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Review

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REVIEW

Targeting Hypoxia to Improve Non–Small Cell Lung Cancer Outcome

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Abstract

Oxygen deprivation (hypoxia) in non–small cell lung cancer (NSCLC) is an important factor in treatment resistance and poor survival. Hypoxia is an attractive therapeutic target, particularly in the context of radiotherapy, which is delivered to more than half of NSCLC patients. However, NSCLC hypoxia-targeted therapy trials have not yet translated into patient benefit. Recently, early termination of promising evofosfamide and tarloxotinib bromide studies due to futility highlighted the need for a paradigm shift in our approach to avoid disappointments in future trials. Radiotherapy dose painting strategies based on hypoxia imaging require careful refinement prior to clinical investigation. This review will summarize the role of hypoxia, highlight the potential of hypoxia as a therapeutic target, and outline past and ongoing hypoxia-targeted therapy trials in NSCLC. Evidence supporting radiotherapy dose painting based on hypoxia imaging will be critically appraised. Carefully selected hypoxia biomarkers suitable for integration within future NSCLC hypoxia-targeted therapy trials will be examined. Research gaps will be identified to guide future investigation. Although this review will focus on NSCLC hypoxia, more general discussions (eg, obstacles of hypoxia biomarker research and developing a framework for future hypoxia trials) are applicable to other tumor sites.

Lung cancer is the leading cause of cancer mortality worldwide. In 2012, there were an estimated 1.8 million new cases, with 1.59 million deaths (1). Five-year age-standardized survival is 10% to 20% across most countries, displaying little global variation (2). This highlights the inadequacy of current therapeutic strategies. Non–small cell lung cancer (NSCLC) represents approximately 90% of lung cancer cases (3). The majority of patients present with locally advanced or metastatic disease (4).

Oxygen deprivation (hypoxia) is a feature of solid tumors that promotes genomic instability, enhanced aggressiveness, and metastases and is an important factor in treatment resistance and poor survival (5). Hypoxia is an attractive therapeutic target that is yet to be successfully exploited in most cancers, including NSCLC (6). Hypoxia-targeted therapies are associated with a favorable therapeutic ratio because hypoxia is nearly exclusively restricted to cancer cells (6). Hypoxia-targeted therapies could counter hypoxia-induced therapeutic resistance, and this approach is more likely to be successful when combined with radiotherapy or chemotherapy (7). However, NSCLC hypoxia-targeted therapy trials have not yet translated into patient benefit (8). As a result, hypoxia-targeted therapy is not part of standard treatment in this patient group (9). Many factors contributed to this, including poor trial design, lack of applying predictive therapeutic biomarkers, and poor academic-commercial partnership. Further, novel drug radiotherapy combinations are not currently prioritized by the pharmaceutical industry (10).

There is long-standing interest in the development and delivery of hypoxia-targeted therapies in cancer patients.

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However, recent early termination of promising NSCLC clinical studies investigating such strategies (eg, evofosfamide, tarloxotinib bromide) (11,12) highlighted the need for a paradigm shift in our approach to avoid future trial disappointments. This review summarizes the literature on the role of hypoxia, highlights the potential of hypoxia as a therapeutic target, and outlines past and ongoing hypoxia-targeted therapy trials in NSCLC. Evidence supporting radiotherapy dose painting based on hypoxia imaging will be critically appraised. We will examine selected hypoxia measurement techniques, suggest future hypoxia-targeted therapy trials, and address obstacles that need to be overcome prior to hypoxia biomarker validation. Hypoxia imaging and targeted therapies are addressed, but not comprehensively, because these are described elsewhere (6,13).

Review Criteria

PubMed and Google scholar databases were searched for English-language original and review articles published before December 19, 2016, using the following terms: "hypoxia," "radiotherapy," "radiation," "lung cancer," "non–small cell lung cancer," and "biomarker." Reference lists of included studies were hand-searched to identify missing publications. Relevant clinical trials were identified from the ClinicalTrials.gov website and major oncology conferences (American Association for Cancer Research (AACR), American Society of Clinical Oncology (ASCO), European Society for Medical Oncology (ESMO), European Society for Radiotherapy and Oncology (ESTRO) and American Society for Radiation Oncology (ASTRO)). Because of the paucity of NSCLC hypoxia research, hypoxia-related research findings in other cancer sites were reviewed as required. These sections are clearly marked.

