The impact of hypoxia and its modification of the outcome of radiotherapy

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ABSTRACT

Since the initial observations made at the beginning of the last century, it has been established that solid tumors contain regions of low oxygenation (hypoxia). Tumor cells can survive in these hypoxic conditions and are a major factor in tumor radioresistance. This significance has resulted in hypoxia becoming the most cited biological topic in translational radiation oncology. Identifying hypoxic cells in human tumors has become paramount, and the ability to do this has been improved by the help of new imaging techniques and the use of predictive gene profiles. Substantial data confirm the presence of hypoxia in many types of human tumors, although with considerable heterogeneity among individual tumors. Various approaches have been investigated for eliminating the hypoxic population. These include increasing oxygen availability, directly radiosensitizing or killing the hypoxic cells, indirectly affecting them by targeting the tumor vascular supply, increasing the radiation dose to this resistant population, or by using radiation with a high linear energy transfer, for which hypoxia is believed to be less of an issue. Many of these approaches have undergone controlled clinical trials during the last 50 years, and the results have shown that hypoxic radiation resistance can indeed be overcome. Thus, ample data exists to support a high level of evidence for the benefit of hypoxic modification. However, such hypoxic modification still has no impact on general clinical practice. In this review we summarize the biological rationale, and the current activities and trials, related to identifying and overcoming hypoxia in modern radiotherapy.

KEYWORDS: hypoxia, radiation, oxygen modifiers, radiosensitizers, hypoxic cell cytotoxins, vascular targeting agents

THE HYPOXIA PROBLEM
Significance of hypoxia

The first studies suggesting the importance of oxygen in radiotherapy were made as far back as the early 1900s. In 1909, Schwarz demonstrated that the radiation response of skin was decreased if the blood flow in the irradiated area was reduced by compression [1]. One year later, Müller reported that tissues in which the blood flow was stimulated by diathermia showed a more prominent response to radiation [2]. These indirect studies were followed by sporadic experimental and clinical observations indicating the importance of a sufficient blood supply for an adequate radiation response. Eventually this led Gray and co-workers, in the early 1950s, to both postulate that oxygen deficiency (hypoxia) was a major source of radiation resistance [3], and from histological data from patients with carcinoma of the bronchus to suggest that hypoxia can exist in human tumors as a result of a diffusion limitation of oxygen [4].

This concept of oxygen diffusing from blood vessels and being utilized by the tumor cells, thus resulting in diffusion gradients, with cells at the end of these gradients being oxygen-deprived or chronically hypoxic, was the working hypothesis for hypoxia until the 1980s. It was then suggested [5] and shown [6] that tumor hypoxia could also be acute, arising as a result of transient fluctuations in tumor blood flow. The current use of 'chronic and acute' to describe hypoxia in tumors is probably an oversimplification of the real situation [7]. Chronic hypoxia generally refers to prolonged and reduced oxygen concentrations that influence radiation response, but there is evidence that oxygen concentrations that are higher, yet below normal physiological levels, are often found [8], and these may influence malignant progression. Furthermore, reduced perfusion can be both partial as well as total [9], and while cells under the former condition would be oxygen deprived, with the latter they would be starved of oxygen and nutrients and as such their survival and response to therapy would be expected to be different.

Imaging tumor hypoxia

The difficulty has always been in applying definitive techniques that not only show that hypoxia exists in human tumors, but also that such measurements correlate with outcome from radiation therapy. Generally, these approaches involve indirect estimates (for review see [10, 11]). The earliest attempts at identifying tumor hypoxia focused on the vascular supply, since this was the oxygen supplier. Endpoints included: immunohistochemical estimates of intercapillary distance, vascular density, and distance from tumor cells to the nearest blood vessel; oxyhemoglobin saturation determined using cryophotometry, or non-invasively with near infrared spectroscopy or magnetic resonance imaging (MRI); or measurements of tumor perfusion using MRI, computed tomography, or positron emission tomography (PET). Hypoxia was later shown to upregulate gene/protein expression; thus, it was suggested that endogenous markers could be used to identify hypoxia. The principal markers have included hypoxia inducible factor 1 (HIF-1), carbonic anhydrase IX (CAIX), the glucose transporters GLUT-1 and GLUT-3, and osteopontin (OPN). These have been applied either individually or combined with other endogenous markers as gene signatures. More popular techniques involve measurements of the binding of exogenous markers. This can be achieved following immunohistological analysis of biopsied sections, using pimonidazole or EF5. It can also be done non-invasively with PET, single-photon emission computed tomography (SPECT), or MRI analysis of radioactively labeled nitroimidazoles (i.e. [^{18}F]-labeled misonidazole or fluoroazomycin arabinoside (FAZA); [^{123}I] labeled azomycin arabinoside); or PET imaging of [$^{60-64}$Cu]-ATSM (copper (II)-diacetyl-bis (N4-methylthiosemicarbazone). The most direct method involves determining oxygen partial pressure (pO$_2$) distributions with polarographic electrodes.

