Radiosensitizers, Radioprotectors and Bioreductive Drugs

A. in choosing to combine a radiosensitizer, radioprotector or bioreductive drug with radiotherapy, you are making an inherent assumption: that the tumor contains one or more resistant, dose-limiting subpopulations of cells

1] typically, **the most likely culprits are: inherently radioresistant cells, rapidly proliferating cells and/or hypoxic cells**, and you would select the appropriate chemical modifier depending on which is perceived to be the biggest threat

a) however, a couple of things need to be kept in mind:

1. *seldom is it known exactly which resistant type(s) of cells are present in a particular patient's tumor, either because there's no way to detect such cells (historically the case), that it is impractical to use laboratory-based assays on a regular basis, and/or that such assays have not been fully validated*

2. even if the resistant moiety *is* known, *usually, it still ends up being the response of the critical normal tissue that is ultimately dose-limiting*; hence the goal in developing any new chemical modifier should always be to differentially target the tumor or normal tissue, but not both

3. even *the best chemical modifier in the coherent universe will not work if the resistant subpopulation of tumor cells that it targets is absent from a particular patient's tumor* - ***hence the critical need for biomarkers!***

2] the "lingo" of the chemical modifiers field

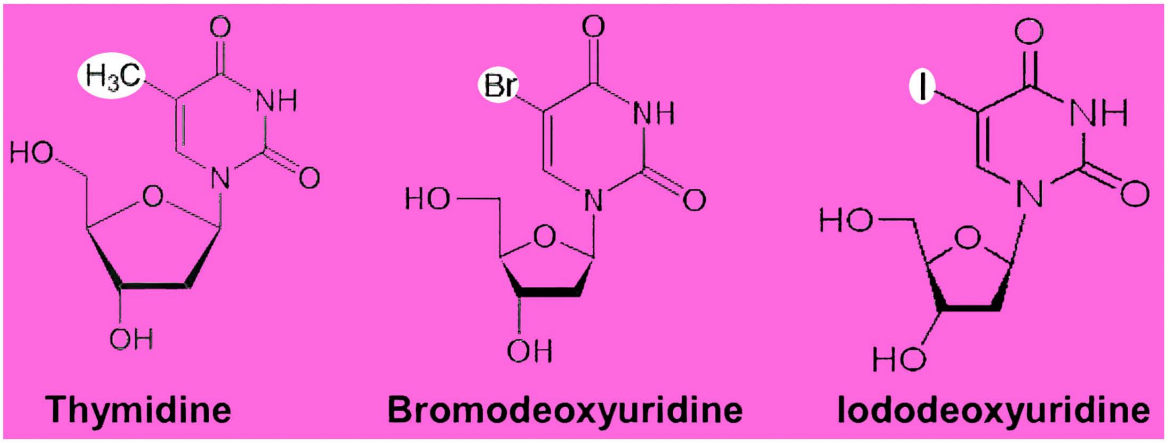
a) there are both generic and specific definitions of terms such as "radiosensitizer" or "radioprotector"

1. an **apparent** radiosensitizer is any drug that, regardless of mechanism of action, when combined with radiation yields a greater effect than the additive toxicities of either treatment given alone...period; this is typically the *clinical* definition of a radiosensitizer (e.g., "concurrent 5-FU or cis-platinum with radiation acts as a radiosensitizer")

2. however, a **true** radiosensitizer meets a stricter criteria, namely that the combination of the drug and radiation also yields a greater-than-additive effect, but that the the chemical modifier alone has little or no toxicity associated with it in the absence of the radiation; this tends to be the *radiobiological* definition (e.g., "misonidazole is a true hypoxic cell radisensitizer")

B. ***Halogenated Pyrimidines*** - (true) radiosensitizers of rapidly proliferating and some inherently radioresistant cells (if such cells also happen to be rapidly-proliferating)

1] a class of drugs known as ***halogenated pyrimidines***, including ***bromodeoxyuridine (BUdR)*** and ***iododeoxyuridine (IUdR)***, have unique chemical structures such that cellular DNA synthesis and repair enzymes think they are the DNA precursor thymidine instead

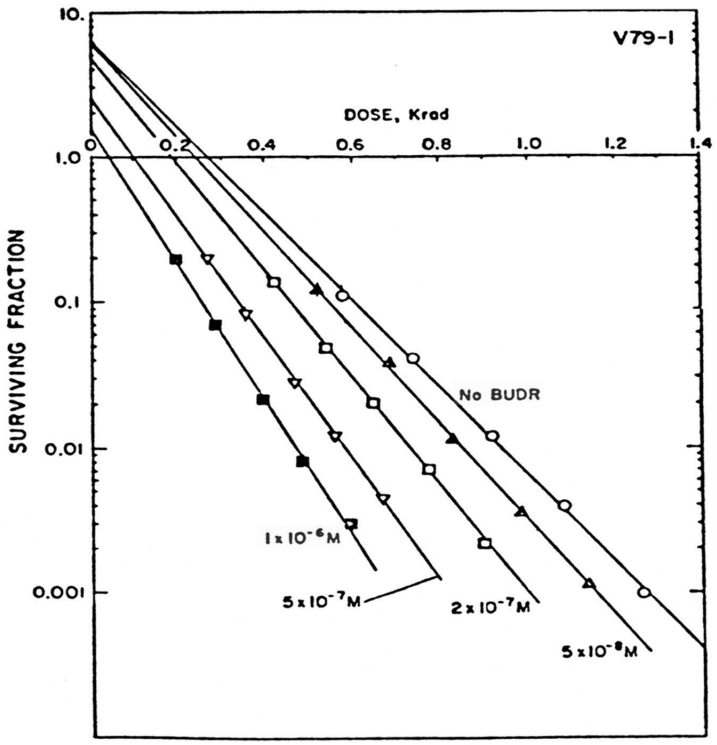


The structures of thymidine, BUdR and IUdR (left to right). Note that the chemical structures are very similar - no wonder the DNA replication machinery gets confused!

a) because of this, any cell that actively goes through S phase in the presence of BUdR (or IUdR) with have the drug inserted into its DNA in place of thymidine; therefore, ***incorporation of the drug only occurs in rapidly proliferating cells...ANY rapidly proliferating cell, normal or tumor***

b) and, as luck would have it, ***the presence of BUdR or IUdR in DNA "destabilizes" it, such that it becomes more susceptible to radiation damage and can't be repaired as well – this causes the cells that take it up to be radiosensitized, increasingly so the greater the amount of drug present in the DNA***

2. because the amount of radiosensitization by halogenated pyrimidines increases with increasing incorporation into DNA, it follows that, for them to be maximally effective as radiosensitizers, ***the drugs must be administered to the patient prior to radiotherapy, and preferably, for as long as practically possible in advance***



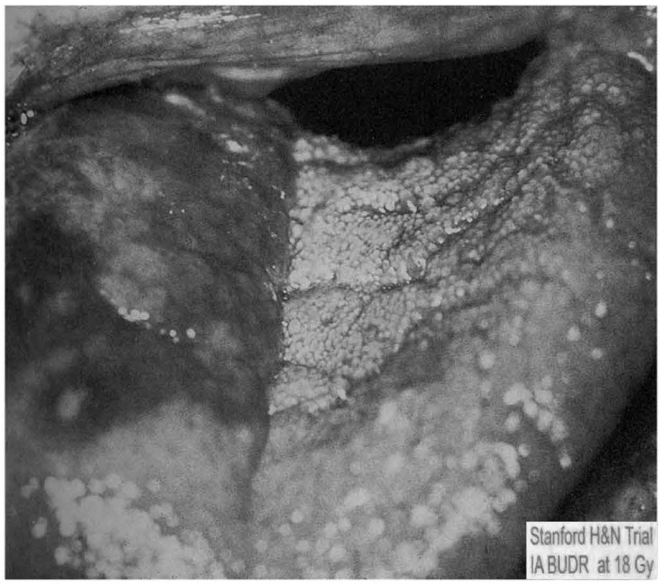
Main effect of incorporating a halogenated pyrimidine into cellular DNA?

A stepwise reduction of the shoulder of the radiation survival curve (as a function of amount of drug incorporated).

X-ray survival curves of *V79-1* cultured cells after growth in BUDR for days. Standard errors in survival are omitted unless larger than the points plotted. (Courtesy of Mohler and Elkind, *Expil. Cell Res.*)

2. clinical trials of halogenated pyrimidines in several tumor types were disappointing - the main problem was that proliferating cells in normal tissues were also sensitized, so there was no therapeutic gain

a) a classic example of this came from a mid-1960's trial at Stanford in patients with head and neck cancer *(in retrospect, not a good choice of site!)*



Photograph of the tongue of a patient at Stanford who received a BrdU infusion in the right carotid artery plus radiotherapy to the entire oral cavity. Note the vigorous mucositis on the right side only (left side of photograph). Courtesy of M. A. Bagshaw and R. L. S. Doggett.

Phillips, Radiation Research *158*: 389-417, 2002

C. **Apparent Radiosensitizers** - several classes of cytotoxic chemotherapy agents also behave as radiosensitizers under certain conditions, although exactly what it is they are "targeting" and how they work often remains unclear

Summary of cytotoxic agents and their mechanisms of action

Class of chemotherapy	Drug example	Mechanism of action
Alkylating agents (cell cycle independent—affect all phases of the cell cycle)	Cyclophosphamide Temozolomide Chloroambucil	Three distinct mechanisms associated with DNA damage: Formation of cross-bridges Mismatch of nucleotides Attachment of alkyl groups to DNA bases
Antimetabolites (affect the S phase of the cell cycle)	Fluorouracil Capecitabine Gemcitabine Pemetrexed Methotrexate	Antimetabolites affect the S phase of the cell cycle by inhibiting the assembly of nucleic acids Classified as: Antifolates Purine analogues Pyrimidine analogues Nucleoside (sugar-modified) analogues
Taxanes (affects G2/M phase of the cell cycle)	Paclitaxel and Doxetaxel	Bind to the β subunits of tubulin Results in: An increase of tubulin polymer mass Formation of microtubule bundles Inhibit microtubule depolymerisation

See Appendix for a list of *all* classes of chemotherapy agents and their mechanisms of action

2] in fact, there are apparent radiosensitizers that aren't even chemotherapy drugs at all!

a. Case in point: the (mostly ill-fated) story of metformin as a radiation sensitizer

(1) why would **metformin**, a diabetes drug, be a radiosensitizer?

a] the best explanation is that metformin reduces insulin signaling through the PIK3 and RAS signaling pathways, thus (indirectly) impairing cancer cell growth and proliferation

(2) based on observational studies suggesting improved overall survival in cancer patients taking metformin (because they had diabetes) concurrent with radiation and/or chemotherapy, clinical trials were initiated in lung and breast cancer; the results:

a] metformin did nothing for patients who didn't already have diabetes

b] among those who did have diabetes, the only cohort that showed any improvement was a small (~15%) subset of women with HER-2 disease

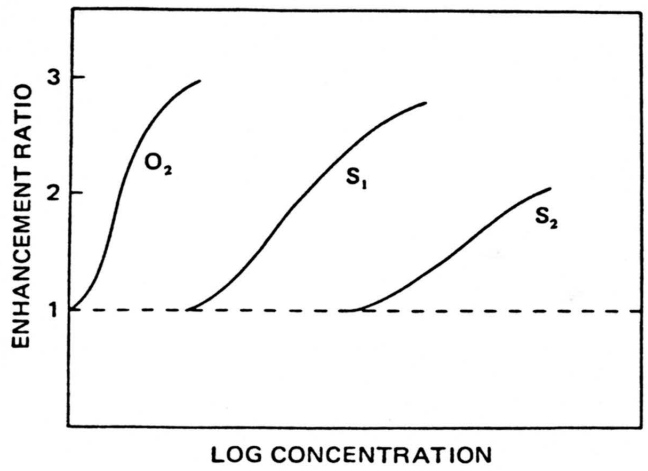
D. Hypoxic Cell Radiosensitizers

hypoxic cells were always an attractive target for cancer drug development thanks to the built-in tumor specificity (i.e., normal tissues generally do not contain hypoxic cells), and the concern that even a tiny fraction of clonogenichypoxic cells in a tumor would render it effectively incurable with radiation therapy

1] What's the best hypoxic cell radiosensitizer there is, hands down? Answer: **Oxygen!**

a. but what is meant by "best"?

