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NEW DEVELOPMENTS IN RADIATION THERAPY FOR HEAD AND NECK CANCER: INTENSITY MODULATED RADIATION THERAPY AND HYPOXIA TARGETING

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Abstract

Intensity modulated radiation therapy (IMRT) has revolutionized radiation treatment for head and neck cancers (HNC). When compared to the traditional techniques, IMRT has the unique ability to minimize the dose delivered to normal tissues without compromising tumor coverage. As a result, side effects from high dose radiation have decreased and patient quality of life has improved. In addition to toxicity reduction, excellent clinical outcomes have been reported for IMRT. The first part of this review will focus on clinical results of IMRT for HNC.

Tumor hypoxia or the condition of low oxygen is a key factor for tumor progression and treatment resistance. Hypoxia develops in solid tumors due to aberrant blood vessel formation, fluctuation in blood flow and increasing oxygen demands for tumor growth. Because hypoxic tumor cells are more resistant to ionizing radiation, hypoxia has been a focus of clinical research in radiation therapy for half a decade. Interest for targeting tumor hypoxia have waxed and waned as promising treatments emerged from the laboratory, only to fail in the clinics. However, with the development of new technologies, the prospect of targeting tumor hypoxia is more tangible. The second half of the review will focus on approaches for assessing tumor hypoxia and on the strategies for targeting this important microenvironmental factor in HNC.

INTENSITY MODULATED RADIOTHERAPY (IMRT)

Overview of IMRT

IMRT is a refinement of three-dimensional conformal radiotherapy (3DCRT). It utilizes a computerized treatment planning system along with a sophisticated delivery machineries to tailor the radiation dose to the tumor target.^{1,2} By subdividing a broad radiation beam into smaller pencil beams and by varying the intensities of these pencil beams, a conformal dose distribution is generated. Tumor coverage is improved, particularly in situations where the disease lies in close proximity to critical normal tissues. Given its highly conformal dose distribution, several advantages are noted when compared to conventional RT techniques.^{3–5} First, by decreasing the dose to the normal tissues, RT-related toxicities are minimized.

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Second, by conforming the doses to the irregularly shaped tumor, dose escalation is possible with IMRT, which can potentially lead to improvement in local control. Third, for each daily fraction, IMRT can give a higher dose to the gross tumor volume (GTV), resulting in a more effective biologic dose.⁶

Although IMRT has several advantages in comparison with conventional RT, one must not overlook several important issues. First, because of the varying degrees of intensities in the pencil beams, a greater target dose inhomogeneity is noted for IMRT. "Hot spots" are found within the GTV where nerves and blood vessels reside that can potentially result in unwarranted complications.⁷ Efforts should be made by the treating physician and treatment planner to minimize these "hot spots." Fortunately, no higher rates of late effects have been reported to date. In fact, published reports have shown outstanding clinical results, particularly with decreased xerostomia, leading to improved quality of life.^{8–11} However, longer follow up is needed to validate these findings. Second, due to the sharp dose fall-off gradient between the target and the surrounding normal tissues, accurate target delineation is absolutely essential.^{12–14} Inaccurate coverage of the target volume may lead to marginal misses since the treatment planning system will not treat areas that are not drawn on the computed tomography (CT) slices. Precise target delineation should be a multidisciplinary effort, integrating all clinical and radiographic findings. Available tools such as fusion of magnetic resonance imaging (MRI) and/or positron emission tomography (PET) to the treatment planning CT can be performed to improve the accuracy of target delineation.¹³

A more challenging question is what constitutes the clinical target volume (CTV) in order to encompass all potential microscopic spread. Several papers examining the precise definition of cervical nodal levels have been published and can serve as guidelines to help the radiation oncologists to delineate the CTV.^{5,15} Efforts by HNC experts from the Radiation Therapy Oncology Group (RTOG), European Organization for the Research and Treatment of Cancer (EORTC), and the Danish Head and Neck Cancer (DAHANCA) cooperative groups have generated a consensus guideline for the non-surgically violated node negative neck.¹⁵ This consensus can be downloaded from the RTOG website (www.rtog.org). However, the primary focus of these atlases is on the delineation of the nodal levels for the uninvolved, non-surgically violated neck. Such a consensus is also necessary for the node positive disease and the postoperative neck, which was recently published by a core group from the first consensus conference.⁵ Essentially, patients with gross nodes or those with extracapsular extension should have larger CTV than those who did not and the entire surgical bed should be included.

Treatment Planning and Radiation Delivery

IMRT can be divided into forward-planning (FP) and inverse-planning (IP).¹⁶ FP requires an experienced treatment planner who through multiple trials and errors designs the treatment fields for a given tumor.¹⁷ The planning process begins with the planner determining the beam directions, shapes, beam weighting. A desired dose distribution then results after multiple iterations and dose calculations by the treatment planner. FP can be used to treat HNC.¹⁸ However, to maximize the full potential of IMRT for complex tumors, IP is required. IP starts with the treatment planner and physician setting desired clinical objectives for the GTV, CTV, and the nearby normal tissues. With clinical objectives specified mathematically, a computerized algorithm determines the beam parameters that will yield the desired dose distribution for the target. The computer will undergo thousands of iterations to find the best solution. This cannot be achieved without a reliable and sophisticated planning system. Several commercial and noncommercial treatment planning systems are available and they are, in general, comparable.^{1,19}

