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REVIEW ARTICLE

Optimising hypoxia PET imaging and its applications in guiding targeted radiation therapy for non-small cell lung cancer: a scoping review

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Abstract

Introduction: Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death. Definitive treatment includes chemotherapy and radiation therapy. Tumour hypoxia impacts the efficacy of these treatment modalities. Novel positron-emission tomography (PET) imaging has been developed to non-invasively quantify hypoxic tumour subregions, and to guide personalised treatment strategies. This review evaluates the reliability of hypoxia imaging in NSCLC in relation to various tracers, its correlations to treatment-related outcomes, and to assess if this imaging modality can be meaningfully applied into radiation therapy workflows. **Methods:** A literature search was conducted on the Medline (Ovid) and Embase databases. Searches included terms related to 'hypoxia', 'positron-emission tomography', 'magnetic resonance imaging' and 'lung cancer'. Results were filtered to exclude studies prior to 2011, and animal studies were excluded. Only studies referring to a confirmed pathology of NSCLC were included, while disease staging was not a limiting factor. Full-text English language and translated literature examined included clinical trials, clinical cohort studies and feasibility studies. **Results:** Quantification of hypoxic volumes in a pre-treatment setting is of prognostic value, and indicative of treatment response. Dosimetric comparisons have highlighted potential to significantly dose escalate to hypoxic volumes without risk of additional toxicity. However, clinical data to support these strategies are lacking. **Conclusion:** Heterogenous study design and non-standardised imaging parameters have led to a lack of clarity regarding the application of hypoxia PET imaging in NSCLC. PET imaging using nitroimidazole tracers is the most investigated method of non-invasively measuring tumour hypoxia and has potential to guide hypoxia-targeted radiation therapy. Further clinical research is required to elucidate the benefits versus risks of dose-escalation strategies.

Introduction

The hypoxic problem in NSCLC

Hypoxia is a common feature of solid malignancies and has posed a therapeutic challenge since Gray et al.[1] first discussed its association with radioresistance in 1953. Defined as a 'state of low oxygen tension', hypoxia can be classified as either acute (limited perfusion of oxygen) or chronic (limited diffusion of oxygen).[2]

Cell death by ionising radiation is predominately caused by an indirect pathway, whereby secondary electrons resulting from primary x-ray interactions, react with cellular oxygen to create oxidative radicals, which subsequently induce DNA damage, culminating in cell death.[2] Hypoxic conditions hinder this pathway, resulting in reduced DNA damage and an increased radioresistant effect. Escalated radiation therapy doses are required to achieve tumour control. Increased tumour hypoxia is associated with more aggressive malignancy, poorer

locoregional control, and negatively impacts overall survival (OS).[2,3] This is due to the stimulation of complex signalling pathways, which interact to drive angiogenesis, alterations in tumour metabolism, and metastasis.[3] As an example, hypoxia stimulates expression of hypoxia-inducible factor-1α (HIF-1α), a gene that promotes multiple cascades facilitating tumour growth, invasion and metastasis.[2,4]

Lung cancer accounts for 25% of all cancer deaths worldwide, with non-small-cell lung cancer (NSCLC) accounting for approximately 90% of all lung cancers, and the presence of tumour hypoxia is associated with poorer treatment response.[5–7] Smoking is a significant aetiological risk factor in NSCLC and is a known stimulant of HIF-1α.[8] Five-year survival probability decreases with increasing pathological stage, and treatment options become limited to only chemotherapy and/or radiation therapy.[5,9] Targeting tumour hypoxia is an attractive option but direct measurement with needle electrodes and oxygen sensors, although accurate, is invasive and limited to sites accessible for surgical insertion.[10] The complications and challenges associated with the application of these methods in NSCLC indicate a need for non-invasive hypoxia measurement strategies, such as imaging, to subsequently personalise treatment strategies.

Imaging hypoxia in NSCLC with PET

Conventional ^{18}F-fluoro-2-deoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) imaging is already a mainstay in the diagnostic work-up, and staging of lung cancer, and is a well-established prognostic marker.[11,12] Various radionuclide hypoxia PET tracers are now being evaluated to determine if a reliable, repeatable and clinically meaningful non-invasive method of quantifying hypoxic tumour subregions is feasible using PET/CT for NSCLC, and how this information can be used to improve often unfavourable outcomes for this patient population.

^{18}F-fluoromisonidale (^{18}F-FMISO) is perhaps the most widely researched radionuclide tracer used to quantify hypoxia across various tumour sites and is often referred to as the 'gold standard' in hypoxia imaging.[13,14] Current research focuses on establishing if ^{18}F-FMISO, and other novel tracers can be considered reliable imaging biomarkers to predict treatment outcomes, specifically by correlating imaging and hypoxia-related factors in NSCLC.

Individual imaging parameters impact how PET imaging measures and quantifies hypoxia, and therefore must be well-defined and standardised. The parameters, and threshold values which define tumour hypoxia vary for different tracers, their pharmacokinetics, and indeed the conditions of image acquisition.[15] The activity of radiotracer administered, timing from tracer administration to image acquisition, scanning duration and other factors will impact PET image quality and how images can be interpreted.[16,17]

Aim

The primary aim of this review is to evaluate if PET can be used to reliably image hypoxia in NSCLC. This will be achieved by examining the evidence on how PET imaging correlates with treatment outcomes, as well as the correlation between PET imaging and immunohistochemistry (IHC) analysis. The factors influencing radionuclide tracer uptake and its impact on hypoxia imaging in NSCLC will be discussed.