Hypoxia Biology

Arterial oxygen tension is 10.66 to 13.33 kPa, with levels in normal tissues ranging from 5.33 to 8 kPa and a value of 5.73 kPa in the lung (14). Hypoxia is characterized by an oxygen tension below physiological normoxia (≤2.03–3.04 kPa) (15). Hypoxia is intimately related to a number of "hallmarks of cancer": resisting cell death, inducing angiogenesis, and reprogramming energy metabolism (16). "Intermediate" hypoxia (0.13–2 kPa) plays a role in enhancing cancer aggressiveness and metastases but does not interfere with radiation-related cell death. "Radiobiological" hypoxia (impairing radiation-induced cell death) occurs at oxygen levels below 0.13 kPa. With further decrease in oxygen (<0.02 kPa), cancer cells demonstrate survival-oriented mutations and maximal resistance to ionizing photon irradiation (15,17).

Hypoxia is often characterized as acute or chronic (Figure 1). In the simplified chronic hypoxia description, a necrotic center represents an anoxic core containing cancer cells beyond the capillary diffusion distance of oxygen while viable cancer cells exist in an environment of decreasing hypoxia away from the center (15). Acute hypoxia occurs in areas adjacent to blood supply due to transient vessel occlusion. This is due to vessel fragility and increased interstitial pressure resulting from tumor cell proliferation outstripping new capillary growth. In reality, the two hypoxia types coexist, resulting in spatial heterogeneity within (intratumor) and among tumors (intertumor) (18). Hypoxia is temporally dynamic. This is explained by acute hypoxia reversibility and, to a lesser degree, dynamic changes in chronic hypoxia. The complex interplay of tumor reoxygenation and vascular dynamics results in "intermittent or cycling" hypoxia, which is thought to be associated with numerous biological effects (19). Evidence also supports an alternative hypoxia characterization concept that depends on a predictable extended longitudinal hypoxia gradient resulting from a decreasing oxygen gradient in arterioles as they traverse tumors (as opposed to chaotic blood flow) (20,21).

Hypoxia inducible factor–1α (HIF-1α) is a pivotal transcription factor interlinked with many downstream molecular hypoxia consequences. In response to falling oxygen levels, HIF-1α promotes expression of genes involved in essential survival processes, for example, angiogenesis, suppression of apoptosis, motility, and invasion (22). In NSCLC, HIF-1α expression is associated with resistance to radiotherapy (23,24), chemotherapy (23,25), and epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) (26). Three separate meta-analyses confirmed the negative impact of HIF-1α tumor expression on overall survival (OS) in NSCLC patients (5,27,28). Carbonic anhydrase-IX, a target of HIF-1α induced in response to hypoxia, is also prognostic in NSCLC patients (29).

Targeting NSCLC Hypoxia: The Potential to Improve Outcome

Because hypoxia is linked to NSCLC therapy resistance, researchers hypothesized that hypoxia-targeted therapies should improve clinical outcomes. There are several approaches to target hypoxia: increasing hypoxic cell radiation sensitivity (eg, misonidazole, nimorazole), increasing oxygen delivery (eg, carbogen and nicotinamide, efaproxiral), decreasing oxygen consumption (eg, metformin, atovaquone), specific targeting of hypoxic cells using hypoxia-activated cytotoxic prodrugs (eg, tirapazamine, evofosfamide, tarloxotinib bromide), hypoxia molecular target inhibitors (eg, aryl sulfonamides targets HIF-1α gene products) (6,30), and various other hypoxia-related mechanisms (eg, nitroglycerin, BKM120). However, learning lessons from failed head and neck cancer trials (31), clinical benefit from hypoxia-targeted therapies in NSCLC will only be realized if the following assumptions are met: 1) tumor hypoxia is present, 2) hypoxia is cure-limiting, and 3) hypoxia-targeted therapies are effective in countering the deleterious hypoxia effects. Detectable hypoxia (based on different imaging definitions) is present at baseline in around 50% to 80% of stage I–IV NSCLC patients according to several positron emission tomography (PET) studies using hypoxia-specific radiotracers (32–38). The validity of the other assumptions is outlined below.