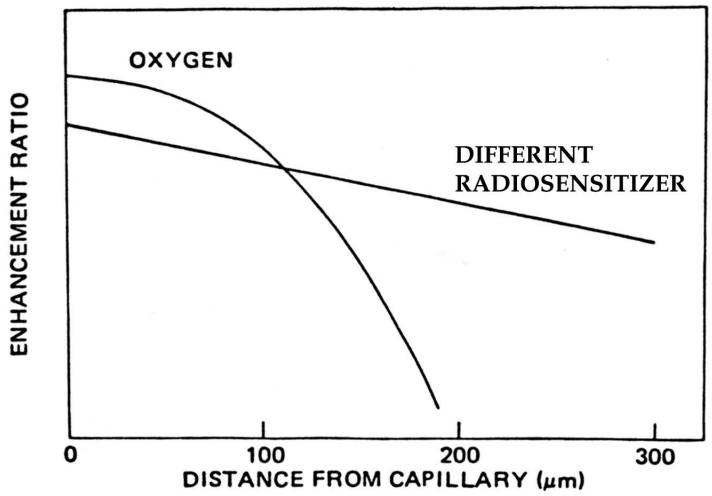
1) ***"best" usually refers to sensitizer EFFICIENCY, that is, which sensitizer produces the most sensitization for the smallest administered dose***



Demonstrating the concept of sensitizer efficiency.

In comparison to two other putative hypoxic cell radiosensitizers S1 and S2, oxygen is clearly the most efficient, that is, produces a high degree of radiosensitization at very low concentrations.

2) however, ***oxygen's problem is that it is not the most EFFECTIVE sensitizer; in other words, it is very rapidly consumed by cells for other purposes (respiration and metabolism), such that, good as it is, it does not penetrate very far into tumor tissue, and many cells are therefore left oxygen-deprived (and radio-resistant)***



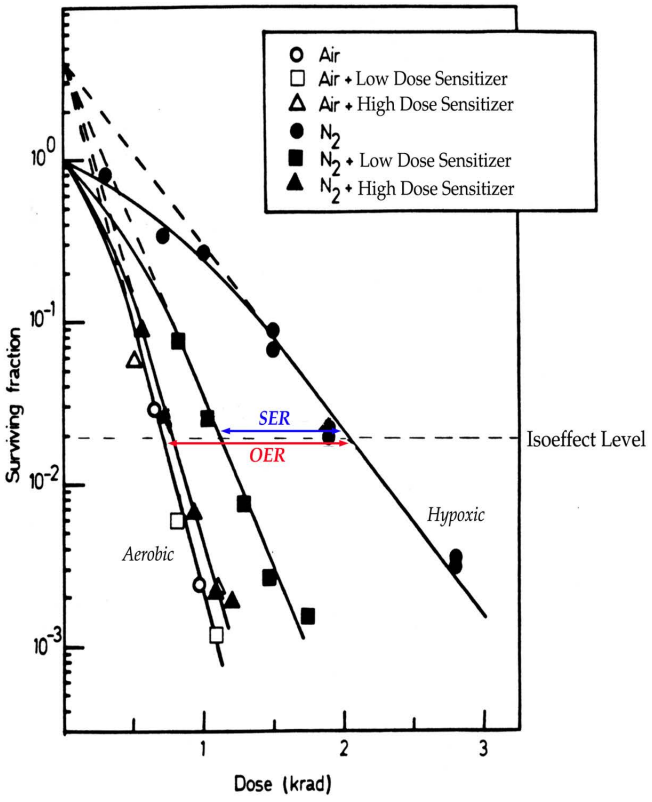
Demonstrating the concept of sensitizer effectiveness.

The rapid consumption of oxygen for other cellular processes means that it cannot reach many cells; these become hypoxic and radiation resistant.

Therefore, the development of *novel* sensitizers that are NOT consumed and can reach further into tumor tissue may be more desirable clinically for combating resistance, *even if such drugs are nowhere near as efficient as oxygen.*

b. this being the case, how is the net goodness or badness of a particular hypoxic cell sensitizer measured radiobiologically?

1) Answer: the **Sensitizer Enhancement Ratio or SER**, almost identical in concept to the oxygen enhancement ratio



Survival data for aerated and hypoxic Chinese hamster cells x-irradiated in the presence of various concentrations of misonidazole (Ro-07-0582). At a concentration of 10 mM of this drug the radiosensitivity of hypoxic cells approaches that of aerated cells. The response of aerated cells is not affected by the drug at all. (From Adams GE, Flockhart IR, Smithen CE, Stratford IJ, Wardman P, Watts ME: Radiat Res 67:9–20, 1976)

$$\text{OER} = \frac{\text{Dose of radiation under hypoxic conditions}}{\text{Dose of radiation under aerobic conditions}}$$

...to yield the same biological endpoint

$$\text{SER} = \frac{\text{Dose of radiation under hypoxic conditions}}{\text{Dose of radiation under hypoxic conditions, and in the presence of a radiosensitizer}}$$

...to yield the same biological endpoint

Hypoxic Cell Radiosensitization Strategy #1: Deliver More Oxygen to the Tumor

1] Many attempts have been made over the last 50+ years to radiosensitize hypoxic cells by trying to "re-oxygenate" them; although a few clinical trials using such methods have been successful in specific sites, most have met with failure overall.

a. **Pre-radiotherapy correction of patient anemia** - using blood transfusions

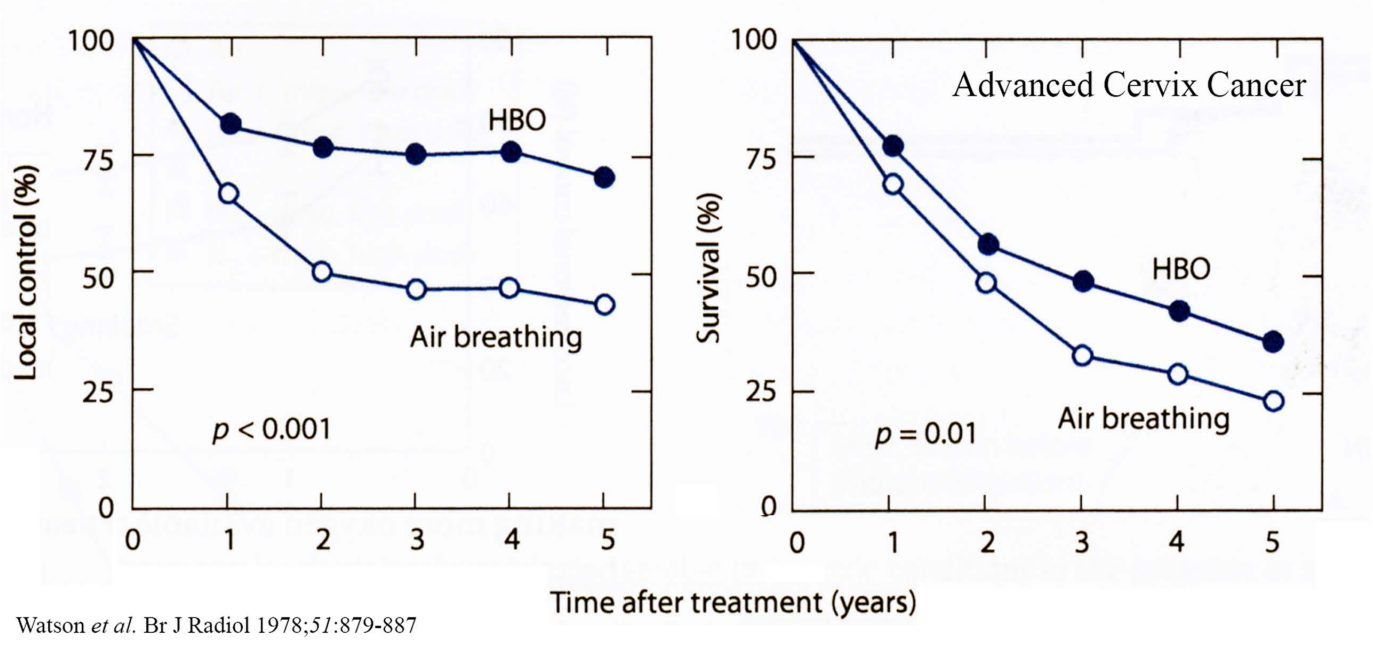
1) which tumor sites did anemia correction yield *some* improvement in treatment outcome?

Cervix and Head and Neck

b. **Hyperbaric oxygen breathing during most or all radiotherapy treatments** - cumbersome, complicated dosimetry, claustrophobic and explosion hazard

1) which tumor sites did **HBO breathing** yield *some* improvement in treatment outcome?

Cervix and Head and Neck

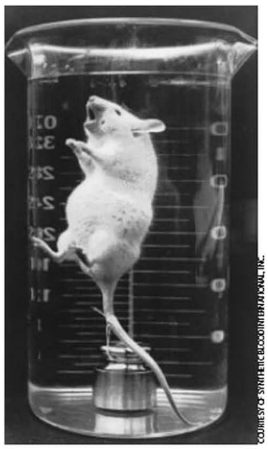


Watson *et al.* Br J Radiol 1978;*51*:879-887

c. **Use of artificial blood substitutes with higher-than-hemoglobin oxygen carrying capacity** - the lead compound of this class is called Fluosol-DA®, a perfluorochemical emulsion (chemically related to Teflon!) that can be transfused in place of normal blood during the entire course of radiotherapy

1) Fluosol is capable of carrying so much oxygen in fact, that it can actually be breathed...even though it's a liquid

2) Clinical studies with Fluosol? The side effects profile (liver toxicity in particular) was discouraging for anything more than a one-time use, and it would have required a several-time use in order to produce any tumor radiosensitization



Obviously willing rodent simply thrilled to be submerged in Fluosol and allowed to breath it for extended periods.