The secondary aim of this review is to discuss if imaging hypoxia can influence treatment delivery in NSCLC, and positively impact on progression-free survival (PFS) and OS, while also sparing organs at risk (OARs).

Methodology

Medline (Ovid) and Embase databases were searched using terms related to 'hypoxia', 'positron-emission tomography', 'magnetic resonance imaging', and 'lung cancer'. Terms were adapted according to the relevant databases, and the full search strategy is detailed in Tables 1 and 2. One additional paper was identified after handsearching the reference lists of papers found from the database search.

Results were transferred into an Endnote 20 library where duplicate material was excluded. Literature pre-dating 2011 was omitted to ensure that all material was relevant to current practice and research. The final search was conducted on January 9th 2023. Title and abstract screening omitted material unrelated to NSCLC, non-imaging studies, and animal studies. Studies with fewer than 10 NSCLC patients were excluded to increase statistical power. The PRISMA flowchart in Figure 1 outlines the search strategy.

Full-text English language and translated clinical trials, clinical cohort studies and feasibility studies were all included for analysis. Meta-analysis, review articles, book chapters, case studies and conference abstracts were excluded. Prospective and retrospective analyses were included. Studies had to include patients with pathologically confirmed primary NSCLC of any stage who had PET/CT imaging with any of ^{18}F-FDG, ^{18}F-FMISO, ^{18}F-fluoroazomycin arabinoside (^{18}F-FAZA), ^{18}F-flortanidazole (^{18}F-HX4), ^{18}F-fluoroerythronitroimidazole

Table 1. Embase search.

Search no.	EMBASE search terms
#1	'positron emission tomography'/exp
#2	((pet OR 'positron emission tomographic') NEAR/2 (scan* OR imag*)):ti,ab
#3	('positron emission tomography' OR PET OR 'positron tomography' OR 'positron-emission tomography'):ti,ab
#4	'nuclear magnetic resonance imaging'/exp
#5	(mri OR 'nmr imaging' OR 'magnetic resonance imag*' OR 'magnetic resonance tomography' OR 'magnetization transfer imaging' OR 'mr imaging' OR 'nuclear magnetic resonance imag*'):ti,ab
#6	#1 OR #2 OR #3 OR #4 OR #5
#7	'hypoxia'/exp/mj
#8	(hypoxia OR 'diffusion anoxia' OR hypoxic):ti,ab
#9	(oxygen NEAR/2 deficienc*):ti,ab
#10	#7 OR #8 OR #9
#11	'lung cancer'/OR 'respiratory tract tumor'/exp OR 'lung tumor'/exp
#12	((bronchus OR lung OR pleura OR 'respiratory tract' OR trachea) NEAR/3 (cancer OR tumour* OR tumor* OR carcimoma* OR metast*)):ti,ab
#13	#11 OR #12
#14	#6 AND #10 AND #13

Table 2. Medline (Ovid) search.

Search no.	MEDLINE (OVID) search terms
#1	exp Positron-Emission Tomography/
#2	((pet OR positron emission tomographic) adj2 (scan* OR imag*)).ti,ab.
#3	(positron emission tomography OR PET OR positron tomography OR positron-emission tomography).ti,ab.
#4	exp Magnetic Resonance Imaging/
#5	(mri OR nmr imaging OR magnetic resonance imag* OR magnetic resonance tomography OR magnetization transfer imaging OR mr imaging OR nuclear magnetic resonance imag*).ti,ab.
#6	#1 OR #2 OR #3 OR #4 OR #5
#7	exp Hypoxia/
#8	(hypoxia OR diffusion anoxia OR hypoxic).ti,ab.
#9	(oxygen adj2 deficienc*).ti,ab.
#10	#7 OR #8 OR #9
#11	exp Respiratory Tract Neoplasms/
#12	((Bronchus OR epiglottis OR larynx OR lung OR nose OR pleura OR respiratory tract OR trachea) adj3 (cancer OR tumour* OR tumour* OR carcinoma* OR metast*)).ti,ab.
#13	#11 OR #12
#14	#6 AND #10 AND #13

(^{18}F-FETNIM), ^{62}copper-diacetyl-bis(N4-methylthiosemicarbazone) (^{62}Cu-ATSM), ^{62}copper-pyruvaldehyde-bis(N4-methylthiosemicarbazone) (^{62}Cu-PTSM), ^{64}copper-diacetyl-bis(N4-methylthiosemicarbazone) (^{64}Cu-ATSM) and ^{18}F-fluoromethyl-choline (^{18}F-D4-FCH) tracers. Not all studies included treatment interventions, but studies referring to surgery, chemotherapy and radiation therapy were included where applicable.

A MINORS quality assessment of the articles in this review is available in Table 3. The scoring scale has been adapted where applicable, as many imaging studies do not include a follow-up period, and the global ideal score is 12 instead of 16 for non-comparative studies, and 20 rather than 24 for comparative studies where follow-up is not included.

Results and Discussion

Impact of timing and acquisition parameters on imaging hypoxia in NSCLC

Acquisition parameters can impact image quality and interpretation of ^{18}F-FDG PET.[17] If a similar theory is applied for novel hypoxia PET tracers, the discord between various studies in terms of choice of radionuclide tracer, scan acquisition protocols and quantification methods becomes a valid point of critique.

