Clin Transl Imaging (2017) 5:497–505
https://doi.org/10.1007/s40336-017-0252-9

EXPERT REVIEW

Impact of hypoxia in head and neck cancer radiotherapy

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Received: 29 August 2017 / Accepted: 3 October 2017 / Published online: 24 October 2017

Abstract
Background Radiotherapy plays an important role in the treatment of pharyngo-laryngeal and oral cavity head and neck squamous cell carcinoma (HNSCC) either as single modality, in combination with concomitant chemotherapy or epithermal growth factor inhibitor, or as adjuvant treatment after curative surgery. Various biological factors have been shown to impact on tumour response to radiotherapy, and among them tumour hypoxia.
Aim This article reviews the clinical data on measurement of tumour hypoxia and summarizes the various options to counteract it, with a special emphasis on the use of hypoxic cell radiosensitizers, radiation dose escalation, and the proper selection of patients who may benefit from such therapeutic interventions.
Results It has been shown that up to 25% of HNSCC express pO_2 value below 2.5 mm Hg, and modification of tumour hypoxia has been demonstrated to improve loco-regional control and overall survival. In particular, the concomitant use of nimorazole, a hypoxic cell radiosensitizer, has been demonstrated in a seminal DAHANCA trial to significantly improve outcome in patients with head and neck SCC. More recently, among the patients included into this trial, a 15-gene hypoxia classifier indicated that only those patients with hypoxic tumours benefited from the combined radiotherapy and nimorazole treatment.
Conclusions An ongoing trial is validating both the use of nimorazole and the gene classifier for patients treated with concomitant chemo-radiotherapy.

Keywords Tumour hypoxia · Nimorazole · FAZA PET · Radiotherapy · Hypoxic molecular signature

Introduction

Radiotherapy plays an important role in the treatment of pharyngo-laryngeal and oral cavity head and neck squamous cell carcinoma (HNSCC) either as single modality, in combination with concomitant chemotherapy or epithermal growth factor inhibitor, or as adjuvant treatment after curative surgery [1]. For early stage disease (i.e., stage I–II), 5-year relative survival rates in the order of 42–74% are typically observed, whereas for advanced stage disease (i.e., stage III–IV) treated with altered fractionation radiotherapy, or combined chemo-radiotherapy, 5-year relative survival rates reach a range of 24–38% [2]. For p16-positive oro-pharyngeal SCC, which represents a raising entity especially in Northern European countries and North America, loco-regional control in the order of 70–95% is typically observed [3].

Over the past few decades, a progressive improvement in patient outcome has been observed, which mainly results both from an improved radiation delivery and from a better biological modulation of radiation effect through altered fractionation and drug combination, although a change in the epidemiology of the disease and a tumour stage migration

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through better patient work-up has also been incriminated [4].

As far as radiation delivery is concerned, the progressive introduction of intensity-modulated radiation therapy (IMRT) and the use of multimodality imaging for target volume and organs at risk delineation translated into a better tumour coverage while minimizing the radiation dose delivered to the surrounding normal tissues [5]. In the near future, further progresses may come from a better integration of molecular imaging to identify tumour sub-volumes that may require additional radiation doses (i.e., dose painting), and/or from treatment adaptation tracing changes in patient anatomy during treatment. Proton therapy, which in some patients generates even more exquisite dose distribution, could potentially further improve patient outcomes.

As far as modulation of radiation effect is concerned, multiple randomized controlled trials have shown that the use of altered fractionation regimens, concomitant platinum-based chemotherapy, or concomitant blockage of the EGFR receptors through specific antibodies improved the loco-regional control probability by 8–15%, translating into a modest but a significant improvement in overall survival [6–8]. Although never directly validated, it is reasonable to consider that altered fractionation could be particularly suitable for stage II disease, whereas stage III and IV require concomitant use of drug and radiotherapy with or without altered fractionation.

