PUSHING THE FRONTIERS OF RADIOBIOLOGY: A SPECIAL FEATURE IN MEMORY OF SIR OLIVER SCOTT AND PROFESSOR JACK FOWLER: REVIEW ARTICLE

Tumor oxygenation and cancer therapy—then and now

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ABSTRACT

In 2012, cancer affected 14.1 million people worldwide and was responsible for 8.2 million deaths. The disease predominantly affects aged populations and is one of the leading causes of death in most western countries. In tumors, the aggressive growth of the neoplastic cell population and associated overexpression of pro-angiogenic factors lead to the development of disorganized blood vessel networks that are structurally and functionally different from normal vasculature. A disorganized labyrinth of vessels that are immature, tortuous and hyperpermeable typifies tumor vasculature. Functionally, the ability of the tumor vasculature to deliver nutrients and remove waste products is severely diminished. A critical consequence of the inadequate vascular networks in solid tumors is the development of regions of hypoxia [low oxygen tensions typically defined as oxygen tensions (pO_2 values) < 10 mm Hg]. Tumor cells existing in such hypoxic environments have long been known to be resistant to anticancer therapy, display an aggressive phenotype, and promote tumor progression and dissemination. This review discusses the physiological basis of hypoxia, methods of detection, and strategies to overcome the resulting therapy resistance.
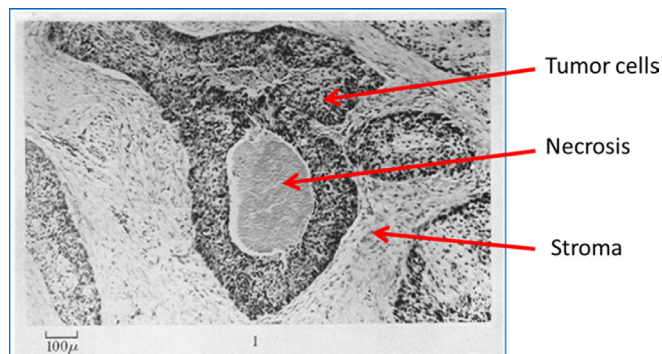
THE OXYGEN EFFECT

Radiation therapy has been used since the end of the 19th century as a means to control tumor burden, both with curative and palliative intent. Following the discovery in 1896 by German physicist Wilhelm Conrad Roentgen, X-rays were used for diagnosis, and within 3 years radiation was used to treat cancer. To test the status of the radiotherapy machine, radiologists would expose the skin of their arms to the instrument. The telltale burn would indicate the instrument was at the proper setting. Unfortunately, the frequent exposure to radiation resulted in many of these individuals developing leukemia. Interestingly, it was reported by Schwarz that clamping the skin to reduce blood flow to the arm decreased the skin irritation effects of ionizing radiation.[1] However, it was not until the 1920 and 30s that the absence of oxygen was found to be the culprit behind the lack of radiation-induced damage.[2] In the 1950s, Hal Gray's research team explored the potential impact of oxygen in radiotherapy *in vitro* and *in vivo*.[3–5] The landmark publication of Thomlinson and Gray, which evaluated histological structures of human lung cancers (Figure 1), concluded that, "there must exist a falling gradient in oxygen tension between the periphery and the centre of each tumour cord".[6] These findings implied that tumor cells

near the limit of oxygen diffusion would survive at lower than normal oxygen tensions, rendering them resistant to radiation therapy.

Initially, evidence of the impact of oxygen on radiation sensitivity was discovered in 1956, utilizing experiments that exposed *Escherichia coli* to radiation while in hypoxic conditions.[7] The same radiation-enhancing effect of oxygen was soon observed in mammalian cells, with the use of the clonogenic cell survival assay developed by Puck and Marcus.[8] In the 1970s, Adams demonstrated that oxygen had to be present during or within submilliseconds after radiation damage in order to observe the "oxygen effect".[9] Radiation causes DNA damage in cells, most importantly double strand breaks, by both direct and indirect effects.[10] In the presence of oxygen, free radical attack enhances radiation-induced DNA damage such that tumor cells lacking oxygen were found to be 2.5–3 times more resistant to radiation than their normoxic counterparts.[10] This phenomenon occurred primarily for oxygen tensions < 20 mm Hg, with little additional radiation sensitizing effect at values > 20 mm Hg.[11] The "oxygen effect" was not confined to tumor cells in tissue culture. Pre-clinical studies in mice[12] demonstrated that even tumors as small as 0.6 mm^3 may

Figure 1.Fixed section of a human squamous cell carcinoma of the bronchus stained by hematoxylin and eosin. Modified with permission from Thomlinson and Gray.[6]

