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Past approaches and future directions for targeting tumor hypoxia in squamous cell carcinomas of the head and neck

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ABSTRACT

Recurrent squamous cell carcinoma of the head and neck (SCCHN) carries a poor prognosis. Tumor hypoxia (TH) has been implicated as one of many factors contributing to SCCHN recurrence. TH leads to radiation resistance by reversing radiation-induced DNA damage. Effective strategies to overcome TH may improve outcomes in patients with SCCHN. We searched the English literature on PubMed and

Abbreviations: ARCON, accelerated radiotherapy with carbogen and nicotinamide; cAMP, cyclic adenosine monophosphate; CA4P, combretastatin A-4 phosphate; CAIX, carbonic anhydrase IX; CNS, central nervous system; CT, computed tomography; CTLA4, cytotoxic T-lymphocyte associated antigen 4; DCE, dynamic contrast-enhanced; DMXAA, 5, 6-dimethylxanthenone-4-acetic acid; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; EPO, erythropoietin; ESA, erythropoietin stimulating agent; FAZA, [18F]fluoroazomycin arabinoside; FETA, [18F]fluoroetanidazole; FETNIM, [18F]fluoroerythronitroimidazole; FMISO, [18F]fluoromisonidazole; Gd-DTPA, gadopentetate dimeglumine; GLUT, glucose transporter; HBO, hyperbaric oxyge; HER2, human epidermal growth factor receptor-2; HIF-1α, hypoxia inducible factor-1α; HPV, human papilloma virus; HSP, heat shock protein; IRE, inositol-requiring enzyme; LRC, loco-regional control; MHC, major histocompatibility complex; MIC, MHC class I chain-related; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; mTOR, mammalian target of rapamycin; NK, natural killer; NO, nitric oxide; OS, overall survival; PET, positron emission tomography; PD-L1, programmed death ligand-1; RCC, renal cell carcinoma; RNA, ribonucleic acid; SCCHN, squamous cell carcinoma of the head and neck; TH, tumor hypoxia; UPR, unfolded protein response; VDA, vascular disrupting agent; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.

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reviewed the reference sections of key articles related to TH (publications spanning from the early 1900s to the present). We summarized the underlying theory of TH in SCCHN, methods for quantifying it, and the numerous therapies developed to modulate it. We included articles that set the foundation of TH as a theory and the most relevant articles published within the last 15 years related to TH quantification and therapeutic targeting. Despite extensive research, targeting TH in SCCHN has not become a part of routine clinical practice in North America, and we analyze the pitfalls in hypoxia research that have led to this failure. We propose that future studies should test a combined approach of targeting the immune system in addition to cellular pathways rendered aberrant in TH and should include development of novel surrogate markers of TH and/or TH imaging.

1. Introduction

Tumor hypoxia (TH) in human malignancies is associated with inferior patient outcomes (Nordsmark et al., 2005). TH is present in most, if not all, squamous cell carcinomas of the head and neck (SCCHN), yet TH-directed therapy has not become a routine part of clinical care despite extensive research. To summarize the current understanding of TH in SCCHN and to propose a way forward, we focus this review on three main areas: 1) the evidence supporting TH's role in radioresistance; 2) methods for quantifying TH; and, 3) existing and emerging therapies targeting TH in SCCHN, with an examination of why many of these treatments have failed. We propose a way to overcome failure of TH-targeted therapy by improving our understanding of TH-induced immunomodulation. Immune-based therapy in combination with TH-targeted drugs is an area ripe for further research.

2. Theory of tumor hypoxia and radioresistance

TH's influence on radiation response as a theory was first proposed by Schwartz in 1909 when he used vascular compression to induce "anemia" in tissues. Radiation associated skin toxicity was reduced, allowing delivery of a higher dose to deep tumors (Kaplan, 1979). Thomlinson and Gray later hypothesized that TH impacted radiation response by conferring radioresistance (Gray et al., 1953). Analysis of cylindrical tumor sections suggested that oxygen diffused a maximum distance of 150–200 μm from a blood vessel (later determined to be a maximum of 70 μm) (Hall, 2000). They reasoned that cells located beyond this distance are anoxic and therefore undergo cell death; however, cells near this necrotic zone, at the outer limit of oxygen diffusion, are hypoxic, relatively radioresistant, retain the ability to form new tumors, and potentially could survive after a course of radiation, leading to tumor repopulation and disease relapse.

Elegant work by Powers and Tolmach (1963) generated cell survival-radiation dose curves under hypoxic conditions using three groups of lymphosarcoma-bearing mice exposed to room air or 100% oxygen during radiation. Using mathematical extrapolation from the cell survival curves of each group, an average of 15% radioresistant hypoxic cells was found in tumors radiated under air-breathing (normal) conditions. This experiment provided *in vivo* evidence of the existence of hypoxic, radioresistant cell populations (Powers and Tolmach, 1963; Moulder and Rockwell, 1984).

TH likely confers radioresistance because of "radical competition." As depicted in Fig. 1, DNA radicals generated by radiation exposure are "fixed" in a damaged state by oxygen forming peroxides with DNA, leading to cell death. In the absence of oxygen, DNA radicals may be reduced by hydrogen donation from sulfhydryl groups on glutathione or other thiol compounds, leading to radioprotection and cell survival (Zeman, 2009). Certain compounds, such as nitroimidazoles, mimic the effect of oxygen, leading to radiosensitization and cell death even in hypoxic tissues.

Blood vessel density and orientation, shunting within tumors and alterations in red cell membrane physiology in response to hypoxia are factors that contribute to TH (Dewhirst et al., 2008). Hypoxic fractions have been observed to change within a matter of hours in some tumors (Kallman, 1972), implying that transient alterations in the blood supply lead to TH (Brown, 1979). Such alterations in blood supply could increase the fraction of hypoxic cells, leading to detrimental effects on radiation response.

In recent years, many additional TH-induced changes in tumor biology, including alterations in gene expression, receptor signalling via tyrosine kinases, angiogenesis, apoptosis and immune function, have been recognized (Wilson and Hay, 2011). The degree to which modifications in these pathways confer radioresistance has not been well characterized. Therapies investigated to date therefore have been based on limited data obtained in an earlier era. Methods to improve oxygen delivery or mimic the effect of oxygen within tumors as means of overcoming hypoxia-driven radioresistance reflect an earlier, less nuanced view of TH. The failure of most TH-directed therapies to meaningfully alter clinical outcomes might be explained by this lack of understanding, as will be discussed further below.

3. Quantifying tumor hypoxia

SCCHN TH can be quantified with several methods, including oxygen electrodes inserted directly into tumors, staining of tumors with endogenous and exogenous markers, and TH imaging. The ideal technique, yet to be found, would be minimally to non-invasive, does not consume oxygen or disrupt oxygen distribution within the tumor, has high resolution spatially and temporally, and is non-toxic. An overview of these methods is provided in Table 1, and reviews have been published previously (Hoogsteen et al., 2007; Pacheco-Torres and Lopez-Larrubia Ballesteros et al., 2011; Bache et al., 2008). Each detection method has its pros and cons, but none is being used clinically.