Past NSCLC Hypoxia-Targeted Therapy Trials

A systematic review of 10 108 patients treated in 86 randomized trials showed a statistically significant OS (odds ratio [OR] = 0.87, 95% confidence interval [CI] = 0.80 to 0.95) and locoregional control (OR = 0.77, 95% CI = 0.71 to 0.86) advantage in favor of treatments designed to modify hypoxia in multiple tumor sites (39). In this review, lung cancer patients demonstrated similar but statistically nonsignificant trends (likely due to inclusion of underpowered trials) in favor of hypoxic modification for both end points (OR = 0.83, 95% CI = 0.52 to 1.33, and OR = 0.84, 95% CI = 0.61 to 1.17, respectively) (39). As this review only included patients treated with radiotherapy alone, additional past and ongoing NSCLC hypoxia-targeted therapy trials were identified (Table 1). In patients with loco-regional disease, nitroglycerin (40) and efaproxiral (41) showed promising results

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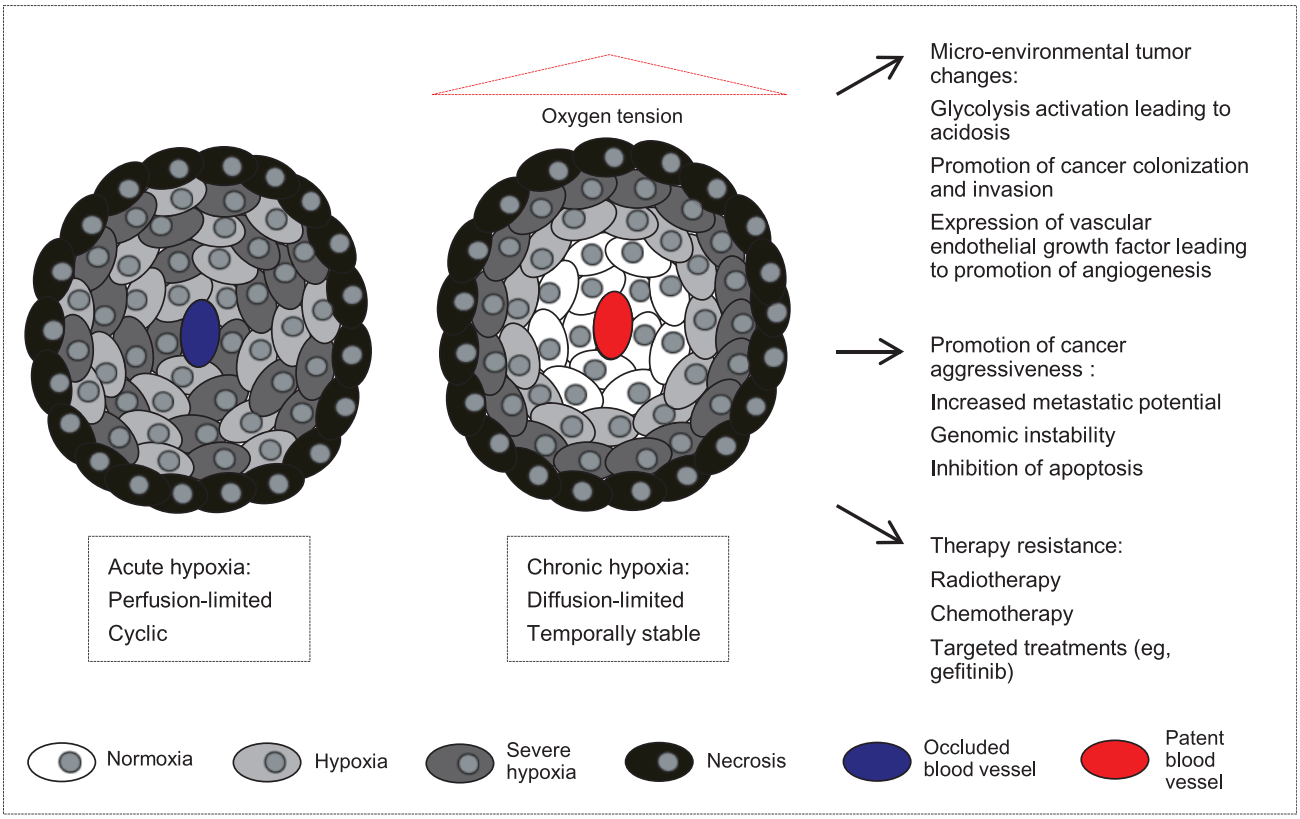

Figure 1. The two main types of hypoxia, acute and chronic, and their impact on non–small cell lung cancer.