Figure 2.(a) Distribution of hypoxia, identified by the bioreductive nitroimidazole agent EF5 (green) and the endogenous hypoxia marker HIF-1 (red), in human cervical carcinoma (SiHa) xenograft with respect to blood vessel localization (CD31, blue). Image courtesy of D. Hedley. (b) Murine mammary carcinoma (4T1) after intravenous injection with Hoechst-33342 (40 mg kg^{-1}, blue) as a surrogate marker for perfusion, and stained with EF5 (red) and CD31 (green). Please see the online version of this publication for colour images.

contain areas of hypoxia that are resistant to radiation treatment, and that such resistance could be mitigated by oxygen breathing prior to tumor irradiation.[13] Indeed, it is now well-established that hypoxia is a common feature of most, if not all, experimental solid tumors, and that there exists a direct correlation between tumor cell survival and the level of hypoxia within a tumor.[11,14]

Support for the hypothesis that oxygen-deficient cells in patient tumors could affect the ability to control malignant disease at the local treatment site comes from three types of radiotherapy investigations; (i) studies of physiological variables associated with hypoxia, (ii) the direct assessments of oxygenation *in situ*, and (iii) outcomes of hypoxia targeted therapeutic interventions.
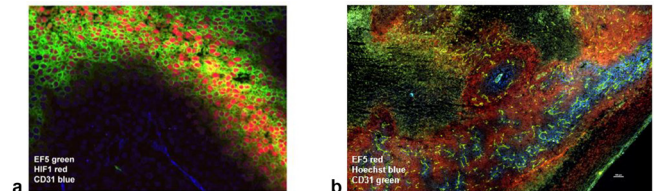
PHYSIOLOGICAL VARIABLES ASSOCIATED WITH HYPOXIA

Tumor oxygenation status was indirectly linked to radiosensitivity, when anemia was identified as a negative prognostic factor in cervical cancer patients receiving radiotherapy in the 1960s.[15] Subsequently, hemoglobin levels were negatively associated with outcomes of patients receiving radiotherapy in a number of cancer types, including uterine cervix,[16] head and neck,[17] bronchus,[18] bladder,[19] and prostate.[20] These observations coupled with the finding that anemic patients treated with radiotherapy responded favorably after receiving blood transfusions[21] strongly supported the belief that oxygenation status influenced the tumor response to radiation treatment.

MEASURING HYPOXIA IN TUMORS

Early measurements of tumor oxygenation began in the late 1960s, using glass electrodes. Studies from this time demonstrated a relationship between tumor oxygenation and response to radiation therapy in both cervical and head and neck cancer.[22,23] The Eppendorf pO$_2$ histograph, also referred to as the polarographic electrode, was introduced in the early 1990s and revolutionized *in vivo* oxygenation measurements.[24] It quickly became the "gold-standard" in oxygen tension measurements because of its ability to directly measure tissue oxygen tensions as low as 1–2 mm Hg and, importantly, because hypoxic measurements made with the electrodes in tumor-bearing mice correlated with the tumor response to radiation.[11] The Eppendorf electrode became widely used to assess oxygen tensions in head and

neck,[25] breast,[24] cervical[26] and prostate cancer.[27] Importantly, several studies confirmed that patients whose tumors were characterized as hypoxic by electrode measurement made prior to radiotherapy, had worse outcomes (local tumor control and/or survival).[28,29]

One shortcoming of the polarographic electrode method of determining oxygen tensions was that it was limited to accessible tumors. Even more concerning was the invasive nature of the procedure, which required insertion of the probe along multiple tracks in order to collect sufficient readings. Consequently, the procedure became unpopular with both patients and physicians. Electrode measurements of oxygen tensions in tumors fell out of favor and were largely discontinued in the early 2000s.

The most widely applied approach for hypoxic tumor cell detection has been the development of nitroimidazole hypoxic markers. Nitroheterocyclic agents, originally developed as radiation sensitizers, contain an RNO$_2$ side chain that becomes rapidly reduced in the absence of oxygen[30,31] and binds to cellular adducts. Detection of these adducts permits quantification of hypoxia by histochemical analysis or flow cytometry.[30] Nitroimidazole-based hypoxic markers, such as EF5[32] and pimonidazole hydrochloride,[33] are capable of identifying regions of hypoxia in tumors (Figure 2) in preclinical[30,33,34] and clinical[34,35] evaluations.

Metabolic markers of tumor hypoxia also have been assessed. Because expression of hypoxia inducible factors 1 and 2 (HIF-1 and HIF-2), Carbonic anhydrase IX (CA-IX), and glucose transporter-1 (GLUT-1) can correlate with areas of pimonidazole staining,[36] these markers have been used to quantify low oxygen tensions.[37] However, the expression of metabolic markers also can be triggered by factors other than hypoxia, including changes in pH, decreased levels of iron and other metabolites, or activation by endogenous ligands. Hence, the use of metabolic markers to identify tumor hypoxia has considerable limitations and consequently, they are more commonly used in pre-clinical assessments of hypoxia than in patient tumor evaluations.