In parallel to these improvements, insights in the biology of HNSCC have highlighted intrinsic factors impacting on radiotherapy outcome, and among them, tumour hypoxia has been identified as a common feature [9]. Hypoxia, a condition of insufficient oxygen supply to support tumour metabolism typically results from a lack of vessel formation during tumour growth (the so-called diffusion-limited hypoxia) and/or from a transient fluctuation of the blood flow within a tumour (the so-called perfusion-limited hypoxia) [10]. In HNSCC, hypoxia can be quantified using various measurement tools, and it has been shown that up to 25% of tumours express pO_2 value below 2.5 mm Hg [11, 12]. Hypoxia is a well-known cause of resistance to radiotherapy, and in patients with HNSCC, it has been demonstrated that any modification of tumour hypoxia improves loco-regional control and survival [13, 14]. In particular, the concomitant use of nimorazole, a hypoxic cell radiosensitizer, has been demonstrated in a seminal DAHANCA trial to significantly improve outcome in patients with HNSCC [15]. Interestingly, a 15-gene hypoxia classifier indicated that only those patients with hypoxic tumours benefited from the combined radiotherapy and nimorazole treatment [16]. However, the DAHANCA trial was conducted in patients treated by radiotherapy alone, and the benefit of hypoxic cell sensitizer in HNSCC patients treated by concomitant chemo-radiotherapy can only be inferred. An ongoing EORTC trial (EORTC 1219) is in progress in several European and Australian centres. This trial will not only confirm that nimorazole is improving patient outcome in addition to chemo-radiotherapy, it will also further validate the use of a gene signature of hypoxia for patient selection.

Radiation dose escalation is another complementary alternative to increase hypoxic cell kill, which has been already proposed by few authors [17, 18]. Although attractive, such approach is potentially limited by an increased risk of late toxicity when higher dose delivery on large volumes exceeds the tolerance of surrounding normal tissues. Hence, the concept of tailored dose increment, i.e., the so-called "dose painting", aiming at specifically boosting the radiation dose to those hypoxic voxels individualized by molecular imaging [19, 20].

In this framework, the following sections review the clinical data on measurement of tumour hypoxia, and summarize the various options to counteract it, with a special emphasis on the use of hypoxic cell radiosensitizers, radiation dose escalation, and the proper selection of patients who may benefit from such therapeutic interventions.

Measurement of tumour hypoxia

One of the quantitative methods for identifying tumour hypoxia is the Eppendorf polarographic electrode, which uses an oxygen sensor at the tip of a needle, which is inserted at different locations into a tumour to measure absolute pO_2 values. When using such a device, it was shown that, virtually, all tumours express hypoxia but with a high degree of heterogeneity—not just between tumours but also within each individual one [11, 21]. Although it gives an absolute measurement of the oxygen partial pressure within a tumour, there are several disadvantages of this invasive technique. First, the method is limited to superficial and easy accessible tissue, such as neck nodes or cervix cancers. Second, it is not possible to determine, if the measurements are from areas with viable cells or conversely necrotic areas. The equipment is nowadays only available in a few centres as the Eppendorf electrode is not in production anymore.

An indirect method for measuring hypoxia is the injection of compounds that under hypoxic conditions which becomes reactive and binds to the hypoxic cells. Among the exogenous markers, the nitroimidazole compounds are the most studied, and binding of pimonidazole has been correlated with hypoxia in experimental tumour models [22]. In HNSCC, pimonidazole binding was correlated to local tumour control after radiotherapy [23], but the same trend was not found in cervix cancer [24]. Assessment of hypoxia using pimonidazole requires biopsies for immunohistochemical staining of the marker, which also limits its utility in routine clinical settings.

An alternative to the invasive electrode measurements or the exogenous hypoxia markers are the endogenous biomarkers, which are the generic terms for molecules that are physiologically expressed at low oxygenation levels. Testing for the expression of endogenous markers of tumour hypoxia often requires a biopsy, and therefore, most studies have been performed using pre-treatment diagnostic biopsies. Several hypoxia-related biomarkers have been investigated: the Hypoxia-Inducible Factor-1 α (HIF-1 α) considered as the master transcriptional regulator of cellular response to hypoxia [25], the Carbonic Anhydrase-9 (CA-IX), a zinc metalloenzyme that catalyses the reversible hydration of carbon dioxide and which is involved in the acid–base balance of the cell [26], the Glucose Transporter-1 (GLUT-1 α) that regulates glucose uptake through the cell membrane and which is increased during hypoxic conditions [27], or the Vascular Endothelial Growth Factor (VEGF), responsible for tumour vasculogenesis and angiogenesis, especially during hypoxic conditions [28]. The disadvantages of endogenous biomarkers are that they require tumour biopsies, which makes dynamic measurements difficult or even impossible. Furthermore, upregulation can be observed even under oxic conditions as some of these endogenous biomarkers are affected by other changes in the microenvironment, such as the pH. Although prognostic, only a few of them may have a predictive potential.