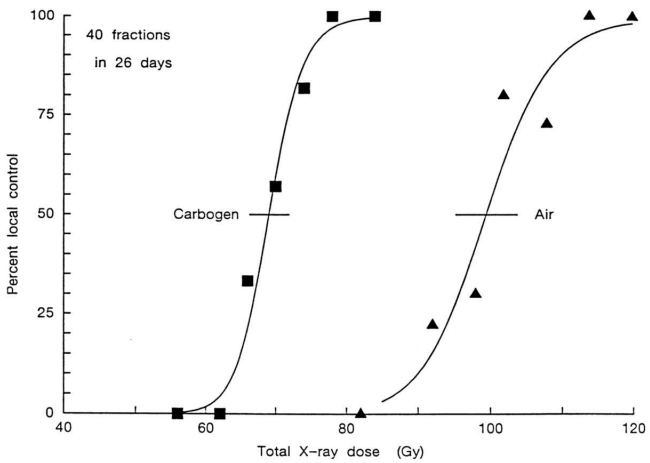
Supposedly, has also been used by US Navy Seals during deep dive submarine rescue and salvage missions. Newsflash: they don't like it either.

Pop culture reference: "The Abyss"

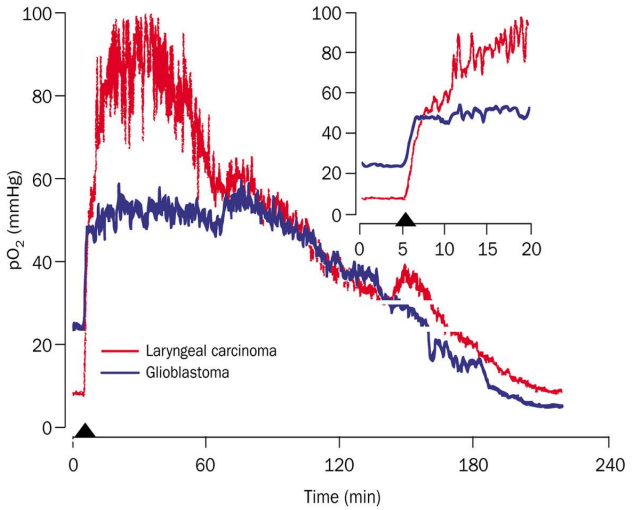
d. **Use of combined high O_2 content breathing with vasoactive agents** - in the hopes of combating both chronic and intermittent hypoxia via improving tumor blood flow

(a) ***carbogen*** is a gas mixture composed of 95% oxygen and 5% CO_2 that helps deliver more oxygen to chronically hypoxic cells in tumors, both by carrying more oxygen than room air, and by increasing blood flow somewhat; patients breath carbogen via a face mask attached to a gas cylinder for a few minutes before, and during, each radiation fraction

(b) ***nicotinamide*** is a B vitamin and nutritional supplement that in higher doses increases tumor blood flow to intermittently hypoxic cells



Dose–effect curves for mouse mammary tumours given 40 fractions of radiotherapy in 26 days to CBA mice breathing either (▲) air or (■) carbogen. (Redrawn from Rojas et al, 1996, by permission of Elsevier Science Inc., 655 Ave. of the Americas, New York NY 10010-5107.)



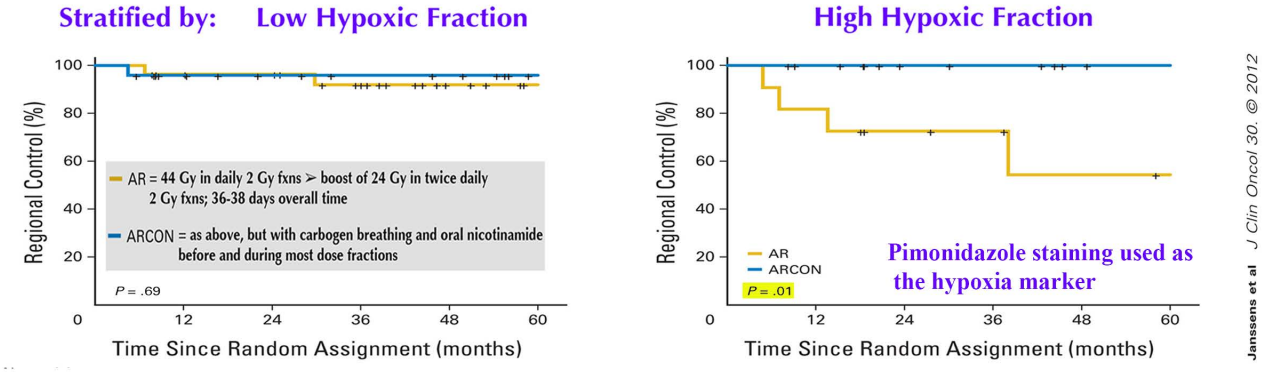
THE LANCET Oncology Vol 3 December 2002

Continuous pO_2 measurements by use of a fibreoptic probe with luminescence-based optical O_2 sensor. Measurements in two human tumours xenografted in nude mice during inhalation of carbogen: squamous-cell carcinoma of the larynx (red) and glioblastoma (blue). Triangle indicates start of carbogen breathing. Several minutes are required before highest pO_2 values are reached. After 1 h of inhalation of carbogen, there is a decrease in tumour oxygenation.

1) randomized clinical trials that tried the carbogen + nicotinamide approach to sensitizing hypoxic cells in tumors, WITH pre-stratification of tumors into low and high hypoxic fraction groups:

ARCON:

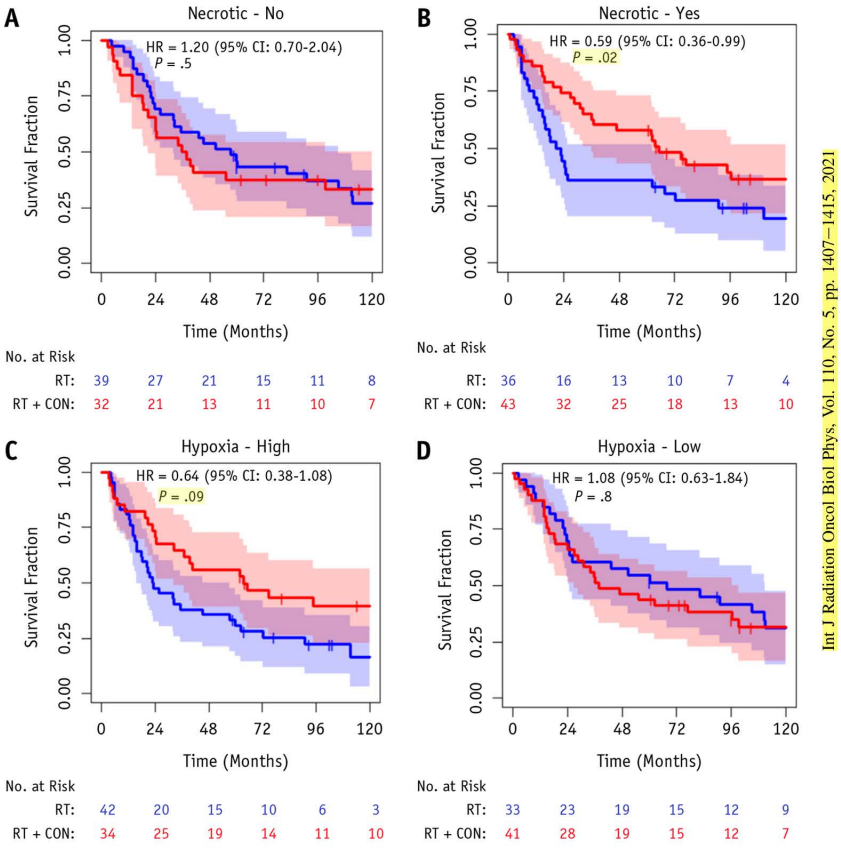
T2-4 SCC of the Larynx/Glottis
~350 patients, Phase III



ARCON ("accelerated radiotherapy with carbogen and nicotinamide") significantly improved 5 year regional control in patients with larynx/glottic cancer...but only in tumors preselected as having a high hypoxic fraction

BCON:

T2-4a muscle-invasive, and high grade non-muscle invasive, bladder cancer
333 patients, Phase III radomized trial across 3 institutions



"Hypoxia" determined in two ways:

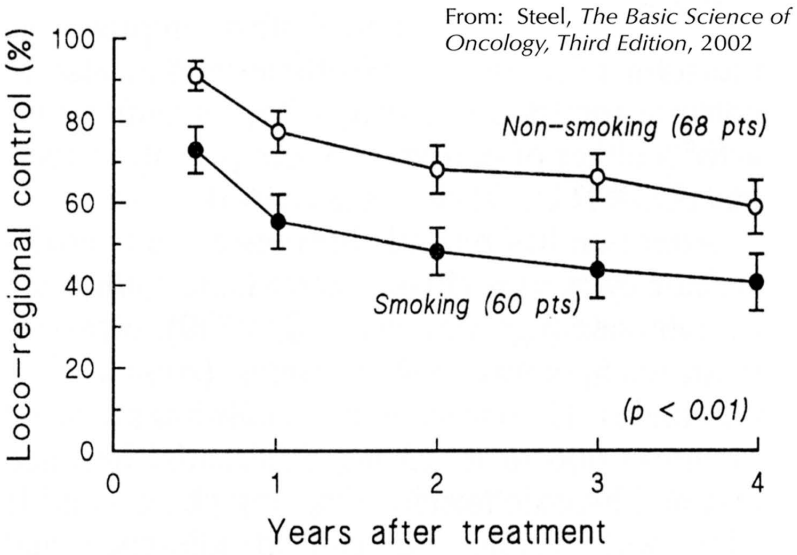
1) by using tumor necrosis as a surrogate for hypoxia; and
2) based on a 24 gene hypoxia signature

<u>Radiation only group</u> = 64 Gy in 32 fractions over 6.5 weeks, or 55 Gy in 20 fractions over 4 weeks

<u>Carbogen + nicotinamide group</u> = as above, but with oral nicotinamide given 1.5-2 hours before each dose fraction, and carbogen breathing for 5 min before and during each dose fraction

Improved overall survival noted in the two groups deemed "more hypoxic"

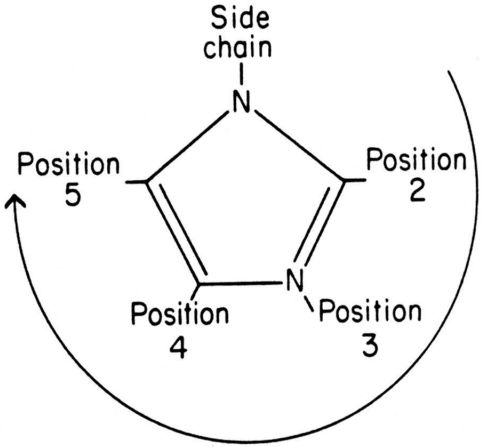
e. also worth noting is that activities that ***reduce*** oxygen delivery to tumors – like continuing to smoke during radiotherapy – can have a negative impact on treatment outcome