Each technique has its limitations. Although oxygen is delivered to the tumor cells via the vasculature, deficiencies in either vascular development, the oxygen-carrying capacity of the blood, or perfusion are not the only reasons why hypoxia develops [7]. Endogenous markers can be upregulated in response to stress factors that are not hypoxia related; even under normoxic conditions, expression has been reported to be induced by reactive oxygen species, cancer cell–specific mutations, and activation of signal transduction pathways [12]. Exogenous markers require time to identify hypoxic regions so probably only detect chronic hypoxia, not acute, and there are clear resolution issues with the various imaging approaches [11]. The techniques that can produce direct estimates of tumor oxygenation are generally invasive, thus not suitable for all tumors and are unlikely to be used on a routine clinical basis [10, 11]. Nevertheless, data using all the different approaches have not only shown that hypoxia exists in tumors, but that its presence can have a significant negative influence on patient outcome following radiation therapy [11]. This is illustrated in Fig. 1 for Eppendorf oxygen electrode measurements, PET-FAZA scans, and gene signature estimates in patients with head and neck squamous cell carcinomas. Such results clearly support the concept of finding clinically applicable approaches to overcoming tumor hypoxia.

APPROACHES FOR DEALING WITH HYPOXIA
Increasing oxygen delivery

The most obvious approach to the hypoxia issue is to increase tumor oxygen delivery. Indeed, this was applied relatively early in patients by allowing them to breathe high-oxygen-content gas under hyperbaric (typically 3 atmospheres) conditions [16]: hyperbaric oxygen was expected to saturate the blood with oxygen more than normobaric conditions. Positive clinical outcomes were obtained, as shown in Table 1. However, the complexity of the procedure and patient compliance led to this approach being stopped [17]. Pre-clinical studies had also shown that the radiosensitizations produced by normobaric oxygen and carbogen (95% oxygen + 5% carbon dioxide) were quite substantial [18, 19], so clinical trials using carbogen were initiated. The early studies failed to show any dramatic improvement in outcome [20, 21], which may have resulted from a failure to achieve the optimum pre-irradiation gas breathing time; experimental studies have shown this to be critical for enhancing radiation response and that it varies from tumor to tumor [19, 22, 23]. Later studies in which short pre-irradiation breathing times were applied gave conflicting results, with either a benefit [24] or no improvement obtained [25]. The failure in the latter study may have been the result of a size limitation, whereas the former positive study may have been due to the fact that nicotinamide was included in the treatment regime. While carbogen breathing will most likely affect chronic hypoxia it is likely to have limited influence on acute hypoxia. Pre-clinical studies have now demonstrated that nicotinamide is an effective treatment for preventing transient fluctuations in tumor blood flow and thus reduces acute hypoxia, although the mechanism is not known [26]. Those results led to the suggestion and subsequent demonstration that effective hypoxic modification would be possible by combining nicotinamide (to target acute hypoxia) with a modifier of chronic hypoxia [27]. Those studies and others eventually led to the clinical evaluation of carbogen with nicotinamide in patients with bladder cancer in the BCON (Bladder, CarbOgen, and Nicotinamide) trial [28], and in head and neck cancer patients in the ARCON (Accelerated Radiation, CarbOgen, and Nicotinamide) trial [29]; both studies reported positive improvements in outcome.

