Chapter 26 – The Biology and Exploitation of Tumor Hypoxia

12/12/2024

Outline

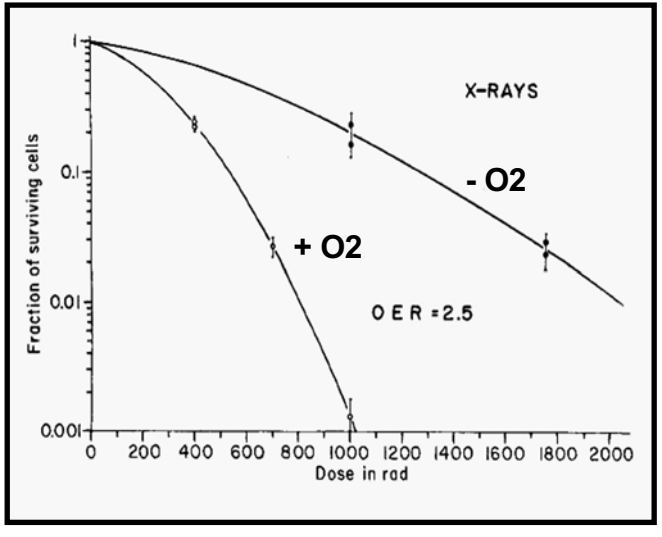
b. Radiosensitizers, bioreductive drugs, and radioprotectors
   i. Definition of therapeutic window
   ii. Tumor radiosensitizers (e.g., oxygen) and mimics (e.g., nitromidazole)
   iii. Normal tissue radioprotectors (e.g., amifostine)
   iv. Biological response modifiers (e.g., IL-2 and IFN)
   v. DNA repair inhibitors (e.g., PARPi, ATMi, ATRi, Chk1/2i)

- Radiosensitizers
  - The Halogenated Pyrimidines → No longer included in the Textbook
  - Radiosensitizing Hypoxic Cells
    - Hyperbaric Oxygen
    - Carbogen
    - Nicotinamide
    - Hypoxic-Cell Radiosensitizers

    } Used with RT
- Hypoxic Cytotoxins — Used alone or with RT; much like traditional chemotherapy
- Exploiting Tumor Hypoxia Summary
- “New Stuff”

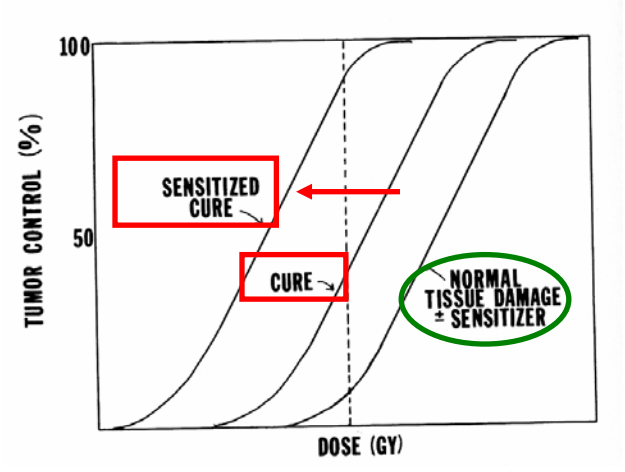
Radiosensitizers

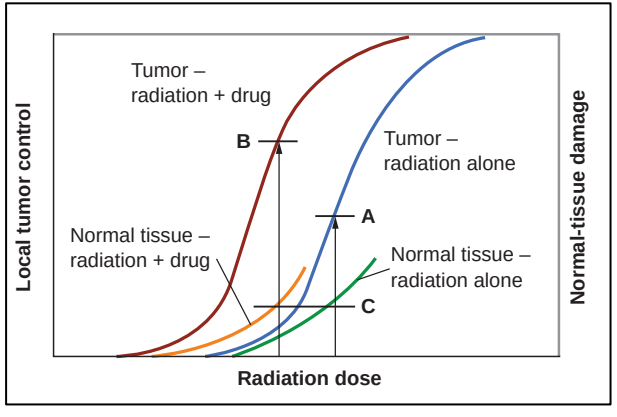
Definition – chemicals or pharmacologic agents that ↑ **the lethal effects of radiation** if administered **in conjunction with radiation**



The simplest and perhaps the most powerful radiosensitizer is O_2

Basic Strategy of Radiosensitizer





The goal would be to ↑ the tumor control probability for a given level of normal tissue complication

Ideally, a sensitizer would have no effect on normal tissue, but only potentiate tumor kill

In reality, as long as the drug increases tumor control **to a greater extent** than it increases normal tissue, damage, it will result in a **therapeutic gain**

Types of Radiosensitizers

Halogenated Pyrimidines

Basis of differential effect – tumor cells cycle faster and incorporate more drug than the surrounding normal tissues

Hypoxic-Cell Sensitizers

Basis of differential effect – hypoxic cells occur only in tumors and not in normal tissues

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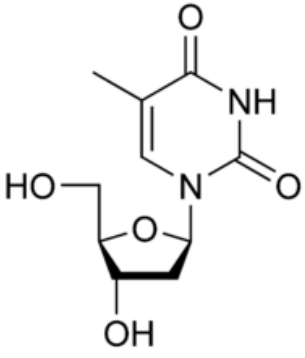
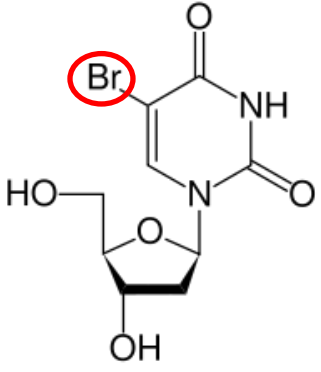
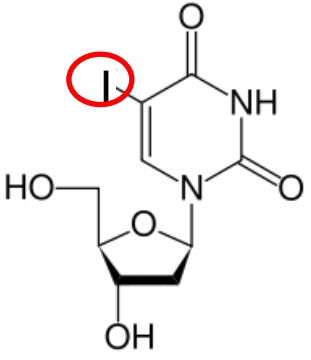
Halogenated Pyrimidines



Thymidine



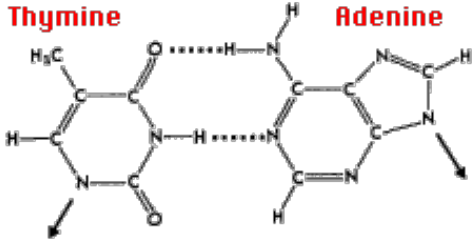
5-bromodeoxyuridine

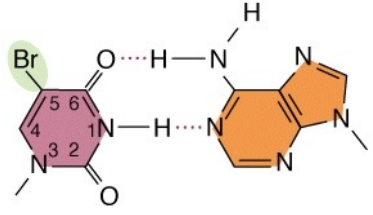


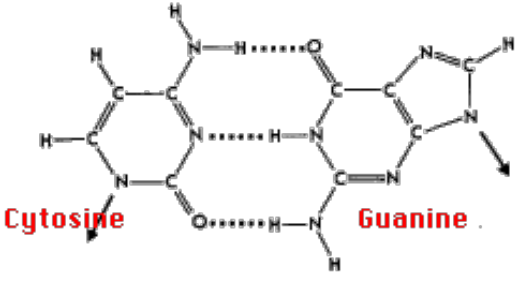
5-iododeoxyuridine

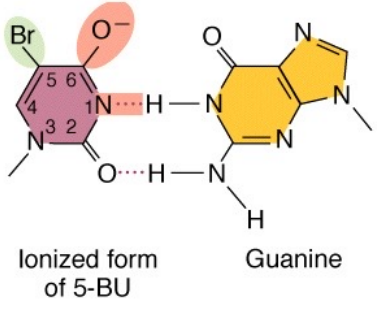
The halogenated pyridimines are analogues of thymidine, having a halogen substituting the methyl group

Halogenated Pyrimidines









Incorporated halogenated pyrimidines **"weakens"** the DNA chain, making the cells more susceptible to damage by **ionizing radiation** or **UV light**

Sensitization to Radiation

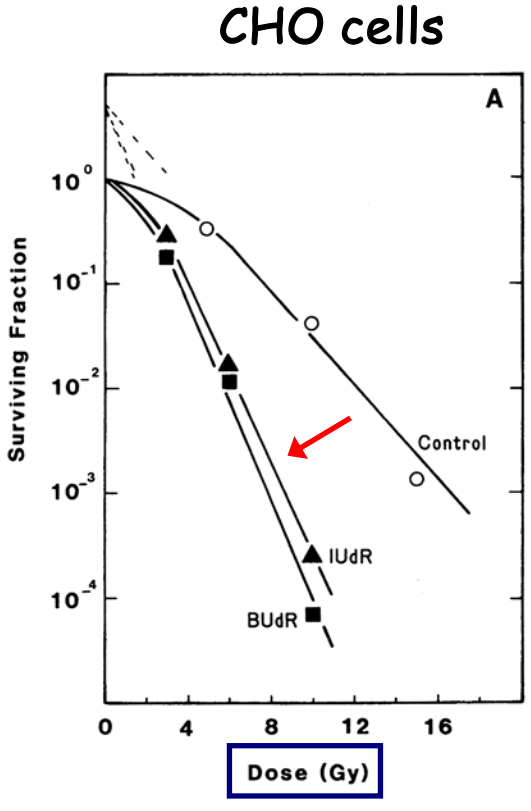
The extent of radiosensitization is proportional to the **percentage of incorporated halogenated pyrimidine**

⇩

The halogenated pyrimidines must be made available to cells for several cell generations so that an appreciable quantity of the analogue may be incorporated into the DNA

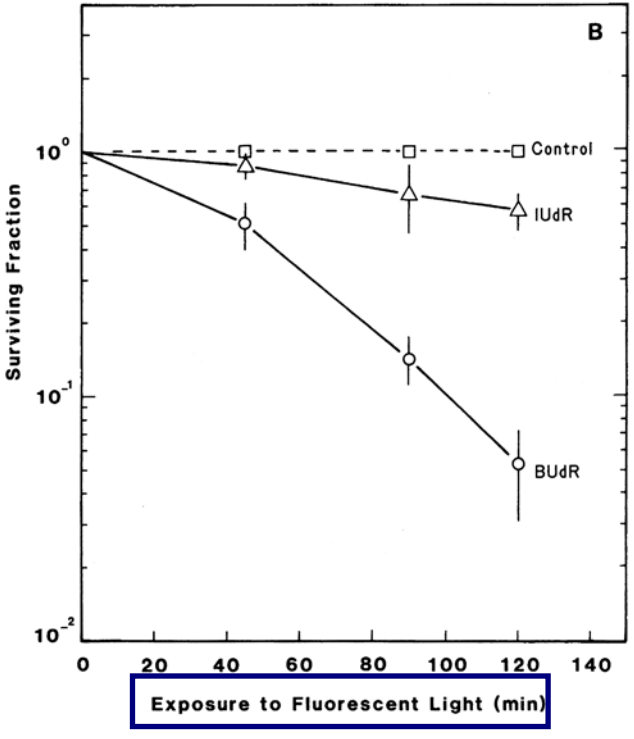
The utility of halogenated pyrimidine as a radiosensitizer is based on the premise that **tumor cells cycle faster and therefore incorporate more of the drug than the surrounding normal tissue**

Sensitization to Ionizing Radiation



BUdR and IUdR are both effective sensitizers to x-rays in mammalian cells

Sensitization to Fluorescent Light



BUdR is a much more efficient sensitizer to fluorescent light

As a clinician, which one would you pick for clinical use?