A more recent development in the attempt to establish tumor oxygenation profiles is the determination of genomic signatures;

Figure 3.Patient with a FAZA PET positive oropharyngeal cancer. Reprinted with permission from Mortensen et al.[39] FAZA is a PET tracer developed as a hypoxia identification method for preclinical and clinical application.[39] FAZA, ^{18}F-Fluoroazomycin arabinoside.
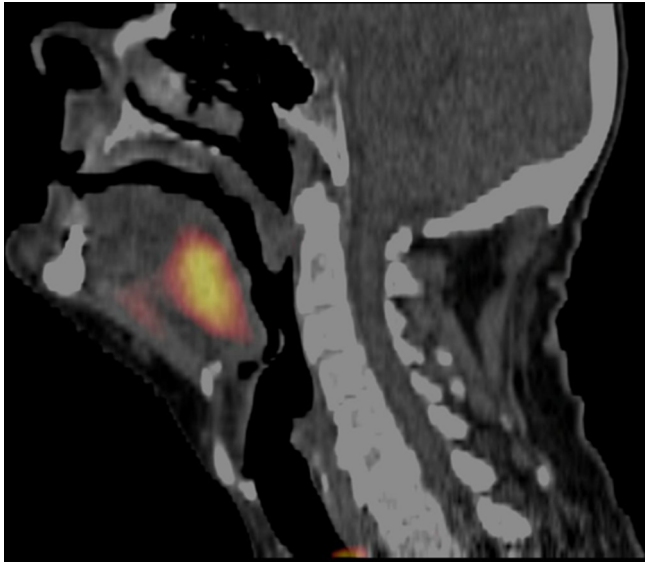
a topic discussed in detail by West et al in this current issue of *Br J Radiol*. However, there are concerns. As of 2015, there were 32 different gene signatures in use, with no generally accepted "gold-standard" hypoxia signature.[38] A further complicating issue is that the majority of genomic information was obtained from late-stage cancer patients. This raises the question of whether such information translates to early-stage patients treated with a curative intent. Furthermore, it is conceivable that the genome changes in time, as the tumor progresses and/or responds during the course of the treatment.

A significant impediment to the clinical application of many of the hypoxia detecting approaches has been the invasive nature of probes or the need to utilize biopsy samples to determine the extent of hypoxic marker expression. To address this issue, various nitroimidazole hypoxia markers have been developed for non-invasive detection methods that can determine spatial and temporal changes in oxygenation, such as PET (Figure 3), MRI, or CT imaging.[39,40] Recent results with non-invasive imaging are highly encouraging, particularly because they allow longitudinal evaluations of tumors throughout the course of therapy. However, these techniques are less precise than invasive techniques such as the oxygen probe,[41] as PET, MRI and CT do not allow detailed image resolution at distances relevant to the oxygen diffusion distance (100 μm).

Heterogeneity is a major issue regardless of hypoxia measurement technique. Gene expression signatures are based on biopsies and therefore, are representative of a very small sampling of the tumor. Eppendorf electrode measurements do sample multiple locations to account for tumor heterogeneity; however, they also measure only a small part of the tumor unless multiple needle tracks are employed. Imaging techniques, while having

the benefit of being non-invasive, have difficulty distinguishing between necrosis and hypoxia and are unable to measure fluctuating (acute) hypoxia. Furthermore, imaging resolution is too low to detect the small regions of hypoxia that frequently occur at the microenvironmental level.

While there has been a great deal of interest in methodologies designed to identify patients whose tumors may contain hypoxic cells, many of these methods show conflicting and inconsistent results when simultaneously assessed. For example, there are reports that demonstrate no correlation between oxygen electrode measurements and CAIX, GLUT-1 measurements, or pimonidazole staining.[42,43] Inconsistencies have also been observed between PET and oxygen electrode measurements[41,42,44–46] and direct comparison between hypoxic signatures and other means of determining tumor oxygenation are lacking. Thus, many unsolved questions remain regarding when and how to measure tumor hypoxia.

HYPOXIA AND PATIENT RESPONSE TO RADIOTHERAPY

Despite the difficulties associated with determining the oxygenation status of tumors, it is clear from studies employing a range of methodologies that hypoxia exists in human tumors and that it can influence radiation response.[47] Results have shown hypoxia to have a significant influence on both local tumor control and overall survival in a variety of tumor types.[39,48–50] Interestingly, in cervical cancer patients who were treated with surgery or radiotherapy,[28] all patients who had tumor pO_2 values < 10 mm Hg (even those treated only with surgery) had a significantly lower overall and disease-free survival than patients whose tumors were better oxygenated.[26] These findings not only corroborated *in vitro* data on the effect of hypoxia on radiation sensitivity, but also suggested a role for hypoxia in tumor aggressiveness. This belief was subsequently confirmed in prostate cancer studies in which hypoxia correlated with local recurrence and early biochemical relapse, supporting a role for hypoxia in radioresistance, increased aggressiveness, and enhanced metastatic potential.[29,51,52] Indeed, hypoxia now is considered an independent predictor of disease progression, treatment failures, and greater metastatic potential in sarcoma, cervix, breast, and prostate cancer.[28,29,52–55]