Influence of smoking during treatment on the outcome of radiotherapy in patients with advanced head and neck carcinoma. The local control probability was significantly poorer in patients who continued to smoke during radiotherapy, probably due to reduced oxygen delivery to the tumour. Results from a prospective study in patients treated with curative radiotherapy alone

Hypoxic Cell Radiosensitization Strategy #2: Oxygen-Mimetic (True) radiosensitizers

1] the most studied class of compounds that mimics oxygen's ability to "fix" radiation damage, while at the same time NOT being rapidly consumed by cells for other purposes, are the ***nitroimidazoles***

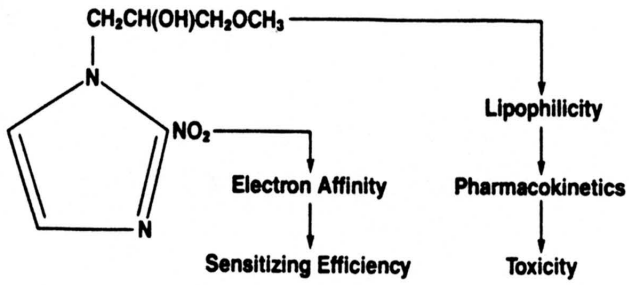


Generalized chemical structure of the nitroimidazoles.

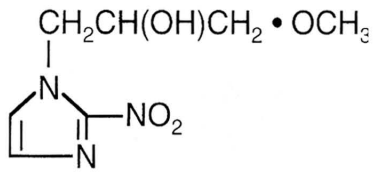
The chemical position of the nitro group ($-NO_2$) on the imidazole ring determines the degree to which these drugs radiosensitize hypoxic cells by fixing radiation damage. The position of the nitro group confers a chemical property called "electron affinity" to the compound, and this property in turn determines how likely it is to participate in free radical reactions.

It was determined early on that 2-nitroimidazoles (example: misonidazole) are superior hypoxic cell radiosensitizers to 5-nitroimidazoles (example: metronidazole).

Meanwhile, the composition of the molecule's carbon and oxygen side chain determines its relative solubility in either water (hydrophilicity) or lipids (lipophilicity). This in turn affects the drug's pharmacokinetics *in vivo*.

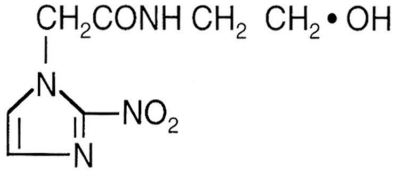


a. although historically, the first nitroimidazole tested for hypoxic cell radiosensitization was metronidazole, ***the most widely studied nitroimidazole, both in the laboratory and clinic, was misonidazole;*** further, as we learned more and more about the action of these drugs, newer, "tweaked" versions engineered for specific properties were likewise synthesized and tested



Misonidazole

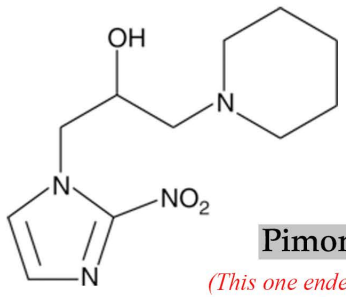
The "first generation"



Etanidazole

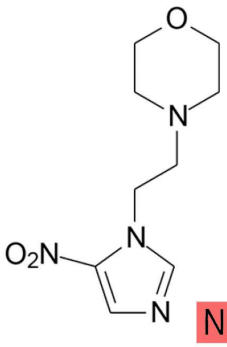
The "second generation"

The "third generation"



Pimonidazole

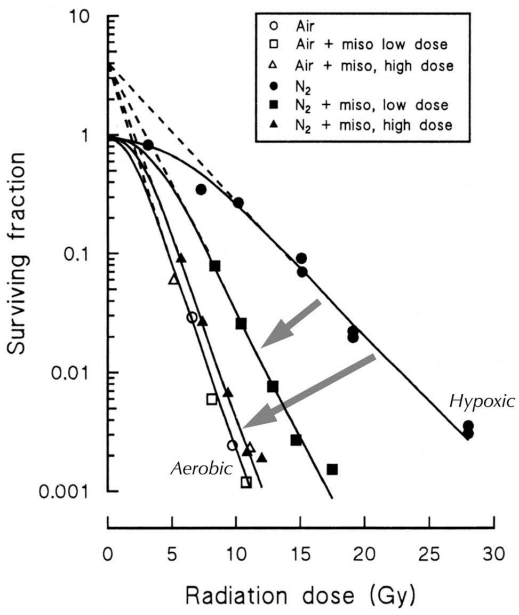
(This one ended up working much better as a hypoxia marker than a hypoxic cell radiosensitizer!)



The "fourth generation"
(and the only one still in use clinically...in Europe)

Nimorazole (Note: 5-nitroimidazole)

b. pre-clinical results with misonidazole were very encouraging, so hopes were high that it would turn out to be a blockbuster in the clinic...



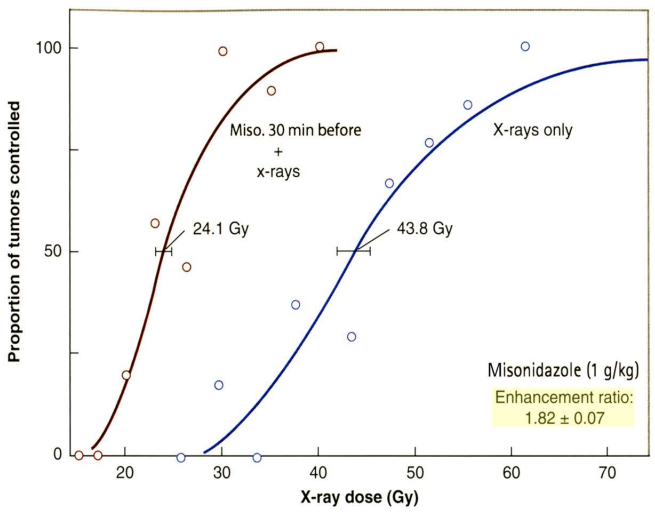
Survival curves for aerated and hypoxic Chinese hamster cells irradiated in the presence or absence of misonidazole. Low dose: 1 m*M*; high dose: 10 m*M*.

Miso was very effective at sensitizing hypoxic cells maintained in culture. It caused no change in aerobic cell radio-sensitivity (as expected), and for hypoxic cells, yielded an SER comparable to the OER at a concentration of 10 mM.

Miso also gave good-to-excellent SER's for experimental mouse tumors *in vivo*.

(One caveat though, somewhat lost in the excitement at the time: look at the dose of miso used, that is, 1 mg/g...do the math and figure out how much that would correspond to for a typical human being.)

Br. J. Cancer *30*: 560-565,1974



...unfortunately, not only did miso NOT turn out to be a blockbuster in the clinic, it was, by most accounts, a total dud, except in a few notable cases

1. at best, only about 8 out of 39 prospective clinical trials worldwide in various tumor sites even hinted at a benefit for misonidazole, and only 4 showed a statistically significant improvement

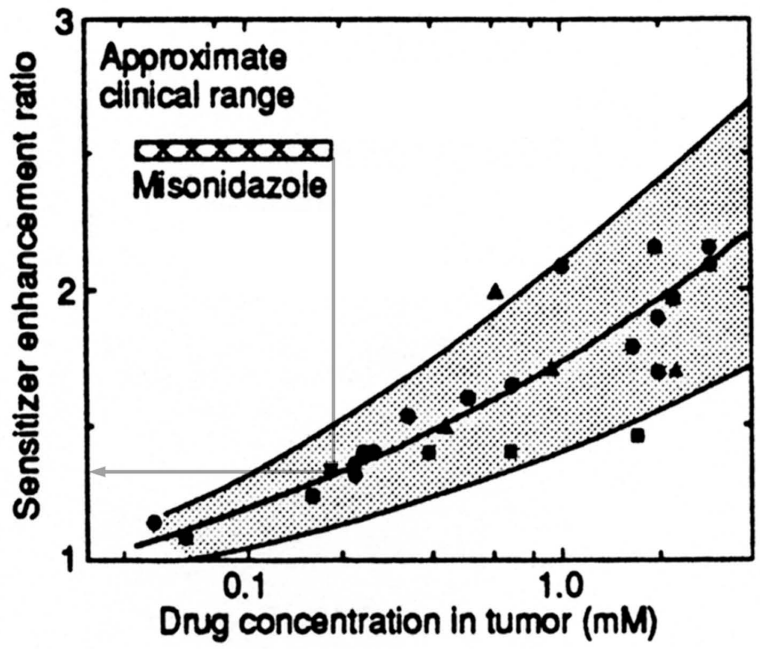
a) which tumor sites benefited from the use of misonidazole in combination with radiotherapy?

Cervix and Head and Neck

c. **the "post-mortem" on misonidazole began promptly as soon as the clinical trial results began to trickle in...what had gone wrong?**

1. Problem #1 - **no pre-selection of patients with tumors known to contain hypoxic cells and therefore most likely to benefit; the technology was simply not available at the time**

2. Problem #2 (the main one) - **the concentrations of miso achievable clinically were severely limited by an unexpected normal tissue toxicity noted in humans, but undetectable in the animal models, namely *peripheral neuropathy***

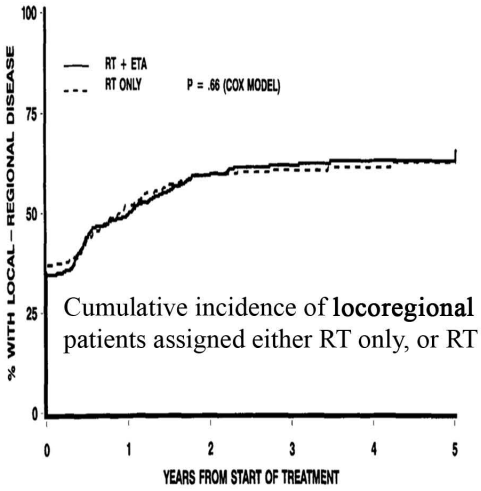


Sensitizer enhancement ratios (SER), obtained for a number of different animal tumors treated with single radiation doses, plotted as a function of the sensitizer concentration in the tumor.