IUdR is preferred clinically because it avoids the light-induced rash produced by BUdR

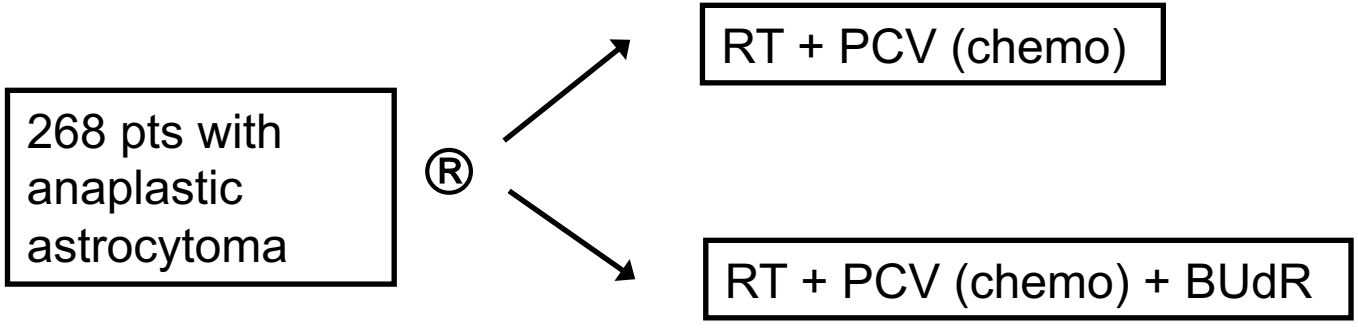
Clinical Application – Tumor Sites

The halogenated pyrimidines was initially tested in patients with **H&N** cancer – tumor response was good but normal-tissue damage was unacceptable (because the normal skin is also actively proliferating!!!)

High-grade glioma represents a more suitable tumor site because the surrounding normal brain is relatively quiescent in terms of proliferation

Following some encouraging phase I/II trials, a phase III clinical trial was conducted (**RTOG 94-04**)

RTOG 94-04



Patients randomized to treatment with BUdR were treated using a continuous 96-h i.v. infusion at a dose of 0.8 g/m^2/d. This infusion began the week before initiation of RT, on a Thursday or Friday before the first Monday of RT. Treatment continued during Weeks 1–5 of RT, starting on Day 4 or 5 of RT each week, for a total of six infusions. During the time of BUdR plus RT, patients were instructed to wear protective sunscreen (SPF >30) on all exposed areas of the skin.

RTOG 94-04

Conclusion – no survival advantage of adding BUdR

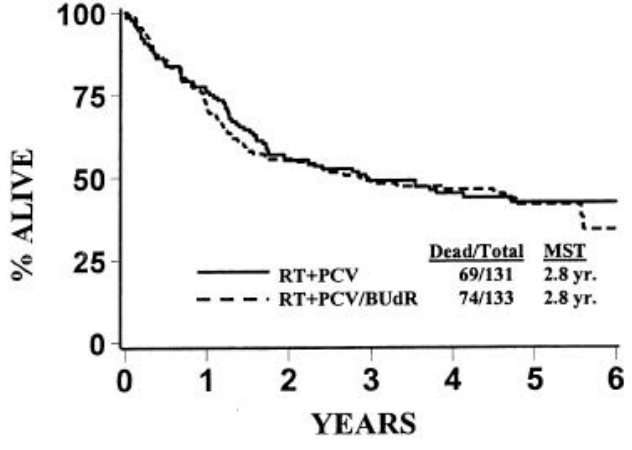


Fig. 2. Survival by treatment (intent-to-treat; $p = 0.76$, log–rank test).

The decision to begin a controlled Phase III trial with BUdR was made in 1990 and was based on extensive laboratory and clinical data. The laboratory data were compelling (2–4). As a result of thymidine substitution in DNA,

The results using BUdR in the dose and schedule of this study were disappointing. A Phase III trial is now ongoing

A radiobiologically sound idea and even compelling laboratory data do not necessarily translate into clinical benefit

Prado 2004

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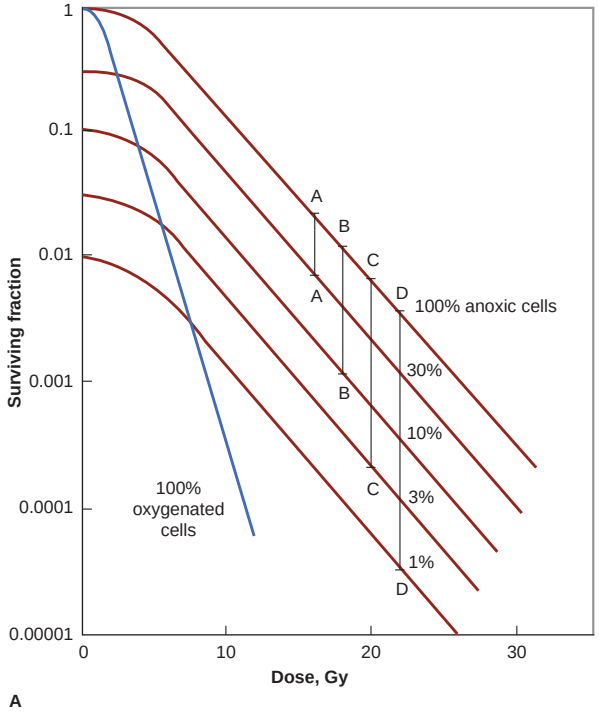
Tumor Hypoxia – Experimental Models

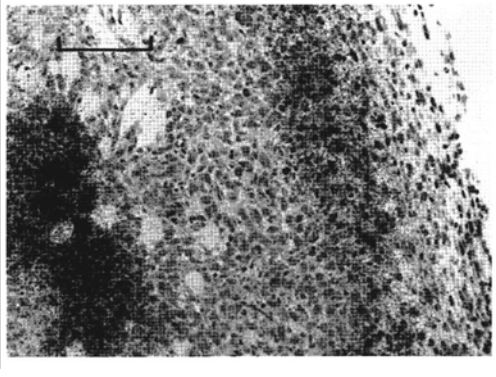


In animal models, hypoxic fractions in a tumor can be measured with **paired survival curves** (see Chapter 6)

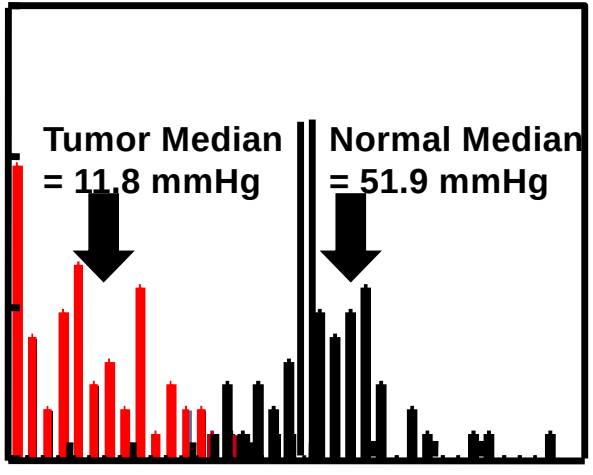
In a survey of all published studies on hypoxic fractions of transplantable tumors in animals, hypoxic fractions range from 0% to 50%, with a tendency for many results to average about **15%**

Hypoxia in Human Tumors

Hypoxic cells are also present in certain human cancers



Radiolabeled misonidazole in **small cell lung cancer**



pO_2 of **H&N cancer**

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Hyperbaric Oxygen

Patients were sealed in chambers filled with **pure O_2** raised to 3 atmosphere

Limitations

Large fractions were used because of the time and effort involved in the procedures

Unsuitable for patients with claustrophobia

Risk of fire

Hyperbaric Oxygen

Clin. Radiol. (1978) **29**, 333–338

CLINICAL TRIALS OF RADIOTHERAPY IN HYPERBARIC OXYGEN AT PORTSMOUTH, 1964–1976

IRENE S. CADE and J. B. McEWEN

From the Department of Radiotherapy and Oncology, St Mary's General Hospital, Portsmouth

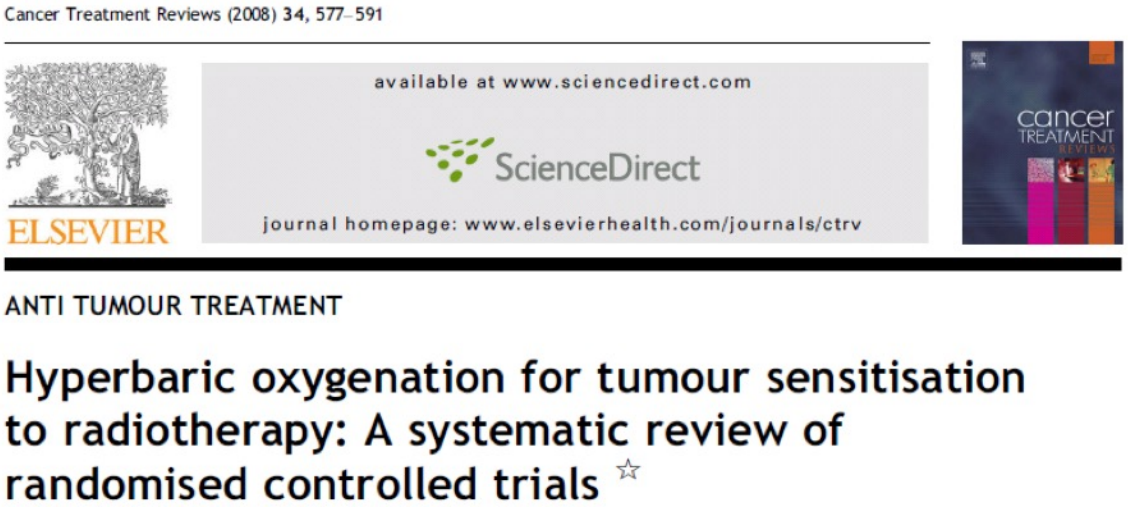
Technique. — The rationale of swallowing and of Valsalva's manoeuvre is explained and rehearsed. These measures relieve most cases of ear pain but, should they fail, a rapid lowering of the pressure by the operator at the controls followed by a slow build up to the previous level comforts the rest. We have

Both groups are treated on cobalt teletherapy machines. Two parallel opposed fields are used to include the known tumour with a margin, and of a maximum size of 150 cm^2. The patient turns from the supine to the prone position within the Vickers chamber. No anaesthetic is used. Sedation is rarely necessary. All are treated as out-patients except those from the Channel Islands and the Isle of Wight.

The rate of compression is 3 lb/in^2 min taking 10 min to reach 3 atm absolute. A saturation period of 15 min is allowed before radiotherapy during which time the chamber is moved from the pressure room to the treatment room and the first field set up.

Treatment times rarely exceed 3 min per field. It is advisable to limit the total time in the chamber to 40 min in order to minimise the incidence of oxygen convulsions. For this we require a high energy source giving 135 rad/min at 1 m. Decompression can be rapid, but we take 5–8 min with carcinoma bronchus to avoid pneumothorax in the presence of a grossly narrowed bronchus.

