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EXPERT REVIEW

Is there a role for [^{18}F]-FMISO PET to guide dose adaptive radiotherapy in head and neck cancer? A review of the literature

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Abstract

Purpose Hypoxia is a major cause of radioresistance in head and neck cancer (HNC), resulting in treatment failure and disease recurrence. ^{18}F-fluoromisonidazole ([^{18}F]FMISO) PET has been proposed as a means of localising intratumoural hypoxia in HNC so that radiotherapy can be specifically escalated in hypoxic regions. This concept may be challenging to implement in routine clinical practice however, given that [^{18}F]FMISO PET is costly, time consuming and difficult to access. The aim of this review was to summarise clinical studies involving [^{18}F]FMISO PET and to appraise the evidence for its role in guiding radiotherapy treatment in HNC.

Methods A comprehensive literature search was conducted on PubMed and Web of Science databases. Studies investigating [^{18}F]FMISO PET in newly diagnosed HNC patients were considered eligible for review.

Results We found the following important results from our literature review: (1) Studies have demonstrated a correlation between [^{18}F]FMISO PET and other hypoxia biomarkers, although the results are not consistent enough to propose a proxy biomarker of [^{18}F]FMISO PET. (2) [^{18}F]FMISO PET uptake changes during a course of radiotherapy treatment, suggesting that imaging should be repeated during treatment. (3) Tumour recurrences do not always occur within the pretreatment hypoxic volume on [^{18}F]FMISO PET. (4) Dose modification studies using [^{18}F]FMISO PET are in a pilot phase.

Conclusions Our results show that currently there is insufficient evidence to propose [^{18}F]FMISO PET for radiotherapy dose adaptation in HNC in a routine clinical setting. Part of the challenge is that hypoxia is a dynamic phenomenon, and thus areas identified on a single scan may not be representative. At present, it is anticipated that [^{18}F]FMISO PET will remain useful within the research setting only.

Keywords [^{18}F]FMISO · Hypoxia · Head and neck cancer

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Introduction

Hypoxia has long been identified as a negative prognostic factor in head and neck cancer (HNC) [1]. It renders cancer cells more resistant to therapies [2] and confers an aggressive phenotype on the tumour. A major treatment modality for locally advanced HNC is radiotherapy, but 30–40% of cases will relapse within 5 years [3]. Most recurrences occur within the high dose region of the irradiated field [4], due to the presence of radioresistant clones within the tumor cell population. Hypoxia is a key driver of radioresistance. It has been shown in vitro that hypoxic cancer cells require three times the delivered radiation dose to achieve the same therapeutic effect [5]. Given the deleterious consequences of hypoxia in head and neck cancer, it is important to develop a means to identify this phenomenon

upfront, so that treatment can be tailored to overcome it. PET (positron emission tomography) imaging of tumors using hypoxia specific tracers is an attractive method of assessing hypoxia. It is non-invasive, can be easily repeated and provides spatial information across the whole tumor. The last point is especially relevant to locally advanced HNC, where it is feasible to deliver a dose escalation specifically to the hypoxic region identified within a tumour [6]; a concept known as 'dose painting'. Of all the hypoxic tracers developed, ^{18}F-fluoromisonidazole ([^{18}F]FMISO) is the most widely investigated in head and neck cancers.

Numerous clinical studies have been conducted in HNC patients using [^{18}F]FMISO PET imaging, evaluating different aspects, such as correlation with other hypoxia biomarkers [7–9], the ability to prognosticate in HNC patients [10], and the variation in uptake during serial imaging [11, 12]. Although there is a wealth of published literature, it is difficult to summate their outcomes as the studies are heterogenous with regards to study design, image analysis (for example evaluating standardized uptake value $(SUV)_{max}$ versus tumour to background ratio (TBR)) and research question. A significant barrier to clinical adoption of [^{18}F]FMISO PET is expense and access to the tracer. In most countries, [^{18}F]FMISO is not commercially available and is produced only at a handful of research institutions, making it costly to obtain [13]. Furthermore, its lipophilic nature leads to slow clearance of the tracer from blood/normal tissues which makes its use time consuming (as PET images need to be acquired 2—4 h after tracer injection) and results in images with a low signal to background ratio.

Several studies have reported a prognostic association between [^{18}F]FMISO uptake pretreatment and survival outcomes [10, 12, 14, 15]. A meta-analysis [16] of 4 trials that included a total of 120 HNC patients who had pretreatment [^{18}F]FMISO imaging and subsequently received curative radiotherapy was recently reported. The patient populations varied in T stage, tumour volume and human papilloma virus (HPV) status. Nevertheless, a multivariate analysis (which included T stage and HPV status) found that baseline [^{18}F]FMISO uptake had a significant impact on locoregional control ($p = 0.04$) and overall survival ($p < 0.04$) in HNC patients treated with radiotherapy. Despite its role as a potential prognostic biomarker, [^{18}F]FMISO PET has not yet transitioned into the clinic. Clinical use may be more likely if the results of [^{18}F]FMISO PET imaging were to change management in patients, such as by individualizing radiotherapy treatment.

Radiotherapy dose escalation has been investigated as a means to overcome radioresistance [17] with different target volumes proposed to receive dose escalation, such as the FDG avid tumour volume [17] or the radiotherapy planning target volume (PTV) [18]. The hypoxic volume on [^{18}F]FMISO PET imaging has also been suggested as a candidate for radiotherapy dose escalation [6], given that hypoxia is a key driver of radioresistance. Several planning studies have demonstrated that it is technically possible to increase the radiotherapy dose to the [^{18}F]FMISO hypoxic volume without exceeding normal tissue tolerance doses [6, 19, 20]. However, whether [^{18}F]FMISO will progress into the clinic to routinely guide radiotherapy treatment for HNC patients remains to be seen. The justification and evidence to support radiotherapy dose escalation to [^{18}F]FMISO PET based hypoxia need to be considered, along with the practicalities of conducting these scans.

The purpose of this narrative review is to summarize the key findings from clinical studies of [^{18}F]FMISO PET in HNC patients so far, to help understand its utility and whether it could realistically be used to guide radiotherapy 'dose painting' in HNC. To achieve this, four key questions will be addressed:

(1) Is there a surrogate and 'easy to perform' biomarker for the [^{18}F]FMISO hypoxia phenotype on imaging? This would help select patients for [^{18}F]FMISO imaging and avoid the cost and inefficiency of scanning all patients.
(2) Is [^{18}F]FMISO PET imaging reproducible and repeatable?
(3) Do locoregional recurrences occur within the initial hypoxic volume on [^{18}F]FMISO PET, such that we could justify dose escalation to the hypoxic volume specifically?
(4) What have we learned so far from dose escalation/de-escalation studies?

