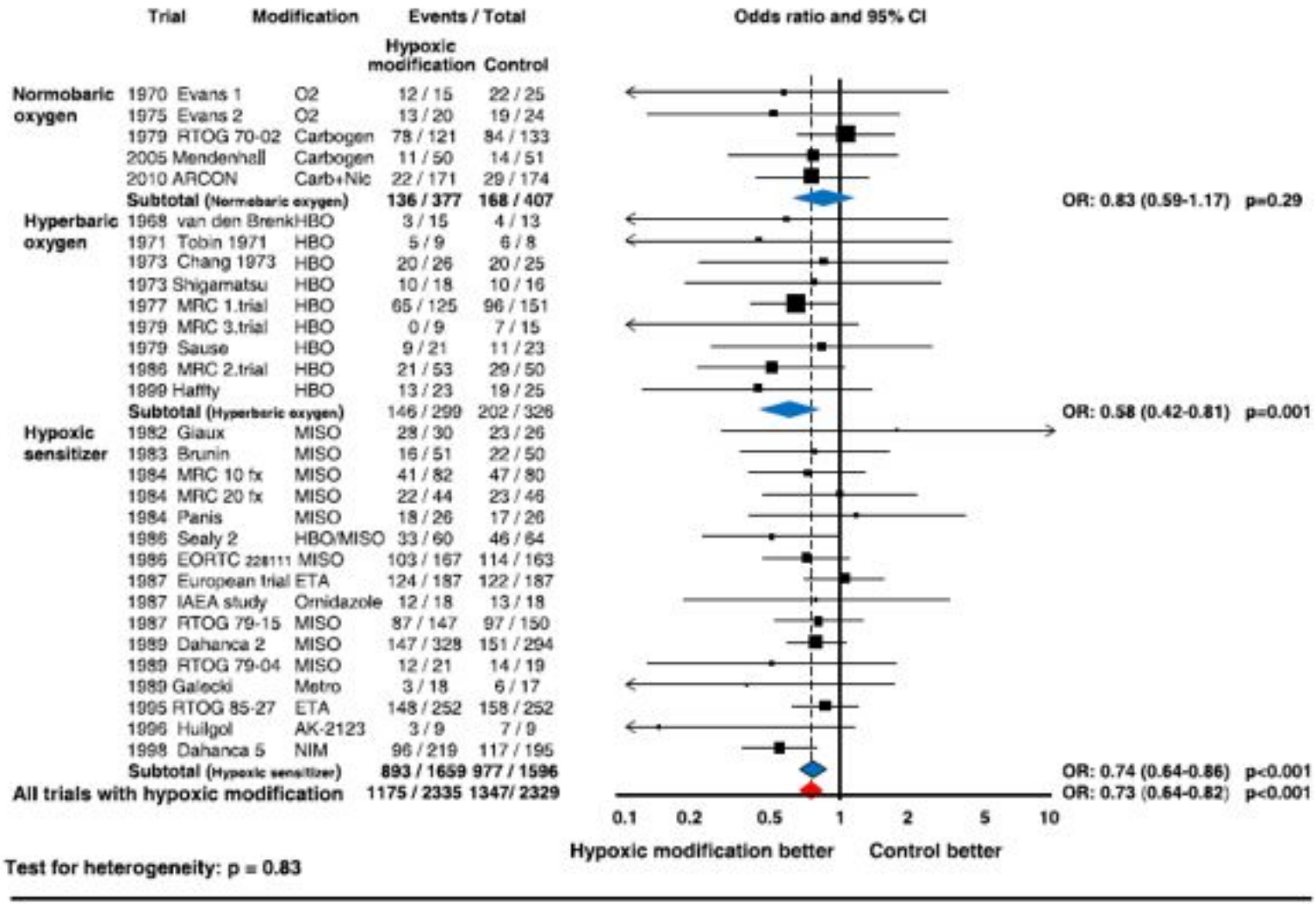
Endpoint: Disease specific death



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Endpoint: Overall death

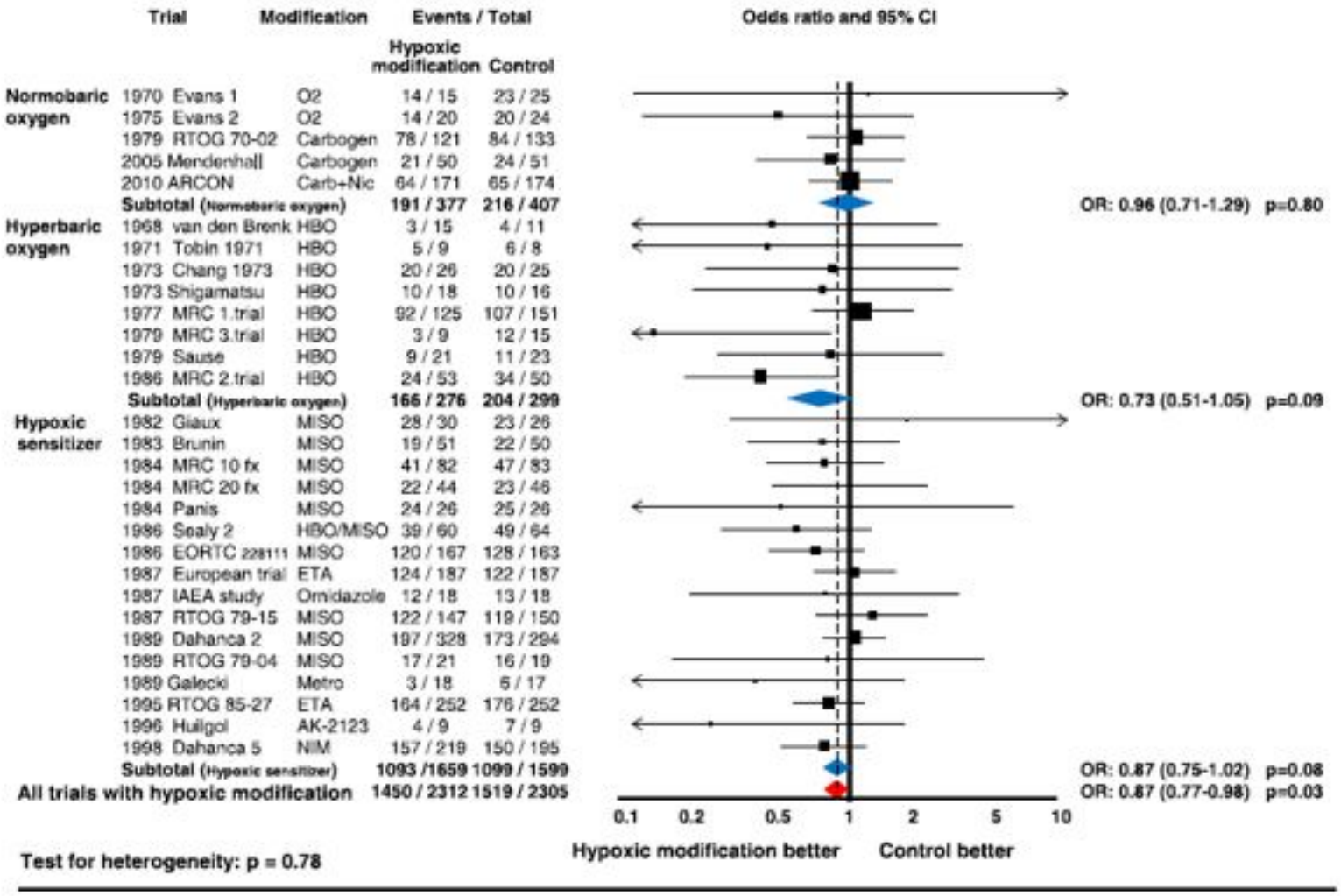

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Table 2
Effect of hypoxic modification of radiotherapy of HNSCC given with different dose per fraction schedules.

Fractionation pattern	Endpoint and Odds Ratio (95% CI)		
	Loco-regional failure	Disease specific death	Late radiation related morbidity
Hypo-fractionation[a]	0.56 (0.40–0.77) $p > 0.001$	0.62 (0.44–0.86) $p > 0.001$	1.83 (1.05–3.18) $p > 0.03$
Conventional fractionation[a]	0.77 (0.67–0.89) $p > 0.001$	0.78 (0.67–0.90 $p > 0.001$	0.90 (0.71–1.14) $p > 0.39$

[a] The same fractionation pattern has been applied in hypoxic modification and control arms.