With regards to radiation delivery, different methods of beam intensity modulation are available.^{2,16} In the past, wedge filters and/or compensators have been used. Recently, a more

sophisticated delivery system utilizes the multileaf collimator (MLC), situated in the aperture of the linear accelerator, to deliver radiation in a “step-and shoot fashion.” The MLC steps to a predetermined configuration and delivers the pencil beam. The MLC then steps to a different configuration and shoots another pencil beam. MLC system can also function in a “sliding window” fashion to deliver the radiation through the constant movements of different shapes of the MLC across the target.²⁰ Another treatment delivery system is called Tomotherapy where radiation is delivered in a constant rotation of the gantry (linear accelerator head) using a concept similar to spiral CT scan.²¹ In general, these delivery systems are comparable to one another.²²

Clinical results

There are increasing reports published from different institutions on the use of IMRT for HNC.^{23,24} Most reports focused on the decreased rates of xerostomia in HNC with IMRT.^{23,25} Besides sparing the parotid glands, IMRT can also spare the oral cavity, which houses several minor salivary glands. Investigators have also used IMRT to spare the constrictor muscles in attempt to minimize swallowing difficulties as a result of intense RT treatments.²⁶ In addition, reduction of mucositis have also been reported, although this is based on retrospective reviews.²⁷ Several centers also reported excellent locoregional control. Most of these series reported on their experience in treating a mixture of different sites within the head and neck.^{25,28} Experience from Stanford University on 69 head and neck cancer patients has shown a remarkable locoregional control of 92% with a median follow-up of 25 months.²⁵ Sites included were oropharynx, oral cavity, larynx, hypopharynx, and unknown primary. In addition, in most series, definitive IMRT treated patients are reported along with post-operative treated patients.^{12,13} Although the results are promising, longer follow-up is needed to validate these findings, particularly with regards to the locoregional control and late complication. One interesting finding is the shift of the failure pattern with the predominant failures being distant rather than local. This changing pattern of failure can also be attributed to the use of concurrent chemotherapy with RT. It is beyond the scope of this article to discuss in detail these findings. Below, the authors present the most updated clinical results, broken down by the disease site.

Nasopharynx—Due to the multiple critical normal tissues surrounding the nasopharynx, such as the optic structures, brain stem, and salivary glands, IMRT is especially useful in treating these tumors. The earliest published dose comparison study on IMRT with conventional RT was reported by Xia et al. from University of California-San Francisco Medical Center (UCSF).³ Superior tumor coverage was shown with IMRT when compared to all other conventional techniques. In addition, the dose delivered to the normal tissue was decreased with IMRT. Other dosimetric analyses corroborated these findings.^{29,30} Since then, several centers have reported decreased rates of xerostomia in nasopharyngeal cancer (NPC) patients treated with IMRT.^{11,31–33} There is even phase III evidence which confirms the advantage of IMRT in improving xerostomia compared to conventional RT. Besides the dosimetric advantages, several centers also reported excellent early clinical outcomes in NPC for IMRT.³⁴

The most mature IMRT clinical data came from UCSF.¹¹ The 4-year local progression-free and regional progression-free rates for 67 loco-regional advanced NPC patients were 97% and 98%, respectively. An update with more patients (n=118), continued to show excellent locoregional control.³⁵ Several centers from Hong Kong also have shown similar findings.^{31,32} A recent experience from MSKCC reported a 91% locoregional control rate for IMRT treated NPC with a median follow-up of 35 months.³³ (Table 1) The RTOG completed a phase II trial of IMRT with or without chemotherapy for non-stage IVC NPC. Preliminary data showed decreased xerostomia when compared to historical RTOG trials where conventional

RT was used. An example of a NPC patient treated with IMRT is shown in figure 1, showing excellent conformation of the isodose lines to the tumor while sparing the optic chiasm and the brain stem.

With the integration of IMRT and chemotherapy, centers are reporting a changing pattern of failure for NPC. Although the locoregional control is in excess of 90%, the distant metastases rates can be as high as 30%. Present regimens of cisplatin-based chemotherapy may not be effective in preventing distant metastases. Increased vascular endothelial growth factor-A (VEGF-A) has been associated with poor prognosis in HNC.³⁶ One study showed significant elevation of serum VEGF in patients with metastatic NPC.³⁷ In another series, VEGF overexpression was noted in 67% of the NPC patients and elevated VEGF expression in Epstein Barr Virus (EBV) positive tumors was associated with more recurrence, advance nodal stage, and lower overall survival.³⁸ Therefore, the RTOG is currently conducting a phase II trial adding bevacizumab to IMRT and chemotherapy for locoregionally advanced NPC to address this issue.