Hyperbaric Oxygen – Clinical Outcomes



Cancer Treatment Reviews (2008) 34, 577–591

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/ctrv

ELSEVIER

ANTI TUMOUR TREATMENT

Hyperbaric oxygenation for tumour sensitisation to radiotherapy: A systematic review of randomised controlled trials ☆

The meta-analysis involved 2,286 patients in 19 randomized trials

Hyperbaric Oxygen – Clinical Outcome

H&N cancer

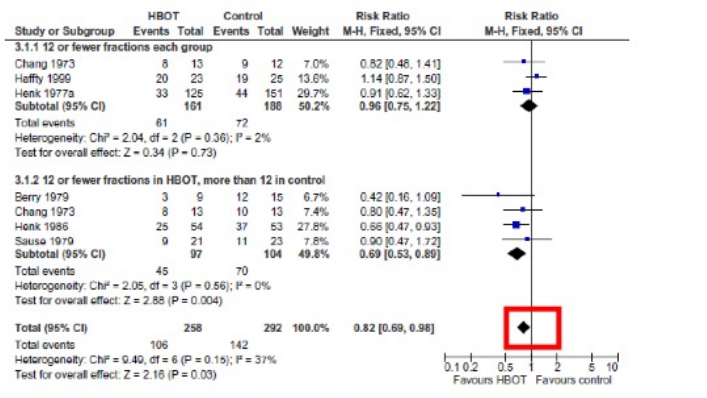


Figure 1 Mortality at 5 years following HBOT for cancer of the head and neck. Subgroup analysis by fractionation scheme.

H&N Cancer

There was a reduction of mortality at 1 and 5 years and improved local control at 3 months

Cervical Cancer

For cervical cancer, there was an improved local control at 2 years, but not at 1, or 5 yrs; and there was no survival benefit at 1,2,5 years

Other Tumor Sites

No local control or survival benefit in bladder, bronchus, rectum, esophagus or glioblastomas

Hyperbaric Oxygen – Toxicity

Study or Subgroup	HBOT Events	HBOT Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Brady 1981	7	29	7	29	19.4%	1.00 [0.40, 2.49]
Haffty 1999	12	23	7	25	18.5%	1.86 [0.89, 3.91]
Henk 1986	4	54	2	53	5.6%	1.96 [0.38, 10.27]
Sause 1979	4	21	3	23	7.9%	1.46 [0.37, 5.78]
Sealy 1986	27	60	7	64	18.7%	4.11 [1.94, 8.74]
Ward 1979	8	31	6	47	13.2%	2.02 [0.78, 5.26]
Watson 1978	20	161	6	159	16.7%	3.29 [1.36, 7.98]
Total (95% CI)		**379**		**400**	**100.0%**	**2.35 [1.66, 3.33]**
Total events	82		38			

Heterogeneity: Chi² = 7.03, df = 6 (P = 0.32); I² = 15%

Test for overall effect: Z = 4.80 (P < 0.00001)

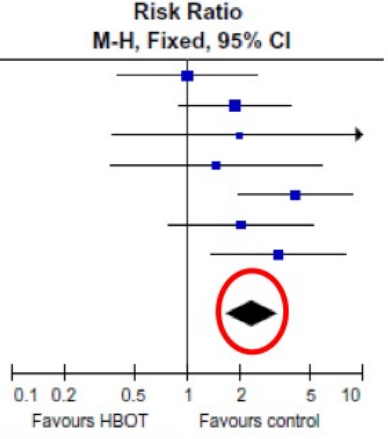


Figure 2 Forest plot of the chance of developing severe radiation tissue damage.

HBO was associated with acute CNS oxygen toxicity (seizures) and a significant increase in severe late radiation injury

HBO thus fell into disuse

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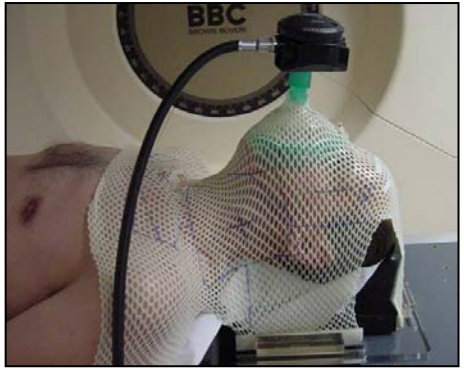
Carbogen Breathing

Carbogen



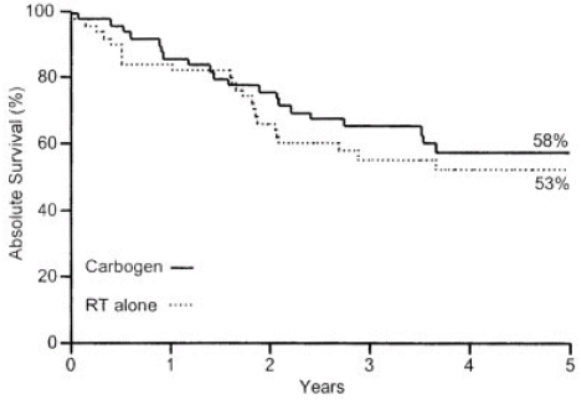
Pure oxygen causes vasoconstriction
This is avoided if a small amount of CO_2 is added (= carbogen)

Initially: 95% O_2 + 5% CO_2
Adjusted: 98% O_2 + 2% CO_2

Carbogen + Hyperfractionated RT in H&N Cancer – U of Florida

TABLE 2
Five-Year Outcomes (Treatment Groups Defined by Intention to Treat)

Parameter	Treatment group: Radiotherapy (n = 51) %	Treatment group: RT + carbogen (n = 50) %	P value
Local control	83	88	0.5155
Locoregional control	81	83	0.7174
Distant metastasis-free survival	82	86	0.5184
Cause-specific survival	73	77	0.5866
Overall survival	53	58	0.4856



CONCLUSIONS. The addition of carbogen breathing to definitive RT did not appear to improve the likelihood of local control significantly. However, because of the limited size of the current study, the authors cannot definitively conclude that carbogen breathing is ineffective. ***Cancer*** **2005;104:332–7.**

Mandenhall 2005

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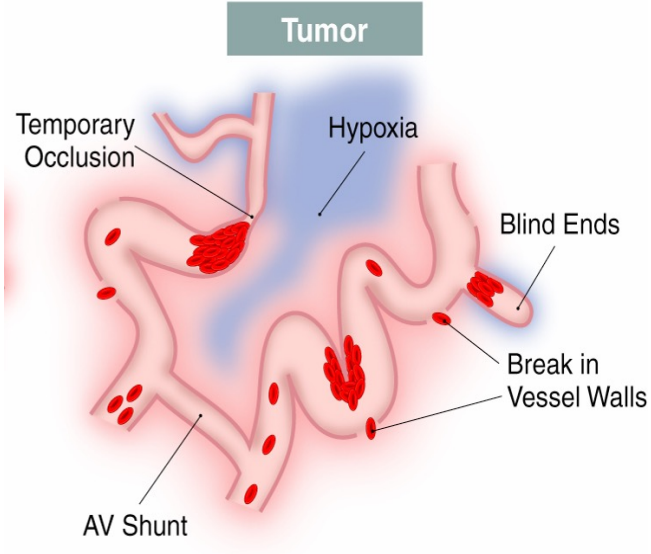
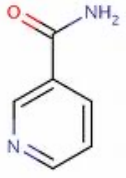
Chronic and Acute Hypoxia



There are 2 components of hypoxia – **chronic hypoxia** (diffusion limited) and **acute hypoxia** (perfusion limited)

Nicotinamide



Prevents transient fluctuations in tumor blood flow

Administration
Orally, liquid formulation
1 h before irradiations

Dose
Initially: 80 mg/kg
Adjusted: 60 mg/kg

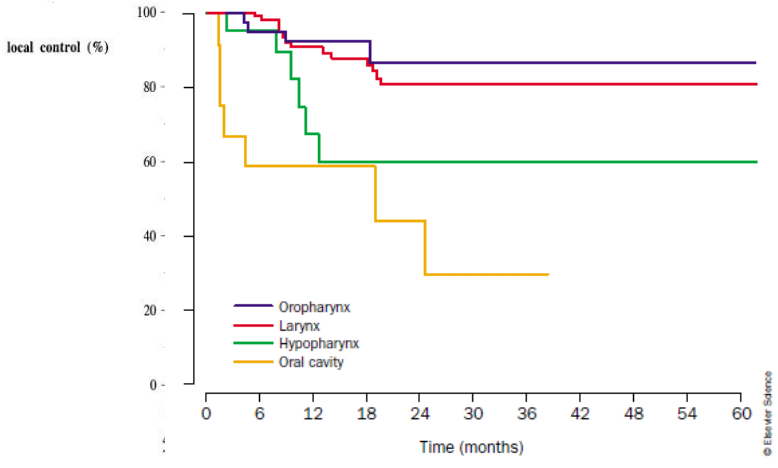
ARCON

ARCON – Accelerated Hyperfractionated Radiation Therapy while Breathing Carbogen and with the Addition of Nicotinamide

Rationale

Accelerated treatment – to minimize tumor proliferation
Hyperfractionation – to minimize late effects
Carbogen breathing – to overcome chronic hypoxia
Nicotinamide – overcome acute hypoxia

ARCON – Phase II Study



Head & Neck cancer patients

Conclusion: ARCON yields high local and regional control rates in advanced head-and-neck carcinomas, and compliance is satisfactory and morbidity acceptable. The local control rate of 80% for T3 and T4 larynx carcinomas offers excellent possibilities for organ preservation.

Phase II trial does not prove anything; Phase III study is underway in Europe

Kannders 2002

ARCON

ELSEVIER

ARCON: accelerated radiotherapy with carbogen and nicotinamide in head and neck squamous cell carcinomas. The experience of the Co-operative Group of Radiotherapy of the European Organization for Research and Treatment of Cancer (EORTC)☆

Jacques Bernier[a,*], Juliana Denekamp[b], Anamaria Rojas[c], Emilio Minatel[d], Jean-Claude Horiot[e], Han Hamers[f], Paolo Antognoni[g], Olav Dahl[h], Pierre Richaud[i], Martine van Glabbeke[j], Marianne Piérart[j]

RADIOTHERAPY & ONCOLOGY
JOURNAL OF THE EUROPEAN SOCIETY FOR THERAPEUTIC RADIOLOGY AND ONCOLOGY

Conclusion: Future ARCON trials should target selected head and neck tumor localizations and stages, and a lower nicotinamide dose is needed to reduce severe upper gastro-intestinal toxicity.