Oxygen electrodes, while providing a direct measurement of TH, may miss regional variations in oxygen tension or redistribute/alter oxygenation within the tumor due to their large size. Non-invasive methods such as exogenous and endogenous marker staining avoid this concern. Exogenous TH detection methods involve administration of nitroimidazoles, compounds that form adducts with thiol groups on amino acids in hypoxic areas, prior to tumor biopsy or surgical excision. Endogenous TH detection consists of immunohistochemical staining of TH markers, including hypoxia inducible factor-1α (HIF-1α), erythropoietin, carbonic anhydrase IX (CAIX), glucose transporters (GLUT-1, GLUT-3), osteopontin and erythropoietin (EPO). Radiographic methods for measuring TH are less invasive than electrodes and exogenous/endogenous techniques, holding promise for more widespread clinical use.

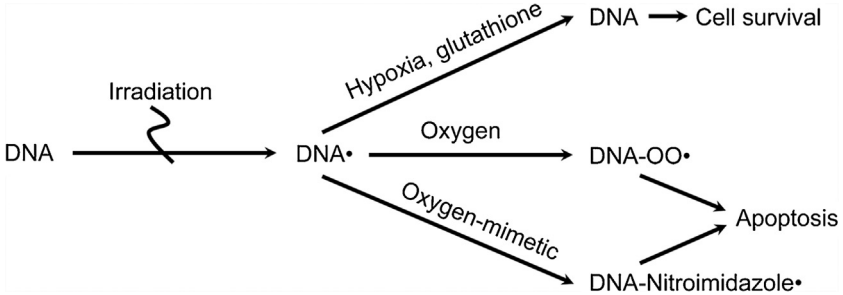

Fig. 1. Oxygen stabilizes DNA damage induced by radiation by forming peroxide compounds with DNA, leading to apoptosis. Hypoxia, intracellular thiol compounds and/or exogenously administered compounds (such as glutathione) reduce DNA radicals, leading to cell survival. Nitroimidazoles mimic oxygen's effect, leading to DNA peroxide formation and apoptosis. Adapted from Zeman EM. Biologic basics of radiation oncology. In: Gunderson L.L., Tepper J.E., editors. Clinical Radiation Oncology. Philadelphia: Churchill Livingstone; 2009. p 3–46.

Table 1
Methods to detect tumor hypoxia and correlation with clinical outcomes in published studies.

Method	Marker	Outcome	Reference
Oxygen electrode	Intratumor oxygen concentration	Inferior OS	Nordsmark et al. (2005); Rudat et al. (2000); Rudat et al. (2001)
Exogenous tumor staining	Pimonidazole	Inferior LRC and DFS	Kaanders et al. (2002c)
	EF5	Inferior DFS	Evans et al. (2007)
Endogenous tumor staining	HIF-1α, CAIX, OPN	Inferior LRC, OS	Bache et al., (2008)
	GLUT-1, GLUT-3	Inferior DFS, OS	Baer et al. (2002); Mineta et al. (2002)
	DLL4	Improved RFS	Kuhnert et al. (2011)
	EPO	Inferior OS	Lin et al. (2012)
	Hypoxia gene signature	Inferior LRC, MFS, RFS	Buffa et al. (2010); Betts et al. (2013); Toustrup et al. (2011); Clatot et al. (2014)

DFS, disease free survival; LRC, loco-regional control; MFS, metastasis free survival; OS, overall survival; RFS, recurrence free survival.

A novel approach to studying TH endogenously is array based profiling of hypoxia gene signatures. Buffa and colleagues developed a gene expression signature enriched for hypoxia genes, which correlated with metastasis-free and recurrence-free survival in a cohort of SCCHN patients (Buffa et al., 2010). Betts and others reported the results of a study wherein tumor samples from 80 SCCHN patients were subject to gene analysis using a Taqman Low Density Array (developed based on data from Buffa et al. and comprised of 26 previously published hypoxia genes expressed in SCCHN). Gene signature results were compared to pimonidazole staining. The authors concluded that gene expression profiling was sensitive for detecting TH and had lower intra-tumor heterogeneity than was observed with pimonidazole staining (Betts et al., 2013).

Gene signature profiling may be useful for predicting treatment response. Toustrup and others validated a 15-gene hypoxia classifier using *in vitro* and *in vivo* methods. It was then validated using 323 biopsies from SCCHN patients enrolled in a Danish study of radiation with nimorazole or placebo. Patients with "more hypoxic" tumors based on gene signature profile who received nimorazole had better 5-year loco-regional failure (54%) than patients with "more hypoxic" tumors randomized to placebo (21%, $p = 0.0001$). The authors believed that the gene signature set could identify patients with hypoxic tumors who might benefit from hypoxia modification (Toustrup et al., 2011). Clatot and others validated a gene expression profile in which the over-expression of 9 genes, including 3 related to TH (HIF1α, CAIX and DLL4), correlated with inferior metastasis-free survival (Clatot et al., 2014). Ongoing prospective trials are evaluating hypoxia gene expression profiling in SCCHN patients undergoing chemoradiation (Bethesda, 2013). Although gene profiling as a predictive biomarker for response to TH-modifying therapy is appealing, cost and reproducibility may be barriers to widespread clinical use. Significant advances in the technology of studying gene expression profile in recent years may lower the cost in the near future to allow clinical trials to further evaluate the value of such approach to design targeted treatment against TH, similar to what has been performed for genetic assays used in early stage breast cancer (Blohmer et al., 2013), and non-small cell lung cancer (Roth et al., 2014).

Radiographic methods for measuring TH are less invasive than exogenous or endogenous techniques and eventually may become more widely used. Positron emission tomography (PET) imaging, when combined with nitroimidazoles chemically modified to contain a radionuclide group, can be used to quantify TH. [^{18}F]fluoromisonidazole (FMISO), [^{18}F]fluoroazomycin arabinoside (FAZA), [^{18}F]fluoroetanidazole (FETA) and [^{18}F]fluoroerythronitroimidazole (FETNIM) are common nitroimidazole agents administered with PET. Hypoxic areas in human tumors *in vivo* are visible using such PET tracers (Krause et al., 2006). Lung, SCCHN and prostate cancers were among the first tumor types shown to contain regional hypoxia by FMISO-based imaging (Rasey et al., 1996). In one study of SCCHN patients, pre-treatment FMISO PET predicted survival (Rajendran et al., 2006). It also predicted response to radiation and local progression-free survival (Kikuchi et al., 2011). Limitations of FMISO-based imaging include slow uptake into tumors, lack of contrast with background tissue and accumulation of radioactive byproducts (Gronroos and Minn, 2007). One study of FETNIM-based imaging in SCCHN reported a trend toward inferior OS with higher FETNIM uptake prior to radiation therapy (Lehtio et al., 2004). FAZA-based imaging was studied in 11 patients with SCCHN. FAZA uptake was confirmed in 7 of 11 primary tumors and 3 of 11 lymph node metastases, although uptake in the kidney and hepatobiliary tree and a mean tumor-to-muscle uptake ratio of 2.3 ± 0.3 were seen as limitations to use of this tracer (Souvatzoglou et al., 2007). No tracer is felt to be superior for radionuclide-based PET imaging; tracers are selected based on their availability and tumor type. For example, FMISO appears most promising in SCCHN, whereas FAZA may have a greater role in central nervous system (CNS) tumors (Chitneni et al., 2011).