Of note, we have not included studies investigating the prognostic potential of [^{18}F]FMISO PET, given this has been addressed in a meta-analysis

Methods

We performed a comprehensive search of Pubmed and Web of Science Databases up to 15 November 2023 to identify relevant articles published from 1992 onwards. The year 1992 was chosen as it is when the first-in-man imaging of [^{18}F]FMISO was reported [21]. We searched for academic scientific papers whose abstracts included any of the following terms: 'fluoromisonidazole', 'misonidazole', 'F-MISO', '[^{18}F]FMISO, '^{18}F-MISO' or 'FMISO'. Studies were included which investigated [^{18}F]FMISO PET imaging (either at baseline or during treatment) in newly diagnosed HNC patients. The articles were reviewed for applicability to our 'key questions' and overall, 40 publications were selected for inclusion. Figure 1 shows the PRISMA flowchart for study selection.

Data were consistently recorded for each study and included the number of patients, [^{18}F]FMISO imaging parameters, time course of [^{18}F]FMISO scanning and the time point during treatment when scans were conducted. The key findings of the studies are summarized here as a narrative review as this was felt to be the most suitable format to provide an overarching view of [^{18}F]FMISO in HNC.

Results

A comprehensive literature review was undertaken and the details and focus of the resulting studies are illustrated in Fig. 2. The majority (58%) were studies correlating [^{18}F]FMISO PET either with tissue or blood hypoxic biomarkers, or other imaging biomarkers. Figure 3 maps out the studies geographically. The 40 studies included in this review were performed at just 13 institutions, across 8 countries, and 18 of the published studies were from Germany alone.

The studies have been summarised in evidence tables (see Tables 1, 2, 3, 4, 5, 6, 7 and 8), with some entered twice if they addressed 2 topics (for example repeat imaging *and* correlation with ^{18}F-fluorodeoxyglucose (^{18}F-FDG)). The results are discussed in detail below, in line with our 'key questions'.

Correlation of [^{18}F]FMISO PET with other biomarkers—is there a surrogate?

[^{18}F]FMISO PET scans are both expensive and time consuming to conduct (as images are acquired 2–4 h after tracer injection), therefore an easily accessible 'surrogate' hypoxia biomarker, which could help select patients who would benefit from [^{18}F]FMISO imaging, would be desirable. Alternative biomarkers include hypoxia-associated protein immunohistochemistry (IHC), hypoxic gene expression, oxygen electrode measurement (all tumour based) and serum osteopontin. Since all patients undergo a biopsy and blood