Transport of oxygen in the blood supply is via hemoglobin; thus considerable attention has been applied to finding various methods to target hemoglobin, thereby improving oxygen delivery to tumors. The most obvious approach is to increase hemoglobin levels. Attempts to do this using transfusion produced conflicting results, with either an increase [30] or no effect [31] on radiation response reported. Increasing hemoglobin concentration by stimulation with erythropoietin (EPO) has also been investigated [32]. Pre-clinical studies showed that this was an effective method for overcoming anemia and for improving radiation response; however, although it was also successful in correcting anemia in patients, those that received EPO and radiation had a poorer outcome than patients who were irradiated without EPO. This negative outcome has been attributed to the fact that EPO is a growth factor and thus probably stimulated tumor growth.

Other approaches for improving oxygen delivery that have been investigated include the use of artificial blood substitutes that can carry more oxygen than hemoglobin [33] and manipulators of the oxygen unloading capacity of blood by modifying the oxy-hemoglobin dissociation curve [34]. Although these approaches improved tumor oxygenation status and radiation response in pre-clinical studies, none reached controlled clinical testing. More recent studies suggest the potential of increasing the oxygen diffusion distance by inhibiting cellular oxygen consumption with metformin [35]. Although
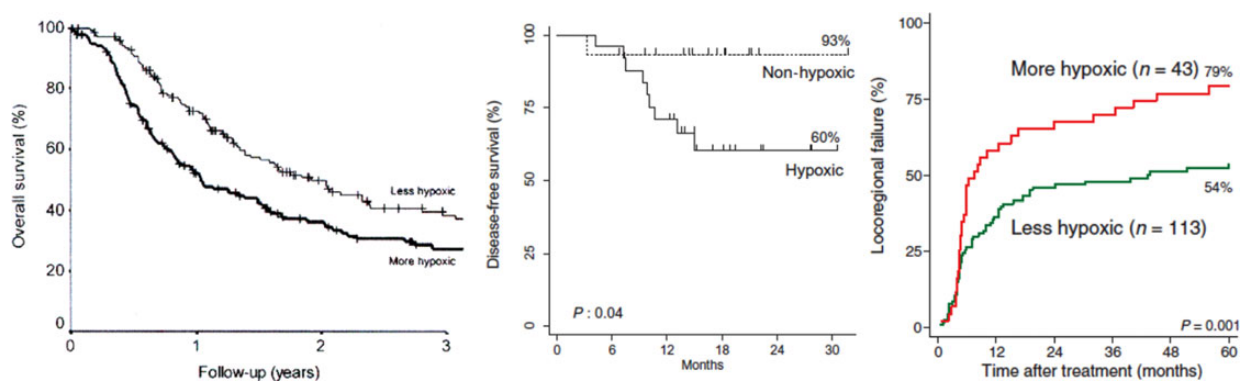
Fig. 1. Demonstration that the presence of hypoxia measured in head and neck squamous cell carcinomas prior to radiation therapy had a negative influence on outcome. (A) Overall survival in 397 patients in which oxygen estimates were obtained with the Eppendorf electrode. The patients were divided into two groups based on whether the percentage of oxygen values ≤2.5 mmHg were below (less hypoxic than) or above (more hypoxic than) the median value of 19%. (B) Disease-free survival in 40 patients that had received an injection of [^{18}F]-labeled FAZA 2 h prior to PET imaging. They were separated into having hypoxic or non-hypoxic tumors based on whether the tumor-to-muscle ratio was above or below 1.4, respectively. (C) Locoregional failure in 156 patients in which a 15-gene hypoxia classifier was applied to biopsy material to separate the tumors into more or less hypoxic. Composite figure from a number of sources [13–15].

Table 1. Medical Research Council (MRC) multicenter randomized trials with hyperbaric oxygen (HBO)

Site and study	No. of patients	Endpoint	Response		Statistical significance
			HBO	Air	
Head and neck carcinoma					
MRC 1st trial (1977)	294	Control (5 years)	53%	30%	$P < 0.01$
MRC 2nd trial (1986)	106	Control (5 years)	60%	41%	$P < 0.05$
Uterine cervix carcinoma					
MRC (1978)	320	Control (5 years)	67%	47%	$P < 0.001$
MRC (1978)	320	Survival (5 years)	37%	25%	$P < 0.01$
Bronchogenic carcinoma					
MRC (1978)	51	Survival (2 years)	15%	8%	n.s.
MRC (1978)	123	Survival (2 years)	25%	12%	$P < 0.05$
Carcinoma of the bladder					
MRC (1978)	241	Survival (2 years)	28%	30%	n.s.