PET combined with other imaging modalities may give additional insight into the tumor microenvironment and the presence of TH. Gadopentetate dimeglumine (Gd-DTPA)-based dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) allows for assessment of blood perfusion, permeability of tumor vasculature, and fluid volume approximations (Yankeelov and Gore, 2009). When combined with FMISO PET and studied in 13 node-positive SCCHN patients, FMISO uptake negatively correlated with tumor perfusion as assessed by DCE-MRI. This result further supports the hypothesis that tumors contain hypoxic areas, and that such areas are created, at least in part, by poor perfusion (Jansen et al., 2010).

Novel nitroimidazole compounds in combination with magnetic resonance spectroscopy (MRS) are being studied. SR-4554 and TF-MISO, both fluorinated nitroimidazole derivatives, have been studied *in vivo* in a variety of tumors. SR-4554 signal intensity correlated with pO_2 measurements in animal models, and has entered Phase I clinical trials, where it has been well tolerated and demonstrated retention within tumors (Pacheco-Torres and Lopez-Larrubia Ballesteros et al., 2011; Seddon et al., 2002; Lee et al., 2009). TF-MISO has not yet been studied in humans, but pre-clinical data suggest it is retained in hypoxic tumors (Procissi et al., 2007). One advantage of magnetic resonance techniques is better spatial resolution of TH than PET provides.

Several studies have attempted to correlate hypoxia tumor imaging results with immunohistochemical markers of hypoxia, but have yielded conflicting results. In one study of 15 SCCHN patients treated with radiation, no correlation between FETNIM imaging results and endogenous expression of hypoxia markers such as HIF-1α and GLUT-1 was found (Gronroos et al., 2014). Other studies have found correlations between FMISO results and HIF-1α staining (Norikane et al., 2014; Sato et al., 2013). Studies to examine the optimal timing of hypoxia imaging during radiation for SCCHN also have been conducted, based on the hypothesis that hypoxia imaging results can be used to increase radiation doses to hypoxic tumors not responding during treatment (Bollineni et al., 2014). However, larger validation studies of these techniques will be needed before they can be implemented in routine clinical practice.

To summarize, hypoxia imaging techniques are not validated sufficiently and their use should be limited as investigational tools. Including hypoxia imaging in future studies of TH-targeted therapies would be useful to further validate this technique in identifying patients for TH-directed therapy, as well as to help confirm an investigational drug's activity on hypoxia pathway(s). Cost-effectiveness of hypoxia imaging is an important factor to consider when considering widespread clinical application of the technique.

4. Current techniques for targeting TH

A systematic review and meta-analysis of studies including 4805 patients with SCCHN found significant improvements in LRC (OR 0.71, 95% CI 0.63–0.80, $p < 0.001$) and OS (OR 0.87, 95% CI 0.77–0.98, $p = 0.03$) with hypoxic modification combined with radiation (Overgaard, 2011). This evidence suggests hypoxic modification probably alters SCCHN tumor biology in clinically meaningful ways. Hypoxic modification strategies that have been investigated in clinical trials are listed in Tables 2 and 3, and will be reviewed here. Note that none of the strategies listed in Tables 2A and 3 have become part of routine clinical practice.

4.1. Hyperbaric oxygen

Hyperbaric oxygen (HBO) is believed to improve oxygen delivery to hypoxic tissues, leading to radiosensitization and therapeutic effect (Table 3). Patients breathe 100% oxygen at a pressure above 1 atmosphere in an enclosed chamber for 20–30 min prior to radiation. The technique was abandoned in the 1980s largely due to space and physical requirements of HBO-based radiation and because oral agents targeting TH became available. Enthusiasm also was dampened by an accident involving explosive decompression of a chamber (Tobin, 1971). Any benefit to HBO likely is offset by increased risk of seizures and radiation tissue injury (Bennett et al., 2005).

Table 2
Existing approaches to targeting SCCHN tumor hypoxia.

Therapeutic Target	Therapy
Increase tumor oxygen delivery	Hyperbaric oxygen Hyperthermia Blood transfusion ESAs Pentoxifylline
Hypoxic cells	Tirapazamine
Radiosensitization	Nitroimidazoles
Combination	ARCON

ARCON, accelerated radiotherapy with carbogen and nicotinamide; ESAs, erythropoietin stimulating agents.

4.2. Hyperthermia

Several studies show hyperthermia favorably impacts TH. Randomized trials of hyperthermia with or without radiation in SCCHN patients showed improved LRC (OR 2.88, 95% CI 1.28–3.39) for combined treatment. Three trials demonstrated improvement in OS, but one trial reported an increase in late toxicity (Horsman and Overgaard, 2007; Datta et al., 1990; Valdagni and Amichetti, 1993; Huilgol et al., 2010). Newer hyperthermia delivery techniques are being studied, which may prompt additional clinical trials in SCCHN (Hurwitz, 2010).

4.3. Blood transfusion

Up to 64% of SCCHN patients were classified as anemic in one trial (Lee et al., 1995) and anemia is almost universal during a course of chemoradiation (Rosen et al., 2003). Lower oxygen carrying capacity theoretically could worsen TH and increase radioresistance. Pre-treatment and/or post-treatment anemia is prognostic for LRC and OS in SCCHN, with an approximate 10% decrease in loco-regional control for every 2 g/dL decrease in hemoglobin (Hu and Harrison, 2005). Radiosensitivity was improved in anemic mice given blood transfusions (Hirst and Wood, 1987; Hill et al., 1971). However, clinical trials evaluating the benefit of blood transfusion prior to or during radiation, though few in number, do not support it as a means to improve loco-regional control, disease-free survival or overall survival, as shown in Table 3. Pre-radiation blood transfusion has not been adopted in routine clinical practice and is not being studied actively at this time.

4.4. Erythropoietin stimulating agents (ESAs)

As with blood transfusion, preclinical models demonstrated improvement in anemia-related radioresistance with ESA use (Stuben et al., 2003). A retrospective analysis of 191 patients with SCCHN treated over a 10-year period with chemoradiation and surgery found that patients given recombinant erythropoietin (EPO) for a pre-chemoradiation hemoglobin value less than 14.5 g/dL had significantly higher 2-year LRC and OS than patients with similar hemoglobin values not given EPO (Glaser et al., 2001). Subsequently, 2 randomized trials and a systematic review found inferior outcomes with ESA use in SCCHN, as shown in Table 3. The presence of EPO receptors on SCCHN and their function as growth

Table 3
Clinical studies of select tumor hypoxia-modifying treatments in SCCHN.