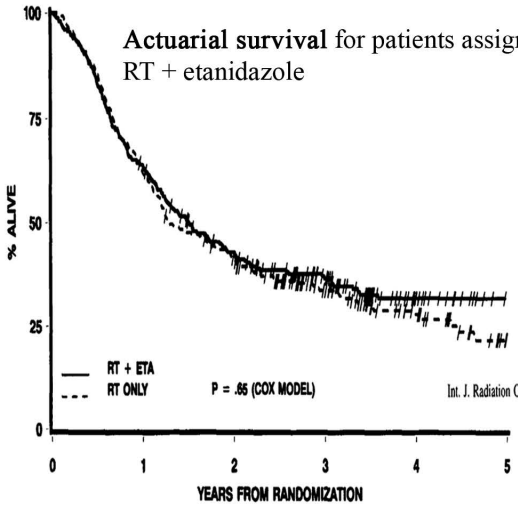
The line is drawn to pass through the data for misonidazole and the shaded region represents the range of values of SER observed for sensitization of hypoxic cells in vitro. The approximate ranges of drug concentrations that are obtainable in human tumors are illustrated by the bars. Modified from Hill (1986)

a) what to do about the peripheral neuropathy problem?

(1) Answer: design new nitroimidazoles that are *less* lipophilic (by changing the compounds' side chains), and therefore less likely to concentrate in neural tissues and cause neuropathy



Cumulative incidence of **locoregional failure** for patients assigned either RT only, or RT + etanidazole

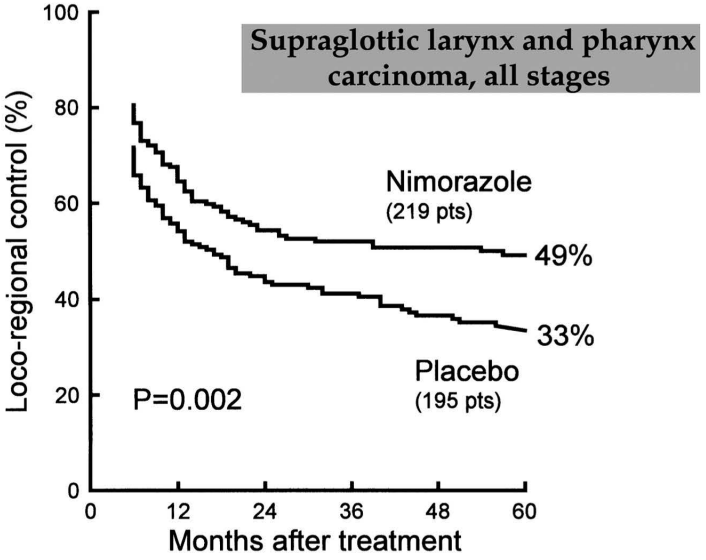


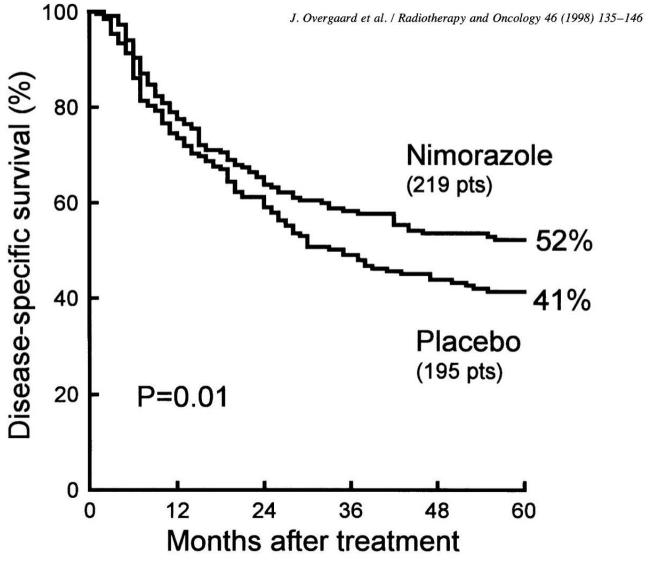
Actuarial survival for patients assigned either RT only, or RT + etanidazole

Int. J. Radiation Oncology Biol. Phys., Vol. 32, No. 3, pp. 567–576, 1995

The bad news was that a large RTOG trial comparing radiotherapy plus etanidazole to radiotherapy alone for patients with advanced head & neck cancer showed no difference between treatment arms. ***This negative clinical trial effectively hammered the final nail in the coffin of hypoxic cell radiosensitizers in the United States.***

That said, the trial did demonstrate that "engineering" nitroimidazoles to be less lipophilic *did* result in a significant decrease in the incidence and severity of peripheral neuropathy. This served as a useful proof of concept (if nothing else).





The good news was that the addition of the drug nimorazole to radiotherapy (or chemorads) significantly increased both local control and disease-specific survival in patients with supraglottic larynx and pharynx cancer.

Today, nimorazole is the standard care for the treatment of these cancers in most of Europe, especially Denmark, where these trials were initially conducted (the DAHANCA 5 trial).

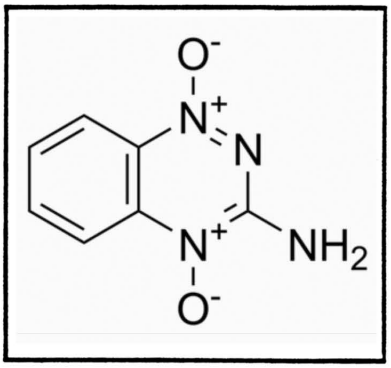
Hypoxic Cell Radiosensitization Strategy #3: Forget about sensitizing hypoxic cells, just kill them outright!

1] compounds with the property of being selectively toxic to hypoxic cells are termed ***bioreductive drugs***; these can be considered apparent hypoxic cell radiosensitizers, because they have the net effect of eliminating an otherwise radioresistant population of tumor cells, making the tumor as a whole more sensitive to ionizing radiation

a. a few classes of drugs have been identified that have the ability to kill hypoxic cells, although they vary in both potency and selectivity for hypoxia versus aerated cells; ***they are called "bioreductive" because their action depends on the drug being metabolized to a toxic intermediate only under hypoxic conditions***

1) both the nitroimidazoles and several quinone antibiotics (e.g., mitomycin C) have this property, but they are not particularly potent, and are only a few-fold selective for hypoxic cells

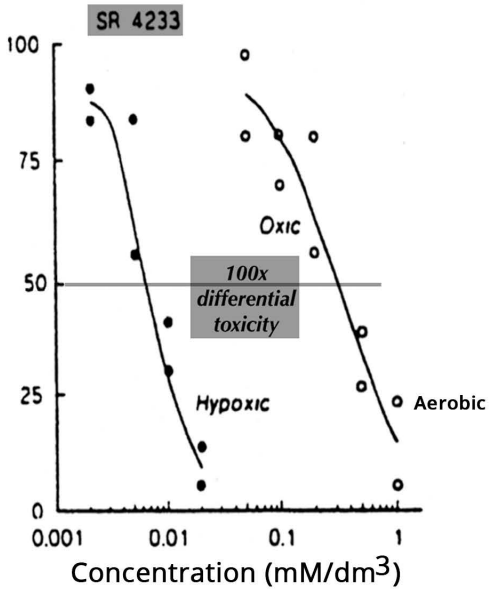
b. the lead compound of this series of bioreductive drugs is an organic nitroxide called ***tirapazamine***



3-amino-1,2,4-benzotriazine 1,4-dioxide

(old name: SR 4233; trade name: Tirapazamine)

1) in preclinical studies, tirapazamine showed very high selective toxicity to hypoxic cells in culture



Cancer Res 2008; 68: (1). January 1, 2008

TPZ

↓ *bioreduction to toxic intermediate*

Single-strand breaks,
base damage,
DNA-protein cross-links

↓ *interferes with replication/repair*

Stalled or collapsed replication forks
Topoisomerase I and II poisoning

↓ *damage sites remain "open"*

DSBs

Tirapazamine's mechanism of action involves bioreduction under hypoxic conditions to a toxic, oxidizing free radical that directly or indirectly (by poisoning Topo II) produces DNA double strand breaks.

In the presence of oxygen, this toxic free radical is imediately back-oxidized to the far less toxic parent compound...hence its lack of much toxicity toward well-aerated cells.

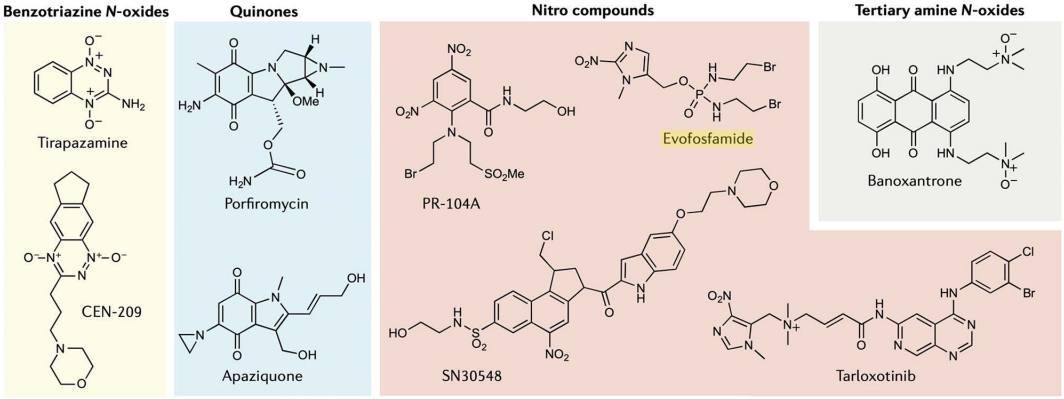
c. Clinical Experience with Tirapazamine

1. the results of a number of Phase I and II clinical trials combining radiation, cisplatin or gemcitabine, and tirapazamine seemed promising in advanced H&N cancer, advanced or recurrent cervical cancer, and in small and non-small cell lung cancer (but not glioblastoma)

2. **Phase III trials with tirapazamine in combination with radiation, chemotherapy or both were largely a bust however, but again, there was no preselection of tumors assessed as "significantly hypoxic"**

d. Tirapazamine: The Next Generation

1. the development of new bioreductive drugs has continued despite tirapazamine going belly-up; in particular, research has focused on "hypoxia-activated prodrugs (HAPs)", i.e., bioreductive drugs that are only metabilized under hypoxic conditions, but that then release a secondary payload, such as an alkylating agent



Structures of widely studied examples of the main HAP classes. The initial radicals of benzotriazine N-oxides such as tirapazamine and CEN-209 fragment to release cytotoxic oxidizing radicals, whereas fragmentation of the nitro radicals of evofosfamide and tarloxotinib generates a DNA-crosslinking agent and a pan-ErbB inhibitor, respectively.