Some endogenous markers can be measured in plasma and thus do not require tissue biopsies, thus opening for the possibility of consecutive more dynamic measurements. The best-studied plasma marker so far is the tumour-associated glycoprotein, osteopontin. Plasma osteopontin correlates inversely with tumour oxygen levels measured by the Eppendorf electrode [29] and has been shown to individualize HNSCC patients who benefit from treatment with the hypoxic radiosensitizer nimorazole given concomitantly to curative radiotherapy [30]. In patients with HNSCC, additional series have suggested that pre-treatment high levels of plasma osteopontin might be prognostic for poor outcome after surgery, radiation alone, or chemo-radiotherapy [31, 32].

Nitroimidazole compounds like misonidazole (MISO) and azomycin arabinofuranoside (AZA) have been fluorinated by the positron emitter ^{18}F, and used for hypoxic imaging with Positron Emission Tomography (PET). Typically, these tracers detect the presence of hypoxia when pO_2 drops below 10 mmHg [33]. Among the various PET tracers synthesized, ^{18}F-Misonidazole (MISO) is the most commonly used, but more recently, other PET tracers such as ^{18}F-FAZA, ^{18}F-FETNIM, ^{18}F-EF3, ^{18}F-EF5, and ^{18}F-HX4 have also been introduced in the clinic [34–38]. ^{18}F-FAZA has several advantages including an easy production with high specific activity, a chemical stability after injection, a specific metabolism in hypoxic cells, and a rapid clearance of unbound tracer from non-hypoxic tissues leading to high tumour-to-background ratios compared to other tracers (Fig. 1) [35]. The potential benefit of these radiolabeled drugs is the minimally invasive approach and the possibility for doing more dynamic measurements. Clinical results in HNSCC have shown that tumours with high tracer uptake have a significantly poorer outcome [36–38]. Others more perfusion-related indirect methods of measuring hypoxia like dynamic contrast-enhanced MRI are under development and seem promising [39, 40].

Modification of tumour hypoxia with nitroimidazole compounds and ARCON

Hypoxic modification by nitroimidazoles has been investigated in a large number of clinical studies. Nine different drugs, i.e., misonidazole, metronidazole, benznidazole, desmethyl-misonidazole, etanidazole, pimonidazole, nimorazole, ornidazole, and RSU 1069, have reached clinical testing. However, most of these studies have inconclusive results due to a relative small number of patients enrolled in these studies. The most potent of these drugs is the 2-nitroimidazole, misonidazole. In experimental systems, 10 mM of misonidazole was able to radiosensitize the hypoxic cells to a degree approaching aerated cells [41]. Five large randomized trials have been performed with misonidazole, as a radiosensitizer, and the results have largely been disappointing. This has been partly explained by some of the studies being very heterogeneous in their design or underpowered; furthermore, the administered dose of misonidazole was limited due to the risk of neurotoxicity [42–46]. The largest of these studies, DAHANCA-2, randomized 626 patients to split-course radiotherapy and either misonidazole or placebo, and found a benefit of the radiosensitizer [45]. The primary endpoint, the 5-year loco-regional control, reached 40% in the misonidazole arm versus 32% in the placebo arm ($p < 0.05$) for patients with pharyngeal or supraglottic carcinomas. However, as misonidazole is a lipophilic compound, accumulation was observed in fatty tissue (especially in women) causing neuropathy in 26% of the patients in the misonidazole group. This side effect was found to be so unacceptable in some patients that the drug was considered too toxic for further clinical development. The 5-nitroimidazole, nimorazole, was found to be less sensitizing than misonidazole, but also less lipophilic and thus with a more tolerable toxicity for concomitant administration during radiotherapy [15, 46]. In the DAHANCA-5 study, between 1985 and 1990, 422 patients with pharyngeal and supraglottic SCC were treated with the conventional fractionated radiotherapy 66–68 Gy, 2 Gy/fx, 5fx/week, and randomized to either concomitant nimorazole on treatment days or placebo [15]. In total, 414 patients were eligible for analysis. Overall, the nimorazole