ARCON

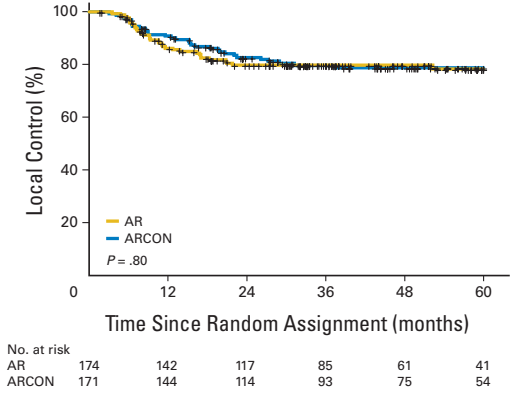
VOLUME 30 · NUMBER 15 · MAY 20 2012

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Accelerated Radiotherapy With Carbogen and Nicotinamide for Laryngeal Cancer: Results of a Phase III Randomized Trial

Geert O. Janssens, Saskia E. Rademakers, Chris H. Terhaard, Patricia A. Doornaert, Hendrik P. Bijl, Piet van den Ende, Alim Chin, Henri A. Marres, Remco de Bree, Albert J. van der Kogel, Ilse J. Hoogsteen, Johannes Bussink, Paul N. Span, and Johannes H. Kaanders



Patients allocated to the ARCON arm received carbogen (98% oxygen + 2% carbon dioxide, 4 minutes before and during daily fractions) and oral nicotinamide (60 mg/kg, 1 to 1.5 hours before each fraction) concurrently with radiotherapy.[22,23] During the boost, nicotinamide was given only before the first fraction of the day. To prevent nausea, domperidone (10 mg, three times a day) was given.

44 Gy / 2 Gy daily ⟶ 24 Gy / 2 Gy BID

Tumor hypoxia was measured in biopsy specimen after pimonidazole infusion

What is the future of targeting hypoxia as a therapeutic strategy? In our view, there is still room for a significant benefit to be realized in a subset of patients with advanced head and neck cancer. Demonstration of any benefit will be challenging as it will likely require that eligibility for future trials be restricted to those with HPV-negative tumors, and that the trials be further enriched for those most at risk of locoregional recurrence as a result of tumor hypoxia as identified by an appropriately validated test. It is also vital that the control arm for future trials should reflect the best available standard of care.

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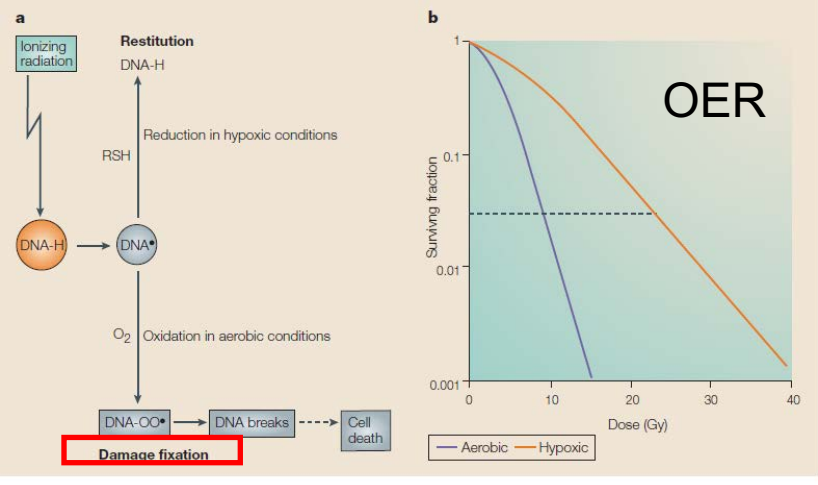
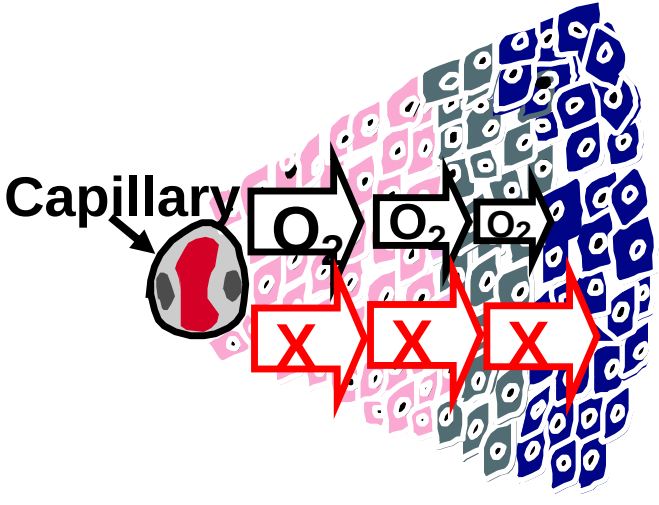
} “forcing O_2 into the tumor”

Mimics O_2 to fix DNA damage

Hypoxic-Cell Radiosensitizers





Hypoxic cell radiosensitizers are electron affinic compounds that **mimic O_2** in their ability to fix radiation damage

This compound should be chemically stable and **not subject to rapid metabolic breakdown** as in the case of O_2

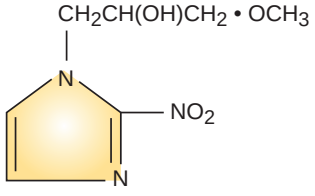
Additional Essential Properties of Hypoxic Radiosensitizers

It has to selectively sensitize hypoxic cells at a concentration that would result in **acceptable toxicity to normal tissues**

It must be **highly soluble in water or lipids** and must be capable of **diffusing a considerable distance** through a non-vascularized cell mass to reach the hypoxic cells (i.e., 200μm from capillary)

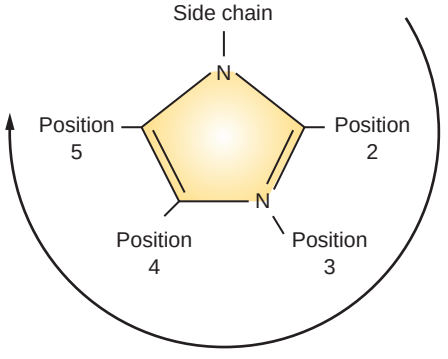
It should be **effective** at the relatively low **daily doses of a few Gy** used in conventional fractionated radiotherapy

Misonidazole



Misonidazole

Side chain determines position 1

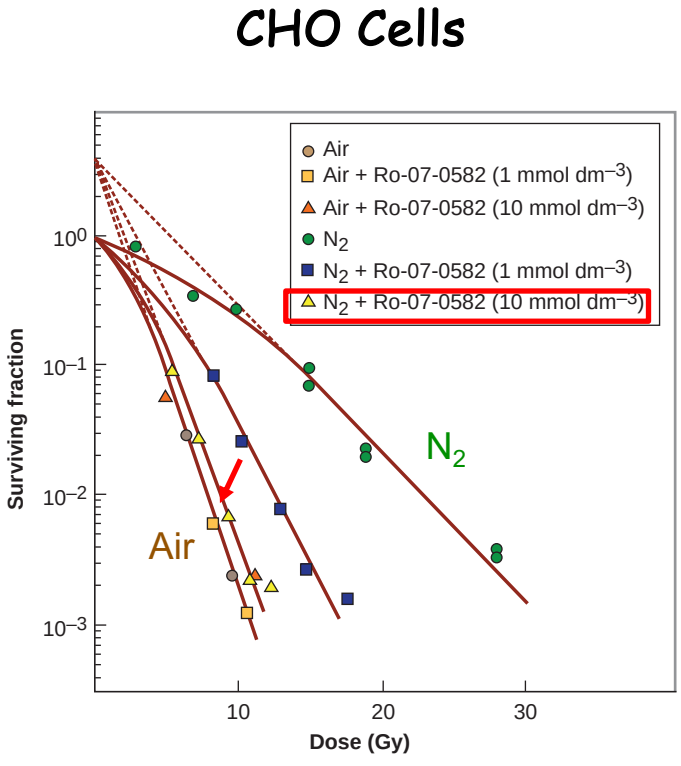


Misonidazole is a 2-nitroimidazole

The sensitizing efficiency is directly related to the **electron affinity** of the compounds

In general, 2-nitroimidazole has highest e-affinity compared to others

Misonidazole – *In Vitro* Sensitization



Misonidazole produces substantial sensitization with cells in culture

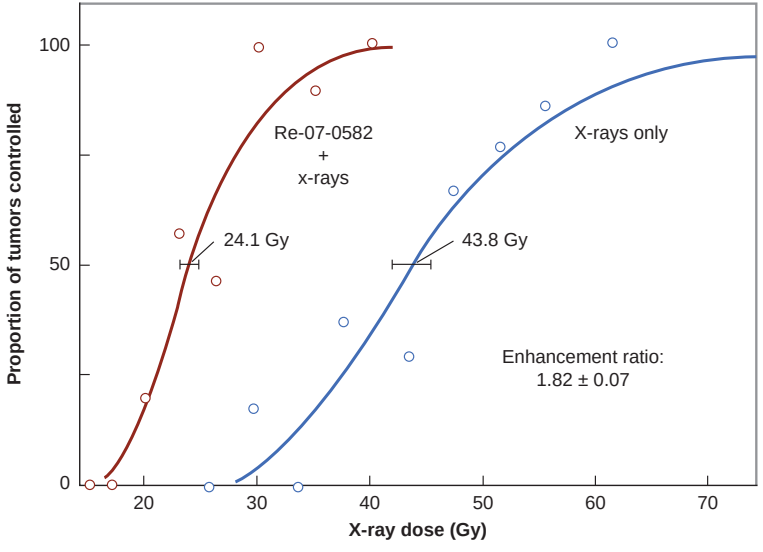
At a concentration of 10 mM, the radiosensitivity of hypoxic cells approaches that of aerated cells

The response of aerated cells is not affected by the drug at all

Misonidazole – *In Vivo*

Misonidazole also has a dramatic effect on tumors in experimental animals

Mouse Mammary Tumor



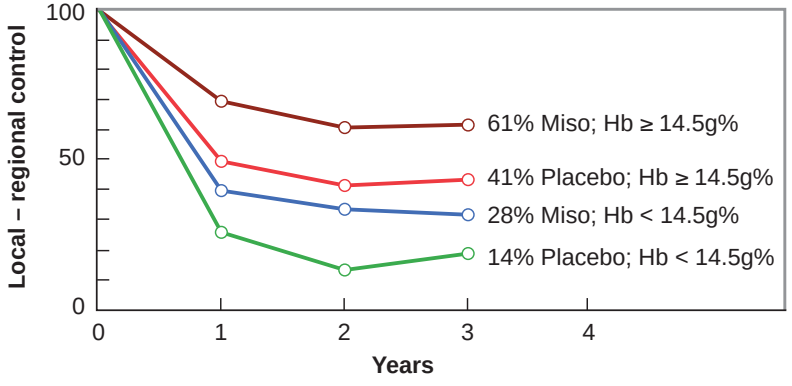
Should the radiation be delivered before or after the administration of misonidazole?

TCD_{50} is reduced by 1.82-fold in single dose experiment

Do you expect the enhancement ratio to be larger or smaller with multifractionated regimen?