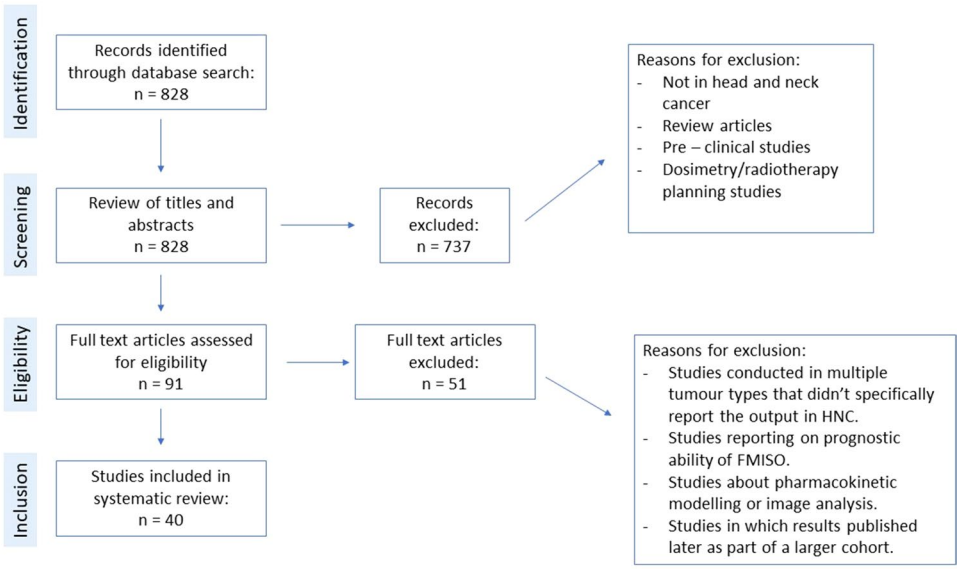

Fig. 1 PRISMA flowchart for study selection

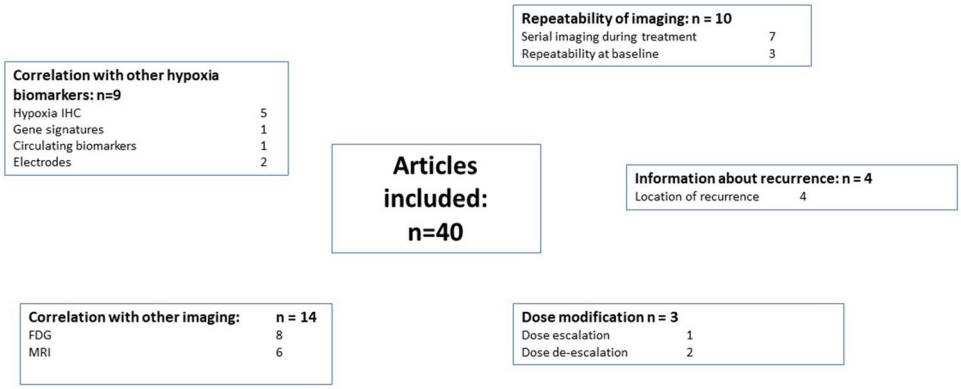

Fig. 2 Studies included in this narrative review

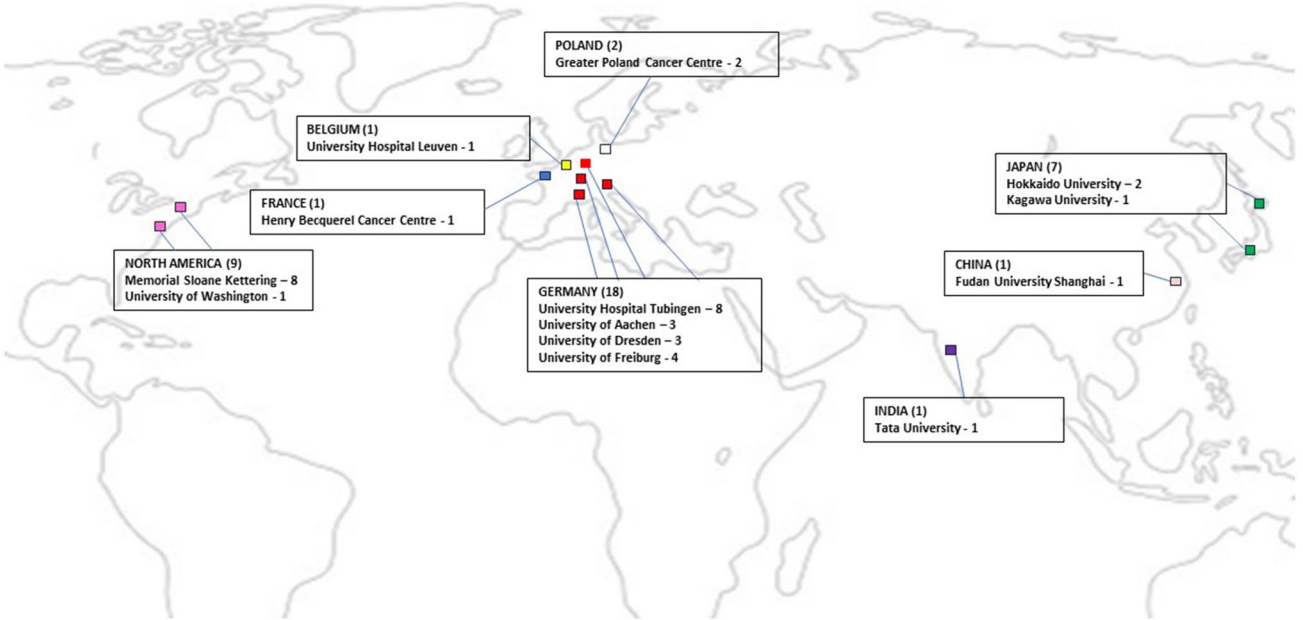

Fig. 3 Geographical illustration to show where the studies have been conducted

tests at diagnosis, it would be ideal if one of these investigations could be applied to select patients for [^{18}F]FMISO PET imaging. The studies correlating [^{18}F]FMISO PET with other hypoxia biomarkers are summarised in Tables 1 and 2.

Studies correlating hypoxia protein immunohistochemistry with [18F]FMISO uptake

Immunohistochemical markers of hypoxia include hypoxia-inducible factor 1-alpha (HIF1α), carbonic anhydrase 9 (CaIX) and glucose transporter 1 (Glut-1). These are endogenous proteins whose expression is upregulated in hypoxic conditions [22] and can be measured using IHC on tumour biopsy specimens. Table 1 outlines the six studies [8, 13, 19, 23–25] which assessed the relationship between hypoxia on [^{18}F]FMISO PET and IHC markers of hypoxia. Overall, the results are mixed, with three studies [8, 13, 23] reporting a positive correlation between hypoxia IHC markers and hypoxia on [^{18}F]FMISO PET, and two studies [19, 25] concluding that there was no association. Nicolay et al. [24] conducted the largest study, assessing the relationship with hypoxia IHC (both CaIX and HIF1α) and [^{18}F]FMISO PET conducted at different time points (week 0, 2 and 5) in 49 patients undergoing chemoradiotherapy. There was no correlation between either HIF1a or CaIX expression and [^{18}F]FMISO hypoxia in treatment-naïve patients. They did however find an association between hypoxia IHC and 'adverse hypoxia dynamics' on [^{18}F]FMISO PET, i.e., delayed resolution of hypoxia on PET scans during radiotherapy treatment.

It appears that there may be a relationship between HIF1α /CaIX/Glut-1 expression and [^{18}F]FMISO imaging, but current evidence is insufficient to propose a proxy biomarker of [^{18}F]FMISO uptake. This question could be answered more fully using archival tumour samples from previous [^{18}F]FMISO trials to assess correlation with HIF1α or CaIX. Ideally, quantification of hypoxia IHC would be consistent; at present some studies look at 'positive' versus 'negative' expression using a cutoff level, whereas others assess the degree of protein expression as a continuous variable resulting in difficulties interpreting the results of the various studies. It should be noted that a caveat of using tumour IHC is sampling bias. Hypoxia is typically heterogeneously distributed across a tumour, so a single biopsy sample may not be representative of the whole tumour. Furthermore, the markers measure different aspects of hypoxia and may not correlate with each other. For example, HIF1α expression represents the transcriptional changes that occur in response to a chronically hypoxic tumour microenvironment whereas [^{18}F]FMISO uptake is a direct indicator of intracellular hypoxia, both acute and chronic. Hence, the lack of any correlation should not be interpreted as[^{18}F]FMISO having an inferior ability to detect hypoxia.

Studies correlating other hypoxia biomarkers with [^{18}F]FMISO uptake

The other hypoxia biomarkers which have been studied in relation to [^{18}F]FMISO imaging are oxygen electrode measurements, gene signatures, and plasma hypoxia markers. The

Table 1 Studies correlating [^{18}F]FMISO uptake with tissue based hypoxia protein immunohistochemistry

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Hypoxia biomarker	Results
Chen et al. 2012 [23]	9	2 h	SUVmax TMR	Pretreatment	Ca IX (positive vs negative)	CaIX expression is correlated with [^{18}F]FMISO uptake. Specificity 50%, sensitivity 71.4%
Norikane et al. 2014 [8]	24	2 h	TBR HV (threshold TBR 1.2)	Pretreatment	HIF1a (level of expression)	Weak correlation between HV and HIF1a expression ($r=0.4$, $p=0.037$) No correlation between TBR and HIF1a
Sato et al. 2017 [13]	23	4 h	SUVmax, TMR, HV (threshold TMR of 1.25)	Pretreatment	HIF1a (positive vs negative)	[^{18}F]FMISO TMR significantly associated with HIF1a expression (odds ratio 22.5, $p=0.005$)
Nicolay et al. 2020 [24]	49	2.5 h	HV (threshold TMR 1.4) Normalised SUVmax	Week 0, 2 and 5 of radiotherapy	HIF1a CaIX (level of expression)	HIF1a and CaIX expression showed no significant correlation with [^{18}F]FMISO hypoxia at week 0 HIF1a expression significantly correlated with increase in tumour hypoxia at week 2 HIF1a and CaIX expression significantly correlated with deferred decrease in hypoxia between weeks 2–5 ($r=0.330$ and 0.416 respectively for HIF1a and CaIX)
Kunder et al. 2021 [25]	14	3 h	HV (threshold TMR 1.5) FHV (ratio of HV to GTV on planning CT) SUVmax SUVmean	Pretreatment	HIF1a CaIX Glut-1 (level of expression)	No significant correlation between HIF1a/CaIX/GLUT-1 expression and [^{18}F]FMISO uptake
Lee et al. 2016 [19]	11	150 min	TMR (> 1.2 interpreted as hypoxic)	Pretreatment	HIF1a (level of expression)	No correlation between HIF1a expression and level of [^{18}F]FMISO uptake