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in phase II studies and warrant further evaluation in randomized phase III trials. In advanced NSCLC (stage IIIB–IV), tirapazamine and nitroglycerin failed to show a survival advantage in combination with chemotherapy in randomized phase III trials (42–44). The only exception is the CATAPULT 1 study, which recruited 446 NSCLC patients (17% stage IIIB and 83% stage IV). Statistically significant improvement in median OS (median OS = 34.6 weeks, 95% CI = 29.4 to 39.6 weeks, vs median OS = 27.7 weeks, 95% CI = 24.3 to 31.3 weeks, $P = .0078$) and response rate (response rate = 27.5%, 95% CI = 21.7 to 34.0, vs response rate = 13.7%, 95% CI = 9.4 to 19.0, $P < .001$) were reported in tirapazamine and cisplatin–treated patients compared with those treated with cisplatin alone, but with relatively high rates of adverse events were reported (including hearing loss, reversible intermittent muscle cramping, diarrhoea, skin rash, nausea, and vomiting) (45). The substandard control arm is an obvious study caveat; this positive result was not replicated in phase III trials that investigated tirapazamine in combination with standard, platinum-based doublet chemotherapies (44,46).

Evidence supports the presence of cure-limiting hypoxia in NSCLC patients, with indication of benefit when hypoxia-targeted therapy is combined with radiotherapy, but not standard chemotherapy. Several factors could obscure detection of hypoxia-targeted therapy efficacy. First, lack of patient selection: patients with little or no tumor hypoxia will not benefit from hypoxia-targeted therapies. Second, poor radiotherapy quality: nearly all radiotherapy trials involved two-dimensional (2D) delivery techniques (except one phase II trial) (40) or lacked radiotherapy quality assurance (2D radiotherapy quality was reviewed in only two trials) (41,47). The importance of radiotherapy quality assurance was demonstrated in a pivotal head and neck cancer trial investigating tirapazamine in addition to chemoradiotherapy. Benefit from the tirapazamine/chemoradiotherapy combination was upheld only when analysis was restricted to patients treated as per radiotherapy protocol (48). Future trials should incorporate 3D or intensity-modulated radiotherapy delivery, with a robust quality assurance program. Last, suboptimal hypoxia-targeted therapy dose: misonidazole has higher electron affinity (hence, radiosensitizer activity) compared with newer radiosensitizers (eg, nimorazole). Misonidazole was used in older studies in combination with radiotherapy in lung cancer (49–54). However, dose-limiting peripheral neuropathy limits sufficient dose escalation to achieve therapeutic effects. Similarly, suboptimal treatment dose is a potential explanation for lack of tirapazamine benefit in a phase III trial (44).

Future Priorities for NSCLC Hypoxia-Targeted Therapy Trials

Critical review of scientific evidence, careful appraisal of lessons learned from hypoxia studies, and in-depth understanding of lung cancer biology and trial landscape are necessary to design future hypoxia-targeted therapy trials with the highest success chances. Researchers are less likely to report discouraging (eg, PR-104) or negative (eg, AQ4N) trial results as compared with successful trials (55). Trialists need to be aware of these results to avoid unnecessary repetition of similar trials in the future (55). Furthermore, continued investigation of failed drugs is discouraged, even within nontherapeutic studies (eg, preclinical

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Table 1. Published and ongoing hypoxia-targeted therapy trials (phase I–III) in NSCLC