Misonidazole – Danish Trial

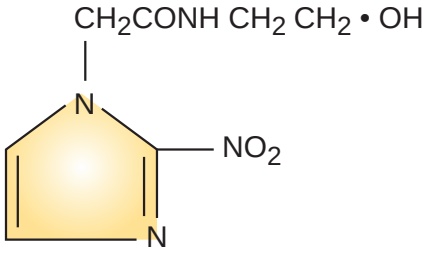
Of the 20 or so randomized trials comparing radiation ± misonidazole, the only positive study is the Danish trial, and in **subgroups** only



Only male, with pharyngeal cancer and high Hgb level benefited from addition of misonidazole – post-hoc subgroup analysis requires validation

Misonidazole resulted in the significant **peripheral neuropathy** in 26% of patients

Etanidazole



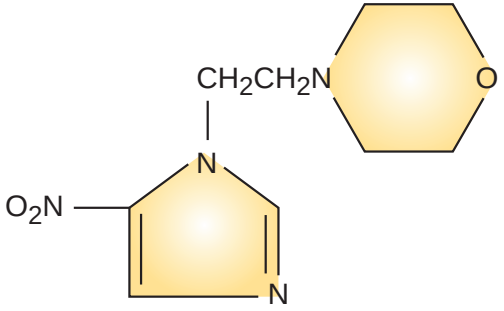
Etanidazole

Etanidazole is also a 2-nitroimidazole, therefore **equally effective as a sensitizer** as misonidazole

Etanidazole has a shorter *in vivo* $t_{1/2}$ and a lower partition coefficient, so that it penetrates poorly into nerve tissue and does not cross the BBB → **less toxic than misonidazole**

Unfortunately, clinical trials with etanidazole showed **no benefit** when added to radiotherapy

Nimorazole



Nimorazole

Nimorazole is a **5-nitroimidazole**, therefore **less effective as a sensitizer** than misonidazole or etanidazole

Nimorazole is **much less neurotoxic** → very large doses can be administered

Addition of nimorazole to conventional radiotherapy was shown to be beneficial in H&N cancer in Danish trial

Nimorazole

DAHANCA 5-85
SUPRAGLOTTIC and PHARYNX CARCINOMA

STRATIFY:

T-size
Region
Sex
Hemoglobin*
Institution

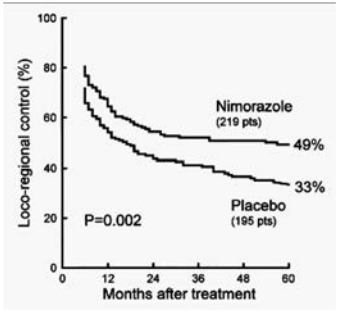
RANDOMIZE

RT + Nimorazole (219 pts)
(NIM: 1.2 g/m2 x 30)
(RT: 62-68 Gy/ 31-34 fx, 5 Fx/wk)

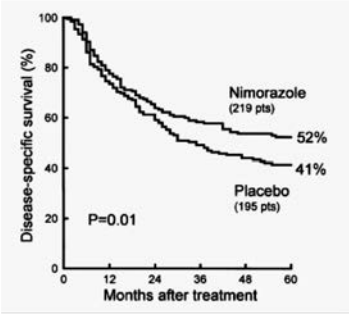
RT + Placebo (195 pts)
(RT: 62-68 Gy/ 31-34 fx, 5 Fx/wk)

g/m^2 or 75 g in total. Patients were instructed to take the capsules 90 min before radiation and the time was written on a schedule which was checked by the radiotherapy technician. Almost all radiation fractions were given with less than 15 min derivation from the planned 90 min.

Local control



Disease-specific survival



Toxicity – drug-related side-effects were minor and tolerable primarily consists of transient nausea and vomiting

Bottom Line – Nimorazole significantly improves the effect of radiotherapeutic management of supraglottic and pharynx tumors and can be given without major side effects

Overgaard 1998

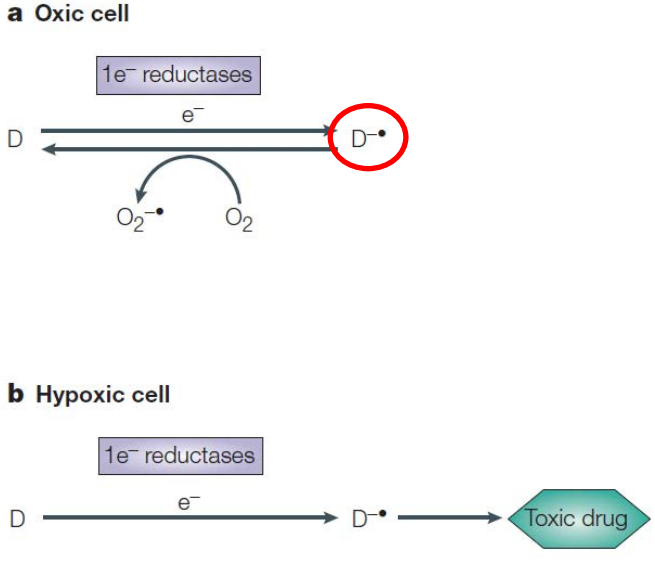
Outline

- Radiosensitizers
  - The Halogenated Pyrimidines
  - Radiosensitizing Hypoxic Cells
    - Hyperbaric Oxygen
    - Carbogen
    - Nicotinamide
    - Hypoxic-Cell Radiosensitizers
- **Hypoxic Cytotoxins**
- Exploiting Tumor Hypoxia Summary
- “New Stuff”

Hypoxic Cytotoxin

Hypoxic cytotoxins are drugs that **selectively kill hypoxic cells**

They are **prodrugs** – addition of **an electron** converts it to a radical



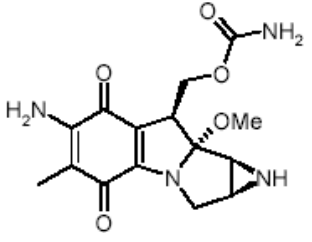
In oxic cells, the unpaired electron in the prodrug radical is rapidly transferred to O_2, forming superoxide and regenerating the initial prodrug (= futile cycle)

In hypoxic cells, the prodrug radical accumulates; itself can be the cytotoxin, or more commonly, it undergoes further reactions to form the ultimate toxic species.

Hypoxic Cytotoxins – 5 Classes

Quinone Antibiotics

Prototype – **mitomycin C**
Mechanism of toxicity – crosslinks DNA when bioreduced
Used as a **chemotherapy agent** for many years
The aerated-hypoxic differential is relatively small



Nitroaromatic Compounds

Unsuitable for clinical use due to substantial normal tissue toxicity

Benzotriazine di-N-oxides

Lead compound – **tirapazamine** (TPZ)

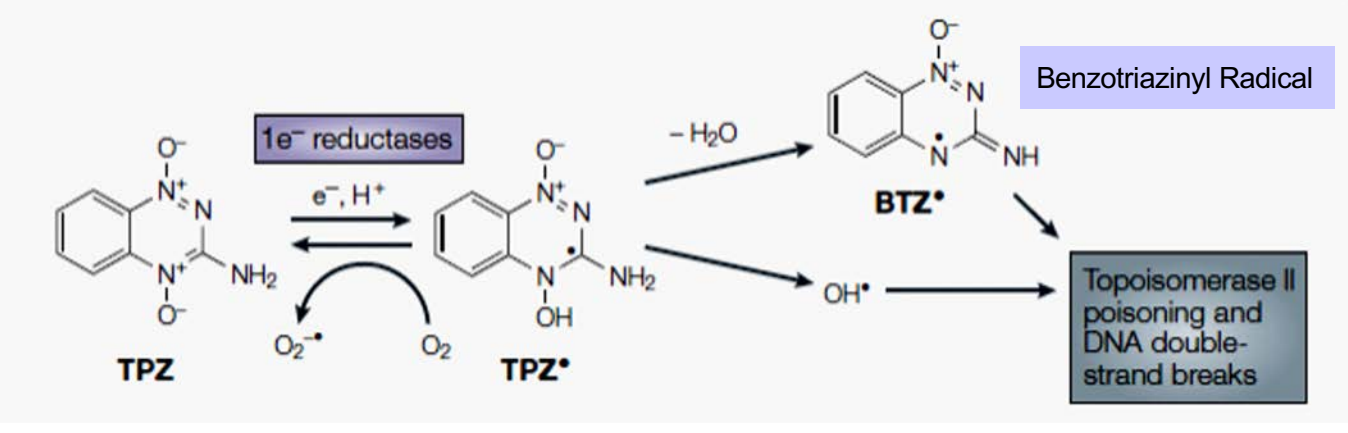
Dinitrobenzamide Modified Nitrogen Mustard

2-nitroiidazole Attached to Dibromo Isophosphoramide

Tirapazamine

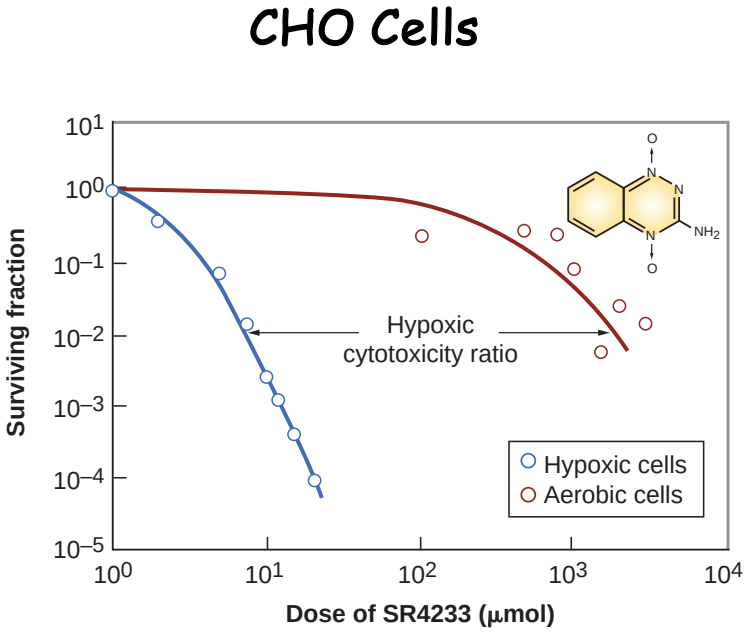
Mechanism of Cytotoxicity

Bioreduction is favored under the hypoxic condition



When O_2 is present, the TPZ radical is back-oxidized to the parent compound, producing a superoxide radical which might be responsible for the muscle cramps seen in patients given the drug