CHRONIC AND ACUTE HYPOXIA

Hypoxia can be broadly classified as chronic or acute (Figure 4).[56] Chronic hypoxia is a frequent characteristic of cancer cells residing at the limit of oxygen diffusion.[6] This diffusion-limited hypoxia occurs as tumor cells proximal to the blood vessel metabolize available oxygen, leaving the distal cells in a state of oxygen deprivation.[6,47,57] Acute hypoxia[58] is characterized as short-term oxygen deprivation due to transient fluctuations in blood flow that may occur when vessels are temporarily partially or totally occluded. Typically, tumor cells may experience acute hypoxia as relatively short hypoxic-oxic cycles lasting for several minutes to a few hours.[47,57] However, designating tumor hypoxia simply as acute or chronic is likely a significant oversimplification. Hypoxia in tumors is dynamic, varying temporally and in severity, and influenced by a variety of factors including blood

Figure 4. Illustration of tumor cells growing as a cord around blood vessels from which they obtain oxygen and nutrients. The left side illustrates oxygen diffusion and utilization from the vessel resulting in the development of chronically hypoxic cells at the outer edge of the cord. The right side shows perfusion through the vessel that has been transiently compromised and results in the development of acute hypoxia; examples of the types of flow/oxygen changes reported during a 60-min period are illustrated in the four panels below. Reprinted with permission from Horsman et al[56].
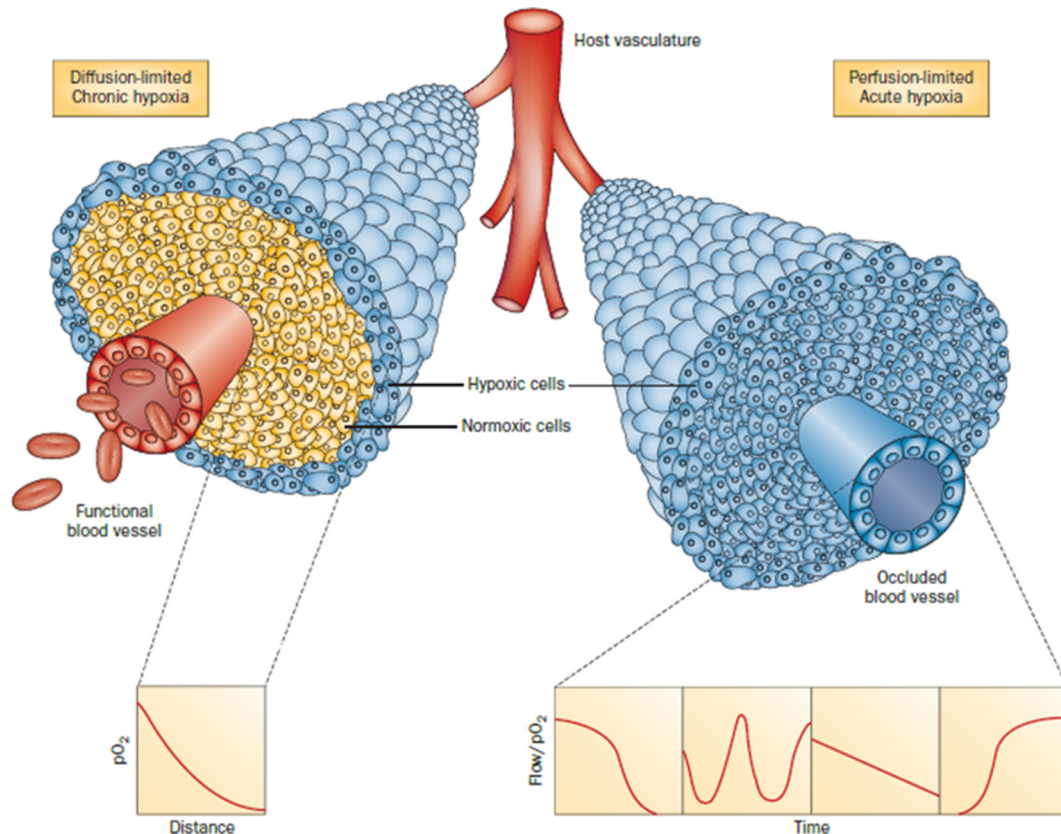
flow variation, cellular oxygen consumption rates, and differences in inherent tumor cell sensitivities to oxygen deprivation.