Therapy	Study Design	No. Patients	Outcome	Limitations	Reference(s)
Hyperbaric oxygen	Systematic review of trials	2286	5-year mortality RRR 0.82 ($p = 0.03$); NNT = 5; 5-year RR improved LC 0.77 ($p = 0.01$)	Included studies comparing conventional fractionation RT (without HBO) to HBO with hypofractionated RT; hypofractionation may have produced hypoxic sensitization; unclear from available evidence if HBO with conventional RT has a clinical benefit; heterogeneity of TH within tumors may limit effect of HBO on disease biology	Bennett et al. (2005)
Blood transfusion	RCT: transfusion for pre-radiation hemoglobin <13 g/dL (women) or <14.5 g/dL (men) versus no transfusion prior to RT	414	No improvement in LRC, DFS, OS; patients without anemia had improved LRC	A sub-randomization of a RCT comparing nimorazole plus RT to RT alone; may lack power	Hoff et al. (2011a)
Blood transfusion	Pooled analysis of 2 RCT: transfusion for pre-radiation hemoglobin <3 g/dL (women) or <14.5 g/dL (men) versus no transfusion prior to RT	1166	No improvement in LRC, DFS, OS	Low hemoglobin may be marker of a worse overall clinical condition that cannot be compensated by blood transfusion	Hoff et al. (2011b)
ESAs	RCT: EPO vs. no EPO for hemoglobin ≤13.5 g/dL (men) or 12.5 g/dL (women) prior to RT	148	3-year LRC, LRPFS, OS not improved; trend for better LRC in control arm	Study prematurely closed (futility determined at interim analysis); trial not designed specifically to evaluate inferior outcomes with ESA	Machtay et al. (2007)
ESAs	RCT: epoetin beta vs. placebo for hemoglobin <120 g/L (women) or 130 g/L (men) prior to and during RT	351	Inferior LRPFS (RR 1.62, $p = 0.0008$); inferior OS (RR 1.39, $p = 0.02$) with epoetin beta	Patients undergoing both definitive RT and adjuvant RT (for both completely and incompletely resected disease) included	Henke et al. (2003)
ESAs	Systematic review of RCT	1397	Inferior OS (OR 0.73, $p = 0.005$); inferior LRPFS (OR 0.63, $p = 0.0002$) with EPO	Inferior outcomes with EPO may be from treating to a higher hemoglobin concentration	Lambin et al. (2009)
ARCON	Single arm, single institution prospective study	51	2-year actuarial LC 92%; 2-year actuarial OS 85%	16% of patients discontinued inhaled carbogen and 33% discontinued nicotinamide during the study (nausea/vomiting)	Kaanders et al. (1998)
ARCON	Single arm, single institution prospective study	215	3-year actuarial LC better for larynx and oropharynx (80% and 88%) than for hypopharynx and oral cavity (69% and 37%)	31% discontinued nicotinamide during the study (nausea/vomiting)	Kaanders et al. (2002b)
ARCON	Multi-institution RCT of accelerated radiotherapy alone versus ARCON	345	No improvement in 5-year LC, laryngeal preservation, DFS and OS; 5-year regional control improved with ARCON (93% versus 86%, $p = 0.04$).	Requirement for and timing of neck dissection for non-CR after RT not specified; regional failure rates may be inaccurate if patients undergoing required "salvage" neck dissections after RT were not counted as failures; heterogeneity of TH within tumors may limit effect of ARCON on disease biology	Janssens et al. (2011)
ARCON	Translational side study of a multi-institution RCT; pimonidazole administered prior to primary tumor biopsy	79	Regional control improved and trend toward improved 5-year DFS among ARCON-treated patients with hypoxic tumors (86% versus 40%, $p = 0.08$)	Small numbers of regional failures and pimonidazole staining of primary tumor (rather than regional nodes) limit conclusions about ARCON effect on controlling hypoxic regional disease	Janssens et al. (2012)

ARCON, accelerated radiotherapy with carbogen and nicotinamide; CR, complete response; DFS, disease free survival; ESAs, erythropoietin stimulating agents; HBO, hyperbaric oxygen; LC, local control; LRC, loco-regional control; LRPFS, loco-regional progression free survival; NNT, number needed to treat; OS, overall survival; RCT, randomized controlled trial(s); RR, relative risk; RRR, relative risk reduction; RT, radiation therapy.

and angiogenesis factors may explain these findings (Kjellen et al., 2006; Ribatti et al., 1999). Another possibility is that tumor oxygen delivery actually is impaired when hemoglobin levels are increased to 14–15 g/dL as a result of microcirculation blockage by relative polycythemia (Machtay et al., 2007). ESAs are not recommended for SCCHN patients being treated with curative intent radiation.

4.5. Agents targeting tumor blood flow

Pentoxifylline, a methylxanthine derivative, decreases fibrinogen levels and increases intracellular cyclic adenosine monophosphate (cAMP), thereby altering cell rigidity (Aviado and Porter, 1984; Ward and Pentoxifylline, 1987). Blood viscosity therefore decreases and erythrocyte flexibility increases, thereby theoretically improving tumor blood flow. Most trials evaluated pentoxifylline as a radiosensitizer in combination with oxygen, nicotinamide and radiation, with disappointing results (Johnson et al., 1998; Stewart et al., 1997; Kwon et al., 2000). No clinical trials of pentoxifylline as a radiosensitizer or radioprotector for SCCHN treatment are ongoing presently.

4.6. Targeting hypoxic cells—bioreductive drugs

Compounds that have selective activity in hypoxic environments are referred to as "bioreductive prodrugs." They are reduced in hypoxic environments to an active superoxide state, while remaining oxidized and inert in normoxic environments, resulting in hypoxia-selective cytotoxicity. Such compounds must contain 1 of 5 unique structures (nitro groups, quinones, aromatic or aliphatic *N*-oxides or a transition metal) to be reduced selectively in hypoxic environments (Wilson and Hay, 2011). Examples include tirapazamine (containing an aromatic *N*-oxide group), banoxantrone (aliphatic *N*-oxide group), apaziquone (quinone group) and PR-104 (nitro group). Banoxantrone and PR-104 are discussed further in Part 5. Nitroimidazoles also are classified as bioreductive prodrugs and will be reviewed in more detail below.

Tirapazamine has been investigated in Phase III trials in both SCCHN and cervical cancer. Neither of these trials demonstrated a benefit when the drug was added to cisplatin and radiation in unselected patients (Rischin et al., 2010; DiSilvestro et al., 2014). Another Phase III trial of tirapazamine with radiation in SCCHN was closed early due to excess mortality in the tirapazamine group (Seiwert et al., 2007). It is unclear what contributed to the observed excess deaths since the trial results have not been published.

4.7. Radiosensitization

Nitroimidazoles are classified as bioreductive prodrugs, possessing both cytotoxic and radiosensitizing properties. They contain aromatic heterocycle di-*N*-oxide groups and a lipophilic hydrocarbon side chain. The nitrogen atoms in the molecule, having an electron affinity similar to oxygen, confer radiosensitizing properties. With ionization induced by radiation, the molecule is reduced, in turn delivering oxidative damage to DNA, an oxygen-mimetic effect. Metronidazole, misonidazole and etanidazole were among the first nitroimidazoles studied for clinical use but proved unsuitable (Table 4).

Nimorazole, a 5-nitroimidazole, has demonstrated clinical efficacy in randomized studies. A Danish phase III trial (DAHANCA 5-85) of 414 patients undergoing radiation for pharynx and supraglottic larynx cancer showed 5-year LRC of 49% with nimorazole compared to 33% among placebo-treated patients ($p < 0.002$). A non-significant improvement in OS was observed among nimorazole-treated patients (OR 1.32, 95% CI, 0.84–2.05; 10-year actuarial survival 26% with nimorazole versus 10% with placebo, $p = 0.32$). The most common side effects included nausea and vomiting (26%), flushing (12%) and a transient skin rash (8%) (Overgaard et al., 1998). No randomized studies have compared nimorazole plus radiation to other radiosensitizers, such as cisplatin or cetuximab, plus radiation. Use of nimorazole has not been adopted for treatment of SCCHN outside of Denmark, likely due to a lack of data showing superiority of nimorazole over other chemotherapeutic drugs, as well as to established practice patterns favoring platinum agents as radiosensitizers in most of the world. A randomized study comparing accelerated radiotherapy alone to accelerated radiotherapy plus nimorazole (IAEA-HypoX) is ongoing (Bethesda, 2011).