Tirapazamine



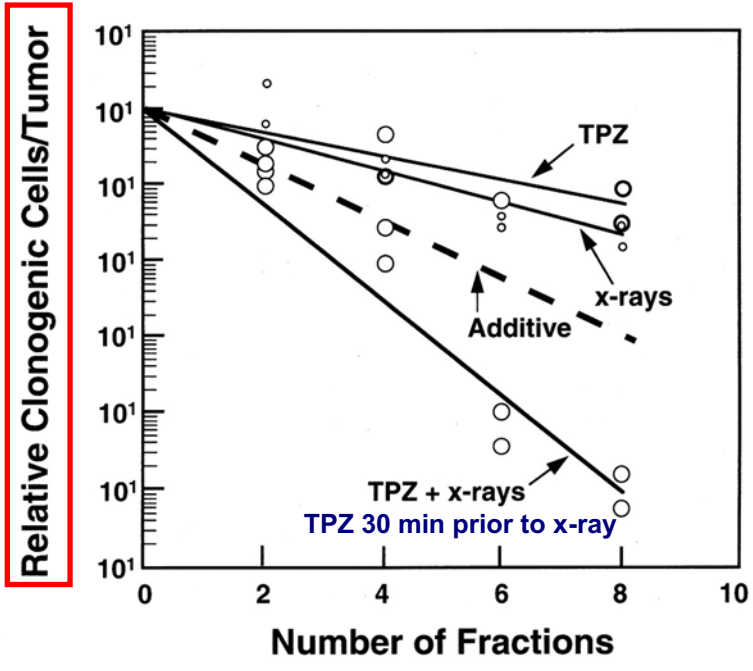
TPZ preferentially kills hypoxic cells

In CHO cells, the hypoxic/oxic cytotoxic ratio is ~ 100

In human cells, the hypoxic/oxic cytotoxic ratio is ~ 20

TPZ + Radiation – *In Vivo/In Vitro*

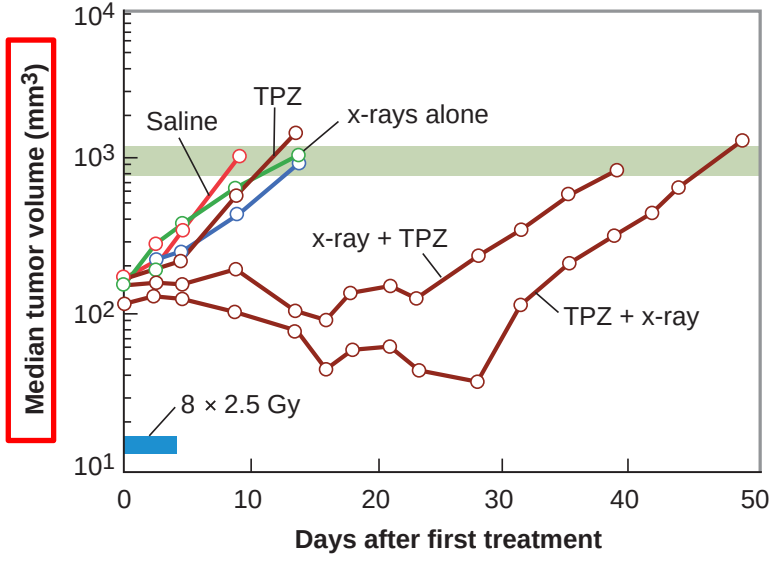
Transplanted mouse carcinoma



Assay – following treatment, the mice sacrificed and cells assayed for **clonogenic survival**

As TPZ kills the most radiation-resistant cells in tumors, TPZ and radiation act as complementary cytotoxins, each one killing the cells resistant to the other, thereby **potentiating the efficacy of radiation on the tumor (synergistic)**

TPZ + Radiation – Growth Delay



The combination of TPZ and x-ray was highly effective

TPZ → x-ray was slightly more effective than x-ray → TPZ

In parallel experiments scoring **skin reaction**, there was no radiosensitization or additive cytotoxicty by the addition of TPZ = **selective cytotoxicity**

TPZ + Radiation – RTOG Trial

Single-Arm, Open-Label Phase II Study of Intravenously Administered Tirapazamine and Radiation Therapy for Glioblastoma Multiforme

By J. Del Rowe, C. Scott, M. Werner-Wasik, J.P. Bahary, W.J.Curran, R.C. Urtasun, and B. Fisher

Trial Design – 124 patients with glioblastoma were treated with 60 Gy + TPZ; TPZ was given 3x/wk for 12 treatments during radiotherapy

Results – survival was equivalent to the control population

Toxicity – the most common toxicities of TPZ were nausea, vomiting, muscle pain and skin reaction

Bottom Line – there is not much clinical data supporting combination of TPZ and radiation

TPZ + Chemotherapy

Tirapazamine Plus Cisplatin Versus Cisplatin in Advanced Non–Small-Cell Lung Cancer: A Report of the International CATAPULT I Study Group

By Joachim von Pawel, Reinhard von Roemeling, Ulrich Gatzemeier, Michael Boyer, Lars Ove Elisson, Peter Clark, Denis Talbot, Augustin Rey, Thomas W. Butler, Vera Hirsh, Ian Olver, Bengt Bergman, Joseph Ayoub, Gary Richardson, David Dunlop, Anthony Arcenas, Robert Vescio, Jean Viallet, and Joseph Treat

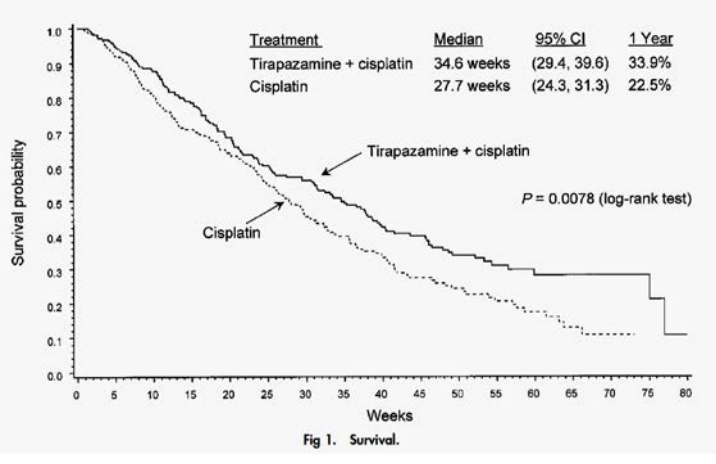


Fig 1. Survival.

TPZ has shown to enhance the activity of **cisplatin** in patients with advanced non-small cell lung cancer and confirms that hypoxia is an exploitable therapeutic target in human malignancies

TPZ + Chemo + Radiation – TROG 02.02

VOLUME 28 · NUMBER 18 · JUNE 20 2010
JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Stage III/IV H&N Ca
861 pts
89 centers in 16 countries

®

RT (70 Gy) + Cisplatin

RT (70 Gy) + Cisplatin + TPZ

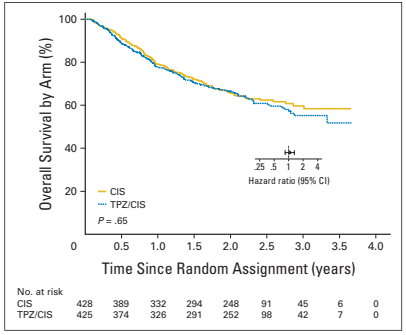


Fig 2. Overall survival by arm. CIS, cisplatin; TPZ, tirapazamine.

Conclusions
We found no evidence that the addition of TPZ to chemoradiotherapy, in patients with advanced head and neck cancer not selected for the presence of hypoxia, improves OS.

and neck cancer. These results were surprising given the promising randomized phase II trial results.[8] Our previous studies of

Rischin 2010; JCO 28: 2989-2995

Outline

- Radiosensitizers
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    - Carbogen
    - Nicotinamide
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- “New Stuff”

Methods of Modification of Hypoxic Radioresistance in Clinical Trials

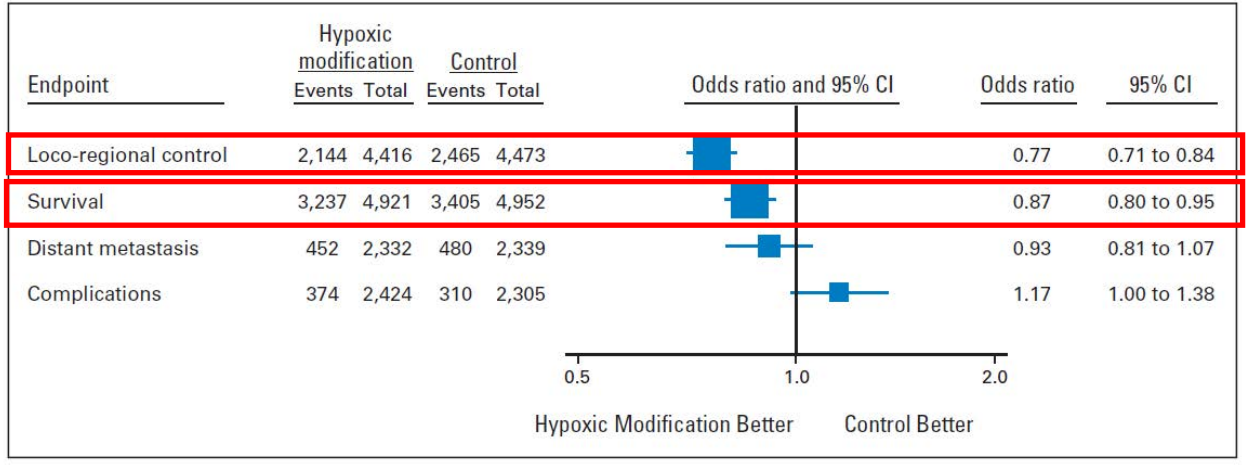
Table 2. Methods of Modification of Hypoxic Radioresistance in Clinical Trials

Increased oxygen delivery by the blood
Hyperbaric oxygen
Normobaric oxygen/carbogen breathing
Nicotinamide
Blood transfusion, erythropoietin
Mimic of oxygen in the radiochemical process
Nitroimidazoles
Destruction of hypoxic cells
Hypoxic cytotoxins
Hyperthermia
Elimination of OER
High LET

Abbreviations: OER, oxygen enhancement ratio; LET, linear energy transfer.

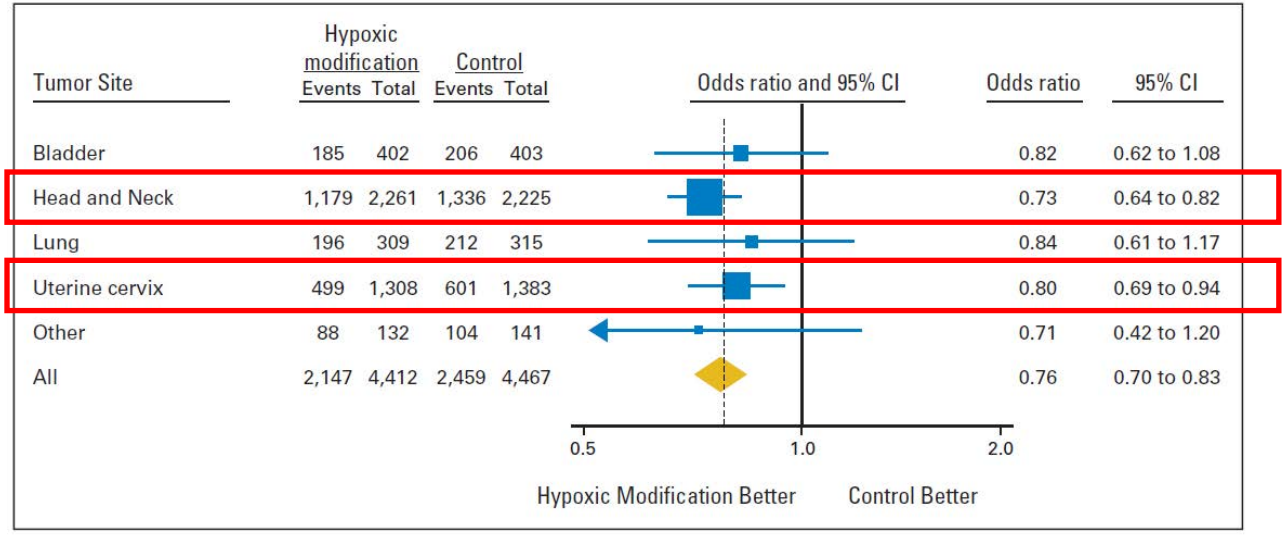
Overgaard's Meta-analysis

Data from 86 randomized trials including 10,108 patients



Overall modification of tumor hypoxia significantly improved the effect of radiotherapy, with an odds ratio of 0.77 for the outcome of locoregional control and with an associated significant overall survival benefit