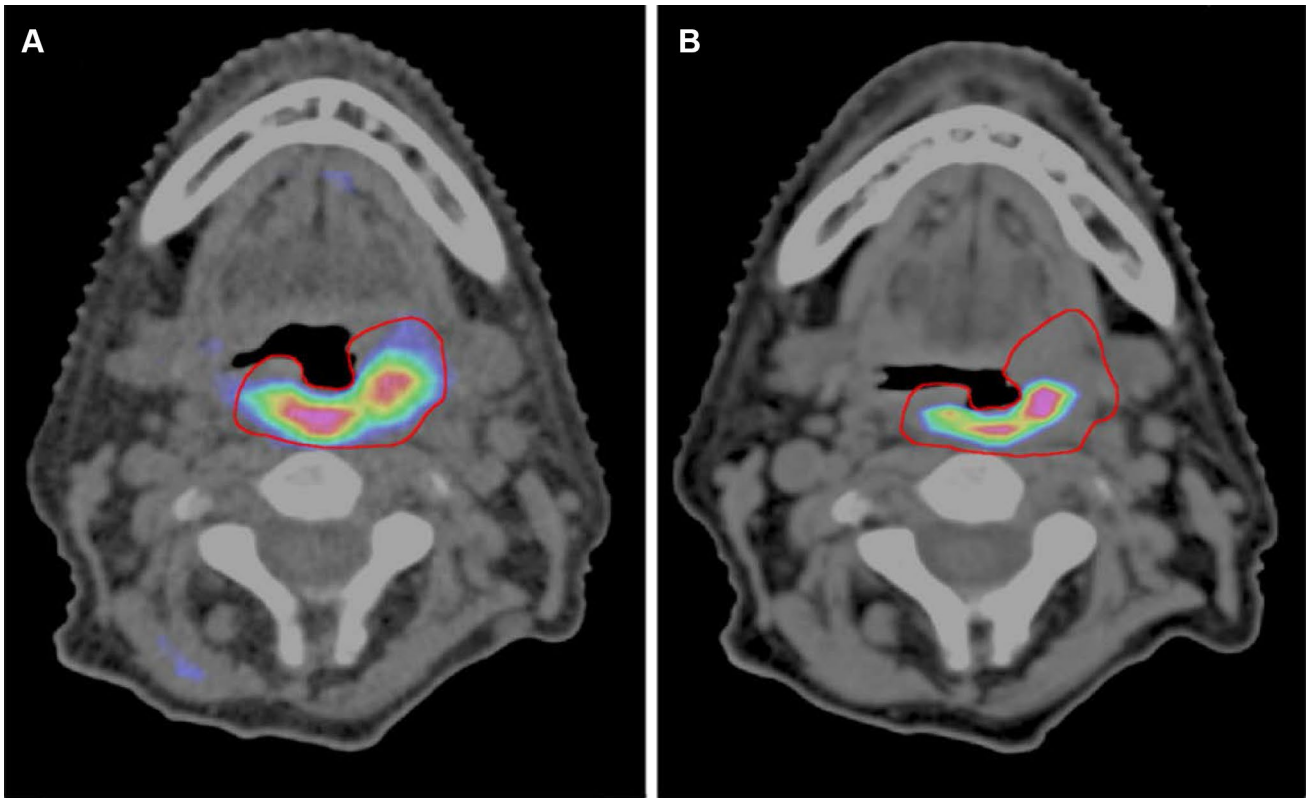

Fig. 1 FAZA PET-CT images of a patient with an oropharyngeal p16-negative squamous cell carcinoma. The patient was imaged 2 h after injection of 10 mCi of FAZA. **a** Baseline image; **b** image taken after a radiation dose of 12 Gy combined with daily nimorazole. The red line depicts the GTV delineated on the planning CT. Courtesy of Lise Saxoe Mortensen