Oropharynx—Although locoregional control rates for oropharyngeal (OP) cancer patients treated with non-IMRT techniques are excellent, both parotid glands are almost always included in the RT portal. As a result, patients suffer from permanent xerostomia, which has a negative impact on nutrition, dentition, communication and emotional well-being. With parotid-sparing IMRT, patient quality of life has improved when compared to conventional RT.³⁹

Dose sculpting and parotid sparing raise a general concern for possible tumor misses with IMRT. However, no clinical reports to date have shown any compromise in local control for OP cancers treated with IMRT. With a median follow-up of 32 months, Eisbruch et al. reported a 3-year locoregional control rate of 94% in 80 OP cancer patients.⁴⁰ Over 90% of the patients had stage III or IV disease. Chao et al reported a local control rate of 87% using IMRT in 74 patients with OP cancer.⁴¹ In this report, 46% had T3/T4 tumors while 76% of the patients had stage III/IV disease. Other centers also reported excellent locoregional control rates. De Arruda et al noted a 100% tumor control rate at a median follow-up of 24 months. An update with a median follow-up of 31 months continues to show excellent local control in this patient cohort.²⁷ (Table 2) A multi-institutional RTOG study, H-0022, using IMRT for early-stage OP cancer has completed accrual. The protocol tests the transportability of IMRT to multiple institutions. Preliminary results have been presented in the American Society of Therapeutic Radiation Oncology (ASTRO) that showed low IMRT violation rates and reduced xerostomia when compared to historical controls. Figure 2 shows an example of an IMRT plan for an OP cancer.

Larynx & Hypopharynx—There are scanty reports on the use of IMRT for laryngeal and hypopharyngeal tumors. Perhaps because these tumors are situated inferior to the parotid glands, using IMRT for parotid sparing are of less importance when compared tumors situated higher in the head. However, IMRT can still offer several advantages in the management of laryngeal and hypopharyngeal cancers. These tumors often present in close proximity to the spinal cord. In order to respect the spinal cord tolerance, conventional treatments require photon/electron matching which results in dose uncertainty at the match line. By using IMRT, a concave dose distribution can be achieved which allows improved tumor coverage while sparing the spinal cord. In addition, significant external contour changes from the head to the low neck can result in dose inhomogeneity and cause unwarranted hot and cold spots within the tumor. IMRT can minimize dose inhomogeneity without the need of tissue compensators. Lastly, for tumors that have a high risk of retropharyngeal nodal involvement, i.e. node positive hypopharyngeal tumors, where target delineation extends to the skull base, IMRT can spare the parotid glands where it would be difficult with conventional techniques.^{10,42} (Figure 3)

With these theoretical advantages, recent studies have reported promising results for IMRT for laryngeal and hypopharyngeal carcinomas. At a median follow-up of 2.2 years, the 2-year locoregional progression-free survival was 84% and the laryngectomy-free survival was 89% for patients treated with IMRT and concurrent chemotherapy.¹⁰ These results were comparable to those seen for the best arm of the intergroup larynx preservation trial.⁴³ In addition, no grade 2 xerostomia was observed in this study. In another report by the Swiss group where IMRT was used to treat hypopharyngeal cancer, 86% of the 29 patients received concurrent chemotherapy and 60% had locally advanced disease.⁴² With a short mean follow-up of 16 months, the 2-year local control and disease-free survival estimates were both 90%.

Paranasal Sinuses (PNS)—Due to the proximity of multiple surrounding critical tissues, IMRT is of particular clinical interest in the treatment of PNS tumors. Numerous treatment-planning studies have demonstrated the superior dose distribution in favor of IMRT for these tumors in comparison to 3DCRT.^{4,44,45} Early clinical results suggest that the theoretical advantages associated IMRT do indeed translate into clinical benefits.^{46,47} Since tumor control is highly dependent on appropriate target volume delineation, which is often difficult for PNS tumors in the post-operative settings, integration of pre-operative imaging studies and inputs from HN surgeons and pathologists are critical for accurate target contouring.

Claus et al. at the Ghent University Hospital has reported on the use of IMRT for 44 patients with PNS cancers.⁴⁸ In general, the prescription dose to the gross disease was 70 Gy, with a maximum dose constraint of 60 Gy to the optic structures. Although the follow-up is relatively short, none of the patients in whom optic pathway was spared had developed severely dry eyes. Locoregional control was excellent for T1-3 tumors but poor for T4 tumors. Other centers have also reported encouraging toxicity reduction when compared to historical controls where the complication rates can be as high as 35%. Chen et al. reported a longitudinal study of 127 patients treated with RT from 1960 to 2005 at UCSF. The incidence of grade 3 and 4 late ocular toxicity among patients treated with conventional RT, 3D CRT, and IMRT was 20%, 9%, and 0%, respectively.⁴⁹ In another series by Hoppe et al, none of the patients who underwent IMRT developed Grade 3–4 late ocular complication.⁴⁶ As more centers gain experience with IMRT, it is anticipated that the late complication rates will continue to decrease. The North American Skull Base Society is currently investigating the role of high dose IMRT and concomitant cisplatin chemotherapy with or without surgery for advanced PNS cancers. The protocol is under development with the goal to determine the feasibility of non-surgical therapy for these tumors in a multi-institution setting.