Design	Drug	Additional treatment	Stage	Status	Major finding(s)
Phase I	PR-104	Concurrent gemcitabine or docetaxel	IV	Completed	Determined dose in phase II trial (175)
	Tirapazamine	Concurrent paclitaxel, carboplatin, and RT	II–III	Closed	Not reported (176)
		Concurrent paclitaxel and carboplatin	n/a	Completed	Determined dose for SWOG 0003 trial (177)
		None	n/a	Completed	Reversible hearing loss and tinnitus were DLTs (178)
		Concurrent cisplatin	n/a	Completed	Doses up to 260 mg/m^2 well-tolerated with cisplatin (179)
		Concurrent cisplatin and vinorelbine	IIIB–IV	Completed	Determined dose in phase II trial (180)
	Evofosfamide	None	n/a	Completed	MTD was 575 mg/m^2/wk and 670 mg/m^2/3 wk (111)
	Banoxantrone (AQ4N)	None	n/a	Completed	MTD was 768 mg/m^2 on days 1, 8, and 15/28-d cycle (181)*
	Apaziquone (E09)	None	n/a	Completed	None of the NSCLC patients had tumor response (182)
	Doranidazole	Concurrent RT	IIIA–IIIB	Completed	Determined dose in phase II trial (47)
	BKM120	Concurrent RT	Any	Open (NCT02128724)	Currently recruiting to 3rd safety cohort (100 mg OD) (135)
	BKM120	Concurrent paclitaxel and carboplatin	IV	Completed	2 of 3 evaluable patients had DLTs (stomatitis, neutropenia) (183)
Phase II	Tirapazamine	Concurrent cisplatin and gemcitabine	IIIB–IV	Completed	ORR = 40% (95% CI n/a), median OS = 8.1 mo (95% CI n/a), median PFS = 6.7 mo (95% CI n/a) (184)
	Tirapazamine	Concurrent cisplatin and vinorelbine	IIIB–IV	Completed	ORR = 47% (95% CI = 32.5% to 61.7%), median OS = 50 wk (95% CI = 37.6 to 58.5 wk) (180)
	Tirapazamine	Concurrent cisplatin	IIIB–IV	Completed	PR in 10 patients = 22.8% (95% CI = 11.4% to 37.9%), median OS = 37 wk (95% CI = 25 to 49 wk) (185)
	Tirapazamine	Concurrent cisplatin	IIIB–IV	Completed	ORR = 25% (95% CI = 11% to 50%) (186)
	Doranidazole	Concurrent RT	IIIA–IIIB	Completed	Median OS for patients who received 21–30 doses of doranidazole 20.9 mo (47)
	Efaproxiral†	Concurrent paclitaxel and carboplatin	IIIA–IIIB	Completed	ORR = 75% (95% CI n/a), median OS = 20.6 mo (95% CI = 14 to 24.2 mo) (41)
	Evofosfamide	Concurrent pemetrexed	IIIB–IV	Terminated early	Interim analysis showed that trial is unlikely to reach primary end point (11,187)
	Tarloxotinib bromide	None	IV‡	Terminated early	Poor ORR (no patient achieved confirmed PR) (12)
	PR-104	Concurrent docetaxel	IIIB–IV	Closed	Results not clinically significant to merit further investigation (188,189)
	Nitroglycerin	Concurrent cisplatin, vinorelbine, and RT	IIIA–IIIB	Completed	Median OS 26.9 mo (95% CI = 15.3 to 38.5 mo) (40)
	Nitroglycerin	Concurrent cisplatin and vinorelbine	IIIB–IV	Completed	Higher disease control in nitroglycerin arm (190)
	Nitroglycerin	Concurrent carboplatin, bevacizumab, and paclitaxel	IV	Completed	No improvement in OS or PFS (109)
	Nitroglycerin	Sequential and concurrent CTRT	IB–IV	Open (NCT01210378)	Preliminary results show reduction in hypoxic fraction (82)
	Nitroglycerin§	Concurrent cisplatin and vinorelbine	IIIB–IV	Completed	ORR = 72% vs 42% (95% CI n/a), $P < .001$, TTP = 327 vs 185 d (95% CI n/a), $P = .002$ in nitroglycerin arm (191)
	ARCON	None	IIIA–B	Completed	Combination therapy toxic and not very effective (81)

(continued)

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Table 1. (continued)