Endpoints were Control (locoregional control) or Survival; n.s. = not significant. Modified from [17].

this agent is already used clinically in treating diabetes and may be associated with decreased rates of some cancer types [36], it is still too early to say whether this will be effective at decreasing tumor hypoxia in patients.

Targeting hypoxic cells

The most extensively investigated approach to the hypoxia problem is the use of agents that specifically target the hypoxic cells. This has been achieved using agents that either directly sensitize the hypoxic cells to radiation or preferentially kill them. In the early 1960s it was shown that the efficacy of hypoxic radiosensitisation was directly related to electron-affinity [37], and that led to *in vitro* studies demonstrating that highly electron-affinic nitroaromatic compounds could preferentially radiosensitise hypoxic cells [38]. These compounds were also found to be effective at enhancing tumor radiation response [39]; these agents are considered to be oxygen mimetics, but unlike oxygen they are not rapidly metabolized by the tumor cells through which they diffuse and thus reach all the cells in tumors, especially the hypoxic cells.

Clinical evaluation was started very early with metronidazole in brain tumors [40], but it was soon replaced by misonidazole and a large number of clinical trials were undertaken [17, 39]. Unfortunately,

most misonidazole trials were unable to generate significant improvements in radiation response, although a benefit was seen in some trials, particularly the Danish Head and Neck Cancer (DAHANCA 2) study [41], as shown in Table 2. Part of the failure to see any benefit was attributed to the fact that the drug doses necessary for effective radiosensitization also produced substantial dose-limiting clinical toxicity. Further clinical studies focussed on identifying more efficient or less toxic hypoxic sensitizers (Table 2). The first of these was a European trial with pimonidazole in uterine cervical cancer, but the preliminary results were disappointing [42]. Etanidazole was then tested in two other multicenter trials in head and neck cancer, but the results showed no benefit [43, 44]. Additional studies with nimorazole, a less efficient sensitizer but less toxic drug, in head and neck cancer patients (DAHANCA 5) showed a highly significant benefit in terms of improved locoregional tumor control and disease-free survival [31]. A more recent International Atomic Energy Agency (IAEA) trial with the 3-nitrotriazole compound sanazole (AK-2123) in uterine cervical cancer also demonstrated a significant improvement in both local tumor control and overall survival [45], while a Japanese randomized trial with the 2-nitroimidazole doranidazole (PR-350) in locally advanced pancreatic cancer reported a significant increase in long-term survival [46]. To date, only one of these drugs has been incorporated into standard radiotherapy treatment—nimorazole in head and neck cancer—and that is only in Denmark, although additional clinical testing is ongoing elsewhere.

Preferentially killing hypoxic cells is another direct approach to targeting hypoxia that has become popular. This has been achieved using non-toxic prodrugs that undergo enzymatic reduction to a cytotoxin under hypoxic conditions. Three basic classes of bioreductive compounds have been developed. These are quinones, nitroaromatics and N-oxides [47, 48]. The first compound recognized to possess bioreductive activity was mitomycin C [49]. Clinical studies designed to test its potential to overcome hypoxia produced conflicting results, with either a benefit or no benefit obtained. A lack of response was probably not surprising because mitomycin C shows only a small differential in cell killing between hypoxic and aerobic cells. Attempts to find more efficient quinones led to the development of porfiromycin and EO9, but again no real additional benefit has been found clinically. Nitroimidazole radiosensitizers such as misonidazole have also been found to be metabolized to cytotoxic products selectively in hypoxic tumor cells [50]. However, these agents were only weakly cytotoxic and moderately selective for hypoxic cells [51]. Nevertheless, the basic concept of using nitroimidazole compounds has led to other agents being developed, including RSU1069, PR-104 and TH-302, the latter compound showing significant selectivity for hypoxic cells [52]; it is currently in clinical development. The lead aromatic N-oxide developed was tirapazamine, and it had a hypoxic-aerobic differential of up to 200 in murine cells and 50 in human cell lines [53]. Although results from Phase II trials generally showed promise, randomized trials were somewhat disappointing. However, the potential