Thyroid—Tumors arising from the thyroid gland are primarily treated by surgical resection and the role of external beam RT is largely restricted to the post-operative setting.⁵⁴ Due to the proximity of the tumor bed to the spinal cord, it has been very difficult to deliver adequate doses without exceeding spinal cord tolerance. Dosimetric studies from different centers have shown improvements in target coverage while reducing the spinal cord dose with IMRT.^{50, 51}

Clinically, IMRT was found to be effective and feasible in a small series by Rosenbluth et al, reporting on 20 patients treated with IMRT between 2001 and 2004.⁵² All patients had non-anaplastic thyroid cancer and many had recurrent or T4N1 tumor. The authors noted 2 local failures and reported a 2-year local progression-free rate of 85%. The 2-year overall survival was 60%. Acute grade 3 toxicities included 7 mucositis, 3 pharyngitis, 2 dermatitis and 2 laryngitis. No significant radiation-related late effects were reported at the time of their analysis. Though impressive, longer follow-up and larger studies are needed to validate these results.

Re-irradiation—Re-irradiation using conventional techniques is limited by the tolerance of surrounding critical structures. Current recommendation, based on the RTOG re-irradiation trials, limits the combined total spinal cord dose (both initial and retreatment) to 50 Gy. Therefore, a major benefit of IMRT over conventional RT techniques is to provide superior coverage for the recurrent tumor while minimizing the dose delivered to the adjacent normal tissues.⁵³ There are few clinical reports on the use of IMRT to retreat recurrent HNC. Lu et al. reported on their experience of IMRT in treating recurrent NPC.⁵⁴ Acute toxicity of the skin, mucosa, and salivary glands was acceptable. Tumor necrosis was noted in 14 patients (29%) toward the end of IMRT. With a median follow-up of 9 months, the locoregional control rate was 100%. Another study included all head and neck sites showed that IMRT was associated with better locoregional progression-free survival than conventional RT on multivariate analysis. In this study, IMRT was intricately related to the ability to deliver higher RT doses, which was associated with better overall survival.⁵⁵ Although promising, longer follow-up, larger and more uniform studies are required to fully assess the clinical utility of IMRT in the recurrent settings.

TUMOR HYPOXIA IN HEAD AND NECK CANCERS

Techniques to measure tumor hypoxia and their clinical significance in HNSCC

Currently, there exist several approaches for detecting tumor hypoxia in HNC. At a recent hypoxia workshop, convened the National Cancer Institute (NCI), the consensus among the experts is that “there is not, and probably will never be, a single clear ‘gold standard’ for *in vivo* hypoxia measurement”.⁵⁶ The workshop report also presented a comprehensive review of different approaches for measuring tumor hypoxia. Briefly, techniques for measuring tumor oxygen can be categorized into 2 groups: direct and indirect. Their advantages and disadvantages are summarized in Table 3.

Direct oxygen measurements in tissues—Direct approaches can be applied to tissue (needle electrodes, fiberoptic probes) or blood (measurements or imaging of oxyhemoglobin saturation and oxygen diffusion). Polarographic needle electrodes (pO₂ histogram, Eppendorf, Hamburg, Germany) provided the first convincing evidence that hypoxia existed in human solid tumors.^{57,58} The sensing electrode, mounted on the tip of a needle, is advanced via a step motor through the tissue, taking rapid measurements (1.4 s) to avoid spurious readings from pressure artifacts. A histogram of oxygen tensions (pO₂) can then be obtained from multiple sampling points along different tracks. Normal tissues typically show a Gaussian pO₂ distribution with the median value between 40–60 mm Hg; whereas tumors invariably show lower pO₂ measurements. Several studies have showed that low tumoral pO₂, defined by either the median value or the hypoxic fraction (% readings below 2.5 or 5 mm Hg), correlated with treatment outcomes in HNC patients treated with RT or chemoradiotherapy.^{59–61} One study also found that tumor pO₂ predicted for pathologically persistent neck nodes in patients undergoing a neck dissection for clinical N2-3 necks after chemoradiation treatment.⁶² Pooled data in 397 HNC patients provided definitive evidence that tumor pO₂ is an independent predictor for survival.⁶³ Although the microelectrode technique directly measures tumor pO₂, it suffers from several drawbacks that make it difficult for general use. These include invasiveness, tumor inaccessibility, pressure dependence, inter-observer variability, failure to distinguish necrosis from hypoxia, and the lack of spatial information. Despite these limitations, it is the most studied approach for assessing hypoxia to date.⁶⁴

Indirect approach – Injectable markers—Indirect approaches use injectable molecular reporters of oxygen as the endpoints. Injectable reporters include 2-nitroimidazole compounds such as misonidazole, pimonidazole (1-(2-nitro-1-imidazolyl)-3-N-piperidino-2-propanolol)⁶⁵, and EF5 (nitroimidazole[2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)]

acetamide).⁶⁶ These compounds form stable adducts with intracellular macromolecules only in hypoxic regions (< 10 mm Hg).⁶⁷ Detection of these adducts with antibodies can provide information on the relative oxygenation at cellular resolution.^{68,69} In general, 2-nitroimidazole markers stain for areas of chronic hypoxia and more sensitive at severe hypoxic conditions than the microelectrode.^{69,70} This approach is limited by the requirement for exogenous drug administration, additional biopsies for staining and quantification of staining. Presently there is minimal clinical data regarding the prognostic significance of these agents in HNC. In a small study evaluating pimonidazole, microvessel density count and carbonic anhydrase IX (CA IX) binding in 42 HNC, pimonidazole staining was more pronounced at distance > 100 μ m from blood vessels than CA IX, suggesting that it is more specific for chronic hypoxia.⁷¹ High pimonidazole staining correlated with a higher risk locoregional relapse in patients treated with RT alone but not in those treated with RT plus carbogen and nicotinamide, which were used to modulate tumor hypoxia.