4.8. ARCON

Accelerated radiotherapy with carbogen and nicotinamide (ARCON) combines high concentrations of oxygen mixed with 2–5% carbon dioxide, with oral nicotinamide 60 mg/kg daily and hyperfractionated radiation (Kaanders et al., 2002a). ARCON increased oxygen enhancement ratios in mammary carcinoma, sarcoma and SCCHN cell lines to a higher degree than in normal tissue (Horsman et al., 1997). Phase I and II clinical trials of ARCON in SCCHN, CNS malignancies and bladder cancer showed that the approach was feasible. A randomized Phase III study of ARCON failed to show meaningful improvements in outcomes compared to accelerated radiation alone (Table 3). However, a recent analysis of 272 histologic specimens of patients enrolled in this trial identified significantly improved 5-year locoregional control and disease specific survival in subjects with low epidermal growth factor receptor (EGFR) expression randomized to ARCON versus accelerated radiotherapy alone, suggesting EGFR expression may be a predictive biomarker for patients likely to benefit from ARCON (Nijkamp et al., 2013). Pre-treatment anemia (hemoglobin $\leq$12 g/dL for women; $\leq$13.6 g/dL for men) also may be a predictor of ARCON benefit (five-year locoregional control 79% with anemia versus 53% without anemia, $p = 0.03$; disease free survival 68% versus 45%, $p = 0.04$) (Janssens et al., 2014). Further study of ARCON in carefully selected patients (such as those with pre-treatment anemia and without EGFR over-expression) may be warranted. A proposed mechanism of ARCON's anti-tumor activity is via downregulation of the HIF-1α pathway (Semenza, 2000). EGFR signalling upregulates this pathway (Nijkamp et al., 2013), thereby suggesting a mechanism whereby tumors with high EGFR expression overcome the effects of ARCON. Animal experiments evaluating the role of EGFR-dependent upregulation of HIF-1α pathway are needed prior to conducting additional human trials with this approach.

5. Emerging techniques for targeting TH

Among the TH-targeting strategies discussed in Part 4, nimorazole is the only agent that has been adopted for clinical use, and then only in Denmark. A variety of emerging techniques for targeting TH are under active investigation (Table 5).

5.1. Agents targeting tumor blood flow

Vascular disrupting agents (VDAs) alter tumor blood flow via activity on the colchicine binding site on tubulin, leading to changes in endothelial cell shape and increased vascular permeability. They also may exert cytotoxic effects on endothelial cells, possibly through activation of apoptosis (McKeage and Baguley, 2010). Weak inter-cellular junctions and abnormalities of smooth muscle and basement membranes in vessel walls render tumor vasculature susceptible to VDAs (Ching et al., 2002; Baluk et al., 2003). Combretastatin A-4 phosphate (CA4P) and AVE8062 are two tubulin-binding VDAs among many in clinical development; 5,6-dimethylxanthenone-4-acetic acid (DMXAA) is a flavone acetic

Table 4
Summary of nitroimidazole compounds demonstrating no meaningful clinical activity in human malignancies.

Nitroimidazole	Clincal Studies	Current Status	Reference
Metronidazole	RCT: 36 patients with supratentorial glioblastomas • Metronidazole plus radiation versus radiation alone • 4.5 month improvement in time to relapse/death with combination • Benefit not better than historic controls	A commonly used antibiotic	Urtasun et al. (1976)
Misonidazole	32 studies; only 5 demonstrated a benefit Neurotoxicity was dose limiting • Up to 80% of patients developed peripheral neuropathy at three years in one trial	Ultimately abandoned for clinical use Modified to [^{18}F]fluoromisonidazole for imaging purposes	Bydder et al. (1989)
Etanidazole	Developed as a superior compound to misonidazole (less lipophilic nature believed to cause less neurotoxicity) Phase I study showed higher doses were possible than with misonidazole Phase III study of 521 patients with SCCHN • No differences in 2-year actuarial survival or 2-year actuarial locoregional control between etanidazole plus radiation versus radiation alone	Ultimately abandoned for clinical use Modified to [^{18}F]fluoroetanidazole for imaging purposes	Lee et al. (1995)

RCT, randomized controlled trial; SCCHN, squamous cell carcinoma of head and neck.

Table 5
Emerging techniques for targeting SCCHN tumor hypoxia.

Therapeutic Target	Therapy
Alter tumor blood flow	VDAs
Hypoxic cells	Banoxantrone Dinitrobenzamide mustards
Radiosensitization	Evofosfamide Bevacizumab
Hypoxia-induced proteins and pathways	HIF-1 inhibitors HSP90 inhibitors CAIX inhibitors Bortezomib mTOR inhibitors
Autophagy	Hydroxychloroquine mTOR inhibitors
Immune modulation	Nitric oxide mimetics Adenosine receptor inhibitors PD-1 inhibitors CTLA-4 inhibitors

CAIX, carbonic anhydrase IX; CTLA4, cytotoxic T-lymphocyte-associated protein-4; HIF-1, hypoxia inducible factor-1; HSP90, heat shock protein 90; mTOR, mammalian target of Rapamune; PD-1, programmed death receptor-1; VDAs, vascular disrupting agents.

acid analogue in clinical development, thought to induce endothelial cell apoptosis and subsequent tumor hemorrhagic necrosis (McKeage and Baguley, 2010; Dvorak et al., 1988). Additionally, DMXAA may eradicate hypoxic regions of a tumor via feedback mechanisms with tumor necrosis factor, allowing tumor reoxygenation and reducing radioresistance (Wilson et al., 1998). When combined with chemotherapy, DMXAA has shown activity in treatment of NSCLC in Phase II trials (McKeage et al., 2008). Pre-clinical sarcoma models of VDAs combined with radiation or thermoradiation have shown improved tumor response (Horsman, 2008; Murata et al., 2001; Li et al., 1998). In these models, extensive necrosis in the center of tumors has been observed in response to VDAs (Li et al., 1998), suggesting that a hypoxic, radioresistant population of cells may be eliminated by the agent, rendering the remaining viable tumor cells at the periphery sensitive to radiation. A mechanism of action such as this justifies clinical trials of this approach, but as of this writing, VDAs combined with radiation are not being studied in SCCHN patients.

5.2. Targeting hypoxic cells—bioreductive drugs

Aliphatic *N*-oxide groups on banoxantrone undergo 2-electron reduction via cytochrome P450 enzymes (specifically CYP3A4), to transform the molecule into a DNA intercalator and topoisomerase II inhibitor known as AQ4. Bioreduction of the drug occurs preferentially in hypoxic tumor environments where P450 enzymes are upregulated. Once bound to DNA, AQ4 is unable to diffuse to areas outside of tumor, thereby lessening toxicity (Papadopoulos et al., 2008). A Phase I dose-finding trial has been completed (Papadopoulos et al., 2008), with studies of the drug in combination with temozolomide and radiation for glioblastoma multiforme ongoing (Bethesda, 2000). The DNA binding properties of the drug suggest it may have radiosensitizing properties, making a trial of AQ4 with radiation for SCCHN an intriguing possibility.