SUV standardised uptake value. *TMR* tumour tp muscle ratio. *TBR* tumour to background ratio. *HV* hypoxic volume. *FHV* fractional hypoxic volume. *GTV* gross tumour volume

Table 2 Studies correlating [^{18}F]FMISO uptake with other hypoxia biomarkers

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Hypoxia biomarker analysed	Results
Gagel et al. 2004 [27]	16	2 h	TMR, SUVmean, SUVmax	Pretreatment	pO2 polarography	Strong correlation between frequency of pO2 readings < 2.5 mmHg, < 5 mmHg, < 10 mmHg and [^{18}F]FMISO TMR (r = 0.759–0.792)
Gagel et al. 2007 [26]	20	2 h	TMR, TBR	Pretreatment	pO2 polarography	Weak to moderate correlation (r = 0.4–0.6) of PO2 polarography with [^{18}F]FMISO hypoxia Exclusion of 4 outliers results in much stronger correlation with [^{18}F]FMISO TMR (R > 0.7)
Zimny et al. 2006 [28]	22	2 h	TMR, TBR	Pretreatment	pO2 polarography	Strong correlation between pO2 polarography and [^{18}F]FMISO TMR (r = 0.78–0.80)
Lock et al. 2019 [9]	42	4 h	HV (TMR > 1.6) TMRpeak	Week 0, 1, 2 and 5	Hypoxia associated genes (n = 58)	Weak correlation between hypoxia associated genes and [^{18}F]FMISO hypoxia (R < 0.44)
Ruhle et al. 2022 [33]	27	2.5 h	HV (threshold TR 1.4)	Week 0, 2 and 5	Plasma hypoxia markers: Osteopontin Galectin VEGF CTGF	Moderate correlation between baseline HV and baseline osteopontin (r = 0.579, p < 0.01) and galectin 3 (r = 0.429, p < 0.05)

SUV standardised uptake value. *TMR* tumour tp muscle ratio. *TBR* tumour to background ratio. *HV* hypoxic volume

Table 3 Studies correlating [^{18}F]FMISO PET with 18F-FDG PET

Authors	No of patients	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Correlates	Results
Zimny et al. 2006 [28]	22	2 h	TMR TBR	Pretreatment	18F-FDG SUV_{max} vs [^{18}F]FMISO TMR 18F-FDG SUV_{max} vs [^{18}F]FMISO TBR	Moderate correlation between 18F-FDG and [^{18}F]FMISO TBR. Correlation coefficient 0.53, $p<0.05$ No correlation between 18F-FDG SUV and [^{18}F]FMISO TMR
Thorwarth et al. 2006 [40]	12	4 h	HF (percentage of voxels with SUV > 1.4) SUVmax SUVavg	Pretreatment	18F-FDG SUV_{max} vs [^{18}F]FMISO SUV_{max}	No significant correlation between 18F-FDG and [^{18}F]FMISO uptake
Rajendran et al. 2004 [35]	26	2 h	HV (threshold TMR 1.2) TBRmax	Pretreatment	18F-FDG SUVmax vs [^{18}F]FMISO HV, assessed globally	Moderate correlation between [^{18}F]FMISO HV and 18F-FDG SUVmax. $R=0.63$
Monnich et al. 2017 [37]	21	4 h	HV (threshold TMR 1.4) * *This study was done to check HV are covered by 18F-FDG volume, wrt consideration of DE*	Pretreatment	$V_{18F\text{-}FDG}$ (highly avid 18F-FDG-avid subvolume) vs [^{18}F]FMISO HV Also looked at coverage of [^{18}F]FMISO HV by $V_{18F\text{-}FDG}$	Moderate correlation between $V_{18F\text{-}FDG}$ and [^{18}F]FMISO HV. $R=0.65$ [^{18}F]FMISO HV well covered by highly 18F-FDG-avid volume (mean coverage is 74.4%)
Crispin-Ortuzar et al. 2018 [43]	75	1.5 and 2.5 h	TBR_{max} Lesion considered hypoxic if $TBR_{max} > 1.4$	Pretreatment	Radiomics features from 18F-FDG PET and accompanying CT Assessed ability of radiomics signature to predict [^{18}F]FMISO TBR_{max}	Radiomics signature combining CT and 18F-FDG-PET features can predict hypoxia (TBR > 1.4) with an AUC 0.83
Kroenke et al. 2019 [38]	38	4 h	HV SUVmean SUVmax	Pretreatment	Voxel based comparison of 18F-FDG vs [^{18}F]FMISO PET Also used texture analysis parameters on 18F-FDG PET to predict [^{18}F]FMISO uptake	Moderate correlation between 18F-FDG and [^{18}F]FMISO uptake using voxel-by-voxel comparison ($r=0.664$, $p<0.001$) Texture analysis parameters did not show improved ability to predict [^{18}F]FMISO uptake, highest correlation coefficient was = 0.524
Nehmeh et al. 2021 [39]	20	2.5 h	SUV_{mean} TBR HV (TBR > 1.2)	Pretreatment	18F-FDG SUV and [^{18}F]FMISO SUV on voxel by voxel basis within hypoxic volume 18F-FDG SUVavg and [^{18}F]FMISO SUVavg across the hypoxia volume	Strong correlation ($R>0.7$) in only 39% primary tumours and 22% lymph nodes Moderate correlation ($R=0.68$, $p<0.01$) between SUVavg on 18F-FDG vs [^{18}F]FMISO PET
Cegla et al. 2020 [41]	36	2 h	SUVmax TLH (HV multiplied by SUVmean)	Pretreatment	18F-FDG TLG (SUVmean x metabolic volume) with [^{18}F]FMISO TLH	Strong correlation between 18F-FDG TLG and [^{18}F]FMISO TLH ($r=0.85$)
Wiedenmann et al. 2018 [42]	10	2.5 h	HV (threshold TMR 1.4) SUVmax	Pretreatment	[^{18}F]FMISO SUVmax vs 18F-FDG SUVmax	Strong correlation between 18F-FDG and [^{18}F]FMISO SUVmax in tumour. $R=0.81$

Table 3 (continued)