group showed a significantly better loco-regional control rate than the placebo group (49% vs. 33%, $p = 0.002$) and this translated into a 5-year disease-specific survival reaching 52% versus 41%, respectively ($p = 0.01$). No statistical difference was found for the 10-year overall survival, although the nimorazole group tended to do better than the placebo group ($p = 0.32$). Toxicity was reported to be manageable with nausea/vomiting being the most prominent side effect in 26% of the patients in the nimorazole group. Recently, toxicity was evaluated retrospectively in the Danish national cohort of 1049 patients treated with curative radiotherapy and nimorazole for head and neck SCC between 1990 and 2013 [47]. They found that overall compliance to the drug was the same (58%) as in the DAHANCA-5 trial (60%) and nausea/vomiting was still the most frequent reason for dose reduction. In fact, 40% of the patients reported any degree of nausea/vomiting, and in total, 22% of the patients had dose reduction due to nausea/vomiting.

The aforementioned nimorazole studies were all performed some years ago and radiation was delivered as 2D-planned radiotherapy. Furthermore, nimorazole has not been tested in a randomized setting in combination with chemo-radiotherapy. At present, three randomized trials are recruiting patients in that setting. The UK-based NIMRAD study aims to recruit 470 patients with locally advanced head and neck SCC treated with IMRT-planned conventional fractionated radiotherapy-only and randomizes patients to concomitant nimorazole or placebo [48]. The study is at present running in 19 different UK institutions and aims for study completion by 2020. Two other international studies are testing the possible benefit of chemo-radiotherapy and concomitant nimorazole. The EORTC 1219 will include 640 patients with p16-negative locally advanced head and neck SCC to be treated with accelerated fractionated chemo-radiotherapy (70 Gy, 35 fx, 6fx/week, and weekly low-dose cisplatin 40 mg/m^2) randomizing to either nimorazole or placebo. At present, the study involves 22 institutions from seven countries and last patient is planned to be enrolled by 2020. An almost similar study is running in parallel in Denmark—the DAHANCA-30 study. The main difference between the EORTC and the DAHANCA study is that nimorazole is already the standard in Denmark. The study will not randomize to chemo-radiotherapy with or without nimorazole, but instead will randomize to omit nimorazole or not in the group of locally advanced HNSCC patients with less hypoxic tumours based on a hypoxic gene profile (see later). Patients with more hypoxic tumours will continue to receive nimorazole as standard. This non-inferiority trial is

estimated to recruit 1262 patients and to be completed at the end of 2019.

It is assumed that the nitroimidazoles primarily target the chronic hypoxia. Another approach for targeting both acute and chronic hypoxia was developed in the Netherlands and consisted of administering carbogen during accelerated radiotherapy (ARCON protocol). Carbogen is a gas mixture of 98% O_2 and 2% CO_2, which increases blood oxygen levels and reduces diffusion-limited hypoxia, plus nicotinamide, which increases the vascular perfusion [49]. This concept was tested in a large randomized phase III trial of ARCON versus accelerated radiotherapy alone in patients with locally advanced SCC of the larynx. However, after inclusion of 345 patients, the 5-year local control rates were identical in both treatments-arms (79% vs. 78%), whereas loco-regional control was in favour of ARCON (93% vs. 86%; $p = 0.04$) [50]. The ARCON regimen is technically complicated to deliver and based on the phase III data, not yet in routine clinical use.

Dose painting and escalation based on hypoxia

Imaging-based dose painting, i.e., the prescription and delivery of a non-uniform dose to the clinical target volume, is a different paradigm for prescribing radiation therapy [19, 51]. The basic idea is to replace, completely or in part, the morphologically or anatomically defined target volumes with a map of the spatial distribution of a specific tumour phenotype that is hypothesized or has been shown to be related to local tumour control after radiotherapy. A dose prescription function is then used to transform this map into a map of prescribed doses that can be used as input to an inverse planning optimizer. Two prototypical strategies have been considered in the literature: sub-volume boosting also known as dose painting by volume (DPBV), where an imaging-defined discrete volume is given an additional "boost" dose, or dose painting by numbers (DPBN), where a dose is prescribed at the voxel level. In the latter case, the prescription function maps a range of image intensities onto a range of doses. Hybrids between the two strategies use a series of nested volumes, often about five or so, with a prescribed dose assigned to each of them.