Design	Drug	Additional treatment	Stage	Status	Major finding(s)
Phase III	Tirapazamine	Concurrent cisplatin	IIIB–IV	Completed	Higher median OS, ORR in tirapazamine arm (45)
	Tirapazamine	Concurrent cisplatin and vinorelbine	IIIB–IV	Closed	Not reported (46)
	Tirapazamine	Concurrent paclitaxel and carboplatin	IIIB–IV	Terminated early	No difference in ORR, PFS, and OS (44)
	Tirapazamine	Concurrent cisplatin	IIIB–IV	Completed	Median OS higher in etoposide arm, 31.4 vs 26.7 wk (95% CI n/a), $P = .038$, ORR comparable (43)
	Nitroglycerin	Concurrent platinum-based doublet	III–IV	Terminated early	No improvement in PFS, OS, and ORR (42)
	Efaproxiral	Concurrent RT	IV	Completed	27% reduction in risk of death in patients with brain metastasis diagnosed more than 1 mo after primary (113)

*Respiratory failure and fatigue were dose-limiting toxicities, but were not experienced at the maximum tolerated dose. One out of two patients with lung cancer had stable disease. AE = adverse event; ARCON = accelerated radiotherapy carbogen and nicotinamide; CI = confidence interval; CTRT = chemoradiotherapy; DLT = dose-limiting toxicity; MTD = maximum tolerated dose; NSCLC = non–small cell lung cancer; OD = once daily; ORR = objective response rate; OS = overall survival; PFS = progression-free survival; PR = partial response; RT = radiotherapy; TTP = time to progression.
†Administered with radiotherapy as part of sequential chemoradiotherapy (following two cycles carboplatin and paclitaxel).
‡EGFR-mutant, T790M-negative patients who failed EGFR TKI.
§Patients were randomly assigned to vinorelbine and cisplatin with or without nitroglycerin.

hypoxia biomarker studies) to ensure clinical translation of results. The choice of therapies that merit further investigation should be based on pharmacodynamic (mechanism of action, target vs off-target activation, bystander effect and half-life) and pharmacokinetic properties (absorption, distribution, metabolism, and elimination), treatment aim, adverse event profile, and previous trial results in NSCLC and other tumor types (Table 2). The interactions between hypoxia-targeted and other anticancer therapies should be unraveled and exploited innovatively in future trials (56). For example, hypoxia-targeted therapies could be appropriately sequenced in a treatment course to overcome the emergence of hypoxia-induced chemotherapeutic resistance (57). Another promising area is the potential to exploit tumor hypoxia for synthetic lethality (lethality due to simultaneous combination of two gene mutations when a single mutation would result in viability) (58). For example, poly(ADP-ribose) polymerase 1 (PARP1) inhibition mediates synthetic lethality of homologous recombination (HR)–deficient hypoxic cells (59) while inhibition of Ataxia Telangiectasia and Rad 3 related (ATR)–mediated signaling in response to DNA replication arrest was shown to be more effective in inducing lethality under hypoxic conditions (60). Further research is warranted to allow clinical translation. "Window of opportunity" studies allow expedited testing of hypoxia-targeted therapies in selected populations (eg, patients with superior sulcus tumors treated with neoadjuvant chemoradiotherapy as part of a trimodality approach) (61). Appropriate interim radiological (eg, response evaluation criteria in solid tumors [62]/hypoxia imaging biomarker response) or pathological (pathological complete response) (63,64) end points could provide early "go/no-go" decisions for subsequent phase III trials (10), limiting patient accrual into ineffective therapy studies. Importantly, it is anticipated that trials are likely to be positive only in selected patients. Translational studies need to be integrated within trials to validate and qualify hypoxia biomarkers (65), leading to the development of biomarker-driven clinical trials. Low baseline hemoglobin (or decline after therapy) can affect tumor oxygenation and clinical outcome in NSCLC patients (66,67). This should be considered as a stratification factor in future trials. Ongoing smoking could result in loss of more than 30% of the oxygen loading capacity of hemoglobin (68). Various approaches are available to test patient compliance to smoking cessation programs (69,70) to ensure rigorous implementation in future trials. Hypoxia-targeted therapy efficacy could be different according to tumor histology (eg, squamous cell vs adenocarcinoma). For this reason, tumor histology should also be considered as a stratification factor in future trials. In NSCLC, we identify four settings where clinical investigation of a potential therapeutic role of hypoxia/ hypoxia-targeted therapies should be prioritized.