Authors	No of patients	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F] FMISO scan	Correlates	Results
Norikane et al. 2014 [8]	24	2 h	TBR HV (threshold TBR 1.2)	Pretreatment	[^{18}F]FMISO HV with 18F-FDG hypermetabolic volume [^{18}F]FMISO T/B max and 18F-FDG SUVmax	Weak correlation between [^{18}F] FMISO HV and 18F-FDG hypermetabolic volume ($r=0.44$, $p=0.046$) No correlation between [^{18}F]FMISO T/B max and 18F-FDG SUV max
Sato et al. 2017 [13]	23	4 h	SUVmax, TMR, HV (threshold TMR of 1.25)	Pretreatment	[^{18}F]FMISO TMRmedian and 18F-FDG SUVmedian	Moderate correlation between [^{18}F] FMISO TMR and 18F-FDG SUV ($r=0.52$, $p=0.007$)
Gagel et al. 2007 [26]	24	2 h	TMR, TBR	Pretreatment	18F-FDG SUVmax and 18F-FDG SUVmean with [^{18}F]FMISO TMR and [^{18}F]FMISO TBR	Weak correlation between 18F-FDG SUVmax and [^{18}F]FMISO TBR ($r=0.430$) only

SUV standardised uptake value. *TMR* tumour tp muscle ratio. *TBR* tumour to background ratio. *HV* hypoxic volume. *TLH* total lesion hypoxia. *TLG* total lesion glcolysis

Table 4 Studies correlating [^{18}F]FMISO PET with MRI

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	MRI modality	MRI parameters assessed	Results
Jansen et al. 2010 [44]	13	2–2.5 h	SUVmax, TBR	Pre treatment	DCE-MRI	DCE MRI parameters (K^{trans}, K_{ep}, V_e)	Strong negative correlation between median Kep and [^{18}F]FMISO SUV (R = -0.58) Hypoxic nodes had significantly lower median Ktrans (p = 0.049) and Kep (p = 0.027) than non hypoxic nodes
Simoncic et al. 2017 [45]	6	2 h, 4 h	TMR, HF (threshold TMR 1.4). Also dynamic [^{18}F]FMISOPET analysis – of uptake rate and vascular parameters	Pre treatment	DCE-MRI	[^{18}F]FMISO uptake and DCE MRI [^{18}F]FMISO vascular parameters and DCE MRI vascular parameters	Strong correlation between [^{18}F]FMISO vascular parameters and DCE MRI vascular parameters
Wiedenmann et al. 2018 [42]	10	2.5 h	HV (threshold TMR 1.4) SUVmax	Week 0, 2 and 5	T2* MRI	T2* mean vs [^{18}F]FMISO SUVmean and [^{18}F]FMISO SUVmax T2* in HV vs non HV	No significant correlation between [^{18}F]FMISO and T2* (p = 0.1157) at baseline T2* smaller in HV than non HV, results borderline significant (p = 0.051)
Wiedenmann et al. 2020 [46]	21	2.5 h	HV (threshold TMR 1.4) SUVmax	Week 0, 2 and 5	Multiparametric MRI – T1/T2/DCE/DWI MRI	T2*, ADC, Ktrans, Kep, Ve	ADC, Ktrans, Ve significantly lower in HV compared to non HV at baseline
Shima et al. 2020 [47]	18	4 h	SUVmax SUVmean TMRmax TMRmean	Pre treatment	DWI MRI	ADC Parameters from diffusion models: K (kurtosis value) Dk (diffusion corrected kurtosis coefficient) α (diffusion heterogeneity) DDC (distributed diffusion coefficient) Dslow (slow diffusion coefficient)	Good correlation between TMRmax and TMR mean with: ADC K Dk DDC Dslow

Table 4 (continued)

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	MRI modality	MRI parameters assessed	Results
Gouel et al. 2023 [48]	16	3 h	HV (threshold TMR 1.4)	Pre treatment	T1/T2/DCE/DWI MRI	T1*, T2*, ADC	ADC, T1* and T2* different in hypoxic versus non hypoxic volumes

SUV standardised uptake value. *TMR* tumour tp muscle ratio. *TBR* tumour to background ratio. *HV* hypoxic volume. *DCE*dynamic contrast enhanced. *DWI*diffusion weighted imaging. *ADC* apparent diffusion coefficient

details of these studies are summarised in Table 2. Oxygen electrodes allow direct measurement of hypoxia by inserting small needles into tumours to measure the partial pressure of oxygen (pO_2). Three studies [26–28] correlated pO_2 readings with [^{18}F]FMISO hypoxia (in a total of 58 patients) and all found a strong correlation. Given that oxygen electrodes are considered the gold standard for detecting intratumoural hypoxia, these results are promising in validating [^{18}F]FMISO PET as a means of detecting hypoxia. They do not, however, provide a practical representative biomarker of [^{18}F]FMISO hypoxia as oxygen electrode measurement is an invasive procedure which requires directly accessible tumours and cannot be used in a routine clinical setting.

Hypoxic gene signatures [29–31] refer to a collection of genes whose expression is upregulated or downregulated in response to hypoxia. They can be measured from a tumour biopsy specimen and are thought to represent the hypoxic phenotype of the overall tumour. Signatures are able to prognosticate in HNC [32] and also predict the benefit of hypoxia modification therapy in HNC [30]. To date, there is only one published study [9] analysing the relationship between hypoxia gene expression and [^{18}F]FMISO uptake in a cohort of 42 HNC patients treated with radiotherapy. Correlations were assessed at baseline and at different time points during radiotherapy. There was a weak association between hypoxic gene signatures and [^{18}F]FMISO uptake at baseline ($r=0.20$) which increased at weeks 1 ($r=0.38$) and 2 ($r=0.43$) during radiotherapy.

The final study [33] in Table 2 investigated the association between [^{18}F]FMISO PET imaging and plasma hypoxia markers (osteopontin, vascular endothelial growth factor (VEGF), galectin-3 and circulating tumour growth factor (CTGF)). The most promising result was obtained with serum osteopontin, a protein whose plasma concentration has been shown to increase in conditions of tumour hypoxia [33]. There was a moderate correlation between osteopontin levels and the baseline hypoxic volume ($r=0.579$), and residual hypoxia on [^{18}F]FMISO PET imaging ($p<0.05$) during treatment. Of note, osteopontin has been shown to inversely correlate with pO_2 in HNC and also to prognosticate and predict benefit from hypoxia modification therapy in HNC [34]. Given that it is easily obtained by a blood test, it could potentially be an ideal 'screening' biomarker to select patients who would benefit from [^{18}F]FMISO PET, but further studies are required to validate this concept.