Regarding dose painting on hypoxic volumes, a few planning studies have been published, using FAZA or FMISO-PET scan to identify hypoxic area to which higher dose could be delivered without significantly exceeding the dose to the surrounding normal tissues [52–56]. Although the concept of dose painting on hypoxic PET voxels is easy to formulate, its implementation raises several methodological issues from image acquisition and segmentation, to dose prescription function and dose adaptation throughout the course of radiotherapy. It is beyond the scope of this review article to discuss these issues in length. In short, compared to CT or MRI, PET camera has a much lower spatial resolution (typically half a centimetre on average for PET versus about 1 mm for CT or MRI) and the amount of signal detected is rather limited, thus translating into a high signal-to-background ratio [57]. On top of these limitations, accurate determination of the volume and shape of the tumour from PET images still remains a challenging task and an incompletely resolved issue [58]. In addition to these limitations, the magnitude of the required radiation dose to control disease in hypoxic regions is still undefined. Simplistic back-of-an-envelope estimates based on in vitro oxygen-enhancement ratios (around 3 for extreme hypoxia) are likely to be gross overestimates of the dose required in human tumours. In a proof-of-concept planning study using F-Miso-derived sub-target volumes, it has been estimated that a 10% dose escalation (above 70 Gy) with dose redistribution could already be associated with a significant increase in tumour control probability [52]. Finally, it has been shown that hypoxia fluctuates during radiotherapy raising the issue of planning adaptation, for which neither the frequency nor the proper implementation has been settled [59]. Furthermore, based on the dynamic of hypoxia during radiotherapy, it has been suggested that a dose escalation protocol using assessment early on during treatment might be more effective than using pre-treatment hypoxia [60].

Notwithstanding the limitations outlined above, three randomized phase II or phase III clinical trial on hypoxia dose escalation has been registered on the ClinicalTrials.gov website (Table 1). One study has supposedly closed its accrual, but no data have been published or reported yet. Preliminary data have been recently released on the treatment of the first 25 patients of the Tubingen trial treated with concomitant chemo-radiotherapy for locally advanced oropharyngeal or hypopharyngeal SCC [61]. The 2-year loco-regional control reached 70 and 44.4% for the patients with hypoxic tumour and dose escalation ($n = 10$), and no dose escalation ($n = 10$), respectively. Five patients with no hypoxic tumour were also included in an observation arm (the same treatment regimen without dose escalation), and the 2-year loco-regional control reached 100%. No difference was observed in the compliance or in early and late toxicity between the two arms of the study.

Patient selection and treatment personalization

As already mentioned, although frequently identified in HNSCC, not all tumours are hypoxic, and in this framework, methods that could identify those individual patients with clinical relevant levels of tumour hypoxia who might benefit from hypoxia-targeted radiosensitization are needed. From the DAHANCA-5 study, it is estimated that six patients need

Table 1 Ongoing randomized phase II and phase III trials on dose escalation on hypoxic sub-volumes

Acronym/ investigator (NCT#)	Tumour location	HPV status	Tumour stage	Molecular imaging	Phase	Study design		Completion date
						Standard arm	Experimental arm	
Xuzhou Medical College, China (NCT# 02089204)	NPC	not relevant	III–IVa	FDG-PET FMISO-PET	III	IMRT + cddp + docetaxel	(1) IMRT + cddp + docetaxel + boost dose on FDG (2) IMRT + cddp + docetaxel + boost dose on FMISO	Dec 2015?
Zips (NCT# 02352792)	Oro, Hyp, Cav, Lar	n.a.	III–IV	FMISO-PET	Rand. II	70 Gy in 7w + 5Fu + mitomycin C or cddp	77 Gy in 7w + 5Fu + mitomycin C or cddp	Dec 2022
ESCALOX (NCT# 01212354)	Oro, Hyp, Cav	n.a.	n.a.	FMISO-PET	III	70/56 Gy in 7w (SIB-IMRT) + concomitant cddp	80.5/70/56 Gy in 7w (SIB-IMRT) + concomitant cddp	Jan 2025

Oro oropharynx, *Hyp* hypopharynx, *Cav* oral cavity, *Lar* larynx, *NPC* nasopharynx, *CH* chemotherapy, *n.a.* non-available, *rand.* randomized

to be treated with nimorazole for the benefit of one of them [15].