These exogenous markers can also be labeled with ¹⁸F and employed as special tracers for hypoxia imaging using PET or SPECT imaging approaches. Hypoxia imaging is discussed in details in the “Novel Imaging Approach” section by Drs Krohn and Yueh.

Indirect approach – Endogenous markers—Endogenous molecular markers for tumor hypoxia represent proteins and genes whose expressions are induced by hypoxic exposure. One of the most studied oxygen response pathways is that mediated by the hypoxia inducible factor-1 (HIF-1), which regulates genes that are involved in cell metabolism, angiogenesis, invasion, metastasis and apoptosis. HIF-1 and several of its downstream targets including Glut-1 (glucose transporter-1), CA IX and VEGF have been widely investigated as prognostic markers in HNC with mixed results (Table 4). In general, elevated expression of these markers portends poorer outcomes in patients treated with non-surgical therapies. The advantage of endogenous markers is that levels of these proteins can be assessed on archival materials, thereby allowing rapid correlation to treatment outcomes. In addition it requires neither the injection of foreign material nor any additional invasive procedure beyond that a biopsy at diagnosis. A significant drawback to these approaches is that these proteins can be regulated by factors other than hypoxia. For example, HIF-1 α expression can be influenced by several non-hypoxic stimuli including nitric oxide, cytokines, trophic stimuli and oncogenes.^{72–75} Comparison of the staining patterns between endogenous and injectable markers showed that the former, in general, stained more diffusely and closer to the blood vessels than the latter, suggesting other modes of induction and activation at a wider range of oxygen concentration.^{71,76} To circumvent this dilemma, suggestions have been made to combine several endogenous markers together to improve hypoxia specificity. For example, gene expression analysis has been used to generate a hypoxia gene signature or a hypoxia metagene to predict treatment outcomes in several solid tumors, including HNC.^{77,78} Using gene expression profiling of 59 HNC, Winter et al generated a hypoxia metagene by identifying genes whose expression clustered with 10 known hypoxia regulated genes.⁷⁸ They found that this metagene was able to predict recurrence-free survival in an independent HNC data set as well as overall survival in another breast cancer series. We have also used a combination of gene expression and proteomic analyses to identify novel hypoxia induced proteins. After confirming their hypoxic inducibility in cell lines and animal models, we investigated their utility in combination with CA IX to predict outcomes by staining a HNC tissue array from with known tumor pO₂. These studies resulted in a panel of 4 hypoxia markers (CA IX, Lysyl oxidase (LOX), Galectin-1 and Ephrin A1) that can be used to predict treatment outcomes in terms of cancer-specific survival.⁷⁹ (Figure 4) These endogenous hypoxia signatures, though promising, need to be validated in larger independent datasets before they can be used in the clinical settings.

Our laboratory has focused on identifying secreted markers of hypoxia that can be rapidly and inexpensively measured in the blood. Two markers that have been tested clinically with mixed

results are VEGF and osteopontin (OPN). Although circulating VEGF levels were elevated in cancer patients^{80,81} and in those with acute hypoxia such as obstructive apnea⁸², the relationship between tumor hypoxia and systemic VEGF levels is unclear. Dunst et al found that serum VEGF levels independently correlated with hypoxic tumor subvolume in 56 HNC patients.⁸³ However, it also correlated with total tumor volume, hemoglobin level and platelet counts. They did not report on the clinical significance of serum VEGF levels in terms of treatment outcomes. In contrast, we did not find a direct relationship between plasma VEGF and tumor pO₂ in 48 HNC patients in our study (unpublished observations). We did however find a small but significant relationship between OPN level and tumor pO₂ in our patient cohort.⁸⁴ This was confirmed by Nordsmark et al.⁸⁵ In addition, plasma OPN was an independent and significant predictor for treatment outcomes in these patients and another independent group of HNC patients.⁸⁶ These results were confirmed by the DAHANCA group in a larger cohort of HNC patients treated with radiation therapy +/- nimorazole, a hypoxic cell radiosensitizer.⁸⁷ Intriguingly, only patients with high pretreatment circulating OPN levels benefited from nimorazole whereas those with low-intermediate levels did not, suggesting that OPN may be used to select patients for hypoxia targeting. Further validation of this marker is ongoing in another set of HNC patients treated with or without Tirapazamine (TPZ), a hypoxic cell cytotoxin.

Hypoxia Targeted Therapy

Since the 1950s, enormous efforts have been devoted to develop strategies to target hypoxia either directly or indirectly. These include techniques to enhance oxygen tissue delivery, generation hypoxic cell radiosensitizers and hypoxic cell cytotoxin, treatment with high linear-energy transfer (LET) radiation that is less oxygen dependence and, more recently, development of inhibitors to signaling pathways that are activated by hypoxia. Although most of these strategies have not achieved general acceptance, a meta-analysis of trials using hypoxic cell sensitizers or hyperbaric oxygen showed a small but statistically significant benefit in terms of locoregional control and survival.⁸⁸ In this part of the paper, we will touch on some past strategies and future approaches for exploiting hypoxia.