Correlation of [^{18}F]FMISO PET with other imaging modalities

Correlation with ^{18}F-FDG PET

^{18}F-FDG PET is a routine investigation for many newly diagnosed HNC patients and hence it is convenient to assess

Table 5 Studies looking at repeat [^{18}F]FMISO PET pretreatment

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Time interval between scans	Results
Okamoto et al. 2013 [50]	11	4 h	SUVmax, TBR, TMR	2 days	Intra class correlation coefficient for SUVmax, TBR and TMR were 0.959, 0.913 and 0.965 respectively Distance between [^{18}F]FMISO maximum uptake location was 4.3 ± 3.0 mm
Lin et al. 2008 [49]	7	160 min	HV (threshold TMR 1.3)	3 days	3/7 patients had similar hypoxic volumes on both scans 4/7 patients had dissimilar hypoxic volumes
Nehmeh et al. 2008 [51]	14	160 min	SUV HV (threshold TMR 1.4)	3 days	6/13 patients had correlation coefficient > 0.5 in HV

SUV = standardised uptake value. TMR = tumour tp muscle ratio. HV = hypoxic volume. TBR = tumour to background ratio

correlation with [^{18}F]FMISO PET. ^{18}F-FDG PET provides assessment of glycolysis in tissue, which is a process affected by hypoxia [35]. HIF1α (activated in areas of low oxygen) upregulates both glucose transporters (GLUTs) and glycolytic enzymes [36], and therefore it is conceivable that ^{18}F-FDG PET could be a surrogate marker of hypoxia. Table 3 details the studies correlating ^{18}F-FDG and [^{18}F]FMISO PET. The majority of these studies showed a weak to moderate correlation between the two imaging modalities [8, 13, 26, 28, 35, 37–39], one showing no association between ^{18}F-FDG and [^{18}F]FMISO PET [40], and two a strong association ($r = 0.81$) [41, 42]. Two other studies [41, 43] that demonstrated a strong relationship used 'second order' features on ^{18}F-FDG PET; one looked at 'total lesion glycolysis' (SUV_{mean} multiplied by metabolic tumour volume on ^{18}F-FDG PET) and found a correlation coefficient of 0.85 with 'total lesion hypoxia' (SUV_{mean} multiplied by hypoxic volume) on [^{18}F]FMISO PET. The other study [43], used a radiomics signature from the CT, and found that this, in combination with ^{18}F-FDG PET, improved the ability to predict for hypoxia on [^{18}F]FMISO PET (with an area under the curve (AUC) of 0.83). In contrast, Kroenke et al. [38] used 'texture analysis' of the tumour on ^{18}F-FDG PET and found that this did not improve the ability of ^{18}F-FDG PET to predict [^{18}F]FMISO uptake.

The outcomes from these studies do not support the use of ^{18}F-FDG PET to select which patients should undergo ^{18}F-FMISO PET for subsequent hypoxia imaging to delineate a hypoxic target volume for radiotherapy dose adaptation.

Correlation with MRI

In recent years, attention has turned to multiparametric MRI (mpMRI) and its ability to provide information about tissue perfusion (dynamic contrast enhanced/DCE-MRI), cellularity (diffusion weighted imaging/DWI-MRI), and oxygenation (transverse relaxation time/T_2*MRI).

These are all processes central to the development of tumour hypoxia. The six studies which have compared mpMRI with [^{18}F]FMISO PET are summarised in Table 4. Two studies [44, 45] identified a relationship between DCE-MRI and [^{18}F]FMISO uptake, with reduced K_{trans} (a measure of perfusion) in the hypoxic volume. Data on ADC are conflicting, with both decreased [46] and increased [47, 48] values being reported in the hypoxic volume. Of these modalities, T_2* MRI is the most direct marker of hypoxia, as it measures the concentration of deoxygenated haemoglobin. The study which compared T_2* MRI to [^{18}F]FMISO PET [42] did not find a correlation, which again can be explained by the fact that they measure different processes; blood oxygenation versus intracellular oxygenations. MRI has the benefit of higher resolution compared to PET and would be ideal to identify tumour hypoxia for radiotherapy planning, but currently we do not have sufficient evidence to propose it for this role.

Is [^{18}F]FMISO imaging repeatable and reproducible?

Studies repeating [^{18}F]FMISO PET at baseline.

Three studies [49–51] repeated [^{18}F]FMISO PET scans within a short time frame (2–3 days) of each other (without interval treatment) to assess repeatability. They are described in Table 5. Okamoto et al. [50] demonstrated that [^{18}F]FMISO imaging is highly repeatable and that the maximum uptake location of [^{18}F]FMISO varied by only 4 mm between two repeat scans. Lin [49] and Nehmeh et al. [51] also reported similar hypoxic volumes in ~ 50%

Table 6 Studies looking at repeat [^{18}F]FMISO PET during radiotherapy treatment

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Results
Bittner et al. 2013 [52]	14	2.5 h	HV (threshold TMR 1.5)	Week 0, and 2	5/14 pts had persistent hypoxia by week 2 2/5 pts showed increased hypoxia at week 2 and 3/5 patients decreasing hypoxia at week 2 Overlap 72% (between weeks 0 and 2) in those with persistent hypoxia
Eschmann et al. 2007 [12]	14	4 h	SUVmean TMRmean	Week 0 After 30 Gy	SUV decreased in 12/14 patients, increased in 2 TMR decreased in 11/14 patients, increased in 3 Deduced a [^{18}F]FMISO 'kinetic curve type' to assess degree of hypoxia – in 11 pts the curve type changed towards less hypoxia, remained unchanged in 3 pts
Lee et al. 2009 [59]	16	2–2.5 h	FMISO positive versus negative	Week 0 and 4	16/18 patients had complete resolution of hypoxia by week 4 scan
Okamoto et al. 2016 [60]	20	4 h	SUVmax TMR HV (threshold TMR 1.25)	Week 0, 3 and post treatment	10/19 patients had resolution of hypoxia by week 3 7/9 patients had resolution of hypoxia from week 3 to post treatment 2/19 patients had persistent hypoxia at end of treatment (NB 1/20 patients did not have hypoxia on their initial scan)
Lock et al. 2017 [61]	50	4 h	TBRmax HV (threshold TBR 1.6)	Week 0, 1, 2 and 5 of treatment	Both HV and TBR reduced from baseline through to week 5 Residual hypoxic volume at week 2 could stratify patients in term of loco regional control
Kazmierska et al. 2020 [62]	33	160 min	SUVmax HV (threshold TMR 1.5) TMR	After 36 Gy	Reduction in hypoxic volume overall ($p < 0.001$) at 36 Gy 20% of patients showed residual hypoxia after 36 Gy TMRmax at baseline correlated with OS ($p = 0.006$) TMRmax after 36 Gy did not predict OS

Table 6 (continued)

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Results
Carles et al. 2021 [53]	35	160 min	HV (threshold TMR 1.4) CP – 'classification parameter' – quantifies variation of hypoxia	Week 0, 2 and 5	HV decreased in 64% at week 2 HV decreased in 80% at week 5 Only 24% of patients showed geographically stable hypoxia during the whole course of treatment Patients with geographically stable hypoxia had a better prognosis in terms of recurrence, metastasis and OS
Riaz et al. 2021 [57]	19	150 min	TMR (> 1.2 interpreted as hypoxic)	Pre treatment Day 6–10 after RT	6/19 pts had no hypoxia at baseline 9/12 pts had resolution of hypoxia by day 6–10
Lee et al. 2016 [19]	33	150mints	TMR (> 1.2 interpreted as hypoxic)	Pre treatment 1 week into RT	26/33 pts showed evidence of hypoxia at primary site at baseline 11/26 pts showed resolution of hypoxia in primary site after week 1