Hypoxia influences many aspects of cancer biology through the activation of transcriptional factors, primarily members of the HIF family.[59] In the presence of oxygen, HIF is quickly degraded.[60] However, in low oxygen tensions, HIF stabilizes and translocates to the nucleus, where it upregulates the expression of a multitude of genes involved in the survival and escape from the hypoxic microenvironment. The phenotypic effects of HIF activation are broad, including epithelial-to-mesenchymal transition, resistance to therapy, enhanced invasive capacities, initiation of angiogenesis, potentiation of metastasis, and modulation of stemness.[61,62] As stemness is involved in cancer therapeutic failure and metastatic disease and the latter is the primary cause of cancer patient deaths,[63] the consequences of tumor hypoxia on cancer therapy outcomes are significant.

Hypoxia also contributes directly to replication stress and genomic instability (for review see Bristow and Hill,[64] Riffle and Hedge[65]). Under conditions of diminished oxygenation, DNA repair pathways including homologous recombination, non-homologous end joining, and mismatch repair are down-regulated, leading to increased genetic instability.[64,66,67] Additionally, hypoxia contributes to microsatellite and chromosomal instability.[68] In terms of radiosensitivity, chronically hypoxic tumor cells generally are more radiosensitive than acutely hypoxic cells;[13,69] a finding typically ascribed to changes in energy metabolism and suppression of DNA repair capacity under prolonged hypoxia.[66,70] Beyond the molecular, the cellular and functional consequences of tumor hypoxia also are strongly dependent on the degree and duration of hypoxia. HIF-1α regulates a large number of genes associated with tumor invasion and progression induced by short-term hypoxia, while HIF-2α expression during prolonged low oxygen tensions drive and maintain stemness.[47,71,72] Since both acute and chronic hypoxia can exist in solid tumors, the presence of one and/or the other and the relative extent to which each occurs, can significantly affect the functional consequences of oxygen deprivation. This is readily apparent when assessing their relative impact on a cancer cell's metastatic behavior. While some studies appear to favor chronic hypoxia as the key factor,[73] others suggest that transient hypoxia mediates the dissemination of tumor cells.[74,75]

IMPACT OF HYPOXIA ON IMMUNE CELLS

Normally, the immune system is effective at recognizing and destroying abnormal cells within the body. However, tumors have the ability to circumvent destruction by the immune system by suppressing or evading immune cells. The field of cancer

immunology has rapidly expanded over the last few years, leading to some very promising discoveries and therapeutics.

In order to maintain homeostasis and prevent an inappropriate response, immune checkpoint proteins regulate the function of immune cells. One method of immune escape utilized by the tumor cells involves suppressing immune cells by interaction with checkpoint proteins. Checkpoint inhibitors, such as CTLA4 or PD-1/PD-L1 inhibitors, have resulted in remarkable improvement in outcomes of patients with certain types of cancer, such as melanoma and lung cancer.[76] Furthermore, combining radiation therapy with immunotherapy may offer a novel treatment strategy in the care of cancer patients.[77] However, recent findings indicate that hypoxic regions in tumors may foster an immunosuppressive environment. PD-L1 is upregulated in response to hypoxia, and is a direct target of HIF-1.[78] Hypoxic zones in tumors are found to be infiltrated with regulatory T-cells,[79] myeloid-derived suppressor cells,[78] and macrophages,[80] cells which promote immune suppression and angiogenesis. HIF-1 is reported to be involved in macrophage-mediated inhibition of T cells. Taken together, current data support the notion that hypoxic microenvironments in tumors may negatively affect this emerging anticancer therapy.

APPROACHES TO MODULATE TUMOR OXYGENATION
The consequences of both chronic and acute hypoxia lead to therapeutic barriers that create a significant hindrance to the efficacy of radiotherapy as well as other conventional anticancer therapies, particularly those requiring blood-borne delivery.[81] Thus, approaches to overcome hypoxia in tumors could increase sensitivity to anticancer therapies and improve overall survival. Initial studies focused on strategies that sought to directly improve oxygenation through methods including modulating blood flow, administering blood transfusions, or high oxygen content breathing. Others have taken advantage of the properties of hypoxic cells to target them with hypoxic cell sensitizers or bioreductive agents. Since high linear energy transfer (LET) radiations have low oxygen enhancement ratio (OER) values, the utilization of such particles in radiotherapy would reduce the relevance of tumor hypoxia. Alternatively, as hypoxic regions in tumors develop because of aberrant vascular networks, vascular targeting approaches, such as antiangiogenic (AAs) or vascular disrupting agents (VDAs), seek to improve tumor oxygenation through blood vessel normalization (AAs) or hypoxic cell elimination via induction of tumor cell death (VDAs). Furthermore, evidence exists that modifiable lifestyle behaviors may influence tumor hypoxia.