Despite extensive research into ^{18}F-FMISO PET, questions remain regarding its application in NSCLC. Sachpekidis et al.[39] concluded that ^{18}F-FMISO PET/CT did not alter the management of their patient group based on the low level of tracer uptake in most lesions. The administered activity was to a maximum of 250 MBq, which is relatively low compared to other studies using this tracer, and image acquisition commenced immediately post-injection (p.i.).[39] Longer acquisition times, and a period of 2–4 h p.i. is preferred to ensure optimum image contrast when using nitroimidazole compound hypoxia-directed tracers.[45,49]

Conversely, Grkovski et al.[34] reported that ^{18}F-FMISO PET/CT had the capacity to clearly delineate subregions of interest within ^{18}F-FDG avid lesions, with serial imaging reliably providing reproducible results, highlighting potential benefits in guiding dose-escalated radiation therapy. Higher tracer activity was administered, and time p.i. was markedly increased compared to Sachpekidis et al.[34,39] (Table 4).

The importance of timing of image acquisition as a prognostic indicator is apparent in studies by Vera et al.[46,47] and Li et al.,[25] who correlated hypoxia as measured on pre-treatment ^{18}F-FMISO PET/CT with poorer survival. Vera et al.[46] found that despite increased radiation therapy dose, disease-free survival (DFS) and OS was significantly reduced for patients with ^{18}F-FMISO avid volumes on pre-treatment imaging. Fractional hypoxic volume as quantified on pre-treatment ^{18}F-FMISO PET/CT was found to be the only prognostic factor significantly correlating with PFS by Li et al.[25]

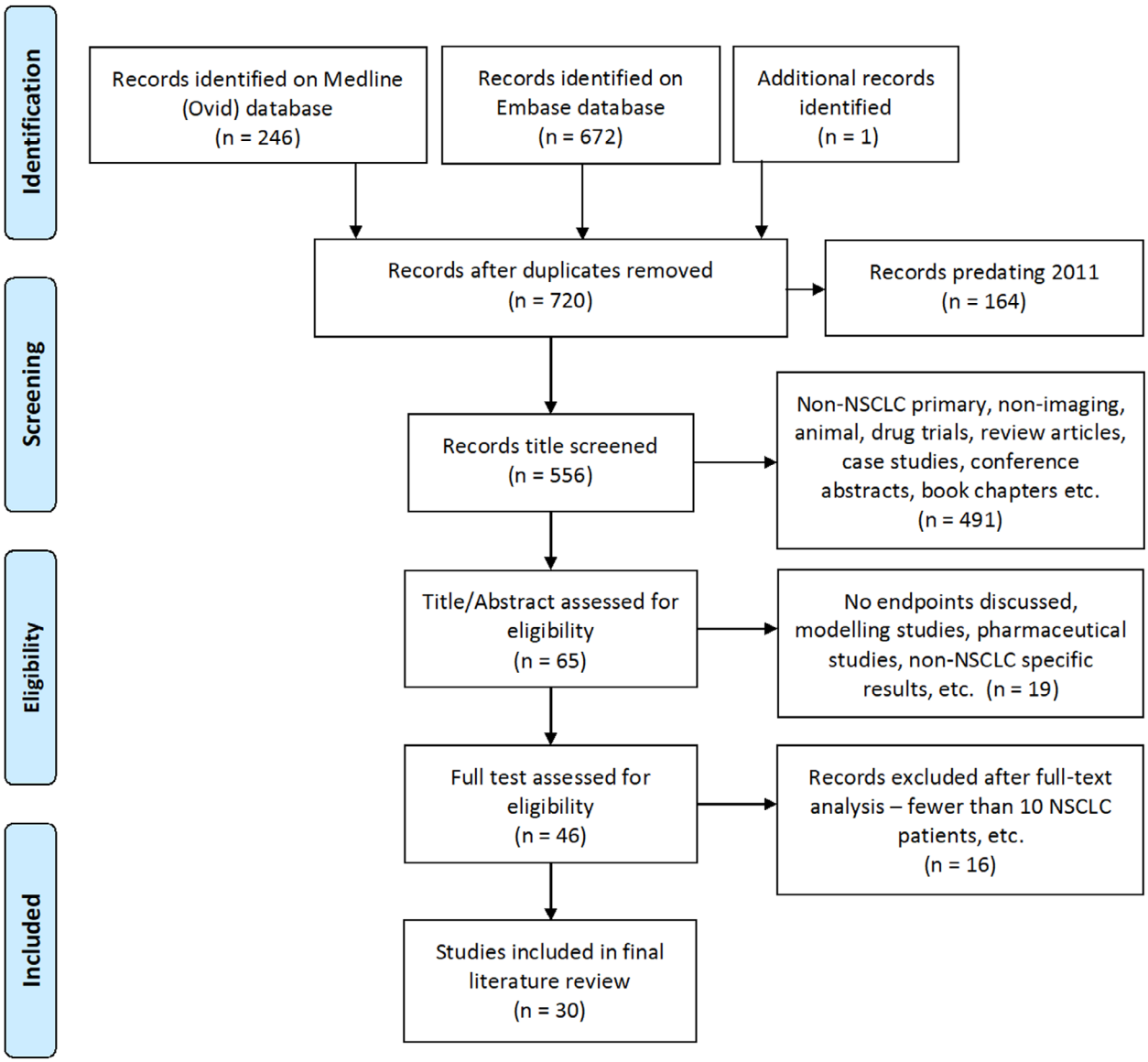

Figure 1. PRISMA flowchart.[63]

Heterogeneity of disease staging, and the range of treatment regimens used in a small patient cohort (six different chemotherapy regimens were used and radiotherapy prescription ranged between 50.4 and 66 Gy) could be considered limitations of this study, but is more likely indicative of common heterogeneity in clinical practice.[25]