Recently, Toustrup et al. identified and validated a molecular gene signature of tumour hypoxia [62]. Nude mice-bearing humans' SCC xenografts were injected with the hypoxic tracer FAZA, and ex vivo visualisation of hypoxic area was performed by autoradiography. Representative hypoxic as well as less hypoxic areas were then excised and examined for twofold (or more) upregulation of 30 pre-defined genes in the more hypoxic areas compared to the less hypoxic areas. Then, these genes were evaluated in a training set of 58 HNSCC with known hypoxia status estimated by the Eppendorf polarographic electrode to generate a gene expression classifier containing 15 genes. This hypoxic gene classifier was later further validated in three different well-defined human cohorts before clinical use [63]. In addition, the classifier was tested using biological material available from the DAHANCA-5 trial, randomizing 422 patients to the conventional treatment with or without the hypoxic radiosensitizer nimorazole. The prognostic impact of the gene test was evaluated in the 156 patients in the radiotherapy-only group. Tumours classified as more hypoxic had a significantly poorer outcome both in terms of 5-year loco-regional control (18% vs. 44%) and disease-specific survival (30% vs. 51%) compared to the less hypoxic tumours, and a non-significant trend for poorer overall survival was observed in the more hypoxic tumours cohort. For the predictive value of the gene classifier, both arms of the trial were included. It was shown that only the more hypoxic tumours did benefit from nimorazole, whereas the tumours classified as less hypoxic did not; the 5-year loco-regional control reached 49% for the more hypoxic tumours associated with nimorazole versus 18% for the more hypoxic tumours with placebo, whereas for the less hypoxic tumours, corresponding figures reached 50 and 44%, respectively [16]. Further stratification of the above-mentioned groups by p16-status as a marker for HPV-related cancers suggested that the benefit of nimorazole was primarily in the p16-negative more hypoxic group of HNSCC. Nevertheless, both p16-positive and p-16-negative SCC can be more hypoxic [37], and both p16-positive and p16-negative more hypoxic SCC do benefit from hypoxic radiosensitization [64]. However, the increased radiosensitivity of the p16-positive oropharyngeal tumours might overcome the benefit of using hypoxic radiosensitization.

The benefit of the 15-gene hypoxic profile for patient selection for nimorazole treatment is at present investigated in the previously mentioned EORTC-1219 and DAHANCA-30 trials. In the EORTC study, all patients will have the gene profile assessed before randomization, but this will not influence the treatment choice, but will be used as a stratification among the two treatment groups. This design will allow testing the predictive value of the hypoxic classifier in the group of patients with the expected largest benefit of nimorazole in a setting with modern IMRT-based chemoradiotherapy. In the DAHANCA-30 trial, the gene test will be performed upfront before randomization. If a patient has a tumour estimated to be more hypoxic, then he will receive nimorazole (which is the current standard of treatment in Denmark). However, if a patient is classified as less hypoxic and estimated as not having benefit of nimorazole, then he will be randomized to receive or not nimorazole. The results of these two trials will help us to define and select which patients should be treated with nimorazole in the future.

Conclusions

Tumour hypoxia is a universal factor that, among other things, affects the response to ionizing radiation. Its measurement can be done by invasive (e.g., polarographic electrode) or non-invasive (e.g., use of PET-labelled tracers). Various options to counteract tumour hypoxia have been tested, and among them, the use of hypoxic cell radiosensitizers and radiation dose escalation has been validated. Ongoing clinical research is focused on a better selection of patients who may benefit from such therapeutic interventions.

Authors' contribution V. Grégoire and J. Eriksen: content planning, writing, and editing.

Compliance with ethical standards

Conflict of interest V. Grégoire and J. Eriksen declare no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by the any of the authors.

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