SUV = standardised uptake value. TMR = tumour tp muscle ratio. HV = hypoxic volume. TBR = tumour to background ratio

Table 7 Studies looking at location of recurrent disease compared to initial [^{18}F]FMISO uptake

Authors	No of patients	No of recurrent lesions in total	Relationship of recurrent volume to initial hypoxic volume
Dirix et al. 2009 [55]	15	9	6/9 recurrent lesions within initial hypoxic volume NB all recurrent lesions occurred within initial 18F-FDG GTV volume
Zschaeck et al. 2015 [11]	25	6	3/6 recurrences within initial hypoxic volume (Generated a stable consensual hypoxic subvolume from week 0 and 2 [^{18}F]FMISO PET. 3/6 recurrences occurred outside this volume) NB There was greater overlap with 18F-FDG GTV volume than [^{18}F]FMISO volume
Nishikawa et al. 2017 [56]	21	9	Uptake of [^{18}F]FMISO (on pretreatment scan) in recurrent region higher than non-recurrent regions ($p < 0.0001$) NB images analysed on voxel by voxel basis Risk of recurrence in a voxel = 30% if TMR > 2.42, AUC 0.591 to predict recurrence
Boeke et al. 2017 [54]	9	9	Significant overlap of recurrence volume with [^{18}F]FMISO positive subvolume within initial GTV Median overlap of recurrence with hypoxic volume = 42%

GTV = gross tumour volume. TMR = tumour tp muscle ratio

of their patients, although there was a significant variation in the location of tumour hypoxia in the other half of patients. The proposed explanation is that [^{18}F]FMISO captures both acute and chronic hypoxia [51], and so stable uptake may be representative of chronic hypoxia only.

Although patient numbers in these studies were small (7–14), the findings lend caution to the concept of dose escalating to a hypoxic volume generated from a single [^{18}F]FMISO PET study in a patient. This is well displayed in the study by Lin et al. [49] (a dose planning study),

Table 8 Dose modification studies based on [^{18}F]FMISO PET imaging

Authors	No pts	Time between tracer injection and image acquisition	[^{18}F]FMISO parameters analysed	Timing of [^{18}F]FMISO scan	Dose modification	Results
De-escalation studies						
Riaz et al. 2021 [57]	19 (15 de-escalated)	150 min	TMR (> 1.2 interpreted as evidence of hypoxia)	Pretreatment Day 6–10 of radiotherapy	Patients without evidence of hypoxia at either pre/intra treatment deescalated to 30 Gy radiotherapy	11/15 pts on de-escalated RT had pCR 4/15 had residual disease 2/4 minimal residual disease 1/4 significant residual disease and tumour regrowth1/4 deviation from protocol and progressive disease
Lee et al. 2016 [19]	33 (10 de-escalated)	150 min	TMR (> 1.2 interpreted as evidence of hypoxia)	Pretreatment 1 week after RT started	Patients without evidence of hypoxia at either pre/intra treatment had RT dose de-escalated to 60 Gy to involved LN	10 pts had de-escalated RT 10/10 patients had no recurrence of disease at 2 yrs
DosDose escalation studies						
Welz et al. 2022 [58]	53 (19 dose escalated)	4 h	HV	Pretreatment	Patients with a hypoxic volume randomized to either standard RT (70 Gy in 35 fractions) to dose escalation up to 77 Gy to HV	Local control rates 84% and 59% in DE vs ST arms respectively, non-significant (p = 0.150)

TMR tumour to muscle ratio. *HV* hypoxic volume. *LN* lymph node. *RT* radiotherapy. *pCR* pathological complete response. *DE* dose escalation. *ST* standard therapy

which showed a decrease in the prescribed uniform dose to the hypoxic volume of up to 12 Gy, between two serials scans (which were separated by 3 days), due to instability of the hypoxic region.

Studies repeating [^{18}F]FMISO PET during radiotherapy treatment

Several studies interrogated hypoxia dynamics during radiotherapy treatment and are displayed in Table 6. All the studies showed that in the majority of patients, reoxygenation occurs and the degree of hypoxia, measured by [^{18}F]FMISO PET, reduces through the course of treatment. These findings suggest that hypoxia observed on a single, pre-treatment, scan may not be sufficiently representative to guide treatment adaptation and serial scans ought to be considered when designing clinical studies of hypoxia guided dose escalation/adaptation Interestingly, although several studies comment on 'residual' hypoxia during treatment, only two formally reported on the geographical stability of the hypoxic volume [52, 53]. Bittner et al. [52] found a 72% overlap of the hypoxic volume from week 0 to week 2, suggesting that 'residual hypoxia' is an appropriate term. In contrast, Carles et al. [53] looked at spatial variation of hypoxia and found that only 24% of patients had geographically 'stable' hypoxia throughout their treatment, and that these patients had a better prognosis in terms of locoregional control. The other studies did not report on the geographical location of the hypoxic region during radiotherapy treatment.

Where do locoregional recurrences occur in relation to the initial hypoxic volume?

Despite the wealth of published studies on [^{18}F]FMISO PET in HNC, only four were identified which correlated recurrence patterns to the initial hypoxic volume (see Table 7). From these, one study [54] concluded that recurrences arise from the original hypoxic subvolumes, with a median overlap of 42% between recurrence volume and the initial hypoxic volume. Two studies [11, 55] showed that a significant proportion of recurrences (33–50%) occur outside the pretreatment hypoxic volume. Nishikawa et al. [56] analysed pretreatment [^{18}F]FMISO PET images from 21 patients with nasopharyngeal carcinoma (of whom nine recurred) to generate a risk model. They found that within the imaged tumour region, voxels with [^{18}F]FMISO tumour to muscle ratio (TMR) > 2.42 predicted a recurrence rate of 30% within the same voxel. The AUC for this prediction model was only 0.59 however, and the authors concluded that the predictive value of pretreatment [^{18}F]FMISO PET was insufficient for up-front dose escalation to the regions with high uptake, i.e. the hypoxic volume.

Overall, these findings suggest that although hypoxia is a known cause of radioresistance, there is not enough evidence to suggest that recurrences arise from the hypoxic regions identified on pretreatment imaging, especially when determined from a single scan. It should be noted, however, that the recurrence data stem from a total of only 70 patients, across 4 studies. Further knowledge is therefore required on disease recurrence and its relation to hypoxic volumes.

What have we learned so far from dose modification studies?