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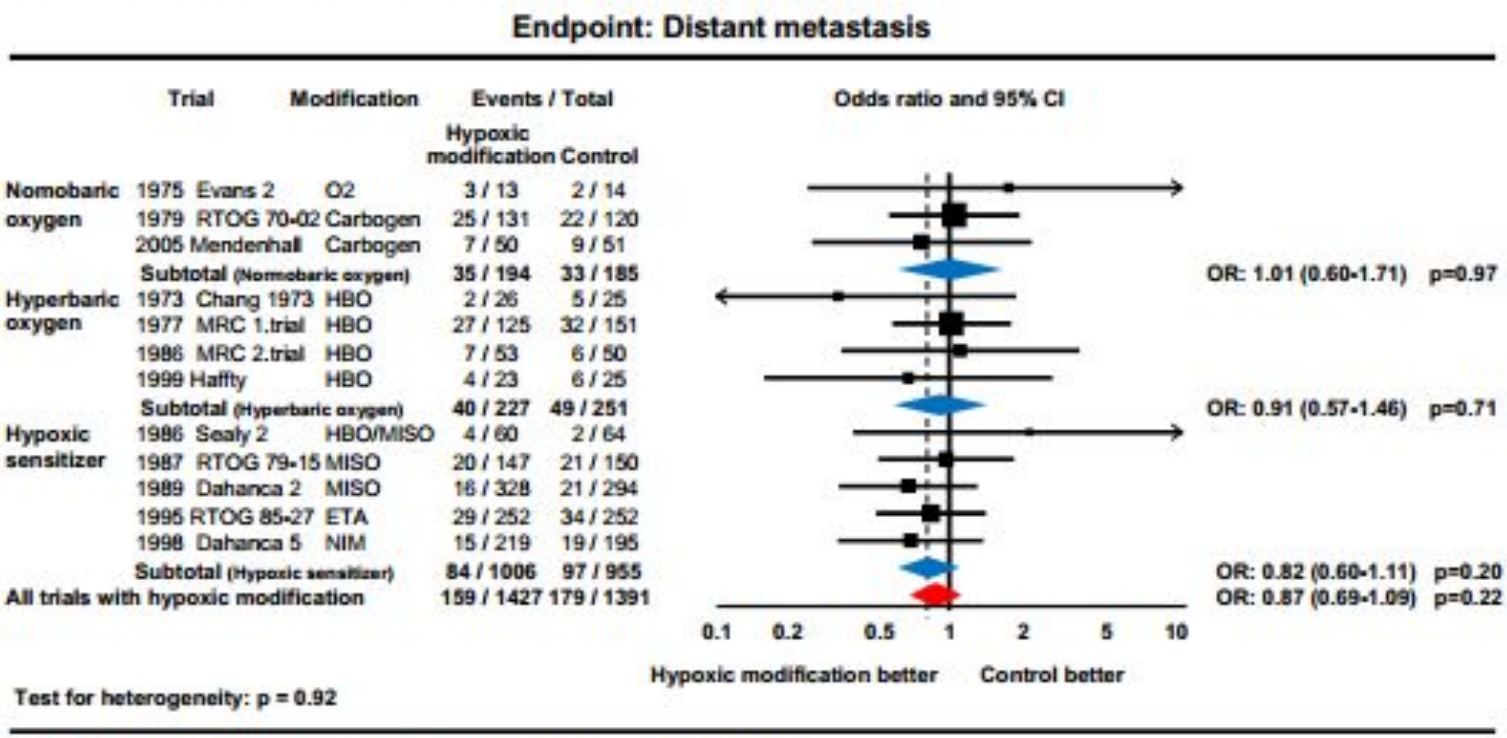