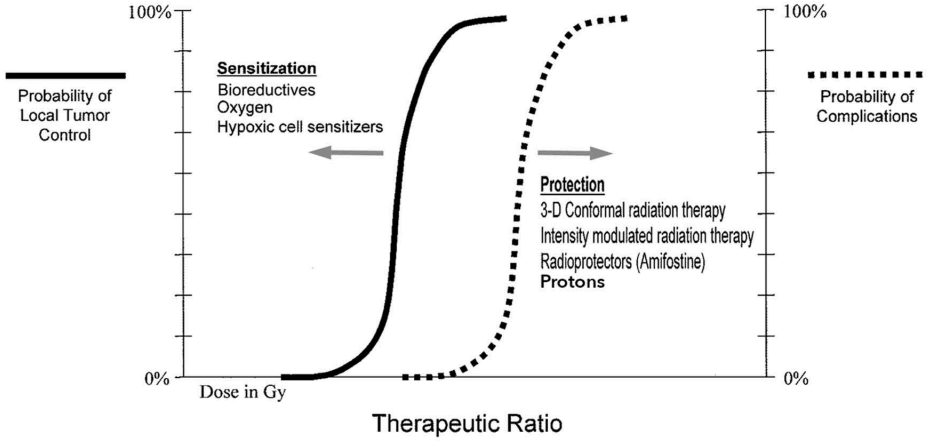
https://doi.org/10.1038/ s41571-021-00539-4

E. **Normal Tissue Radioprotectors** - yet *another* approach to coping with tumor resistance

1] taking the hint from the natural radioprotectors in cells themselves, principally glutathione, it was reasoned that other thiols (compounds containing an -SH or sulfhydryl group) might increase radioprotection if added directly to cells

a) if so, it would be possible to radioprotect normal tissues, i.e., equalize the radiosensitivity of normal tissues and resistant, hypoxic cells; this would allow higher radiation doses to be given, without having to worry as much about normal tissue complications

American Journal of Clinical Oncology • Volume 26, Number 5, October 2003



The sigmoid curves represent tumor control probability and normal tissue complication probability. Below a minimum level of radiation, tumor control probability and normal tissue complication probability are low. There is a steep dose response for tumor control, increasing from 10% to 90% over 5 to 20 Gy. A similarly steep response occurs for normal tissue complication probability. The goal is to spread these curves (open the therapeutic window) by using better schedules of cytotoxic drugs, biologic modifiers of radiation response, and by the incorporation of better image guided treatment techniques.

2] many classes of thiols were studied by the Army (hence the designation "WR" for the drugs, which stands for "Walter Reed Army Hospital"), and the best class of agents turned out to be the aminothiols (which contained an NH_2 group as well as an SH group)

a) the lead compound of this series of drugs is called ***Amifostine*** (pre-clinical designation: WR-2721); it need only be present at the exact time of irradiation to work, in keeping with its free radical scavenging mechanism of action (as such, it's also a "true" radioprotector)

$$NH_2 - (CH_2)_3 - NH - (CH_2)_2 - SH_2\,PO_3$$

Drug is metabolically dephosphorylated to "release" the sulfhydryl group, which is the part of the molecule responsible for radical scavenging

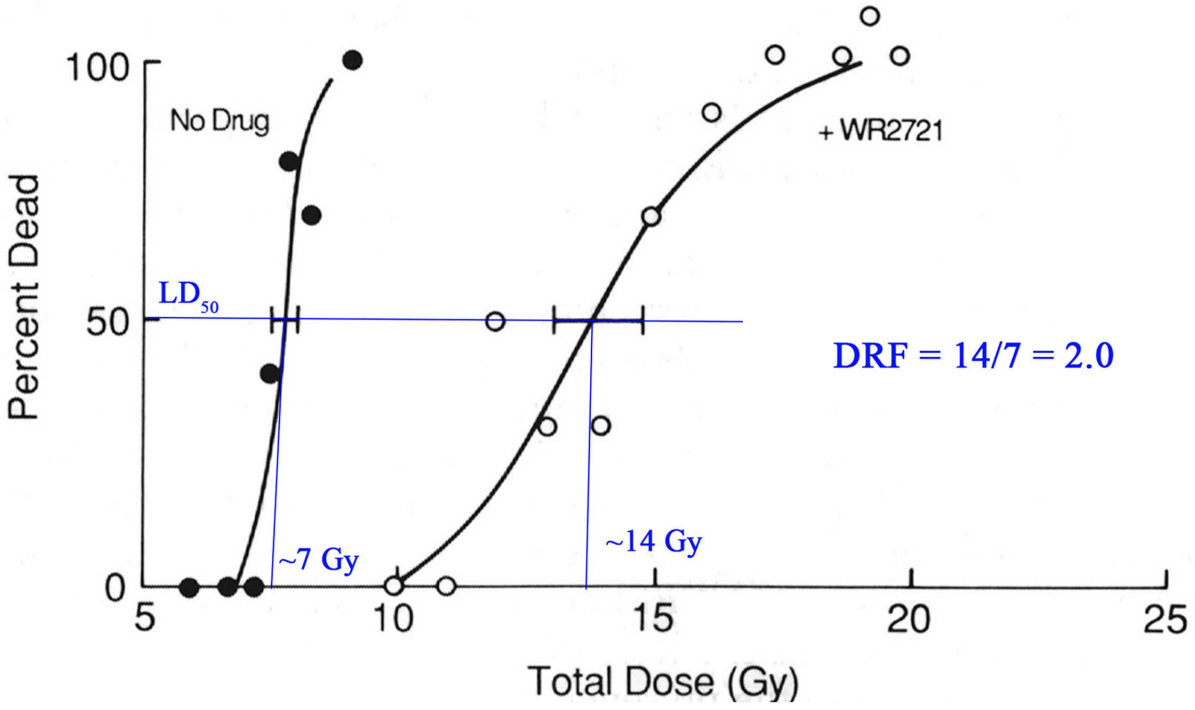
Three Protectors in Practical Use

COMPOUND	STRUCTURE	USE
WR-638	$NH_2CH_2CH_2SPO_3HNa$	Carried in field pack by Russian army (Cystaphos)
WR-2721	$NH_2(CH_2)_3NHCH_2CH_2SPO_3H_2$	Protector in radiotherapy and carried by US astronauts on lunar trips (amifostine)
WR-1607	$CH_3(CH_2)_9NHCH_2CH_2SSO_3H$	Marketed as rat poison (d-CON)

3] How is the extent of radioprotection quantified *in vitro* and *in vivo*? Answer: with another dose ratio, this time called the "dose reduction factor" or DRF

$$DRF = \frac{\text{Dose of radiation in the presence of the drug}}{\text{Dose of radiation in the absence of the drug}}$$

...to yield the same biological endpoint



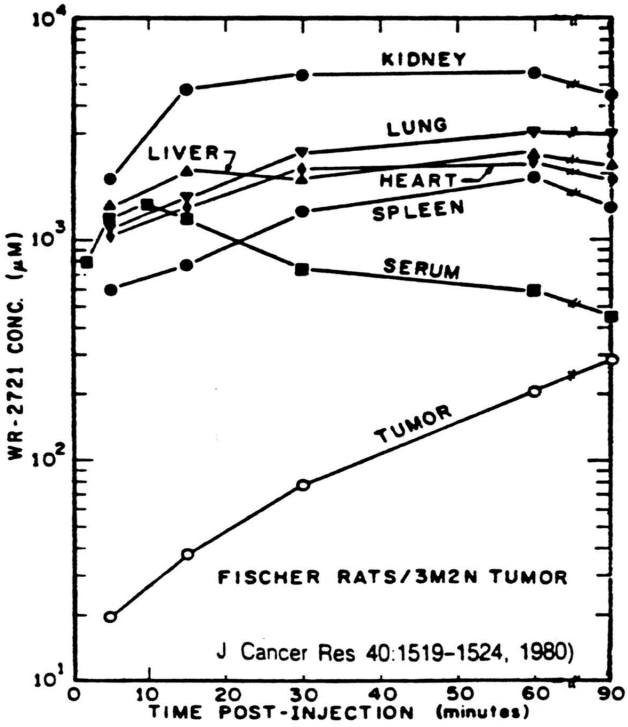
Protection Factors Achieved by Amifostine in Different Normal Tissues

Tissue	Protection Factor
Salivary gland	2.3-3.3
Bone marrow	1.8-3.0
Jejunum	1.5-2.1
Skin	1.4-2.1
Testis	1.5-1.6
Kidney	1.3-1.5
Bladder	1.3-1.5
Lung	1.2-1.4
Heart	>1.0

The CNS is **not** protected by amifostine, which is particularly unfortunate, as that's a tissue we'd really want to protect against radiation injury.

Problem? Presumably, it doesn't get through the blood-brain barrier.

4] How do you achieve a therapeutic gain when amifostine presumably protects tumor as well as normal tissues?



Serum, tissue, and tumor concentration of the radioprotector Amifostine (WR-2721) as a function of time after intraperitoneal administration of the drug (200 mg/kg).

Two main mechanisms of action account for amifostine's selectivity in protecting normal tissues but not tumors:

1. **The pharmacokinetics of amifostine are such that it has rapid (within ~10 minutes) uptake in most normal tissues, but much slower uptake in tumors. This provides a window of opportunity to deliver radiotherapy after normal tissues are protected, but before tumors are.**

2. **In general, normal tissues have higher concentrations of the phosphatases that convert amifostine to its active metabolite (WR 1065) than tumors do.**

5] Clinical Experience with Amifostine

a. ***the main indication for the use of amifostine in the clinic is to reduce the incidence of xerostomia in patients receiving radiotherapy to the salivary glands,*** although it also shows activity against some other typical normal tissue complications, e.g., dysphagia, pneumonitis, *but with no protection of tumors*

b. not unlike the case of tirapazamine sensitizing tumors to certain chemotherapy agents, ***amifostine likewise protects normal tissues (especially bone marrow) against chemotherapy injury, particularly for cis-platinum and cyclophosphamide***

c. in the case of radiotherapy though, amifostine isn't used much anymore, thanks to the advent of IMRT, which better spares salivary gland tissue, so the drug usually isn't needed

F. Apparent Radioprotectors (aka Radiation Mitigators aka Biological Response Modifiers)

1) in recent years, it has become clear that there are other classes of radioprotectors besides the thiols, and that these work by mechanisms of action other than free radical scavenging

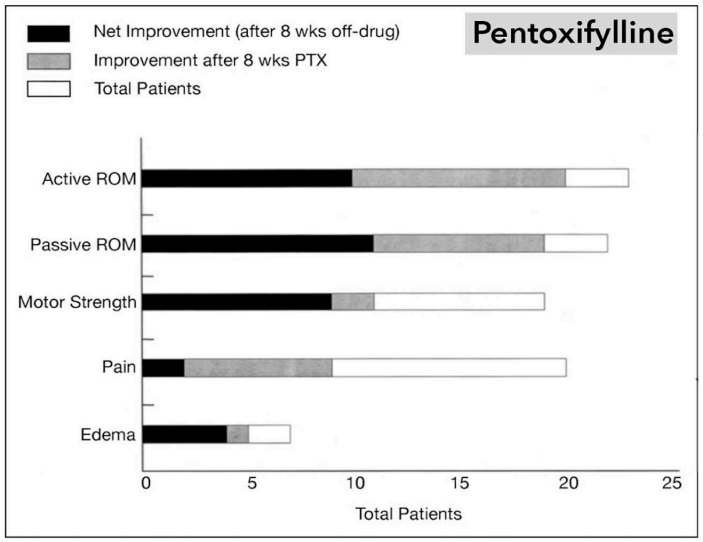
2) classes of agents that are radiation mitigators:

Generalized radiation injury mechanisms and agents that target these mechanisms

Mechanism	Agents that prevent/mitigate the radiation injury mechanism	Agents that treat the radiation injury mechanism
Activation of inflammatory pathways	ACE inhibitors/ARBs Statins Topical steroids Probiotics	Systemic steroids
Vascular endothelial dysfunction	Pentoxifylline Hyperbaric oxygen	Pentoxifylline Hyperbaric oxygen Bevacizumab Anticoagulation
Decreased normal tissue resilience and function	Memantine Pilocarpine Growth factors Supportive care	Methylphenidate Pilocarpine PDE-5 inhibitors Supportive care

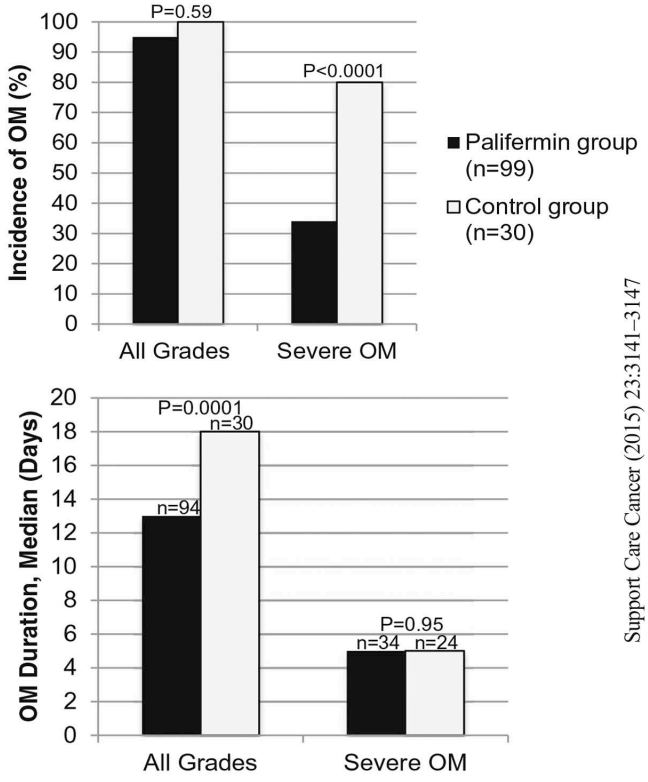
Abbreviations: ACE = angiotensin-converting enzyme; ARBs = angiotensin II receptor blockers; PDE-5 = phosphodiesterase-5; SOD = superoxide dismutase.

for example, cytokines can stimulate repopulation earlier and more vigorously than might otherwise occur, and this could have the net effect of reducing the severity of a normal tissue response to radiation or chemotherapy--like dangerously low blood counts



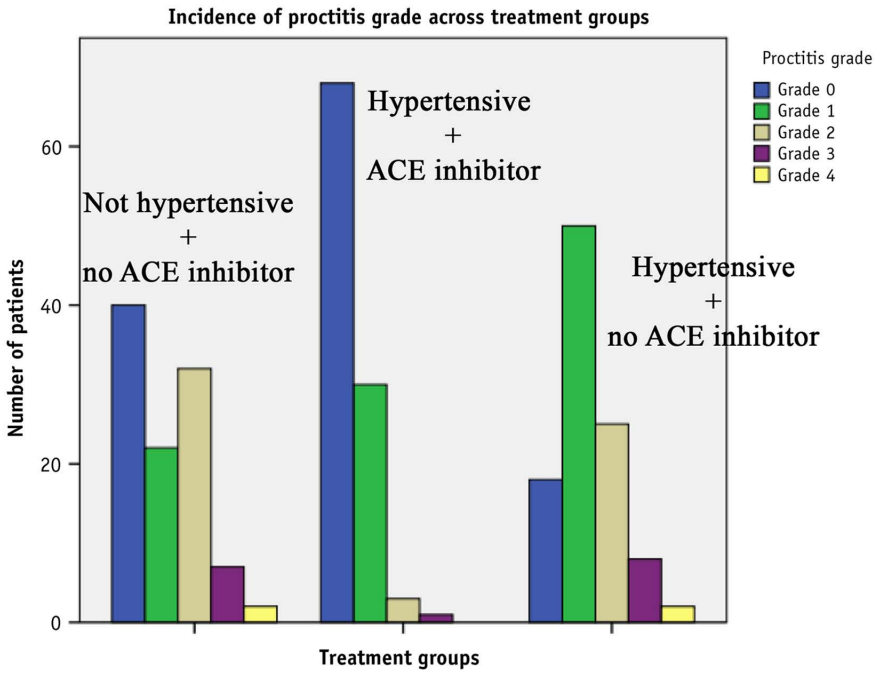
The total number of patients with initial deficit is represented by the entire bar length, and shaded areas include: gray, improvement after 8 weeks of pentoxifylline (PTX) treatment; black, net improvement at 16 weeks, after 8 weeks of PTX treatment followed by 8 weeks off-drug.

Pentoxifylline reduces the symptoms (such as pain, swelling, limited range of motion, etc.) associated with already-established radiation fibrosis. It also reduces the actual amount and density of fibrosis...one of the few treatments capable of actually "reversing" a late effect.



Support Care Cancer (2015) 23:3141–3147

Palifermin (analog of keratinocyte growth factor) reduces the likelihood, severity and maybe the duration, of oral mucositis in patients being prepped for bone marrow transplant



Int J Radiation Oncol Biol Phys, Vol. 94, No. 1, pp. 93e101, 2016

Prostate cancer patients who received radical radiotherapy plus hormone therapy experienced less severe proctitis if they were hypertensive and on ACE inhibitors, compared to hypertensive patients not taking ACE inhibitors, and to non-hypertensive patients not taking ACE inibitors.

Some - but not all - studies in both rodents and humans have also shown this effect for radiation complications in the lung, kidney and brain.

Emerging Science!

	PBO	AVA	AVA vs PBO Relative Δ
SOM incidence through IMRT	64%	54%	16%
SOM duration through f/u, median days	18	8	56%
Gr 4 OM incidence through IMRT	33%	24%	27%
SOM incidence through 50 Gy	45%	28%	38%
SOM incidence through 60 Gy	58%	42%	28%
SOM incidence through f/u	71%	58%	18%
SOM onset, median days	38	49	29%

PBO = placebo
AVA = avasopasem
SOM/OM = (severe) oral mucositis

Clinical trial number: NCT03689712

A new drug called **avasopasem** (drug company designation: GC4419) **is a superoxide dismutase mimetic that has shown efficacy in reducing both the severity and duration of severe oral mucositis.**

It acts by converting superoxide to hydrogen peroxide, thereby shutting off superoxide's ability to activate signaling cascades associated with chronic oxidative stress, which in turn can lead to the development of radiation-induced severe oral mucositis.

Early results (2022) from a Phase III clinical trial in ~400 patients with locally-advanced, non-metastatic oral cavity and oropharyngeal squamous cell carcinomas receiving chemorads (IMRT plus cisplatin). Some signs of improvement across different measures of oral mucositis severity, onset and duration when using avasopasem.

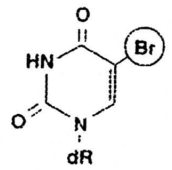
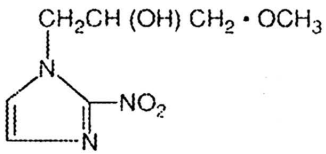
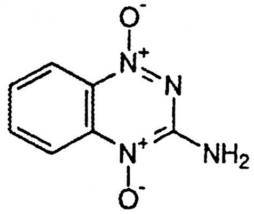
APPENDIX MATERIALS

Contemporary Targets for Radiosensitization

Target	Substance	Radiosensitization of Cell Line/Tumor Entity	Comments
ATM	CP466722	HeLa (cervix carcinoma)	only in vitro results
ATM	KU-55933	various tumor cell lines HeLa, MCF-7, ovary cancer cells, bladder cancer cell, *etc.*	up to now no clinical trial
ATM	KU-60019	glioblastoma and glioblastoma-initiating cells	successor of KU-55933
			increased radiosensitivity in p53-deficient cells
ATR	NU6027	MCF-7 (breast carcinoma)	increased effects in combination with various chemotherapeutic drugs
BCR-ABL	imatinib	RT112 (transitional bladder cell carcinoma), H1299 (lung carcinoma), PANC1 (pancreatic adenocarcinoma), PC3 (prostate adenocarcinoma)	no increased radiation gut toxicity in an animal model with xenotransplantation of PC3
CDK1, 2, 4	flavopiridol (alvocidib)	various cancer cell lines and xenografts	successful clinical studies in combination with standard chemotherapeutic regimens
CDK1, 2, 9	AZD5438	A549, H1299, and H460 (non-small cell lung cancer)	discontinued clinical development due to low tolerability in phase II studies
CDK4/6	palbociclib (PD0332991)	human glioblastoma U87 intracranial xenografts and brainstem glioma mouse model	FDA approval for potential treatment of breast cancer
CHK1	UCN-01	A549 (lung carcinoma), NCI-H460 (large-cell lung carcinoma), K562 (erythroblastoid leukemia cell line), glioblastoma stem-like cells *in vitro* and in xenografts	no effect on BEAS-2B (immortalized normal bronchial epithelial cell line) enhanced radiosensitivity of lung cancer cell lines in combination with celecoxib and of head and neck squamous cell carcinoma by combination with ATRA (8 all-trans retinoic acid)
CHK2	PV1019	MCF-7 (breast carcinoma), U251 (glioblastoma)	radioprotective in mouse thymocytes
CHK2	XL-844	HT-29 (colon carcinoma)	only one in vitro study with irradiation
EGFR	cetuximab	several clinical trials combined with standard chemoradiotherapy	FDA approval only for treatment of locally advanced head and neck cancer in combination with radiation
HDAC	LBH589 (panobinostat)	prostate cancer and glioblastoma cells	obatoclax, inhibitor of BCL-2, for increased radiosensitization of glioblastoma cells resistant to LBH589 and SAHA
HDAC	PCI-24781 (abexinostat)	cervical and colon carcinoma cells, nasopharyngeal carcinoma cells *in vitro* and in xenografts	two phase I studies as mono- or combination (with doxorubicin) therapy in patients with metastatic carcinoma, lymphomas
HDAC	SAHA (vorinostat)	LN18 and U251 (glioblastoma cells), osteosarcoma (OS) and rhabdomyosarcoma cell lines and OS xenografts	two finished phase I trials to determine the maximum well-tolerated dose
HSP90	17-AAG (geldanamycin)	DU145 (prostate carcinoma), SQ-5 (lung squamous carcinoma), T98G and U87-MG (glioblastoma), esophageal cancer cells	enhanced radiosensitization in combination with the PARP inhibitor olaparib; no radiosensitizing effect in normal tissue cells
leftHSP90	17-DMAG	MiaPaCa (pancreatic carcinoma), NSCLC cell lines	no radiosensitizing effect in normal tissue cells; radioprotective in PBMC
leftHSP90	NVP-AUY922, NVP-BEP800, NVP-HSP990	various tumor cell lines: A549, GaMG, HT 1080, SNB19, MIA PaCa-2 and U251	no clinical trial
leftHSP90	STA-9090 (ganetespib)	oropharyngeal squamous cell carcinoma (SCC) tissue samples HCT 116 (colorectal cancer cell line)	effective also in combination with cisplatin and in xenografts combined with capecitabine two ongoing clinical trials in combination with chemoradiation
leftMDM2	nutlin-3a	prostate cancer cell lines, NSCLC cells	activation of p53 resulted in increased senescence
leftMDM2	PXN727	HCT116 (colon cancer cell line)	upregulation of secretion of HSP70
leftMRN-complex	telomelysin (OBP-301)	orthotopic human esophageal cancer xenograft model	ongoing analysis of the safety and efficacy of telomelysin in patients with hepatocellular carcinoma
leftp53	PRIMA-1MET MIRA-1	SCLC cell lines with mutant p53 *in vitro* and as xenografts in mouse experiments	reactivation of p53 and radiosensitization
leftPRKDC	NU7441	C4-2 and PC3 (prostate carcinoma), MCF-7 SW620 (colon carcinoma) cell culture and xenografts	increased radiosensitization of MCF-7 cells in combination with K55933 no effect in PRKDC-deficient V3 cells