PR-104, a dinitrobenzamide mustard, is reduced in hypoxic environments to generate an active mustard moiety. A Phase I study of PR-104 alone and a Phase Ib study of PR-104 in combination with gemcitabine or docetaxel, both in patients with advanced solid tumors, have been completed (McKeage et al., 2011, 2012). In the Phase Ib study, thrombocytopenia with gemcitabine and PR-104 was dose-limiting and prohibited further development of this combination. A recommended Phase II dose was established for docetaxel and PR-104, although the combination requires filgrastim support. At the recommended Phase II dose, 3 of 12 patients had a partial response, and 2 patients had stable disease for at least 18 weeks. Of the 42 patients treated on the trial, 4 partial responses were observed, 3 of which had SCCHN histology (McKeage et al., 2012). No active, accruing trials of PR104 are open presently. Investigations of AQ4 and PR104 in SCCHN may show improved outcomes relative to past studies of bioreductive drugs, given a different anti-tumor mechanism of action than tirapazamine.

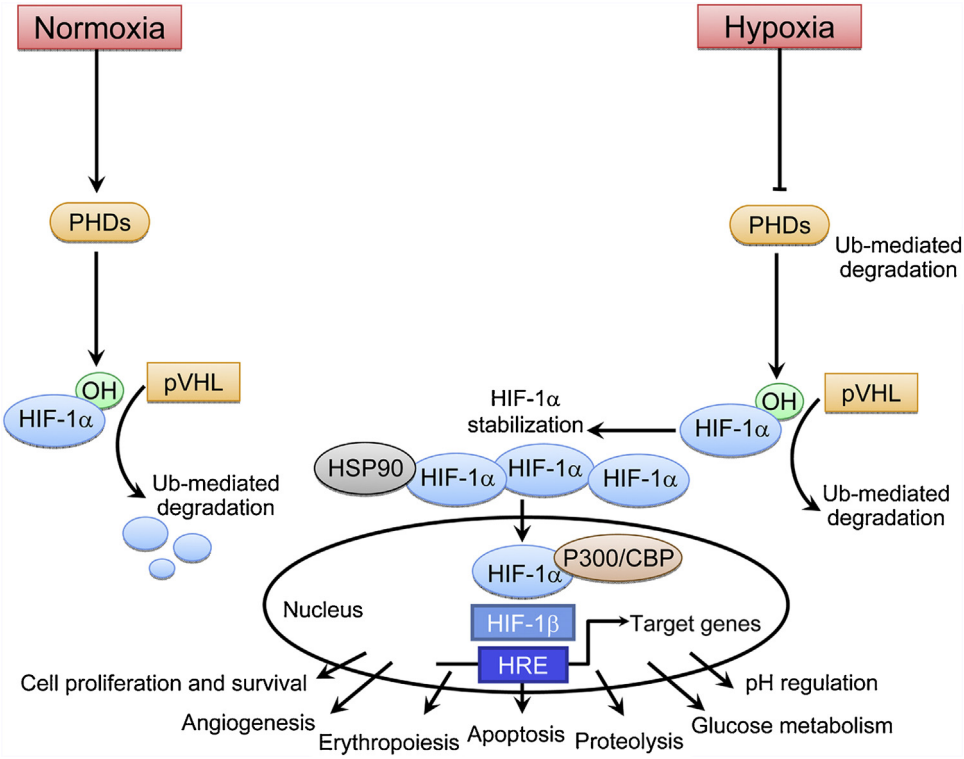

Fig. 2. Schematic of the hypoxia inducible factor 1alpha (HIF-1α) pathway. In the presence of normoxia, prolyl-4-hydroxylase (PHDs) hydroxylates proline residues on the von Hippel-Lindau protein (pVHL), which in turn binds to HIF-1α, targeting it for ubiquitination and degradation. In low oxygen states, HIF-1α accumulates, leading to transcription of genes necessary for cell survival, angiogenesis, pH regulation and cellular metabolism.

From: Carroll VA, Ashcroft M. Targeting the molecular basis for tumour hypoxia. *Expert Rev. Mol. Med.* 2005; 7 (6): 1-16, reproduced with permission.

5.3. Radiosensitization

Evofosfamide (formerly TH-302) is a nitroimidazole derived from an active metabolite of ifosfamide, further substituting bromine into positions typically occupied by chlorine in the molecule. When reduced under hypoxic conditions, a bromo-iphosphoramide mustard agent is released, leading to DNA damage, while the drug remains inert in oxic cells (Hart et al., 2008; Ganjoo, 2010). This agent is an excellent example of the ability of nitroimidazoles to confer hypoxia-selective cytotoxicity. The drug currently is not being studied in SCCHN, but its nitroimidazole properties provide a rationale for studying evofosfamide with radiation in SCCHN and other tumors.

Bevacizumab, a humanized monoclonal antibody specific for vascular endothelial growth factor (VEGF), has radiosensitizing properties. Pre-clinical models demonstrated impaired endothelial cell function in response to bevacizumab, and when combined with radiation, endothelial cell apoptosis increases and tumor angiogenesis decreases significantly (Hoang et al., 2012; Bozec et al., 2008). A Phase II trial of bevacizumab with cisplatin and radiation for Stage III and IV SCCHN has been completed; 42 patients received cisplatin 50 mg/m^2 on days 1, 2, 22, 23, 43 and 44 with bevacizumab 15 mg/kg on days 1, 22 and 43, along with 70 Gy of radiation. At 2 years, progression free survival was 75.9% (95% CI, 63.9–90.1%) and OS was 88% (95% CI, 78.6–98.4%). Toxicity did not appear increased compared to the historical standard of cisplatin and radiation (Fury et al., 2012). Another phase II study of bevacizumab with cisplatin and cetuximab plus radiation for Stage III and IV SCCHN has been published, with acceptable toxicity levels and similar survival outcomes (Fury et al., 2015). In a Phase I study of bevacizumab and cisplatin with radiation, hypoxia imaging using Cu-diacetyl-bis(N4-methylthiosemicarbazone) PET/CT demonstrated significant reductions in mean SUV with bevacizumab therapy prior to radiation and during radiation (Nyflot et al., 2015). It remains unclear from the available data whether the therapeutic effect of bevacizumab is to reduce pathologic blood vessel growth within tumors, thereby leading to enhanced oxygen delivery and tumor responsiveness (Carmeliet and Jain, 2011; Masunaga et al., 2011), or whether it reduces blood vessel recruitment into tumors, thereby further increasing hypoxia and causing additional tumor necrosis (Kruser et al., 2010; Williams et al., 2007). Bevacizumab might best be used along with radiation as de-intensified treatment for favorable risk human papilloma virus (HPV)-related SCCHN. Preclinical models have shown upregulation of VEGF in response to HPV infection (Tang et al., 2007), providing the rationale to study the drug further especially in this patient population.