Past Strategies—The most straightforward strategy to overcome hypoxia is to administer oxygen at pressure higher than room air (usually 3 atmospheres), i.e. hyperbaric oxygen treatment. Although one study showed promising results in HNC patients, the results have been mixed.^{89,90} In retrospect, this strategy only affects chronically hypoxic cells but is not expected to change acute hypoxia. Although a meta-analysis suggests that the use of hyperbaric oxygen breathing during RT can improve local control by 10%, it has not gained general acceptance for clinical use due to inconsistent response, safety issues and high cost for implementation.⁸⁸

A widely investigated hypoxia targeted strategy is to use electron-affinic drugs (nitroimidazoles) to sensitize tumors to radiation. Xenograft studies showed significant radiosensitization with nitroimidazole compounds in tumors without enhancing normal tissue toxicity.^{91,92} For 2 decades, nitroimidazole compounds had been extensively studied by RTOG and DAHANCA groups in HNC as an adjunct to RT with mixed results.^{93–97} Most trials reported disappointing outcomes except for one large study. In this phase III study (DAHANCA 5-85), the addition of nimorazole to RT resulted in improved locoregional control (49% versus 33%, $p = 0.002$) and cancer-related survival (52% versus 41%) when compared to the placebo arm in patients with supraglottic larynx and pharynx cancers.⁹⁶ The main drawback of these compounds is the neurotoxicity associated with multiple doses.^{98,99} This toxicity limits the total amount of drugs that can be administered to achieve maximal efficacy.

Present Strategies

Erythropoietin manipulation: It is hypothesized that increase oxygen delivery via modifications of hemoglobin levels can attenuate tumor hypoxia. Henke et al performed a phase III randomized study of RT +/- erythropoietin (EPO) in HNC. Unfortunately, patients who received EPO had worse locoregional progression-free survival.¹⁰⁰ This could be explained partially by the quadratic regression in the correlation between tumor pO₂ and hemoglobin levels: at levels ≤ 14 g/dL, increasing hemoglobin directly correlates with increasing pO₂; however, at levels above 14 g/dL, an inverse correlation is noted with lower pO₂ associated with increasing hemoglobin, presumably from venostasis.⁶³ More recently, the presence of EPO receptors in tumor cells has also been implicated with worse outcomes in patients receiving EPO on this study.¹⁰¹

Vascular normalization: Increased vascular leakage from immature tumor blood vessels can result in higher interstitial fluid pressure, thereby, worsening tumor hypoxia and impeding effective tumoral drug delivery. Jain et al. popularized the concept of normalization of tumor vasculature through antiangiogenic therapy such as bevacizumab.¹⁰² This concept was supported by clinical data in colorectal, breast and brain cancers, where treatment with bevacizumab, paclitaxel (a chemotherapy with antiangiogenic properties) or AZD2171 (a VEGF receptor tyrosine kinase inhibitor), respectively, was shown to reduce tumor interstitial pressure.¹⁰³⁻¹⁰⁵ This concept has yet to be tested in HNC.

ARCON (Accelerated Radiotherapy with CarbOgen and Nicotinamide): Another promising approach to target hypoxia in HNC is the combined use of the nicotinamide vasodilator and carbogen breathing to increase tumor pO₂. ARCON has produced a 3-year local control rate in excess of 80% for advanced stage T3-4 laryngeal and oropharyngeal cancers.¹⁰⁶ Presently, a phase III clinical trial testing the efficacy of ARCON in laryngeal cancers is ongoing in Europe and will address the effectiveness of this strategy in HNC.¹⁰⁶

Hypoxic cell cytotoxin: An important strategy to exploit tumor hypoxia is the use of bioreductive agents that can selectively kill hypoxic cells. The first bioreductive drug used in clinical trials is mitomycin-C. Pooled data from 2 randomized trials in HNC suggested that the addition of mitomycin-C to RT resulted in statistically significant improvement in locoregional control and cause-specific survival.¹⁰⁷ Another study comparing conventional fractionated RT to the Vienna Continuous Hyperfractionated Accelerated RT regimen (V-CHART) or to V-CHART plus mitomycin-C showed that the best survival and locoregional control rates were observed for the V-CHART and mitomycin-C group.^{108,109} Although promising, mitomycin-C toxicity limits the frequency of drug delivery, making it unlikely to be the ideal drug for exploiting tumor hypoxia.

A widely studied hypoxic cell cytotoxin is Tirapazamine (TPZ). In a randomized phase II trial (TROG), the combination of TPZ, cisplatin and RT was found to be superior to 5FU, cisplatin and RT for locally advanced HNC.¹¹⁰ In contrast, we found that the addition of TPZ to an aggressive regimen of induction and concurrent cisplatin and 5FU with RT did not result in improved outcomes in a small randomized phase II study.¹¹¹ A large multi-institutional phase III trial testing the benefit of adding TPZ to concurrent RT and cisplatin has been completed and the results are pending.