Wei et al.[48] observed that increased standardised uptake value (SUV) and tumour-to-blood activity ratio (TBR) on ^{18}F-FMISO PET/CT correlated with increasing lesion size and higher disease stage. ^{18}F-FMISO and ^{18}F-FETNIM uptake was compared, and ^{18}F-FMISO was found to be a stronger indicator of tumour hypoxia, with higher values observed for both SUV and TBR in NSCLC lesions, while uptake in most normal tissues was comparable.[48]

Pharmacokinetic analysis of pre-treatment dynamic ^{18}F-FMISO PET by Schwartz et al.[41] examined correlations between parameters such as trapping rate, TBR, and tracer delivery in NSCLC and concluded that dynamic ^{18}F-FMISO PET was feasible in NSCLC. However, there was not always a correlation between TBR and trapping rate, and TBR as an independent hypoxia surrogate resulted in underestimation of tumour hypoxia, despite lesions being ^{18}F-FMISO avid.[41] This highlights the use of multiparametric assessment to definitively

Table 3. Minors quality assessment tool.[18]

Author	Ref.	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Total
Study scored as per MINORS scale – global ideal score of 16														
Castello (2018)	19	2	2	1	2	2	1	2	1					13/16
Furukawa (2015)	20	2	0	1	2	0	2	2	0					9/16
Hu (2013)	21	2	0	2	2	0	1	1	0					8/16
Kaira (2014)	22	2	2	1	2	2	2	0	2					13/16
Kaira (2018)	23	2	2	1	2	0	2	0	2					11/16
Kinoshita (2016)	24	2	0	1	2	0	1	0	1					7/16
Li (2018)	25	2	0	2	2	0	1	2	1					10/16
Trinkaus (2013)	26	2	1	2	2	1	2	1	0					11/16
Zhang (2013)	27	2	0	2	2	0	1	2	0					9/16
Study did not include follow-up period—global ideal score is adjusted to 12 instead of 16														
Besson (2020)	28	2	0	2	2	0	n/a	n/a	0					6/12
Bollineni (2013)	29	2	0	2	2	2	n/a	n/a	0					8/12
Di Perri (2017)	30	2	0	1	2	0	n/a	n/a	0					5/12
Dubash (2020)	31	2	0	2	1	0	n/a	n/a	0					5/12
Even (2017)	32	2	0	1	2	0	n/a	n/a	0					5/12
Even (2015)	33	2	0	0	2	0	n/a	n/a	0					4/12
Grkovski (2016)	34	2	0	0	2	0	n/a	n/a	2					6/12
Iqbal (2016)	35	2	0	0	2	0	n/a	n/a	0					4/12
Li (2018)	36	2	0	0	2	0	n/a	n/a	0					4/12
Lindblom (2017)	37	2	0	1	2	0	n/a	n/a	0					5/12
Mandeville (2012)	38	2	2	2	2	0	n/a	n/a	0					8/12
Sachpekidis (2015)	39	2	0	1	1	0	n/a	n/a	0					4/12
Salem (1988)	40	2	0	2	2	0	n/a	n/a	1					7/12
Schwartz (2017)	41	2	0	2	2	0	n/a	n/a	0					6/12
Thureau (2021)	42	2	0	2	1	2	n/a	n/a	0					7/12
Van Elmpt (2016)	43	2	0	0	2	0	n/a	n/a	0					4/12
Zegers (2014)	44	2	0	1	2	0	n/a	n/a	0					5/12
Zegers (2013)	45	2	0	1	2	0	n/a	n/a	0					5/12
Comparative Study – ideal global score of 24														
Vera (2019)	46	2	0	2	2	0	2	2	1	2	2	1	1	17/24
Vera (2017)	47	2	0	2	2	1	1	2	2	2	2	1	1	18/24
Comparative study, no follow-up period – global ideal score is adjusted to 20 instead of 24														
Wei (2016)	48	2	0	2	2	2	n/a	n/a	0	1	2	2	1	14/20

identify hypoxic sub-volumes on PET imaging, especially where there is variation in acquisition protocols, and multiple parameters to quantify hypoxia (Table 4).

^{18}F-FAZA and ^{18}F-HX4 are other nitroimidazole compounds cited as providing superior image contrast when compared to ^{18}F-FMISO, due to faster clearance from normoxic tissues.[50,51] Bollineni et al.[29] and Di Perri et al.[30] compared the uptake patterns of ^{18}F-FAZA to ^{18}F-FDG in NSCLC, but concluded differently regarding the capacity of ^{18}F-FAZA to provide information on tumour hypoxia, and guide dose painting. Bollineni et al.[29] reported that the obvious heterogeneity that was observed between the uptake of the two tracers suggested that they represented different biological processes and that ^{18}F-FAZA could identify hypoxic volumes targetable by dose painting.

Conversely, Di Perri et al.[30] stated that the similarities observed between the uptake patterns of both tracers negated any value of ^{18}F-FAZA, despite PET acquisition parameters being relatively similar to Bollineni et al.[29] However, the thresholds defining metabolic tumour volume (MTV) on ^{18}F-FDG PET/CT differed between the two studies, Bollineni et al.[29] specified a threshold of SUV > 34% of ^{18}F-FDG maximum SUV (SUV_{max}) to define MTV, while Di Perri et al.[30] defined MTV as SUV greater than 50% of SUV_{max}. A greater threshold value may result in a smaller MTV being delineated, which would subsequently have greater correlation with more limited ^{18}F-FAZA uptake patterns. Different parameters were also used to quantify hypoxia. Bollineni et al.[29] defined the hypoxic volume as having a tumour-to-background activity ratio ≥1.2 to 1.4, but Di Perri et al.[30] specified that voxels with a SUV > 1.4 within the gross tumour volume as delineated on CT to define the hypoxic volume.