5.4. Drugs targeting hypoxia-induced proteins

Cells exhibit a well-known pattern of gene activation in response to hypoxia. HIF-1 and CAIX are transcription factors stabilized under hypoxic conditions, leading to transcription of genes involved in cellular metabolism, angiogenesis, invasion and survival. As depicted in Fig. 2, the normoxic state leads to hydroxylation of proline residues on von Hippel-Lindau protein (VHL) by prolyl-4-hydroxylase. VHL in turn binds to HIF-1, targeting the protein for ubiquitination and degradation. In low oxygen states, HIF-1 accumulates, leading to gene transcription (Powis and Kirkpatrick, 2004). p53 also has been implicated in HIF-1 regulation, with loss of p53 leading to decreased MDM-2 mediated ubiquitination and degradation of HIF-1. Heat shock protein HSP90 plays a role in HIF-1 stabilization, since inhibitors of HSP90 lead to increased HIF-1 ubiquitination and degradation (Isaacs et al., 2002). The PI3K/AKT/mTOR pathway also has been demonstrated to regulate HIF-1 expression in some reports, but not others (Jiang et al., 2001; Hudson et al., 2002; Alvarez-Tejado et al., 2001). *In vitro* and in animal models, HIF-1 inhibition by small interfering RNAs or hairpin RNAs

decreased cell migration and angiogenesis and decreased cell survival, among other effects (Bache et al., 2008). Methods of targeting HIF-1 include inhibiting the PI3K/AKT/mTOR pathway, inhibiting HSP90, and small molecules that inhibit HIF-1 stabilization (PX-478, BAY 87-2243) (Isaacs et al., 2002; Treins et al., 2002; Zhong et al., 2000; Minet et al., 1999; Jordan et al., 2005; Ellinghaus et al., 2011). Phase I trials of PX-478 and BAY 87-2243 are ongoing. A Phase I trial of bortezomib (decrease HIF-1 transcriptional activity) and bevacizumab showed a partial response in 4 of 91 patients with advanced, refractory malignancies and 7 patients with stable disease for more than 6 months (Falchook et al., 2014).

HSP90 inhibitor trials have shown mixed results. Phase II trials of patients with castrate-resistant prostate cancer and metastatic melanoma were disappointing (Heath et al., 2008; Solit et al., 2008). In contrast, a phase II trial of the HSP90 inhibitor tanespimycin with trastuzumab for HER2 positive metastatic breast cancer showed a combined 59% rate of complete/partial response or stable disease, with a 22% overall response rate (Modi et al., 2011). Among 3 patients with ALK gene rearrangement-positive advanced NSCLC treated with the HSP90 inhibitor IPI-504, there were 2 partial responses and 1 stable disease (Sequist et al., 2010). HSP90 inhibitors are not being studied in SCCHN patients as of this writing. However, trials of HSP90 inhibitors in SCCHN would be justified owing to their mechanism of action on HIF-1 stabilization in areas of TH.

CAIX is a membrane associated zinc metalloenzyme involved in conversion of carbon dioxide to bicarbonate, regulating intracellular pH in hypoxic cells reliant on glycolysis for energy production. It also may be involved in cell adhesion and signal transduction (Pastorekova et al., 2008). CAIX is overexpressed in solid tumors, including SCCHN, where it has been linked to tumor necrosis, higher tumor stage, and poor treatment outcome (Beasley et al., 2001; Kaluz et al., 2009). HIF-1 binds to G/ACGTG sequences in DNA (hypoxia responsive elements), leading to transcription of CAIX (Kaluz et al., 2009). Phase II clinical trials of G250, a monoclonal antibody against CAIX, in patients with advanced renal cell carcinoma (RCC) demonstrated median survivals up to 15 months, and stable disease in 27–30% of patients (Bleumer et al., 2004; Varga et al., 2003). When combined with daily low-dose IL-2, 23% of advanced RCC patients experienced either stable disease or partial response and mean survival of 22 months (Bleumer et al., 2006). Trials of G250 in the adjuvant RCC setting are ongoing. The drug is not being studied presently in SCCHN, but based on known overexpression of CAIX in SCCHN, a trial to evaluate its efficacy in this patient population may be warranted.

Unfolded protein response (UPR) and mammalian target of rapamycin (mTOR) are two additional pathways altered by TH and are potential targets for therapeutic modification. TH leads to unfolded protein accumulation in the endoplasmic reticulum, prompting activation of pathways to decrease protein synthesis and increase protein destruction (UPR) (Wouters and Koritzinsky, 2008). Bortezomib, HSP90 inhibitors, and inhibitors of inositol-requiring enzyme (IRE)-1 are examples of agents that might target this pathway (Wilson and Hay, 2011). Cells living in energy and nutrient-deficient environments modulate their activity via inhibition of mTOR complex 1, leading to an adaptive response (Koumenis and Wouters, 2006). Inhibition of the pathway with agents such as rapamycin and WYE 125132 has been studied pre-clinically, with tumor control observed *in vivo* (Pencreach et al., 2009; Yu et al., 2010). Clinical studies of these approaches are not yet underway.

5.5. Autophagy

Cellular components, including proteins and organelles, are conserved and recycled in the endoplasmic reticulum by a process called autophagy. Recycling of cellular contents may improve cell survival when metabolic demands are high, as found in TH. This theory is supported by findings from a murine model of SCCHN, where TH induced autophagy, in turn reducing apoptosis (Vigneswaran et al., 2011). *In vitro*, hypoxia, via HIF1, upregulates BNIP3 and BNIP3L, genes critical to induction of autophagy (Wu et al., 2015). 5′ adenosine monophosphate activated protein kinase also is activated under hypoxic conditions, leading to upregulation of TSC1 and TSC2, which downregulate the mTOR pathway, removing a key inhibitor of autophagy response (Papandreou et al., 2008). UPR also mediates autophagy. As stress on the endoplasmic reticulum to refold proteins mounts under hypoxic conditions, autophagy pathways are activated (Suh et al., 2012). Antimalarial drugs such as hydroxychloroquine, and NVP-BGT226, an inhibitor of the mTOR pathway, have been shown to inhibit autophagy, and may have a role to play in SCCHN treatment (Wu et al., 2015). Several clinical trials of hydroxychloroquine in combination with mTOR inhibitors, proteasome inhibitors and other agents are underway in patients with a variety of solid tumors (Bethesda, 2010).

5.6. Immune modulation

Another potential direction for targeting TH may be through immune modulation. Recent evidence suggests that 3 major mechanisms are present in TH leading to tumor immune escape: 1) aberrations in nitric oxide (NO) signalling, which reduces NK-cell and cytolytic T lymphocyte mediated killing; 2) TH-induced modulation of dendritic, NK and T cell activity by adenosine; and 3) promotion of T regulatory cells by tumor associated macrophages and other factors in the tumor microenvironment (Lee et al., 2010).

TH results in impaired NO production (McCormick et al., 2000). Impaired NO signalling appears to increase MHC class I chain-related (MIC) molecule shedding, leading to downstream immune-modulating effects. *In vitro* and *in vivo* models show increased MIC molecule shedding in the presence of TH, leading to downregulated expression of the NK and T cell activating receptor NKG2D (Doubrovina et al., 2003; Siemens et al., 2008). NO mimetics such as glyceryl trinitrate have been shown *in vivo* to block HIF-1α accumulation in hypoxic cells, which in one study led to decreased programmed death ligand-1 (PD-L1) expression and reduced inhibition to cytolytic T lymphocyte killing (Barsoum et al., 2014). Taken together, these studies suggest targeting the nitric oxide pathway may be a mechanism to overcome TH-associated immune escape.