Targeting Hypoxia in Early-Stage NSCLC Treated With Stereotactic Ablative Radiotherapy

Local control following stereotactic ablative radiotherapy for medically inoperable stage I NSCLC is very good (>90%), but metastatic spread is the main pattern of relapse. In a large retrospective analysis of 676 patients, the five-year distant recurrence rate was 19.9% (95% CI = 14.9% to 24.6%; 46% of whom experienced isolated distant recurrence) (71). Evidence from preclinical and modeling studies suggests a larger detrimental effect of hypoxia when large doses per fraction or single dose radiotherapy are delivered, compared with fractionated radiotherapy (72). Radiation doses in excess of 10 Gray can induce severe vascular damage, resulting in reduced perfusion and an impact on the tumor microenvironment (73). Further, there is indication of improved prediction of tumor control probability when models take into account hypoxic cells in NSCLC treated with stereotactic ablative radiotherapy (74,75). Collectively, these findings point toward a tumor hypoxia role as a driver of cancer aggressiveness and metastases in this patient group. Hypoxia-targeted therapies could have a role in this patient group, particularly if we can identify a patient subgroup at higher risk of distant relapse. Early clinical studies should substantiate the relationship between tumor hypoxia and clinical outcome

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Table 2. Summary of relevant hypoxia-targeted therapy features, based on studies conducted in NSCLC and other tumors

Mechanism of action	Drug/company or institution	Clinical status in NSCLC/ concurrent use with radiotherapy	Major adverse event(s)	Comment(s)
Hypoxic radiosensitizer	Misonidazole/n/a (39,49–54)	Phase III/yes	Peripheral neuropathy	Higher radiosensitizer activity but more toxic (compared with nimorazole)
	Nimorazole (Nimoral)/Azanta A/S (Hellerup, Denmark) (192,193)	To be tested/no	Rash, n/v	Estimated SER is 1.3–1.45, well-tolerated
	Doranidazole/POLA Chemical Industries Inc. (Yokohama, Japan) (47,194)	Phase II/yes	Pneumonitis, peripheral neuropathy	Well-tolerated in phase I/II trial
Hypoxia-activated cytotoxic prodrugs	Tirapazamine/SRI International (Menlo Park, CA) (44,45,48,195)	Phase III/no	Muscle cramps, n/v, hearing loss	Activation in moderate hypoxia leads to more toxicity (compared with evofosfamide)
	Evofosfamide/Threshold Pharmaceuticals (San Francisco, CA) (110,111)	Phase II/no	Skin, mucosal toxicity	Negative phase II trial in NSCLC but led to PFS improvement, favorable toxicity profile
	PR-104/Peoacta, Inc. (San Diego, CA) (175,188,189,196)	Phase II/no	Thrombocytopenia, neutropenic fever, fatigue	Produces slight, non–clinically significant tumor response improvement
	Banoxantrone (AQ4N)/Novacea (San Francisco, CA) (55,181,197)	Preclinical/no	Respiratory failure, fatigue, diarrhoea, n/v	Limited single-agent activity, no studies in lung cancer
	Apaziquone (E09)/Spectrum Pharmaceuticals, Inc. (Irvine, CA) (182,195,198,199)	Phase I/no	Reversible proteinuria, salt and water retention	No tumor response in NSCLC
	Tarloxotinib bromide/Threshold Pharmaceuticals (San Francisco, CA) (12)	Phase II/no	No data	Poor tumor response rate
Decrease oxygen consumption	Metformin*/n/a (84,200,201)	Ongoing phase II/yes	Gastrointestinal	Enhanced cytotoxicity when combined with radiotherapy in preclinical models
	Atovaquone/n/a (61)	Early clinical study/no	Headache, n/v, diarrhea	Led to rapid reduction in oxygen consumption in a preclinical study
Increase oxygen delivery	Carbogen and nicotinamide/n/a (81)	Phase II/yes	Flushing, n/v	No improvement in time to progression in phase I/II NSCLC trial
	Efaproxiral/Allos Therapeutics (Westminster, CO) (41)†	Phase II/yes	Transient hypoxemia, pneumonitis, fatigue	Well-tolerated with radiotherapy, promising OS in single-arm phase II NSCLC trial
Multiple/ unclear mechanisms	Nitroglycerin/n/a (40)‡	Phase III/yes	Headache, hypotension	Well-tolerated with CTRT, inconsistent therapeutic efficacy across studies
	BKM120/n/a (135)§	Phase I/yes	Moderate	Results from phase I trial awaited