Table 2. Selected multicenter randomized trials with nitroimidazole radiosensitizers

Site and study	No. of patients	Drug	Endpoint	Response		Statistical significance
				RT + drug	RT	
Uterine cervix carcinoma						
MRC (1983)	183	Pimo	Control (4 years)	64%	80%	$P < 0.01$
			Survival (4 years)	36%	54%	$P < 0.05$
IAEA (2007)	326	Sana	Control (5 years)	61%	46%	$P = 0.005$
			Survival (5 years)	57%	41%	$P = 0.01$
Head and neck carcinoma						
DAHANCA 2 (1989)	626	Miso	Control (5 years)	41%	34%	$P < 0.05$
RTOG 85–27 (1995)	521	Eta	Control (2 years)	40%	40%	n.s.
			Survival (2 years)	43%	41%	n.s.
EORTC (1978)	374	Eta	Control (2 years)	53%	53%	n.s.
			Survival (2 years)	54%	54%	n.s.
DAHANCA 5 (1998)	414	Nim	Control (5 years)	49%	33%	$P = 0.002$
			Survival (5 years)	52%	41%	$P = 0.01$
Pancreatic carcinoma						
JAPAN (2008)	46	Dora	Survival (3 years)	23%	0%	$P = 0.02$

Endpoints were Control or Survival; n.s. = not significant; RT = radiotherapy. The trials were the Medical Research Council (MRC) trial with pimonidazole (Pimo) [42], the International Atomic Energy Agency (IAEA) trial with sanazol (Sana) [45], the Danish Head and Neck Cancer (DAHANCA) trials with misonidazole (Miso) [41] and nimorazole (Nim) [31], the North American Radiation Therapy Oncology Group (RTOG) [43], the European Organisation for Research and Treatment of cancer (EORTC) [44] trials with etanidazole (Eta), and the Japanese trials with doranidazole (Dora) [46].

of such agents has led to the development of related compounds, such as chlorambucil N-oxide and banoxantrone [47].

Probably one of the best approaches for targeting hypoxic cells is the use of hyperthermia, since heat can both sensitize and kill hypoxic cells [54]. Pre-clinical studies have clearly shown that irradiating tumors and at the same time heating them to temperatures up to 43°C can substantially enhance radiation response [54]. This has been attributed to a sensitization affect mediated through an inhibition of radiation-induced DNA repair [55, 56]. However, if a time interval is introduced between the radiation and heat treatments, sensitization is lost, yet an enhancement of radiation response is still observed [54]. This effect has been attributed to heat simply killing those cells that are hypoxic [57, 58]. Regardless of the mechanism for the heat effect on radiation, there is now good clinical evidence that hyperthermia can substantially improve tumor response to radiation in a number of different clinical sites, as illustrated in Table 3.

Table 3. Meta-analysis of randomized clinical trials comparing radiation only with radiation and hyperthermia

Tumor site	No. of trials	No. of patients	Response		Odds ratio (95% CI)
			RT + HT	RT	
Advanced breast	2	143	68%	67%	1.06 (0.52–2.14)
Prostate	1	49	81%	79%	1.16 (0.28–4.77)
Mixed	3	442	39%	34%	1.24 (0.84–1.82)
Head and neck	5	274	51%	33%	2.08 (1.28–3.39)
Rectum	2	258	19%	9%	2.27 (1.08–4.76)
Chest wall	4	276	59%	38%	2.37 (1.46–3.86)
Bladder	1	101	73%	51%	2.61 (1.14–5.98)
Melanoma	1	128	56%	31%	2.81 (1.36–5.80)
Cervix	4	248	77%	52%	3.05 (1.77–5.27)
All trials	**23**	**1919**	**52%**	**38%**	**1.80 (1.50–2.16)**

Endpoints were all locoregional control, RT = radiation, HT = hyperthermia, CI = confidence intervals. Modified from [54].