Overgaard's Meta-analysis



Why is Hypoxic Modification Not Impacting Clinical Practice

VOLUME 25 · NUMBER 26 · SEPTEMBER 10 2007

JOURNAL OF CLINICAL ONCOLOGY

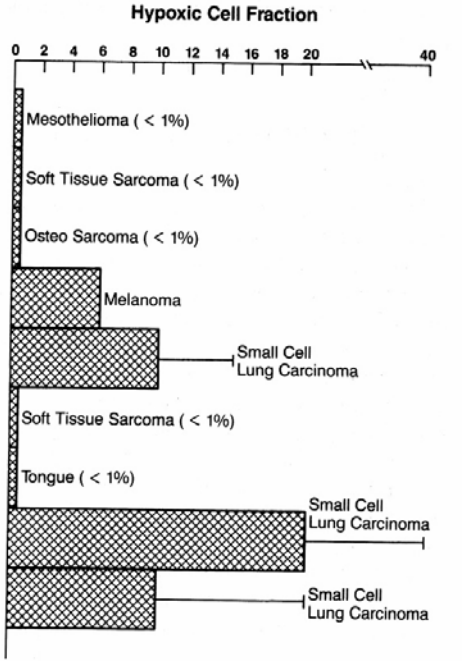
REVIEW ARTICLE

Hypoxic Radiosensitization: Adored and Ignored

Jens Overgaard

both past and more current clinical trials have demonstrated that this hypoxic radioresistance, to some extent, can be overcome and that that may happen by the use of simple and inexpensive drugs, which, unfortunately, do not attract the pharmaceutical industry because of the lack of financial incentive. There might be those who

Patient Selection is An Issue



9 patients administered radioactive-labeled misonidazole

Only 4 of the 9 patients had tumors with a significant proportion of hypoxic cells

⇩

Thus, hypoxia in human tumors may not be as pronounced as that in animal tumors

Only appropriately selected patients whose tumor demonstrates significant hypoxia is likely to benefit from hypoxia modifying therapy
The advancement of hypoxia imaging techniques certainly could make this possible

Question

One would expect to achieve the largest therapeutic gain using a:

A. radiation sensitizer that increases response of tumors and dose limiting normal tissue by the same factor
B. radiation protector that protects the tumor but not the normal tissue
C. high LET ion beam with a tumor type having a large hypoxic fraction
D. radiation protector that decreases the radiation response of both the tumor and normal tissues
E. fractionated course of neutrons that yields an RBE of 3.0 for the tumor and an RBE of 5.5 for the dose limiting normal tissue

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- **“New Stuff”**

Medical Residents Only

“New Stuff”

- Newer Bioreductive Drugs
- Imaging of Hypoxia
- Targeting Tumor Metabolism to Kill Hypoxic Cells
- Unfolded Protein Response

Bioreductive Drugs

- **A prodrug**
- **Metabolites give rise to DNA interstrand cross-links**
- **In Phase II trial**

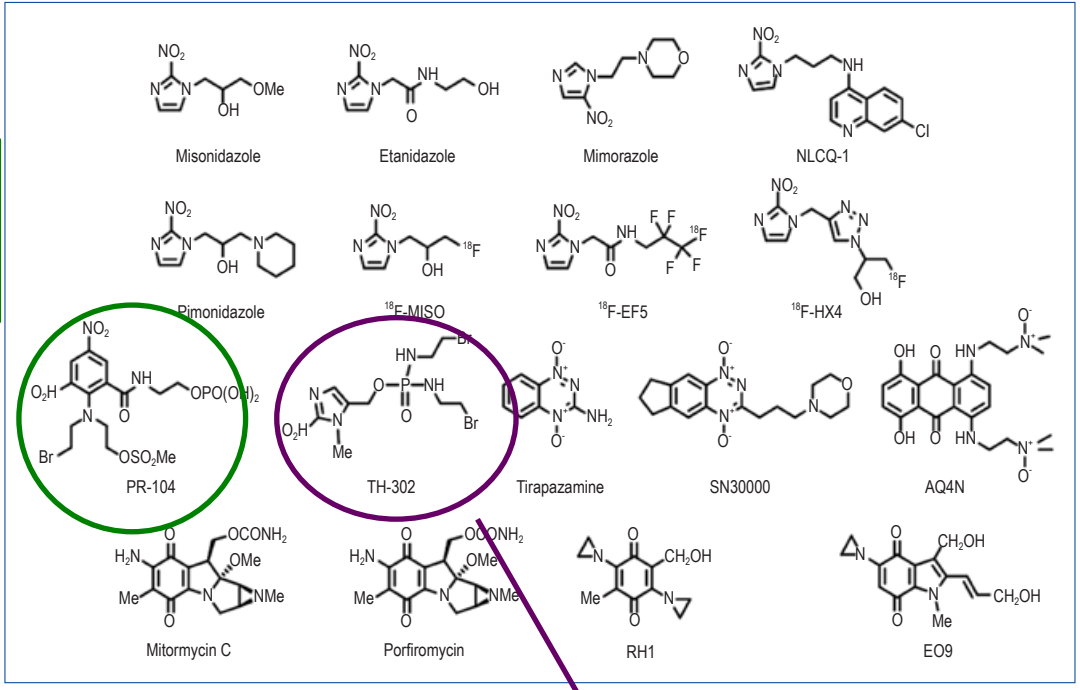


Figure 1. **Structure of the bioreductive compounds.**

- **Metabolites act as a DNA cross-linking agent particularly effective in cell lines deficient in homologous recombination**
- **Combination with chemo is currently in phase II and III trials**

Guise 2014; Chinese Journal of Cancer 33(2): 81-86

Imaging of Hypoxia

Patient selection is the key if any hypoxic directed therapy should work

Table 1. Examples of non-invasive methods for hypoxia determination in living tissues [14-18]

Modality		Technique	Limitations
Optical-based	Phosphorescence	Infusion of water-soluble phosphor probes into the vasculature.	The measurement represents the vascular pO_2, not tissue pO_2.
	Near-infrared spectroscopy (NIRS)	Non-invasive assessment of hemoglobin (Hb) saturation.	The measurement provides information on vascular oxygenation, but not on tissue pO_2.
MRI-based	Blood oxygen level-dependent magnetic resonance imaging (BOLD MRI)	BOLD images reveal the changes in the amount of oxygen bound to hemoglobin in blood owing to deoxyhemoglobin, which is a paramagnetic substance.	The measurement provides information on changes in blood oxygenation, but not on the absolute oxygen concentration in tissue.
	^{19}F-MRI or NMR (nuclear magnetic resonance)	Perfluorocarbons (PFCs) are injected intravenously and their ^{19}F spin lattice relaxation rate (R1) varies linearly with the dissolved oxygen concentration.	The relaxation rate of ^{19}F may depend on other physiological factors present in the tissue and not only on O_2 concentration.
	Electron paramagnetic resonance imaging (EPRI)	Use of implantable paramagnetic particulates or soluble probes, intravenously injected, that physically interact with oxygen.	The molecules may predominantly distribute in the vasculature, thus biasing in part measurements of tissue oxygenation.
	Proton–electron double resonance imaging (PEDRI)	Injection of an external probe that has unpaired electrons and use of a strong EPR impulse.	The molecules may predominantly distribute in the vasculature, thus biasing in part measurements of tissue oxygenation.
	DCE-MRI (dynamic Gd-DTPA-enhanced MRI)	Injection of contrast agent and determination of vasculature perfusion/permeability.	Low specificity, because the measurement provides information on both vascular and tissue oxygenation.
Nuclear-based	Single-photon emission computed tomography (SPECT)	Injection of gamma (γ) emitting radiopharmaceuticals selective for hypoxic tissue. High specificity	Limited resolution dependent on voxel-based distribution of hypoxia.
	Positron emission tomography (PET)	Injection of positron (β+) emitting radiopharmaceuticals selective for hypoxic tissue. High specificity.	Limited resolution compared to MRI and optical methods, but superior to SPECT.

PET Radiopharmaceuticals

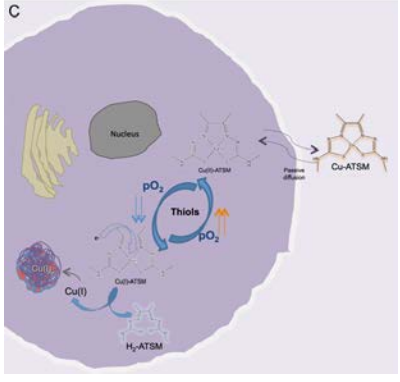
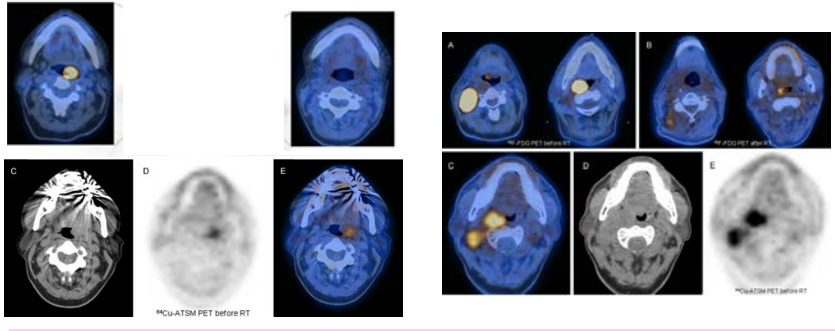


Table 3. Characteristics of copper nuclides utilized in PET imaging and comparison with otherpositron emitters [59, 137-140]

Nuclides	$T_{1/2}$	Production	β⁺ emission (E_{mean})	Other emissions	Range of β⁺ in tissue	Use
Copper-60 (^{60}Cu)	23.7 min	Cyclotron	93% (0.970 MeV)	γ emission 1332 keV 88% 1791 keV 45.4%	4.4 mm	Diagnostic
Copper-61 (^{61}Cu)	3.33 h	Cyclotron	61% (0.500 MeV)	γ emission 282 keV 12.20% 656 keV 10.77%	2.6 mm	Diagnostic
Copper-62 (^{62}Cu)	9.67 min	Generator/cyclotron	97.83% (1.319 MeV)	γ emission 1172 0.74%	6.6 mm	Diagnostic
Copper-64 (^{64}Cu)	12.7 h	Cyclotron	17.6% (0.278 MeV)	γ emission 1345 keV 0.47% β- emission 0.190 MeV 38.7%	1.4 mm	Diagnostic/therapeutic
Fluoride-18 (^{18}F)	109.7 min	Cyclotron	96.7% (0.249 MeV)	β- emission (0.52 keV)	0.6 mm	Diagnostic
Iodine-124 (^{124}I)	4.17 days	Cyclotron	22.7% (0.820 MeV)	γ emission 0.602 keV 62.9% 1690.9 keV 11.15%	3 mm	Diagnostic
Gallium-68 (^{68}Ga)	67.71 min	Generator	88.91% (0.829 MeV)	γ emission 1077 keV 3.2%	2.9 mm	Diagnostic