Meta Analysis - Hypoxic modification of radiotherapy in HNSCC

Meta-analysis of hypoxia in HNSCC

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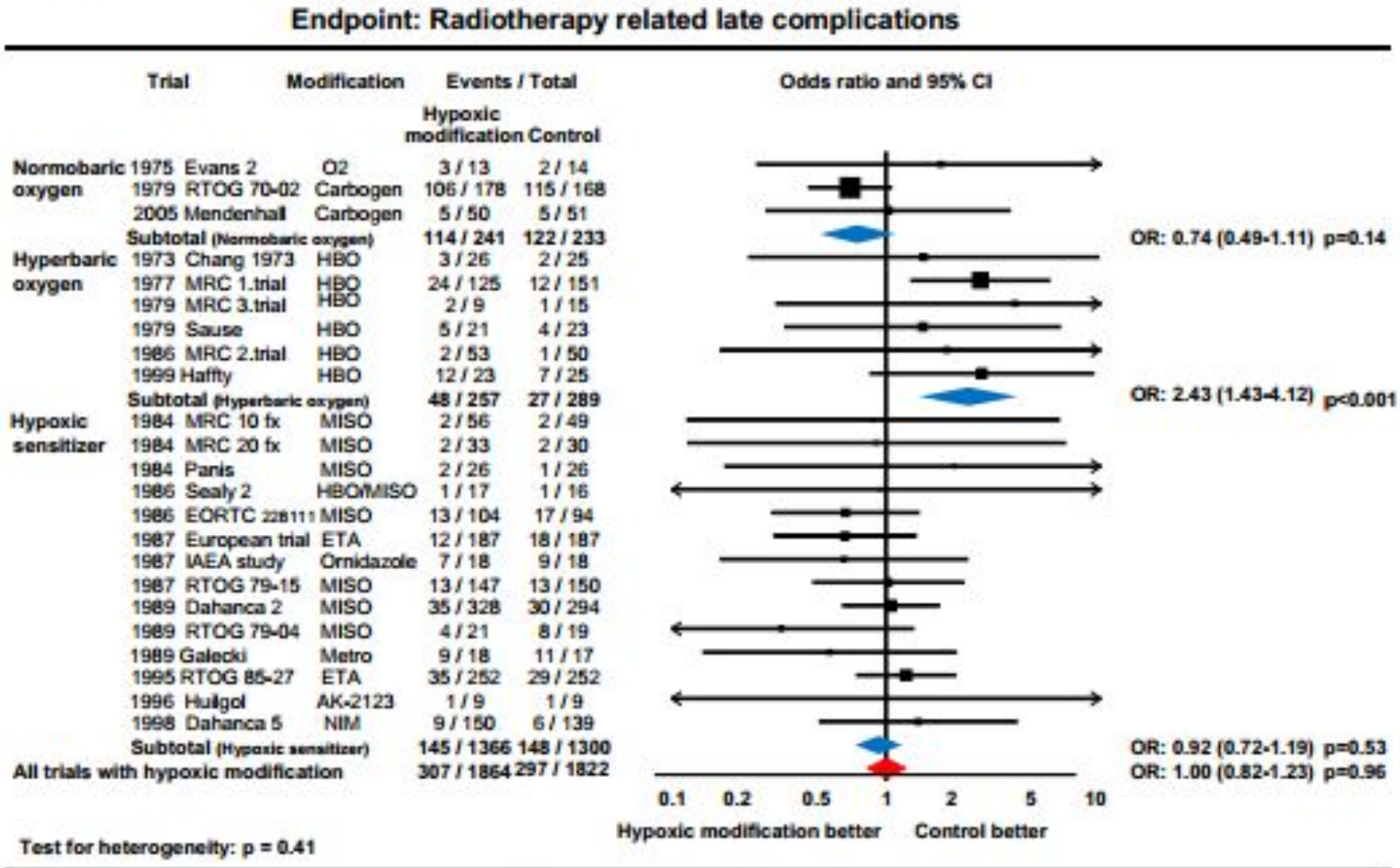

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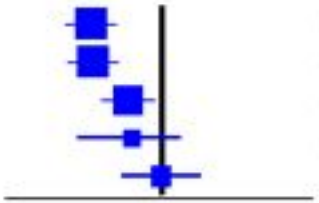
Head and neck cancer - meta analysis - summary

Endpoint	Events / Total		Odds ratio and 95% CI	Odds ratio	Risk Reduction	NNT**
	Hypoxic modification	Control				
Loco-regional control	1203 / 2406	1383 / 2399		0.71 (0.63-0.80)*	8% (5-10%)*	13
Disease specific survival	1175 / 2335	1347 / 2329		0.73 (0.64-0.82)	7% (5-10%)	14
Overall survival	1450 / 2312	1519 / 2305		0.87 (0.77-0.98)	3% (0-6%)	31
Distant metastasis	159 / 1427	179 / 1391		0.87 (0.69-1.09)	2% (-1-4%)	57
Radiotherapy complications	307 / 1864	297 / 1822		1.00 (0.82-1.23)	0% (-3-2%)	>>



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* 95% CI.
** Numbers of patients Needed to Treat to achieve benefit in one patients.

Concluding remarks

Magnitude and cost of hypoxic modification

1. **The magnitude of hypoxic modification resulted in a risk reduction of approximately 8% for loco-regional failure and disease specific death.**

2. **This magnitude was the same as that achieved by accelerated fractionation, but slightly less than that obtained by simultaneous chemoradiotherapy or hyperfractionated radiotherapy.**

3. **This benefit is, however, achieved without any detectable enhancement of radiation related morbidity and as such, it represents a pure long-term gain**

4. **For the primary cancer related endpoints of loco-regional control and disease related survival it was estimated that every time approximately 13 patients were treated did on average one patient benefit from the use of hypoxic modification.**

5. **Since it does not cause any persistent or serious side effects, does it in full justify the use of hypoxic modification, also because the other (economical) related costs are small, especially when compared to the treatment with, e.g. biological modifiers or chemotherapy.**