AIAs
- TIMP
- Thalidomide
- Suramin and analogues
- Fumagillin and TNP470
- Endostatin
- Angiostatin
- Anti-VEGF(R) Ab
- SU5416
- SU6668
- Sunitinib (SU11248)
- PTK787/ZK222 584
- Vandetanib (ZD6474)
- Sorafenib (Bay 43-9006)
- Cytokines
- CAI
- Arginine Deiminase
- Anginex
- AG-013736
- Pazopanib
- Nelfinavir
- Thrombospondin
- Nicotinamide
- Heparin
- EGFR inhibitors
- COX-2 inhibitors
- Chemotherapy

VDAs
- Hyperthermia
- Photodynamic therapy
- LAK cell therapy
- Tumour necrosis factor
- Interleukins
- Interferon-gamma
- Vinka alkaloids
- Colchicine
- Arsenic trioxides
- Dolastatins
- TZT-1027
- Flavone Acetic Acid (FAA)
- DMXAA (ASA404/Vadimezan)
- CYT997
- CA4P (Fosbretabulin/Zybrestat)
- AVE8062
- ZD6126 (ANG453)
- OXi-4503
- MN-029
- Plinabulin (NPI-2358)
- BNC105
- 2-methoxyestradiol
- ENMD-1198
- EPC2407 (MX116407)
- ABT-751
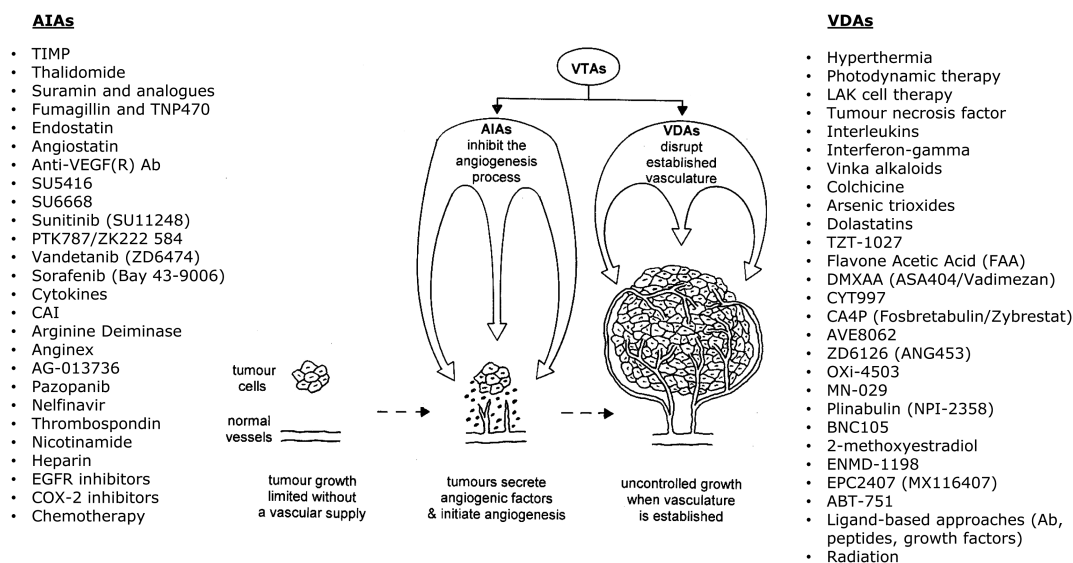- Ligand-based approaches (Ab, peptides, growth factors)
- Radiation

Fig. 2. Schematic illustration that the growth and development of solid tumors requires they form their own functional vasculature to supply essential oxygen and nutrients. Tumors achieve this from the normal host vessels by the process of angiogenesis. Therapeutic targeting of the tumor vasculature can be achieved using various vascular targeting agents (VTAs). These are either angiogenesis-inhibiting agents (AIAs), which can inhibit any one of the steps in the angiogenesis process, or vascular-disrupting agents (VDAs), which damage the already established vasculature. Examples of both types of VTAs are listed. Redrawn from [60].