Trinkaus et al.[26] evaluated pre- and post-treatment tumour hypoxia on ^{18}F-FAZA PET/CT in patients

Table 4. Comparison of scan acquisition parameters for ^{18}F-FMISO PET/CT imaging studies.

Author (year)	No. of patients	Tracer activity administered	Time p.i. to acquisition	Duration of acquisition	Parameters quantifying hypoxia	Ref.
Grkovski (2016)	10	388 ± 15 MBq	163 ± 13 min	10 min, (static)	TBR ≥1.2	34
Li (2018)	29	4.81 MBq/kg	120 min	0.8 sec per rotation	TBR_{mean} > 1.2 Fractional hypoxic volume = percentage of pixels in region of interest with TBR > 1.2	25
Sachpekidis (2015)	13	250 MBq	0 min	60 min, (dynamic) 2 min per bed position, (static)	$SUV_{average}$ = 3.4, and SUV_{max} used to define a singular "^{18}F-FMISO avid" lesion. No threshold value defined.	39
Schwartz (2017)	16	346 ± 33 MBq	Scan 1: 0 min Scan 2: 95 ± 12 min Scan 3: 168 ± 15min	Scan 1: 45 min, (dynamic) Scan 2: 10 min, (static) Scan 3: 10 min, (static)	TBR > 1.2 Fractional hypoxic volume = volume of voxels with TBR > 1.2	41
Thureau (2021)	19	4 MBq/kg	180 ± 10 min	12 min	SUV_{max} > 1.4	42
Vera (2017, 2019)	54	4.5 MBq/kg	240 ± 20 min	Not specified	SUV ≥ 1.4	46,47
Wei (2016)	24	4.81 MBq/kg	120 min	0.8 sec per rotation	No threshold value defined	48

Note: Gray shade represents, questions 9–12 on the MINORs scale were not relevant to the included studies and therefore not scored.

receiving chemoradiation for NSCLC and assessed response to treatment as correlated to pre-treatment hypoxia levels. They found that pre-treatment ^{18}F-FAZA identified smaller subregions of tracer uptake when compared to ^{18}F-FDG uptake, clearly demarcating areas of tumour hypoxia. However, on the basis that intratumoural hypoxia had resolved for most patients on post-treatment ^{18}F-FAZA PET/CT, they suggested that hypoxia may be a less significant factor impacting treatment outcomes in NSCLC than for other malignancies. This statement is poorly supported when only 8 of the 11 patients with baseline hypoxia had follow-up ^{18}F-FAZA PET/CT, of which just one did not experience disease progression.[26] These factors imply that pre-treatment hypoxia is a significant risk factor for disease progression. The problem posed by hypoxia in NSCLC has been widely reported.[52,53]

The activity of ^{18}F-FAZA administered and quantification parameters used varied between the studies of Bollineni, Di Perri, and Trinkaus.[26,29,30] Tracer dose was either administered per kilogram of body mass, or as absolute dose regardless of patients' individual weight. Time p.i. to acquisition was relatively consistent, but scanning duration was not always specified (Table 5). Patient size, injected tracer dose and acquisition time are recognised parameters which impact ^{18}F-FDG PET imaging quality, so should be assumed for more novel tracers, unless otherwise proven.[17]

Zegers et al.[44,45] have investigated the optimal imaging parameters for ^{18}F-HX4 as a hypoxia tracer. Image acquisition at 2 h p.i. was found to be feasible, and scan duration could be reduced from 30 to 10 min without impacting TBR or hypoxic fraction, but acquisition was required to extend to 4 h p.i. for optimal image contrast, particularly with reduced acquisition duration.[45] They also found that SUV_{max} reduced between 2 h p.i. and 4 h p.i., but that maximum TBR increased significantly due to greater clearance of ^{18}F-HX4 from the blood, and reported that activity ratios such as TBR were more reliable hypoxia parameters than SUV_{max}.[45] This finding may explain Trinkaus et al.[26] and Di Perri et al.[30] conflicting reports regarding ^{18}F-FAZA PET/CT.

Hypoxic subregions as visualised on ^{18}F-HX4 PET/CT were located wholly or partially within metabolic tumour volumes quantified on ^{18}F-FDG PET/CT, suggesting that ^{18}F-HX4 PET imaging could guide hypoxia-directed therapy.[44] Lindblom et al.[37] observed comparable uptake between ^{18}F-HX4 and ^{18}F-FMISO, strengthening the potential of ^{18}F-HX4 in guiding hypoxia-targeted therapy. Imaging protocol across these trials was relatively consistent (Table 6). Improved image contrast is the only apparent benefit of ^{18}F-HX4, as the time required for high-quality imaging is not reduced relative to ^{18}F-FMISO.[49]

Another considerable factor when using intravenous tracers is limited perfusion. Perfusion is a contributing factor in the development of tumour hypoxia, whereby

Table 5. Comparison of scan acquisition parameters for ^{18}F-FAZA PET/CT imaging studies.