Int. J. Mol. Sci. 2020;17:102

Cheat Sheet: Selected Chemical Modifiers of Radiation Therapy

Chemical Structure	Name (Type of Compound)	Mechanism of Action	Clinical Status	Other Comments
	5-Bromodeoxyuridine (Halogenated Pyrimidine)	Sensitizer of rapidly proliferating cells by incorporation into DNA during S phase of the cell cycle; incorporation results in decrease or removal of shoulder of radiation survival curve	Current laboratory studies focus on ways to increase radiosensitization by altering the method of drug delivery, e.g., longer continuous infusions versus short, repeated exposures	Requires prolonged exposure prior to irradiation to be effective; also sensitizes rapidly proliferating cells in normal tissues
	Misonidazole (2-Nitroimidazole)	Radiosensitizer of hypoxic cells; principal mechanism of action is mimicry of molecular oxygen's ability to "fix" free radical damage caused by ionizing radiation (and some toxic chemicals)	Disappointing overall, except in selected sites, most notably, head and neck tumors; failure ascribed to insufficient tumor levels of drug because of dose-limiting neurological toxicity	Need only be present at the time of irradiation to be effective; some potential for use as a chemosensitizer and possibly, in combination with intra-operative radiotherapy
	Tirapazamine (Organic Nitroxide)	Bioreductive drug selectively toxic to hypoxic cells; drug is reduced to a toxic intermediate only in the absence of oxygen	Currently in early stages of Phase III clinical trials in both North America and Europe as an adjunct to radiotherapy and in Phase II trials with platinum compounds	Also called SR 4233; shows overlapping toxicity with ionizing radiation by eliminating an otherwise radioresistant population of (hypoxic) tumor cells; little normal tissue toxicity noted to date
NH_2–$(CH_2)_3$–NH–$(CH_2)_2$–SH_2PO_3	Amifostine (Thiol Compound, Free Radical Scavenger)	Radioprotective compound capable of reversing or "restituting" free radical damage caused by ionizing radiation and some toxic chemicals	Human investigations have focused on the use of amifostine as protection against the nephro- and ototoxicity of platinum compounds, and the hematological toxicity of cyclophosphamide	Also called WR 2721 (dephosphorylated active metabolite is WR 1065); selectivity for normal tissues achieved due to slow uptake of drug in tumors; need only be present at the time of irradiation to be effective

SnapShot **Cell**

Cancer chemotherapy

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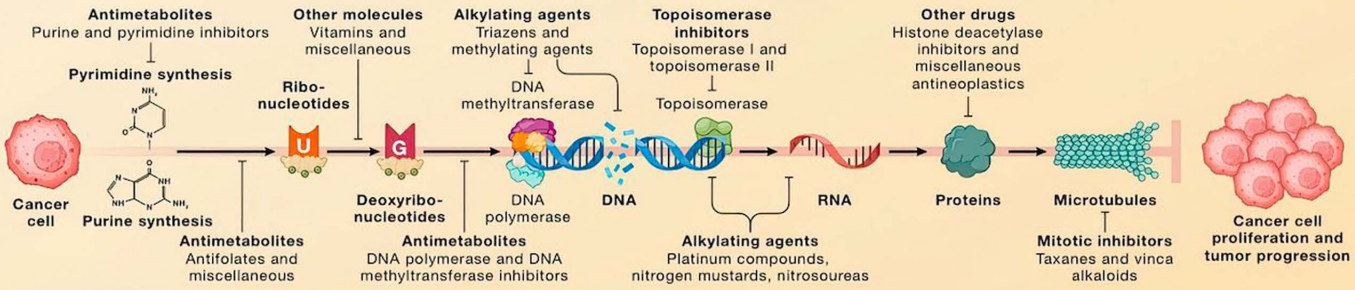

ALKYLATING AGENTS

	Drug	Mechanism of action	Therapeutic applications
Platinum compounds	Cisplatin	① Alkylating agents	Bladder, testicular, ovarian, head and neck, uterus, lung cancer
	Carboplatin		Lung cancer, ovarian cancer
	Oxaliplatin		Colorectal cancer
Nitrosoureas	Carmustine	Alkyl group	Brain tumor, lymphoma, multiple myeloma
	Lomustine		Brain and lung tumor, malignant melanoma, Hodgkin's lymphoma
	Streptozocin		Pancreatic cancer
Nitrogen mustards	Bendamustine	② DNA alkylation, DNA crosslink	Chronic lymphocytic leukemia, B-cell non-Hodgkin's lymphoma, multiple myeloma
	Chlorambucil		Hodgkin's lymphoma, chronic lymphocytic leukemia, giant follicular lymphoma
	Cyclo-phosphamide		Multiple solid tumors
	Ifosfamide		Sarcoma, testicular, ovarian, bronchial, breast, pancreatic, endometrial cancer, lymphoma
	Mechlorethamine	③ Multiple DNA strand breaks	T-cell lymphoma, B-cell lymphoma, chronic leukemia, lung cancer, medulloblastoma
	Melphalan		Multiple myeloma, ovarian cancer, neuroblastoma, melanoma, sarcoma
Triazenes	Dacarbazine	④ Cell cycle arrest and death	Malignant melanoma, Hodgkin's lymphoma, sarcoma
	Temozolomide		Brain tumors
	Procarbazine		Hodgkin's lymphoma

ANTIMETABOLITES

	Drug	Mechanism of action	Therapeutic applications
Pyrimidine antagonists	Fluorouracil	Purine antagonists, Pyrimidine antagonists; DNA incorporation, HGPRT and thymidylate synthase	Colorectal, breast, stomach, pancreatic, head and neck cancer
	Capecitabine		Colorectal, breast and gastric cancer
	Floxuridine		Digestive system cancers
Purine antagonists	6 - mercaptopurine	RNA incorporation	Acute lymphoblastic or lymphocytic leukemia
	Thioguanine		Acute myeloblastic or lymphoblastic leukemia
Antifolates	Methotrexate	Antifolates: Inosine synthesis, Dihydrofolate reductase, Thymidylate synthase; Nucleotide synthesis	Leukemia, breast, skin, head and neck, lung, uterine cancer
	Pemetrexed		Non-squamous non-small cell lung cancer, malignant pleural mesothelioma
	Pralatrexate		T-cell lymphoma
Enzyme Inhibitors	Cladribine	Thymidylate synthase, Ribonucleotide reductase; Gemcitabine, Cladribine, Cytarabine, Clofarabine, Fludarabine, Nelarabine; DNA polymerase; DNA crosslinks, DNA terminator	Hairy cell leukemia
	Fludarabine		B-cell chronic lymphocytic leukemia
	Gemcitabine		Pancreatic, lung, ovarian, breast cancer
	Clofarabine		Acute lymphoblastic leukemia
	Nelarabine		T-cell lymphoblastic leukemia and lymphoma
	Cytarabine		Acute myeloid and other leukemias

MITOTIC INHIBITORS

	Drug	Mechanism of action	Therapeutic applications
Taxanes	Cabazitaxel	Vinca alkaloids: Microtubule polymerization; Taxanes: Microtubule degradation; Mitosis block and cell death	Metastatic castration-resistant prostate cancer
	Docetaxel		Breast, lung, prostate, stomach, head and neck cancer
	(Nab) Paclitaxel		Breast, ovarian, lung and pancreatic cancer, AIDS-related Kaposi's sarcoma
Vinca alkaloids	Vinblastine		Hodgkin's lymphoma, testicular and breast cancer, Kaposi's sarcoma
	(Liposome) Vincristine		Leukemia,lymphoma, neuroblastoma, sarcomas
	Vinorelbine		Lung cancer and breast cancer

ANTI-TUMOR ANTIBIOTICS

	Drug	Mechanism of action	Therapeutic applications
Anthracyclines	Daunorubicin	Anthracyclines; DNA binding, TOP2; Arrest of DNA replication	Leukemia
	(Liposomal) Doxorubicin		Several solid tumors and hematological malignancies, AIDS-Kaposi's sarcoma
	Epirubicin		Several solid tumors and hematological malignancies
	Idarubicin		Acute myeloid/lymphoid leukemia
	Valrubicin		Bladder cancer
Non-Anthracyclines	Bleomycin	Bleomycin, Mitomycin; DNA breaks and crosslinks	Squamous cell and testicular carcinoma, lymphoma, pleural effusion
	Dactinomycin		Several solid tumors
	Mitomycin-C	DNA binding, TOP1, TOP2	Stomach, pancreatic, breast, bronchial carcinoma, solid tumors
	Mitoxantrone	Dactinomycin, Mitoxantrone	Prostate cancer, leukemia, non-Hodgkin's lymphoma, breast cancer, hepatocellular carcinoma

TOPOISOMERASE INHIBITORS

	Drug	Mechanism of action	Therapeutic applications
TOP1 Inhibitors	(Liposomal) Irinotecan	TOP1 inhibitors; TOP2 inhibitors; Block of DNA unfolding and replication	Colorectal, small-cell lung, pancreatic cancer
	Topotecan		Ovarian cancer, small cell lung cancer, cervical cancer
TOP2 Inhibitors	Etoposide (VP-16)		Lung, testicular and ovarian cancer, lymphoma, acute myeloid leukemia
	Mitoxantrone		Prostate, liver and breast cancer, leukemia, non-Hodgkin's lymphoma
	Teniposide		Leukemia

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