Vascular targeting agents

The growth and development of solid tumors requires they develop their own functional vascular supply, which they do from the normal host vasculature by the process of angiogenesis. This importance of the tumor vascular supply makes it an attractive target for therapy, and two major groups of vascular targeting agents (VTAs) have emerged [59], as shown in Fig. 2. One approach involves preventing the development of the tumor vasculature by inhibiting various steps in the angiogenic process (angiogenesis inhibiting agents; AIAs). An alternative method is to use agents that damage the already established tumor vessels (vascular disrupting agents; VDAs). Although both AIAs and VDAs show antitumor effects, they never result in tumor control, even when used in combination [61, 62]. This has led to the suggestion that their potential clinical application would be when combined with more conventional treatments, especially radiation [63]. In fact, numerous pre-clinical studies have now shown that the response of tumors to radiation can be significantly improved when animals are treated with either AIAs or VDAs [61].

With both AIAs and VDAs, hypoxia has been implicated in the mechanism for this enhancement of radiation response. For AIAs, the consensus of opinion is that the improvement in radiation response found in pre-clinical studies is the consequence of normalization of the tumor vasculature resulting in a decrease in tumor hypoxia [64]. While there are certainly pre-clinical studies showing an improved tumor oxygenation status with such treatments, there are just as many studies showing no change and even a decrease in tumor oxygenation [61]. These findings not only make it unclear as to the role of vessel normalization in influencing the combination of angiogenesis inhibitors with radiation, they also suggest that timing and sequencing of the two modalities may be critical for an optimal benefit. With VDAs the situation is less controversial. VDAs damage tumor vasculature and as a result cause a severe reduction in tumor blood flow, leading to ischemia and cell death [61]. The cells that die first are most likely the hypoxic cells that are already oxygen and nutrient deprived, and the improved antitumor responses observed when such agents are combined with radiotherapy probably reflects an additive tumor response resulting from the VDA eliminating treatment-resistant hypoxic tumor cells while the radiotherapy acts against the aerobic tumor cell population. However, there are indications that VDAs may induce hypoxia in cells that were not previously hypoxic and that such cells survive this treatment [61, 65]. This supports the concept that timing of VDA therapy relative to radiation treatment is critical, and that for the best affect the drugs should be given soon after irradiating.
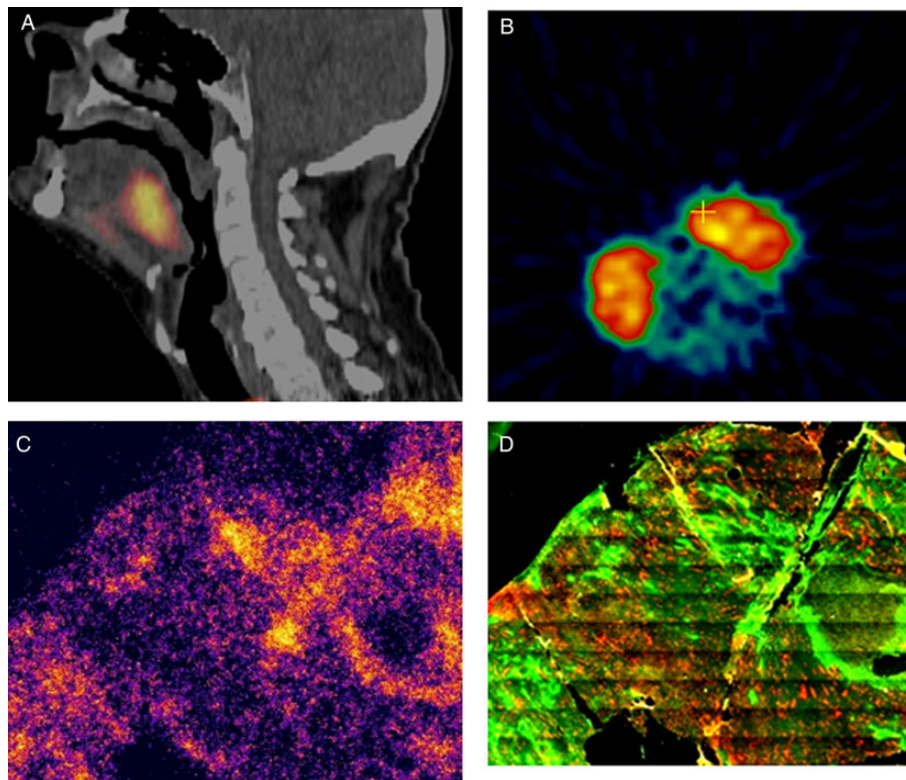