TPZ has several limitations including poor drug diffusion through hypoxic tissues and become activate under less stringent hypoxia, a feature that can cause normal tissue toxicity in poorly oxygenated organs. Therefore, there are strong interests in developing novel hypoxic cell cytotoxins with more specific antitumor activity. Dinitrobenzamide mustards (DNBMs) belong to a new and highly potent class of hypoxic cytotoxins discovered by the Auckland

University group. These compounds have improved properties over TPZ, including activation by more stringent hypoxia and a substantial bystander killing effect. A lead compound of the DNBM family, PR-104 is being studied in phase I-II clinical trials for several solid tumors.^{112,113}

Future directions: Targeting HIF-1: Since HIF-1 is a key transcriptional factor for several hypoxia induced genes, there is a strong interest in developing specific HIF-1 inhibitors. The rationales for targeting HIF-1 have been eloquently outlined in a review by Giaccia et al.¹¹⁴ Although targeting HIF-1 is a conceptually attractive, it's highly challenging; therefore, blocking upstream signaling pathways leading to HIF-1 inactivation or downstream effector functions may represent a more practical strategy. For a thorough review of emerging HIF-1 inhibitors, please refer to Melillo et al.¹¹⁵ To date, inhibitors of several upstream signaling pathways of HIF-1 have either been approved by the FDA for clinical use or are being investigated in clinical trials. Examples of these include mTOR inhibitors (Rapamycin and Temsirolimus) that can suppress mTOR-dependent HIF-1 translation and epidermal growth factor receptor (EGFR) inhibitors (Gefitinib, Erlotinib) or antibodies (Cetuximab, Panitumumab) that inhibit HIF-1 induction by EGFR-dependent pathways.¹¹⁵ Another approach is to inhibit HIF protein accumulation. Chemicals in this class include Topotecan¹¹⁶, 2-methoxyestradiol (2ME2)¹¹⁷, Hsp90 inhibitors (17-N-Allylamino-17-demethoxy geldanamycin [17-AAG] and 17-dimethylaminoethylamino-17-demethoxygeldanamycin [17-DMAG])¹¹⁸, Histone deacetylase (HDAC) inhibitors (Vorinostat)¹¹⁹, YC-1 (3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole) a soluble guanylate cyclase activator¹²⁰, PX478¹²¹, Thioredoxin inhibitors (PX-12)¹²², and flavopiridol, a cyclin dependent kinase inhibitor.¹²³ Although most of these compounds have been identified as anticancer drugs by their other activities, all negatively affect HIF-1 protein levels. One of such drug, topotecan, is being tested in a clinical trial at the NCI to specifically assess its HIF-1 inhibitory function in patients with advanced tumors, refractory to standard therapies.¹¹⁵ A crucial feature of this trial is that the enrolled population is intentionally enriched for tumors with elevated HIF-1 protein expression. Inhibitors that directly target HIF-1 transactivating activity are slowly emerging from high throughput screenings.¹¹⁵ These include echinomycin and chetomin, both of which are currently being evaluated only in animal models.

An interesting proposal to target HIF-1 that has yet to be tested in patients is to take advantage of its involvement in tumor metabolism, which is different from that of normal tissues. Several of HIF-1 target genes are key regulators of tumor metabolism. One of such genes is pyruvate dehydrogenase kinase 1 (PDK1), which limits the amounts of pyruvate entering into the citric acid cycle, thereby decreasing mitochondrial oxygen consumption. Cairn et al showed that inhibition of HIF-1 and PDK1 either genetically or pharmacologically resulted in enhanced oxygen consumption and a transient rise in tumor hypoxia.¹²⁴ They theorize that pretreatment with either HIF-1 or PDK1 inhibitors would transiently decrease tumor oxygenation, which in turns would improve the effectiveness of hypoxic cell cytotoxins such as TPZ. They elegantly showed that HIF-1 inhibitors dramatically increased TPZ anticancer effectiveness when administered before but not after TPZ, and this enhancement is dependent on HIF-1 being functional. This novel strategy for hypoxia modification may circumvent the dilemma of hypoxia heterogeneity and may also alleviate the need for accurately identifying hypoxic tumors. In addition, it highlights the importance of appropriate sequencing of RT and HIF-1 inhibitors as HIF-1 blockade before RT may increase tumor hypoxia and negate RT effectiveness. This is also consistent with the data from Moeller et al who showed that HIF inhibition increased radiation effectiveness when occurred after but not before RT.¹²⁵

CONCLUSIONS

Given the available dosimetric and clinical data, IMRT is an effective treatment modality for HNC. It conforms the dose to the tumor while sparing nearby normal tissues. Toxicity from high dose RT has decreased when compared to historical controls. Clinical reports have shown great promise in terms of tumor control. With advances in anatomic and functional imaging, along with more clinical experience, it is likely that IMRT will lead to further improvements in tumor control, survival and quality of life in HNC patients.

After half a decade of efforts, tumor hypoxia continues to be a therapeutic challenge in HNC. Nonetheless, the prospect of reducing its impact is looking brighter with improved ability of detecting hypoxia and better understanding of its molecular targets for therapeutic exploitation. Testing new leads from the laboratory will require clinical trials with innovative designs that incorporate serial novel non-invasive surrogate endpoints for hypoxia such as molecular makers or imaging methods.