RADIATION DOSING SCHEDULE
In conventional fractionated radiotherapy, small radiation doses are given on a daily basis for periods of weeks or months. Such a treatment schedule may elicit the process of tumor reoxygenation,[82] by which tumor cells that were resistant to radiation due to being hypoxic at the time of irradiation with a given dose become oxygenated and hence, sensitive to radiation when the next dose is given. Consequently, reoxygenation could reduce the impact of hypoxia on conventional fractionated radiotherapy

outcomes. However, the extent to which tumors reoxygenate and the time course of reoxygenation is highly variable among tumors and thus likely to be insufficient to overcome the detriment of hypoxia. Recently, stereotactic body radiation therapy (SBRT), which employs high doses of radiation given in a single or a few treatment sessions, has become increasingly popular. The impact of SBRT on the relevance of tumor hypoxia as a determinant of treatment outcome remains somewhat controversial but pre-clinical studies and limited clinical findings suggest that tumor hypoxia is likely to be more important for SBRT than for conventional fractionation.[83,84] Furthermore, our aforementioned lack of knowledge of the relative contributions of acutely and chronically hypoxic cell fractions in tumors, coupled with the modulation of genomic instability and DNA repair capacities under varying hypoxic conditions, makes predicting the impact of reoxygenation exceedingly difficult.

OVERCOMING ANEMIA
Although anemic patients who received blood transfusions prior to radiotherapy had improved treatment outcomes, issues arose in treating cancer patients with this approach because of the HIV epidemic in the 1980s, when many individuals were unwittingly infected with the virus through blood transfusions.[85] Additional complications of blood transfusions include immunomodulation[86] and a low supply of donated blood. Erythropoietin therapy was developed as an alternative method to stimulate hemoglobin levels in anemic patients. However, in cancer patients, several clinical trials indicated that erythropoietin therapy not only did not improve response to radiation treatment, it was tentatively associated with a decrease in survival.[87,88]

BLOOD FLOW MODULATORS
Modulation of tumor blood flow to increase tissue oxygenation offers another means of reducing tumor hypoxia. Noradrenaline was shown to significantly improved blood flow and drug delivery in a rat model of liver cancer.[89] Benzyl nicotinate improved the radiation response of a preclinical murine tumor model, likely due to an increase in tumor pO_2.[90] A number of other agents evaluated preclinically, including pentoxifylline and nicotinamide, also improved tumor blood flow and response to radiotherapy.[91–93] However, clinical trials of nicotinamide have encountered considerable toxicities, with some patients experiencing nausea and vomiting that was unresponsive to antiemetics.[91]

HIGH OXYGEN CONTENT BREATHING
Hyperbaric chambers
Oxygen delivery to tissues is dependent on several factors, mainly blood flow, oxygen content, and oxygen tension.[94] Recognizing the deficiencies of oxygen delivery in solid tumors, the landmark publication of Thomlinson and Gray[6] ushered in the era of hyperbaric oxygen use in the treatment of cancer. Hyperbaric chambers were designed to alter both oxygen content and partial pressure by treating patients with 100% oxygen at three atmospheres pressure. Under these conditions, the amount of oxygen dissolved in plasma is significantly increased independently of hemoglobin saturation.[95] However, despite initial positive results, hyperbaric chambers were discontinued in favor

of alternate methods due to patient discomfort, cases of oxygen poisoning, and fires that occurred inside the chambers.[96]

Carbogen breathing

100% oxygen breathing has been shown to elicit deleterious and toxic effects in the body, mainly to the central nervous system, lungs, red blood cell morphology, and peripheral vision.[97] Furthermore, in response to this level of oxygen, arteries and arterioles actively vasoconstrict. To mitigate the latter, carbogen (95% O_2, 5% CO_2) was introduced as an alternate breathing gas. Indeed, preclinical investigations demonstrated that carbogen improved tumor oxygenation and response to radiotherapy.[98,99] In the clinic, carbogen largely, and somewhat surprisingly, did not demonstrate improvements in radiotherapy outcomes.[100] However, a trial using carbogen breathing in advanced head and neck cancer patients did suggest that carbogen breathing may be an alternative for patients who are unfit to receive concomitant chemotherapy with the radiation treatments.[101]

Pre-clinical investigations have shown that carbogen effectively reduces chronic hypoxia in tumors, but has little or no impact on acute hypoxia. Nicotinamide reduces acute hypoxia, though the mechanism remains unknown.[102] Thus, to overcome both types of hypoxia, nicotinamide and carbogen breathing were evaluated in combination. In pre-clinical tumor models, the combination showed substantial improvements in radiosensitivity by reducing both acute and chronic hypoxia.[103,104] However, it is important to note that these improvements were time and dose-dependent, making the duration of breathing carbogen, the dose of nicotinamide, and the timing of the radiotherapy crucial to the therapy success.[98,105,106] When evaluated in the clinic, combining carbogen and nicotinamide with conventional or accelerated radiotherapy protocols in bladder and laryngeal cancer demonstrated improvements in outcome.[107,108] However, despite showing promising results, the clinical usage of carbogen as a means to improve tumor oxygenation has fallen out of favor.