Author (year)	No. of patients	Tracer activity administered	Time p.i. to acquisition	Duration of acquisition	Parameters quantifying hypoxia	Ref.
Bollineni (2013)	11	370 MBq total dose	2 h	Not specified	Tumour-to-background activity ratio ≥ 1.2 or ≥ 1.4	29
Di Perri (2017)	14	Median 386 MBq	Median 127 min	30 min, (static)	SUV > 1.4	30
Iqbal (2015)	10	168 ± 39 MBq	Not specified – suggested immediately p.i.	70 min, (dynamic)	Volume of distribution- voxel clustering. No threshold value for hypoxia specified	35
Kinoshita (2016)	45/47	370 MBq total dose	2 h	4 min per bed position, (dynamic)	Tumour-to-muscle activity ratio ≥1.1	24
Thureau (2021)	19	4 MBq/kg	180 ± 10 min	12 min	SUV_{max} > 1.4	42
Trinkaus (2013)	17	185 MBq total dose	2 and 4 h	30 min per scan, (static)	TBR > 1.4	26

Table 6. Comparison of scan acquisition parameters for ^{18}F-HX4 PET/CT imaging studies.

Author (year)	No. of patients	Tracer activity administered	Time p.i. to acquisition	Duration of acquisition	Parameters quantifying hypoxia	Ref.
Even (2017)	34	417 ± 77 MBq	4 h	Not specified	Tumour-to-background activity ratio > 1.2	32
Lindblom (2017)	10	Not specified	4 h	Not specified	TBR > 1.4 Tumour-to-muscle activity ratio > 1.4	37
Van Elmpt (2015)	14	Approx. 440 MBq	4 h	Not specified	TBR >1.2 and >1.4	43
Zegers (2013)	15	423 ± 72 MBq	2 and 4 h	30 min per scan, (static)	TBR > 1.4 Hypoxic fraction was assessed in range TBR >1.1 – TBR >1.6	45
Zegers (2014)	25	429 ± 57 MBq	4 h	20–30 min, (static)	TBR >1.4	44

reduced blood flow leads to reduced oxygenation of tissue.[54] Iqbal et al.[35] hypothesised that it was reasonable to expect that decreased perfusion might limit the efficacy of tracer uptake to such regions, resulting in poor visualisation and underestimation of tumour hypoxia. They found that perfusion and ^{18}F-FAZA uptake did not have a definitively inverse relationship as might be expected and that low tracer uptake may not be a reliable indicator that hypoxia was absent.[35] However, time p.i. was poorly defined, and tracer administered was relatively low (Table 5).

Zhang et al.[27] compared both perfusion and hypoxia based on PET imaging using radioisotopes ^{62}Cu-PTSM and ^{62}Cu-ATSM respectively. They found that uptake of both tracers was increased around the periphery of tumours. As hypoxic volumes are often located more centrally within lesions, away from peripheral tumour vasculature, uptake of ^{62}Cu-ATSM was expected to increase more centrally in tumours.[27] The similarity between uptake of both tracers was contrary to expectations but may support Iqbal et al.[35] and be indicative of restricted tracer uptake in lowly perfused, more acutely hypoxic, or necrotic tissue.

Van Elmpt et al.[43] found a spatial mismatch as might be expected between hypoxic tumour subregions visualised on ^{18}F-HX4 PET/CT, and highly perfused areas as visualised on dynamic contract-enhanced CT (DCE-CT). Similarly, Mandeville et al.[38] found blood flow quantified on DCE-CT, negatively correlated with hypoxic tumour regions as defined by IHC analysis of resected pimonidazole-stained tumour specimens. Iqbal[35] and Zhang[27] assessed perfusion using PET imaging, and the questions raised may have been more conclusively answered if comparative analysis of tumour specimens was possible.

PET imaging of hypoxia and immunohistochemistry analysis

Expression of hypoxia inducible factor-1α (HIF1α) is stimulated under hypoxic conditions, activating genes

that are involved in multiple processes within the tumour microenvironment—glycolysis, cell proliferation, lymphovascular invasion and angiogenesis—all of which are associated with more aggressive malignancy.[3] Recent studies examining the potential correlations between spatial uptake of different PET imaging tracers, and expression of these molecular markers have yielded mixed results.

IHC analysis by Furukawa et al.,[20] and Kaira et al.[22] demonstrated that there was a positive correlation between SUV_{max} on ^{18}F-FDG PET/CT and expression of HIF1α and glucose transporter 1 (GLUT1). Kaira et al.[22] found that increased expression of GLUT1 was an independent negative prognostic factor in OS where adenocarcinoma was the confirmed tumour histology, while expression of GLUT1, disease stage, and tracer uptake were all significantly correlated with poorer PFS. Furukawa et al.[20] also found that SUV_{max} was significantly linked to malignancy-related clinicopathological features and increased expression of HIF1α and GLUT1, which significantly correlated with poorer survival in adenocarcinoma. Kaira et al.[23] further examined ^{18}F-FDG uptake and expression of programmed death-ligand 1, which is linked to HIF1α and GLUT1 expression, in adenocarcinoma NSCLC and reported SUV_{max} as an unfavourable prognostic factor in DFS and OS. Similarly, Castello et al.[19] found that increased disease stage, SUV_{max}, and expression of cytoplasmic HIF1α all significantly correlated with poorer DFS, but not in association to specific tumour histology.