Given that the hypoxic uptake on [^{18}F]FMISO PET carries important prognostic information [16], trials are underway to determine if radiotherapy can be dose de-escalated for patients with a good prognosis (absence or early resolution of hypoxia) and conversely escalated for patients with hypoxic tumours. So far 3 dose modification trials using [^{18}F]FMISO PET as a biomarker have been published; see Table 8.

Dose de-escalation studies

The first [^{18}F]FMISO de-escalation study was published in 2016 [19] on 33 patients with human papilloma virus (HPV)-positive oropharyngeal cancer. The radiotherapy dose to the metastatic lymph nodes was reduced by 10 Gy to 60 Gy in patients who had resolution of hypoxia at week one of radiotherapy. Ten patients had their radiotherapy dose de-escalated and remained recurrence free at two years. The second de-escalation study [57] was in a cohort of 19 HPV-positive oropharyngeal cancer patients who were treated with resection of the primary tumour and radiotherapy to the nodes followed four months later by a neck dissection. The radiotherapy dose was reduced to 30 Gy in 15 patients who had no hypoxia at either pre- or intra-treatment [^{18}F]FMISO PET imaging. Eleven of the 15 patients had a pathological complete response. As these were pilot studies, neither had a comparative cohort in which patients received radiotherapy dose de-escalation despite hypoxia on PET. Given that the tumours were HPV-positive, it is possible their outcomes would have still been favourable, despite the observed hypoxia on PET.

A larger scale de-escalation study (clinical trial identifier NCT03323463; $n = 300$) at Memorial Sloane Kettering is currently underway using [^{18}F]FMISO PET to select patients to receive a de-escalated dose of radiation (30 Gy) if no hypoxia is observed on pre/intra-treatment imaging. In this

study, all patients will receive two cycles of concomitant chemotherapy and surgical resection is no longer mandatory. The results from this trial will help determine if it is safe to deescalate radiation dose in non-hypoxic tumours. However, this trial will not determine if it is the absence of hypoxia (on [^{18}F]FMISO PET) that renders the patients suitable for treatment de-escalation as no randomisation to standard treatment vs. de-escalation is planned.

Dose escalation trial

So far one randomized phase II study has been published [58], which looked at dose escalation to the hypoxic volume alone on [^{18}F]FMISO PET. Patients with a hypoxic volume pretreatment were randomised to receive standard chemoRT (70 Gy in 35 fractions) or escalation of up to 10% with 77 Gy to the hypoxic volume only. The trial closed prematurely due to slow accrual (53 patients over 8 years). Thirty-nine patients had hypoxic tumours, of whom 19 received dose-escalation. The authors reported a non-significant improvement of 25% in local control for the dose escalation arm. Furthermore, of the patients treated with dose escalation, only a 2% mean elevation of radiotherapy dose was achieved, rather than the planned 10%. This trial highlights the difficulties of carrying out large-scale prospective imaging trials with [^{18}F]FMISO. One of the reasons given for poor recruitment was scanner and tracer availability. In addition, the modest 2% dose escalation is much smaller than the 10% frequently quoted in planning studies and highlights the need to use real life patient data.

Conclusion

Hypoxia PET imaging has been proposed for many years as a potential method to specifically target hypoxic tumour regions with higher doses of radiotherapy to improve outcomes. The results of our review are mixed, and whilst some findings support the use of [^{18}F]FMISO PET, a number of challenges are identified:

(1) A number of studies have looked at the correlation between [^{18}F]FMISO PET and other hypoxia biomarkers and, overall, the most promising results were found with tumour HIF1α and serum osteopontin. It should be noted that hypoxia is an umbrella term referring to different biological processes on different assays, i.e., intracellular hypoxia versus interstitial and blood hypoxia, or acute versus chronic hypoxia. As such, the different hypoxia biomarkers described should not necessarily be expected to correlate with each other. Currently, there is no clinically deliverable surrogate biomarker to predict for the [^{18}F]FMISO hypoxic phenotype and that would enable patient selection for [^{18}F]FMISO imaging. This is a significant barrier as the routine use of [^{18}F]FMISO PET scans in all locally advanced HNC patients is hampered by cost and availability.

(2) The results of [^{18}F]FMISO PET imaging are not necessarily repeatable and the location of uptake may vary during treatment. This highlights that hypoxia is a dynamic phenomenon and a single snapshot [^{18}F]FMISO PET imageis unlikely to provide all the information required for radiotherapy dose modification. Some of the presented studies suggest that maybe radiotherapy adaptation should be based on residual hypoxia after the initiation of radiotherapy treatment, at weeks 1–2.

(3) Tumour recurrences do not necessarily occur within the pretreatment hypoxic volume on [^{18}F]FMISO PET. Only a few studies have looked at recurrence patterns with regard to the FMISO hypoxic volume pre-treatment. Future work is needed in this area, both with reference to pre-treatment and intra-treatment scans.

(4) Dose modification studies published thus far are pilot studies and, therefore, do not provide sufficient evidence about the *efficacy* of dose painting or modification based on [^{18}F]FMISO PET imaging. The de-escalation studies have not proved that it was the absence of hypoxia on [^{18}F]FMISO PET which made treatment de-escalation safe. Furthermore, the single dose escalation study illustrated the challenges of carrying out [^{18}F]FMISO PET in a large-scale trial and the difficulties for dose escalation in real-world patients.

In summary, [^{18}F]FMISO PET has been extensively investigated in HNC addressing various research questions. Pre-treatment uptake has been shown to relate to prognosis in HNC patients treated with radiotherapy, highlighting the clinical importance and relevance of hypoxia denoted on [^{18}F]FMISO PET. Planning studies have demonstrated that it is technically feasible to escalate the radiotherapy dose to hypoxic regions, without exceeding normal tissue tolerance doses. The positive correlation with other hypoxia biomarkers (in particular oxygen electrodes) validate [^{18}F]FMISO PET as a reliable means of detecting intra tumoral hypoxia.

The findings from this review suggest, however, that there is insufficient evidence to support dose escalation to a hypoxic region using a single pretreatment scan and further work is required to identify if residual hypoxia would be more appropriate to guide radiotherapy dose escalation. Cost and access to [^{18}F]FMISO PET remain an issue, especially for patients on a curative chemoradiotherapy treatment

pathway for whom additional investigations need to be easily accessible and carried out promptly to avoid treatment delay. If dose escalation is to be investigated as a management strategy to overcome radioresistance, then currently [^{18}F]FDG PET or MRI are more accessible and less costly options to delineate the target volume. The studies included in this review were based on HNC but the findings could potentially be extrapolated to other tumour sites as many of the issues mentioned are related to the biology of hypoxia and the different assays used to measure it.

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Declarations

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