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Figure 1.
An example of a T4N0 nasopharyngeal carcinoma treated with IMRT.

Figure 2.
An example of a T3N0 base of tongue cancer treated with IMRT.

Figure 3.
An example of a T3N1 laryngeal carcinoma treated with IMRT.

Figure 4.

Cancer-specific survival by hypoxia marker score comprised of Galectin-1, Ephrin A1, Lysyl Oxidase, CA IX cytoplasmic and CA IX membrane staining, where a score of 1 was assigned to strong staining for each marker and a score of 0 to negative and weak staining. This has been adjusted for age and hemoglobin levels, 2 other significant factors on univariate analysis.

Table 1

Results of IMRT for nasopharyngeal carcinomas

Author	N	Stage	F/U (month)	Local Control
Lee ¹⁴	67	T1-T4	33	97%
Wolden ³⁶	74	T1-T4	35	91%
Kwong ³⁷	33	T1	24	100%
Kam ³⁴	63	T1-T4	29	92%

Table 2

Results of IMRT for oropharyngeal carcinomas

Author	N	Stage	F/U (month)	Loco-regional Control
Chao ⁴⁶	74	I-IV	33	87%
Eisbruch ⁴⁵	80	I-IV	32	94%
De Arruda ⁶¹	50	I-IV	24	92%

Table 3

Different techniques for assessing tumor hypoxia

Method	Examples	Measure	Spatial resolution	Advantages	Disadvantages
PO2 Histography	Eppendorf electrode OxyLite fiber optic probe	pO2	0.5 mm (thousands of cells)	<ul style="list-style-type: none"> • Direct • Rapid real-time measurements • Validated in human tumors 	<ul style="list-style-type: none"> • Invasive • Tumor inaccessibility • Pressure dependence • Inter-observer variability • Readings affected by necrosis • No spatial information
Exogenous Markers	EF5 Pimonidazole	Chronic hypoxia	1.0 um (single cell)	<ul style="list-style-type: none"> • Highly sensitive • Reproducible 	<ul style="list-style-type: none"> • Require drug injection • Require extra biopsies
Endogenous hypoxia marker	HIF-1 CA-IX Glut-1	Biologic hypoxia	1.0 um (single cell)	<ul style="list-style-type: none"> • Apply to archival tissues • No drug injection • No extra biopsies 	<ul style="list-style-type: none"> • Less hypoxia specific • Variability in staining & interpretation
Secreted markers	OPN VEGF	Biologic hypoxia	N/A	<ul style="list-style-type: none"> • Non invasive • Inexpensive • No biopsy or drug injection • Serial measurements 	<ul style="list-style-type: none"> • Less hypoxia specific • Less tumor specific • Specimen processing critical • Antibody specificity
PET-based hypoxia imaging	¹⁸ F-MISO ¹⁸ F-FAZA ¹⁸ F-EF5 ¹⁸ F-PETNIM ⁶⁰ CuATSM ²⁴ LaZGP	Chronic hypoxia	2–10 mm	<ul style="list-style-type: none"> • Spatial resolution • Serial measurements • RT directed targeting • Reproducible 	<ul style="list-style-type: none"> • Requires dedicated equipments (tracer generation & imaging) • Expensive • Radiation exposure • Tracer synthesis expertise

Table 4
Significance of endogenous markers for hypoxia in head and neck cancers

Author	Marker	# Pts	Tumor site	Treatment	Respond	LRC	Survival	Associated parameters
Aebersold ¹²⁶	HIF-1 α	98	Oropharynx	RT or CRT	Yes	Yes	Yes	Grade (inverse)
Koukourakis ¹²⁷	HIF-1 α , HIF-2 α	75	H&N	CRT	Yes	Yes	Yes	T-stage, MVD, VEGF
Beasley ¹²⁸	HIF-1 α	79	H&N	Surgery			No [*]	Necrosis
Hui ¹²⁹	HIF-1 α , CA IX	90	Nasopharynx	RT or CRT			Yes ^{**}	CA IX, VEGF
Beasley ¹³⁰	CA IX	79	H&N	Surgery			Yes	MVD, necrosis
Koukourakis ¹³¹	CA IX	75	H&N	CRT	Yes	Yes	Yes	MVD, necrosis
Kaanders ⁷¹	CA IX	43	H&N	RT +/- ARCON	No	No	No	Pimonidazole staining
Winter ¹³²	HIF-1 α , HIF-2 α , CA IX	140	H&N	Surgery			Yes ^{***}	
Koukourakis ¹³³	HIF-2 α , CA IX	198	H&N	RT ^{****}		Yes	Yes	Higher T-stage

Yr: year; Pt: patients; H&N: head and neck; RT: radiotherapy; CRT: chemoradiotherapy; ARCON: Carbogen and nicotidamide; LRC: locoregional control; MVD: microvessel density count.

* Improved disease-free and overall survival with HIF-1 α over expression

** The combination of HIF-1 α and CA IX positive staining (hypoxic profile) was associated with worse progression-free survival. Positive individual marker staining did not correlated with survival.

*** Improved disease-specific survival but not overall survival with HIF-1 α & HIF-2 α expression, but not with CA IX

**** RT: Conventional fraction versus continuous hyperfractionated accelerated radiation therapy (CHART)