HYPOXIC CELL SENSITIZERS

Hypoxic cell radiation sensitizers mimic the effects of oxygen to increase radiation-induced DNA damage. Such agents are extremely effective in sensitizing hypoxic cells *in vitro* and in rodent tumors to radiation.[109–111] Several agents reached clinical evaluation. However, many clinical trials encountered dose-limiting toxicities. These precluded administration of the agents at doses sufficient for effective radiosensitization during courses of multidose fractionated radiotherapy.[109,110] Indeed, rodent studies mimicking the clinical treatment regimens showed no or very little improvement in tumor response.[110] To date, the most significant effects of combining hypoxic cell sensitizers with radiotherapy are those reported by Overgaard et al which combined nimorazole and radiotherapy to treat head and neck cancer patients, who experienced improvements in disease-free survival with no major side effects.[18] Consequently, the addition of nimorazole to radiotherapy has now become standard of care for head and neck cancer patients in Denmark.

BIOREDUCTIVE THERAPIES

In contrast to hypoxic cell sensitizers, bioreductive agents selectively kill hypoxic cells. These agents become cytotoxic by redox processes under hypoxic conditions.[112] A number of different classes of agents including quinones, nitroimidazoles, and benzotriazines demonstrate hypoxic selectivity.[113–115] One of the most effective, tirapazamine, causes DNA single- and double-strand breaks under low oxygen conditions, and significantly enhances the efficacy of radiotherapy in *in vitro* and in preclinical mouse models.[116,117] However, several clinical trials investigations combining tirapazamine with chemotherapy or radiotherapy failed to provide an improvement in survival compared to standard care.[118,119] Still, the area of deploying cytotoxic agents to eliminate treatment resistant hypoxic tumor cells remains an active area of research.[120] Given that tumor cells exposed to acute and chronic hypoxic conditions can initiate differences in genomic instability and repair capacities, it would appear imperative that the efficacy of future agents be evaluated under both short term and prolonged oxygen deprivation.

HIGH LINEAR ENERGY TRANSFER

An alternate method to reduce the loss of radiation sensitivity of cells existing in tumors in areas of low oxygen tensions is to use a radiation that is less dependent on oxygen for its efficacy. It is believed that as the LET for radiation increases, the OER decreases.[121,122] Charged particles, such as protons, have a high LET yet provide only a modest improvement in OER.[123] Heavier particles, such as carbon, hold promise for further decreasing the OER. While high-LET radiation can reduce the impact of hypoxia, currently used particle radiotherapy machines cannot completely overcome the detrimental impact of hypoxia on tumor response.

TARGETING THE TUMOR VASCULATURE

Antiangiogenic therapy

In order for a tumor to grow beyond a small size, it must induce its own vascular network.[124,125] However, the neoplastic cell demands are so severe the resultant tumor vasculature is functionally and structurally abnormal.[126] Due to constant stimulation with pro-angiogenic growth factors such as VEGF,[127] the tumor vasculature never achieves maturation, lacks coverage by pericyte or smooth muscle cells, and is leaky and prone to collapse.[128]

Several AA therapeutics have been developed as a strategy to target tumor vasculature.[129] Targeting the vasculature is appealing, as normal cells will not rapidly mutate and develop resistance, as tumor cells do. By blocking the effects of VEGF or other pro-angiogenic growth factors, the vasculature has an opportunity to mature and obtain pericyte and smooth muscle cell coverage in a process known as normalization. This normalization allows more effective blood delivery, reducing tumor hypoxia.[130] As a result, the addition of AA therapy to radiation can improve tumor response.[131] However, such normalization of the tumor vasculature after AA therapy is not a universal occurrence being dependent on the agent, schedule, phase of growth and tumor type.[47]

Vascular disrupting agents

VDAs do not target hypoxic cells, *per se*, rather, this class of drug disrupts established tumor vasculature and elicits a tumor cell death cascade due to prolonged ischemia. VDAs induce vascular shutdown lasting hours to days leading to extensive necrosis in the core and hypoxic regions of a variety of tumor types,[132,133] and consequently, leave behind viable well-oxygenated tumor cells that are sensitive to radiation and other conventional anti-cancer therapies.[47,132,133]

While both AA and VDA treatments can affect tumor oxygenation and therapy response, the combination of the two classes may be required to realize the full antitumor potential of vascular targeting therapy. Preclinical and initial clinical data support the therapeutic potential of such a combination.[133]

MODIFIABLE LIFESTYLE BEHAVIORS

Smoking and obesity

Modifiable lifestyle behaviors are also known impact tumor hypoxia. Patients who smoke cigarettes often have lower oxygen concentrations in blood as well as tumors, due to the reduction in arterial oxygen saturation. Pre-clinical investigations showed that carboxyhemoglobin levels such as those observed in smokers could significantly impair the efficacy of radiation treatment.[134] Non-small cell lung cancer patients, who smoke during radiotherapy have worse outcomes than their non-smoking counterparts.[135] Adipose tissue secretes a variety of cytokines, including many implicated in cancer progression, including TNF-alpha and IL-6.[136] Simple changes made to modifiable behaviors including obesity and smoking have the potential to modulate tumor oxygenation and tumor response to radiation therapy.