Analysis of hypoxia-specific ^{18}F-FETNIM PET/CT by Hu et al.[21] identified a correlation between tumour-to-mediastinum activity (T/Me) ratio and expression of HIF1α and GLUT1, and that increased T/Me ratio was associated with poorer OS in NSCLC. The correlation between T/Me ratio and HIF1α is promising in terms of validating ^{18}F-FETNIM as a hypoxia imaging biomarker, although data related to this tracer in NSCLC are limited. Thureau et al.[42] found no correlation between expression of either HIF1α or GLUT1, and the uptake of the two more investigated hypoxia tracers ^{18}F-FMISO and ^{18}F-FAZA. However, they still asserted that both tracers were 'reliable hypoxia detectors', which appears to be based solely on the strong correlation between both the SUV_{max} and uptake volumes of both tracers but is otherwise poorly rationalised.[42]

Kinoshita et al.[24] compared analysis of resected NSCLC tumour specimens to pre-operative ^{18}F-FAZA and ^{62}Cu-ATSM PET/CT and found that increased uptake of both tracers correlated significantly with poorer OS and PFS, as did tumour-to-muscle activity ratio of ^{18}F-FAZA. IHC analysis for molecular markers HIF1α and GLUT1 was not performed, but instead clinicopathological features such as tumour size, lymphovascular and pleural invasion, and tumour histology, were evaluated relative to tracer uptake. Correlations between tracer uptake and unfavourable features such as larger tumour diameter, increased pathological stage, vascular and pleural invasion were confirmed.[24] Like Furukawa et al.[20] and Castello et al.[19] this methodology provides definitive links between tracer uptake and unfavourable clinicopathological features, rather than expression of molecular markers.

The conflicted findings relating the uptake of hypoxia-specific tracers to IHC analysis, and the apparent superior correlation of conventional ^{18}F-FDG PET imaging with expression of key molecular markers, suggests that ^{18}F-FDG PET has greater potential to quantify tumour hypoxia. However, direct correlation between glucose metabolism and hypoxia has been demonstrated to be weak despite the correlations found between expression of GLUT1 and HIF1α.[29,39] Establishing sound correlations between tracer uptake, and molecular markers or unfavourable tumour features is important to validate the accuracy of these novel tracers when quantifying tumour hypoxia and other clinicopathological features in NSCLC, and planning hypoxia-targeted radiation therapy.

The influence of hypoxia imaging in radiation therapy for NSCLC

Using hypoxia PET imaging to guide targeted dose-escalated radiation therapy is a widely discussed topic. Long-term follow-up of RTOG 0617 confirmed that dose escalation from 60 to 74 Gy in NSCLC showed poorer OS, and more treatment-related deaths for patients treated with higher dose.[55] Radiation therapy was delivered using a 3D-conformal radiotherapy (3DCRT) technique, and increased cardiac dose was suggested to contribute to poorer outcomes for patients in the high dose arm.[55,56]

Modulated treatment techniques now provide superior dose conformity to target volumes, improved OAR sparing, and increased opportunities to dose escalate. Simultaneous integrated boost (SIB) has the capacity to deliver escalated doses to more radioresistant hypoxic tumour subregions, while maintaining a safe dose level to OARs.

Even et al.[33] found that radiation therapy prescription of up to a maximum of 129.6 Gy could be delivered using SIB in 24 fractions when boosting to intratumoral hypoxic regions as visualised on ^{18}F-HX4 PET/CT, before OAR dose constraints were compromised. This is an isotoxic planning study only and demonstrates what can potentially be achieved in terms of dose escalation to tumour subregions, but needs validation in clinical trials. Concomitant chemotherapy has been found feasible with moderately hypofractionated radiation therapy prescriptions

of up to 3 Gy per fraction in NSCLC, but with a significantly increased risk of acute oesophagitis.[57] While this group did take into consideration the increase of dose per fraction and corrected OAR dose constraints to EQD2, such significantly escalated radiation therapy prescription of up to 5.4 Gy per fraction is likely to result in significant further toxicity, impacting the feasibility of concomitant chemotherapy, which is recommended as part of definitive treatment for locally advanced NSCLC.[33,58]

Dosimetric analysis by Li et al.[36] concluded similarly that dose escalation using SIB was feasible without exceeding OAR constraints, albeit to a more conservative dose of 86 Gy. A non-randomised phase II clinical trial confirmed that boosting radiation therapy dose up to 86 Gy to hypoxic subregions as quantified on ^{18}F-FMISO PET/CT was feasible without significant additional early or late toxicity.[46,47] However, no statistically significant benefit was observed in OS after 3 years for patients who received escalated dose, as OS and PFS were significantly poorer for patients with higher ^{18}F-FMISO uptake.[46,47] Treatment was delivered using 3DCRT in 43 fractions over 8.5 weeks and extended overall treatment time is unfavourable considering clonogenic repopulation and repair.[59] Improved survival outcomes may be possible using SIB over 5 weeks as outlined by Li et al.[36]

Efficacy of hypoxia-targeted radiation therapy planned using pre-treatment imaging alone will be impacted where hypoxia is transient. Longitudinal imaging will be required to identify transient hypoxia, and to guide adaptions required during treatment. Based on reproducible results observed on serial pre-treatment imaging, Grkovski et al.[34] reported that longitudinal ^{18}F-FMISO PET/CT imaging has the capacity to quantify intratumoral changes and guide radiation therapy adaptions during treatment. The potential of DCE-CT in providing indirect measurement of tumour hypoxia has already been demonstrated and is more clinically accessible.[38,43]

Future direction in hypoxia imaging in NSCLC

In a move away from PET imaging, Salem et al.[40] reported that serial pre-treatment oxygen-enhanced MRI (OE-MRI) demonstrated repeatable results in hypoxia mapping across primary, nodal and metastatic NSCLC lesions, and that OE-MRI outperformed ^{18}F-FMISO PET in terms of consistency when classifying both hypoxic and normoxic tumours, referencing Grkovski et al.[34] ^{18}F-FMISO study.