Radioactive Cu labeled with ATSM is a very promising PET radiopharmaceutical for hypoxia imaging
Entrapment in the cell reflects the level tissue oxygenation in many tumor types



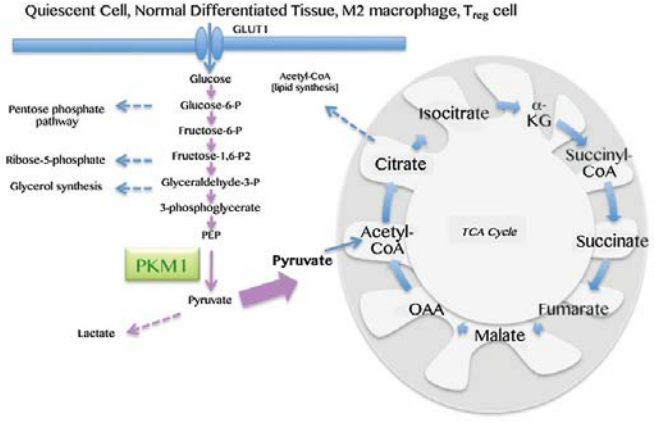
^{18}F-FDG PET

^{64}Cu-ATSM PET

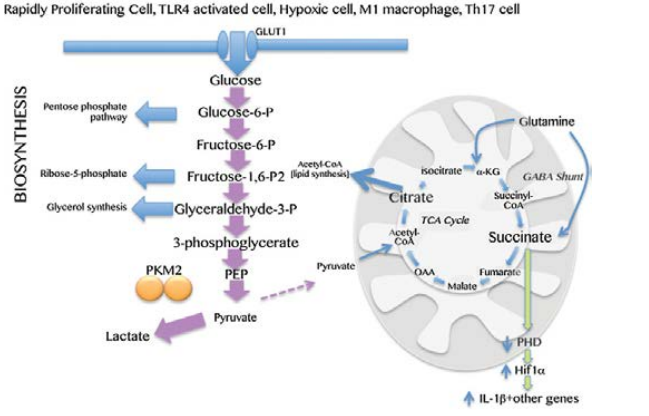
Degree of hypoxia correlates with treatment response

Lopci 2014; A J Nucl Med Mol Imaging 4(4): 365-384

Targeting Tumor Metabolism to Kill Hypoxic Cells – Warburg Effect



Normal resting cells generate ATP through a a combination of glycolysis and mitochondrial respiration by the TCA cycle

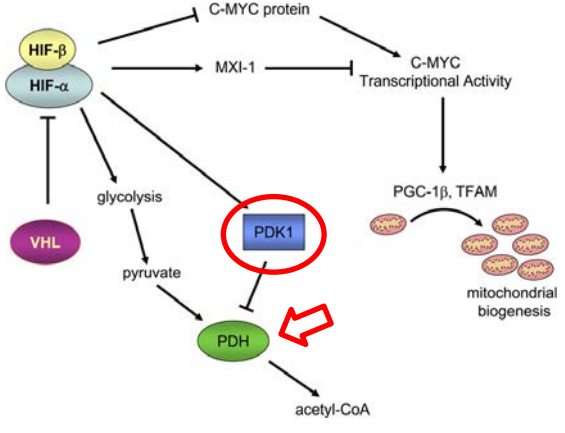


In highly proliferating cells such as tumor cells, ATP and essential components used in biosynthesis are generated by switching metabolism from oxidative phosphorylation to aerobic glycolysis, the so-called **"Warburg effect"**

Increased glycolysis and suppressed mitochondrial glucose oxidation provides cancer cells with a proliferative advantage, conducive with apoptosis resistance and even increased angiogenesis

Targeting Tumor Metabolism

Several studies have implicated a role for HIF-1α as a mediator of the Warburg effect



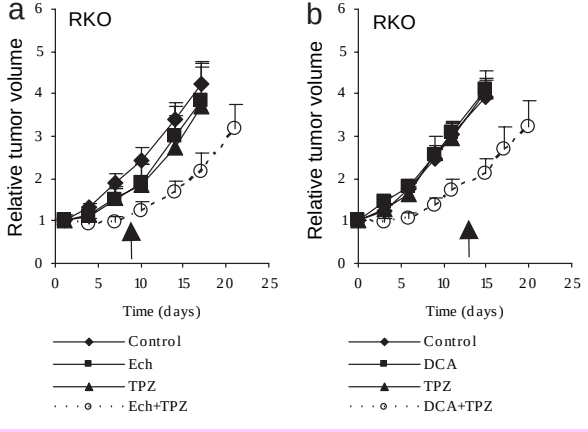
HIF1-α induces pyruvate dehydrogenase kinase 1 (**PDK1**), which inhibits the conversion of pyruvate to acetyl-CoA by blocking the activity of pyruvate dehydrogenase (**PDH**), resulting in diminished mitochondrial respiration, thereby conserving O_2

Inhibition of PDK1 therefore ↑ tumor hypoxia

Hypoxic cytotoxins such as TPZ show increased toxicity as O_2 concentration decreases

⇩

Increasing the number of hypoxic tumor cells by a PDK1 inhibitor should enhance the efficacy of such drugs



Treatment of RKO tumor bearing animals with PDK inhibitors enhanced efficacy of TPZ

Sutphin 2007; Developmental Cell 12: 845-846

Cairns 2007 PNAS 104 (22): 9445-9450

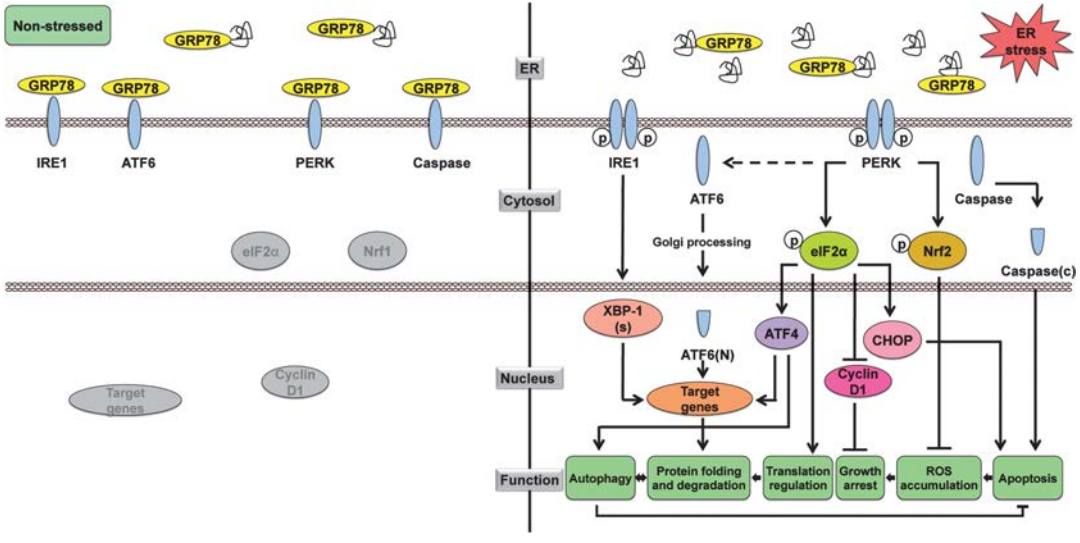
Unfolded Protein Response

Cancer progression is characterized by rapidly proliferating cancer cells that are in need of increased protein synthesis

Therefore, enhanced endoplasmic reticulum (ER) activity is required to facilitate the folding, assembly and transportation of membrane and secretory proteins. These functions are carried out by ER chaperones , such as GRP78

Because of intrinsic alterations of cellular metabolism and extrinsic factors in the tumor microenvironment (such as **prolonged hypoxia**), cancer cells are under ER stress, and they respond to this stress by activating the **unfolded protein response (UPR)**

Unfolded Protein Response



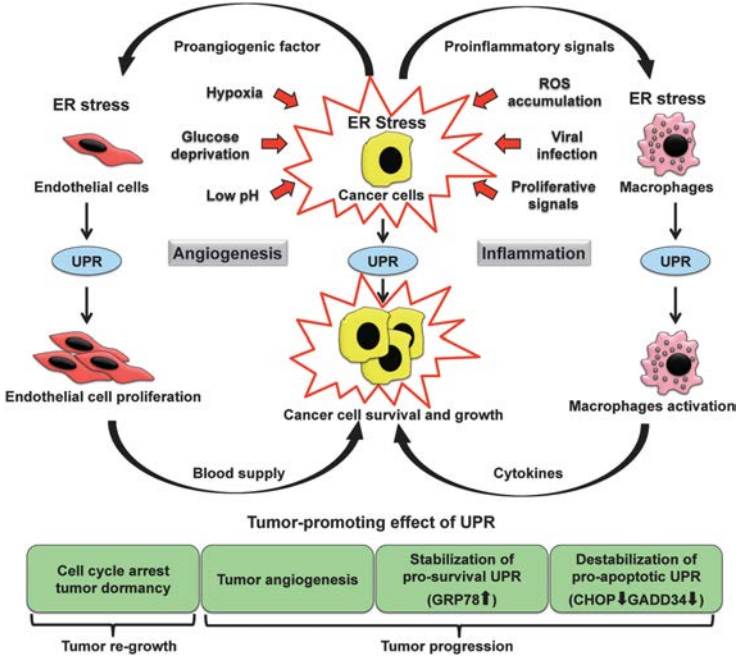
Depending on the severity and duration of ER stress, the signaling branches of the UPR can activate adaptive and pro-survival signals, or induce apoptotic cell death

XBP1 could be inhibited by small molecules that this inhibition resulted in a significant inhibition of tumor growth

When cells are under ER stress, **GRP78** is titrated away through binding to the mis-folded proteins, resulting in the activation of the IRE1α, ATF6 and PERK signaling pathway
IRE1α activates its RNAase activity to cleave the mRNA of XBP1, resulting in spliced form of XBP1
XBP1, ATF6, ATF4 act in concert to induce transcription of target genes mediating protein folding and degradation

Luo & Lee 2013; Oncogene 32: 805-818

Unfolded Protein Response



Adaptive UPR is activated to support tumor cell survival and growth

- Cancer cells are under ER stress because of the growth signaling and factors from the microenvironments
- Cancer cells under ER stress secrete pro-angiogenic factors to stimulate the proliferation of endothelial cells, which in return promotes cancer cell survival and tumor growth
- Cancer cells under ER stress also secrete proinflammatory signals to the stromal cells in the vicinity, mostly tumor-associated macrophages, which in turn are activated and secrete inflammatory cytokines that promote tumor growth, angiogenesis, invasion and metastasis

In summary, perturbance of ER homeostasis has critical roles in tumorigenesis, and therapeutic modulation of ER chaperones and/or UPR components presents potential antitumor treatments.

Question 1

What is the most correct mechanism by which the HIV protease inhibitor nelfinavir may result in radiosensitization?

A. Enhance the effect of ionizing radiation on endothelial cell function
B. Downregulate VEGF expression in tumor cells via hypoxia-inducible factor 1-α
C. Inhibition of the PI3K-AKT-mTOR pathway
D. Activation of the unfolded protein response
E. All of the above