Exercise

Systemic interventions, such as aerobic exercise, may be a possible therapeutic adjuvant to conventional anticancer therapies by improving treatment efficacy, decreasing therapeutic side effects and improving overall quality of life. The idea that exercise may prevent cancer development was first postulated early in the 20th century,[137] and several elegant reviews have focused on physical activity and cancer risk,[138] tumor progression[139] and cardiovascular disease in cancer patients.[140] Preclinical data are beginning to accumulate on the effects of prolonged exercise training on the tumor microenvironment.[139] Results indicate that during exercise, the increased perfusion pressure to the tumor (and the inability of tumor blood vessels to constrict) increases tumor blood flow and reduces tumor hypoxia.[141] Given its potential to modulate tumor physiology, the use of physical exercise in conjunction with conventional anticancer therapies is under active investigation and a recent report indicates that exercise could improve cyclophosphamide efficacy.[142] In addition to the hypothesis that prolonged exercise may normalize tumor vasculature and its favorable immunological effects,[139,141,142] exercise training as an adjuvant to conventional anticancer therapy incurs little risk of negative side effects.

MODULATION OF TUMOR HYPOXIA SUMMARY

Pre-clinical studies have clearly established a variety of interventional approaches that can be utilized to overcome tumor

Figure 5. Effect of hypoxic modification in cancer patients treated with primary radiotherapy. Data are from 86 randomized trials including 10,108 patients with either bladder, cervical, head and neck, CNS, lung or other types of cancer such as esophagus and pancreas. Reprinted with permission from Overgaard[143].
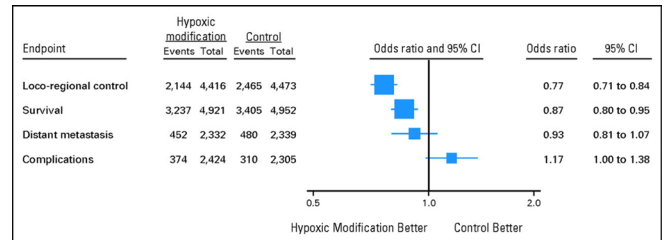
hypoxia and reduce its detrimental impact on conventional as well as emerging anticancer therapies. However, the translation of such approaches to the clinical management of cancer has been difficult. Enthusiasm for developing radiosensitizers and bioreductive agents has declined after multiple trials failed to demonstrate a positive impact. However, said trials did not include assessments of tumor hypoxia in the patients prior to enrolment. As with any targeted therapeutic, presence of the target is essential for therapeutic efficacy. Evaluating an agent in a setting where some of the patients lack the target will result in a high likelihood of failure to achieve statistically significant results. Moreover, as these interventions come with risks, it is imperative to deliver them only to patients who could benefit.

Despite limitations and difficulties, the comprehensive meta-analysis review by Overgaard[143] of more than 80 trials and 10,000 patients showed that hypoxia modification in solid tumors undergoing radiotherapy leads to improved outcomes (Figure 5). Clearly, these findings underscore the need for continued efforts to identify accurately those patients whose tumors may contain treatment refractory hypoxic cells. Most notably, it is essential to develop techniques that can quantify and monitor both acute and chronic hypoxia in solid tumors in patients. Until such an ideal can be achieved, non-invasive imaging that allows longitudinal evaluations of tumors throughout the course of therapy may prove most advantageous despite limitations.

Molecular, cellular, and functional behavior of cancer cells as well as their ability to cope with oxygen deprivation and therapeutic insults, differ greatly depending upon the extent and duration of the hypoxia exposure. It makes sense that therapeutic interventions and molecular targeting strategies aimed at overcoming detrimental effects of hypoxia or seeking to exploit hypoxia-associated cancer cell vulnerabilities are assessed under conditions of both acute and prolonged oxygen deprivation. Once obtained, such information will establish targeted molecules that allow the development of novel therapeutic intervention strategies to provide more effective treatment options for cancer patients.

CONCLUSIONS

Extensive data derived from preclinical investigations now exist to clearly demonstrate that hypoxic microenvironments in solid

tumors significantly impair tumor response to anticancer therapies, promote tumor aggression and progression, and facilitate the spread of cancer cells. Much interest in this topic originated from the scientific investigations of the "oxygen effect" by Sir Oliver Scott and colleagues at the Gray Laboratories in the 1950s that led to the translation from the laboratory to the pioneering application of hyperbaric oxygen in combination with radiation therapy. Furthermore, his research set the stage for numerous subsequent laboratory and clinical investigations seeking to understand and overcome the detrimental role of hypoxia in the treatment of cancer.

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