Imaging at approximately 2 weeks into radiation therapy indicated consistently decreased hypoxic volumes, suggesting that OE-MRI could guide adaptive radiotherapy strategies, timely as MR-Linacs become more available clinically. OE-MRI has the advantage of being more feasible in clinical departments than PET imaging and with an acquisition time of 45 min, already demonstrates a less resource intensive methodology.[40] Used in parallel with ^{18}F-FMISO PET/CT, multiparametric MRI hypoxia parameters have been reliably correlated with hypoxia as measured on ^{18}F-FMISO PET/CT in oropharyngeal cancer.[60]

Dubash et al.[31] recently investigated the uptake patterns of choline PET tracer ^{18}F-D4-FCH in NSCLC. Despite findings that ^{18}F-D4-FCH displayed heterogenous intratumoral uptake, this feasibility study was based on a novel hypothesis regarding choline metabolism by hypoxia transcriptional factors, and there is no conclusive evidence that the observed uptake represented hypoxic regions. Given the evidence presented regarding the accessibility and reliability of MRI hypoxia imaging, further research efforts should focus in this area, or on optimising the use of already established PET tracers, and other imaging modalities.

Even et al.[32] found that DCE-CT used with conventional ^{18}F-FDG PET/CT, provided a reliable indirect measurement of tumour hypoxia in selected NSCLC tumours, which was confirmed through comparison with hypoxia measured on ^{18}F-HX4 PET/CT. This method correctly differentiated between all hypoxic and normoxic lesions but was more accurate for smaller volumes and did underestimate larger hypoxic volumes.[32] The inclusion of DCE-CT was necessary for the correct classification of all lesions compared to ^{18}F-FDG PET/CT alone, demonstrating the value of multiparametric imaging assessment. Besson et al.[28] also demonstrated the potential of multiparametric imaging in NSCLC. An inverse correlation between increased tumour metabolism and perfusion, as quantified on simultaneously acquired ^{18}F-FDG PET and DCE-MRI, was reported in over half the tumours imaged, which was assumed to be indicative of hypoxia.[28]

Even et al.[33] found that dose-boosting to ^{18}F-FDG avid areas also escalated dose to hypoxic areas as there was a large overlap between the metabolically active tumour regions and smaller hypoxic subregions visualised on ^{18}F-HX4 PET/CT, albeit to a lesser extent than if boosting to the hypoxic volumes alone. This strategy used with the addition of DCE-CT or DCE-MRI may enable specific localisation of hypoxic tumour subregions, enabling optimised dose escalation.

Future research into single and/or combined imaging modalities will be challenging. The inclusion of adaptive radiation therapy strategies over the course of fractionated treatment schedules is complex. Not exclusively because of the limited availability of the

imaging modalities discussed here, but also in terms of the time and resources needed to successfully utilise adaptive radiation therapy.

The introduction of MR linear accelerators into radiation therapy departments may further the development and implementation of adaptive planning in the treatment of NSCLC. However, consideration must also be given to studies such as RTOG 0617[55] and the CHART trial,[61] which both found extended overall treatment time to negatively impact tumour control, and overall survival outcomes. Dose escalation would have to be implemented in a way cognisant of this limitation in extending fractionation to increase overall dose, and also with caution in limiting exposure to normal tissues with increased dose per fraction.

The improved overall survival as demonstrated by the CHART trial[61] indicates that accelerated treatment may be worth further investigation and that adaption in radiotherapy services may need to be expanded for specific patient cohorts to provide uninterrupted treatment if the benefits are significantly improved.

Conclusion

The inclusion of hypoxia imaging in radiation therapy treatment planning has the potential to stratify patients for personalised treatment, and to be a prognostic indicator. However, the limitations in terms of the resources required to implement hypoxia PET imaging into standard clinical practice—availability of novel tracers, scanners that facilitate patients being immobilised in treatment positions, and simply the time required—present challenges. Clinical trials are needed to provide definitive answers regarding the implementation of targeted dose escalation, and whether dosimetric studies can actually safely translate into clinical practice to improve outcomes for patients.

Pre-treatment imaging has been shown as a sufficient predictor of treatment response. However, considering the physiological changes that commonly occur in both lung lesions and healthy tissue during treatment, and the potential transiency of hypoxia, pre-treatment imaging alone will not be sufficient to fully exploit hypoxia-targeted treatment strategies. Radiation therapy treatment adaptions in lung cancer have been found to be most apparently required at approximately week 3–4 of treatment, and perhaps proactive intra-treatment hypoxia imaging protocol for NSCLC should take this timepoint into consideration if using longitudinal imaging.[62]

One of the major limitations of this literature review is the variations in imaging methodologies observed across the studies discussed and demonstrates a need for increased cohesion of robust imaging protocols to allow more accurate comparison of study results and facilitate the development of standardised hypoxia imaging guidelines for NSCLC. Uncertainties raised regarding the issue of perfusion and tracer uptake also suggests that reliable hypoxia quantification may require multiparametric assessment, which may warrant the inclusion of a secondary imaging modality such as DCE-CT or MRI, or a robust multiparametric assessment when using an independent modality.

This is a complex, multifaceted area, but investigations to date demonstrate the capacity for further development of hypoxia imaging modalities to fully realise the potential of targeted radiation therapy in NSCLC.

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Conflict of interest

The authors declare no conflict of interest.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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