Fig. 3. (A) FAZA-PET scan from a patient with a head and neck tumor; high FAZA activity was detected 2 h after injection and is shown by the bright spot in the neck region. (B) FAZA activity in two SCCVII squamous cell tumors on the flanks of a C3H mouse; FAZA activity, measured 2 h after injection is again shown by the two large bright areas and was recorded using an animal dedicated micro PET. (C) Autoradiography section of one of the SCCVII mouse tumors showing microregional areas of FAZA activity, which are unlike two large areas in Fig. 3B. (D) The same section as in C, but now stained for binding of the hypoxic cell marker pimonidazole, which was injected 2 h prior to excision; note that the bright areas showing pimonidazole binding are the same as those in the FAZA autoradiography image. Modified from [14, 67].

Radiation-based approaches

It has been suggested that rather than add some form of modifier to eliminate hypoxic cells, it may be possible to overcome hypoxia by using radiation itself. If we can identify the hypoxic regions in tumors, we could then simply increase the radiation to these areas, an approach that is referred to as 'dose painting' [66]. The problem with this approach is in truly identifying the hypoxic regions with current technology. This is illustrated in Fig. 3. Using histological analysis of hypoxic markers, we are able to separate clearly hypoxic and non-hypoxic areas [67]. However, from PET scans of tumors, whether animal or human, the hypoxic region appears as a solid mass; this is simply because the region identified by each individual voxel is large due to resolution issues. As a consequence, areas are being detected that could contain both hypoxic and non-hypoxic cells. Furthermore, the lack of hypoxic imaging in other areas does not mean that hypoxia is not present, rather that the amount of hypoxia in a particular voxel is not large enough to raise the overall threshold value. It has also been suggested that tumor hypoxia is dynamic rather than static, thus hypoxia measured prior to the start of therapy may not be the same as that during therapy. This was indicated in a PET study in head and neck cancer patients in which considerable variability in intratumoral uptake of $[^{18}F]$ misonidazole was reported between repeated scans [68]. However, a more recent pre-clinical study using FAZA-PET [69] and another clinical study in head and neck cancer patients using $[^{18}F]$ misonidazole reported that the uptake between repeated scans was highly reproducible [70]. There is also the question of how much the radiation dose should be increased to have a definitive effect on response. Based on these issues it would seem more pertinent to simply increase the dose to the entire tumor in which hypoxia has been identified and see whether this actually makes a difference to outcome [11].

It has been established that as the linear energy transfer (LET) for radiation increases, so the oxygen enhancement ratio (OER) decreases [71, 72]. Based on historical data, the consensus of opinion has generally been that if radiation of a sufficiently high LET is applied then hypoxia ceases to be an issue. However, a more recent comprehensive summary of results from a range of different *in vitro* experiments [72] has shown that the OER effect only disappears when the LET value is ~500 keV/μm or greater, which is beyond what we can achieve clinically. Thus, although the use of high-LET radiation can certainly reduce the impact of hypoxia, it is unlikely that using current technology it will ever be possible to completely eliminate hypoxia, suggesting that even with high-LET radiation some form of modifier needs to be included in the treatment regime.

CONCLUSIONS

Current interest in cancer therapy focuses on the application of targeted therapies. In that context, hypoxia must be considered the ultimate target. It is a characteristic feature of most human tumors that has a major negative influence on determining tumor response to conventional therapy, as well as being an important factor in influencing malignant progression, both in terms of the aggressive growth of the primary tumor and its ability for metastatic spread. Numerous clinically applicable techniques have been developed that allow us to identify hypoxia in human tumors. Both pre-clinical and clinical studies have shown that hypoxia can be reduced to improve the

outcome of radiation therapy. However, despite the fact that the potential of hypoxia to modify radiation response was first identified 100 years ago, that we have known for some 60 years as to why hypoxia influences radiation response, and that almost 40 years of clinical trials have clearly shown we can successfully modify hypoxia, hypoxic modification has still not been established as a standard treatment with radiotherapy. Why this should be so is unclear, but from a patient point of view this must be considered a totally unacceptable situation that should be addressed.

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