Regarding the second hypothesis, elevated adenosine levels in the presence of TH may favor maturation of dendritic cells that stimulate a Th-0 or Th-2T cell response, possibly leading to compromised anti-tumor cytolytic T lymphocyte activity (Panther et al., 2003). Additionally, elevated adenosine levels in the tumor microenvironment may inhibit T-cell receptor function via cAMP signalling through the adenosine receptors A2AR and A2BR, thus affecting T cell proliferation and secretion of antitumor cytokines (Ohta et al., 2006; Sitkovsky and Ohta, 2005; Sitkovsky et al., 2008). Adenosine also appears to inhibit NK cell activity, decreasing cytokine production and impairing perforin and Fas ligand-mediated cytotoxic activity (Raskovalova et al., 2005; Lokshin et al., 2006). *In vitro* and *in vivo* data show improved NK cell activity against ovarian cancer (Hausler et al., 2011) and breast cancer (Beavis et al., 2013) in the presence of adenosine receptor inhibitors.

A variety of factors in the tumor microenvironment further serve to promote a T regulatory cell response instead of a cytolytic T cell response. TH increases macrophage colony-stimulating factor and CC chemokine ligands 2 and 5, chemoattractants that recruit macrophages to the tumor microenvironment. Once present, macrophages appear to acquire a M2 phenotype, promoting a Th-2T cell response (Bingle et al., 2002; Webb et al., 2007; Baginska et al.,

2013). TH also increases tumor cell production of TGF-β, which has been shown to promote regulatory T cell accumulation and proliferation in the tumor microenvironment and to decrease NK cell activity (Deng et al., 2013; Ghiringhelli et al., 2005).

Our understanding of how TH impacts the immune response to tumors, together with recent clinical successes whereby anti-tumor T cell responses are improved by monoclonal antibody therapy (Hodi et al., 2010), point toward a potential new direction for TH-targeted therapy. To date, the vast majority of unsuccessful therapeutic trials targeting TH have focused on ways of improving radiosensitivity via increased tumor oxygenation, but they have paid little attention to the importance of a blunted immune response in the presence of TH. Perhaps what is required is both improved oxygenation to increase radiosensitivity, as well as immune modulation. Such a combined approach may overcome our prior TH-targeted therapeutic failures. We propose that future work in this area should focus on how an increase in oxygen tension in the tumor microenvironment modifies not only radioresponse but also immune response. By increasing tumor oxygenation and by targeting NO or adenosine pathways, the cytolytic T cell response and NK cell activity may be improved. Tumor associated macrophages and cytokine production might also be altered by such methods. As a first step toward testing this hypothesis, pre-clinical models should evaluate how small inhibitory RNAs to adenosine receptors, nitric oxide mimetic agents, PD-1 pathway inhibitors, CTLA4 inhibitors, and other agents known to modulate immune response pathways improve radioresponse when combined with oxygen mimetics such as nitroimidazoles or HIF-1 pathway inhibitors. If successful, clinical trials evaluating a combined approach then can be designed for a patient population most likely to benefit. This population may include patients with relapsed SCCHN, where TH- and immune-modification may be critically important. Biomarkers such as hypoxia gene signatures or PET/MRI-based hypoxia imaging may help to identify the appropriate patient population for these trials.

6. Concluding remarks

As lamented by Overgaard (Overgaard, 2007), treatments targeting TH are "adored and ignored." Evidence to date demonstrates that simple compounds sensitize hypoxic cells to radiation effects, generating enthusiasm for further research on the part of clinicians and basic scientists. Unfortunately, a lack of funding and interest from drug manufacturers restricts clinical validation of such drugs.

TH biology is more complex than captured by the current models on which such therapies are based and this may explain the preponderance of negative trials. We lack an understanding of which pathways altered by TH are the most critical for driving tumor growth. Additionally, our ability to select appropriate patients for TH-targeted therapy is poor, especially because TH may not be prognostic in all forms of SCCHN. This point is illustrated by recent data suggesting HPV-related SCCHN has a similar incidence of TH compared to HPV-unrelated tumors, yet TH has little impact on the excellent prognosis of these patients (Kong et al., 2009; Trinkaus et al., 2011). Similarly, hypoxia modification in HPV-related SCCHN may not improve outcome (Lassen et al., 2010). Thus it may appear in a pre-clinical model that targeting TH will lead to an improved outcome, yet if the targeted hypoxia pathway is not a key regulator of tumor biology in a given patient, or if the therapy does not hit the target effectively, there would not be an improvement in treatment outcome.

Another potential explanation for the failure of TH-targeted therapy to improve outcomes is the heterogeneity of TH levels within individual tumors. Acute blood flow changes within a tumor may result in tumor cells that are exposed only temporarily to TH, setting up 2 subpopulations of hypoxic cells – those cells that are chronically, severely hypoxic due to oxygen diffusion limitations, and those cells that are temporarily hypoxic due to acute flow alterations (Janssen et al., 2005). Transient TH and subsequent reperfusion may be mutagenic to these cells, resulting in cells that are viable yet also radio- and drug-resistant (Janssen et al., 2005). Therefore, only the most severely hypoxic cells in a tumor may respond to TH-directed therapy, whereas cells exposed temporarily to TH are resistant to drugs or strategies aiming to improve tumor oxygen levels. Targeting TH therefore is relevant only to a subpopulation of the tumor, rendering TH-targeting a relatively weak strategy to fully eradicate a tumor (Wouters and Brown, 1997).

In their editorial written in the aftermath of the failed Phase III study of ARCON, Peters and Rischin highlighted problems with hypoxia research in SCCHN (Peters and Rischin, 2012). In the so-called "HPV era" of SCCHN, it will be especially important to focus hypoxia research on patients for whom it is a key driver of tumor biology, likely those who have HPV-negative tumors. Prior to designing and initiating additional phase III trials in this area, it will be critical to develop validated markers of TH. Biomarkers such as hypoxia gene signatures or PET/MRI-based hypoxia imaging must be included and validated in early phase trials of novel hypoxia-targeting compounds, with the patient population of such trials enriched for those likely to benefit. Ideally, the development of gene signature profiling that can be commercially available, analogous to *Oncotype DX*® in guiding the use of chemotherapy for breast cancer, would significantly increase the chance of successful development of a drug targeting TH. Future studies must have an appropriate control arm, and the rationale for testing a therapy in a phase III setting must be sound.

With additional pre-clinical studies to evaluate a combined immune- and TH- modulating approach, our hope is that the hurdle of effective TH targeting may be overcome. However, until we have a better understanding of which TH pathways are the most critical for driving tumor growth, as well as a deeper knowledge of the immune system's response in the face of TH, it remains unlikely that TH-targeted therapy will be a part of a clinician's therapeutic armamentarium anytime soon. TH, known from multiple studies to impact treatment outcomes, is an important consideration when caring for patients with SCCHN, and ongoing research efforts to measure and target it therapeutically are justified.

7. Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgment

None.

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Biography

Kelly K. Curtis, MD is an Assistant Professor of Medicine in the Mayo Clinic College of Medicine, and a Consultant in the Division of Hematology/Medical Oncology at Mayo Clinic in Phoenix, Arizona. Dr. Curtis graduated from Albany Medical College in Albany, New York in 2004, and completed Internal Medicine residency at Mayo Clinic in Scottsdale, Arizona (2007) followed by Hematology/Oncology fellowship at Mayo Clinic in Scottsdale, Arizona (2010). Dr. Curtis's research interests include cancer prevention through investigation of new smoking cessation aids, treatment of recurrent head and neck cancer with reirradiation and novel chemotherapy agents, and treatment of soft tissue sarcoma.