*Effects could be due to decrease in circulating insulin, activation of adenosine monophosphate-activated kinase (201). CTRT = chemoradiotherapy; DLT = dose-limiting toxicity; NSCLC = non–small cell lung cancer; n/v = nausea and vomiting; PFS = progression-free survival; SER = sensitizer enhancement ratio.
†Decreases oxygen binding capacity of hemoglobin, which leads to release of oxygen to the tumor and improvement in oxygenation.
‡Results in vasodilation, decreased HIF1α and enhanced radiotherapy DNA damage.
§Confirmation of efficacy against tumor hypoxia under investigation in an ongoing phase I trial. Therapeutic effect likely to be mediated through change in tumor microenvironment and perfusion.

(metastatic spread and OS) in early-stage NSCLC patients treated with stereotactic ablative radiotherapy. The next step is to investigate optimal therapeutic interventions, based on hypoxia-targeted therapies.

Targeting Hypoxia in Locally Advanced NSCLC

Loco-regional control is correlated with OS in patients with locally advanced NSCLC (76). Following chemoradiotherapy,

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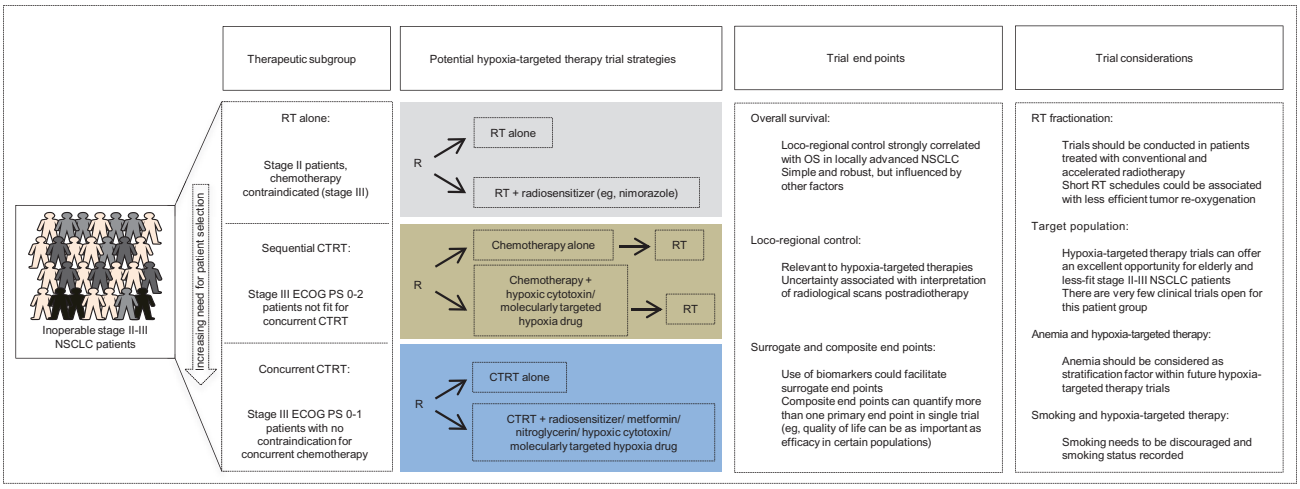

Figure 2. Potential hypoxia-targeted therapy trial strategies and overview of trial end points and considerations in locally advanced non–small cell lung cancer patients. Patients receiving sequential chemoradiotherapy could also be treated with standard chemotherapy, with hypoxia-targeted therapy applied concurrently with radiotherapy. CTRT = chemoradiotherapy; ECOG PS = Eastern Cooperative Oncology Group Performance Scale; NSCLC = non–small cell lung cancer; RT = radiotherapy.

in-field radiation recurrence and distant metastases are the predominant sites of failure (40% and 58% of patients develop local recurrence and distant metastases by three years, respectively) (77). Strategies that intensify standard chemoradiotherapy have failed to improve outcome (eg, radiotherapy dose escalation to 74 Gray with or without cetuximab) (78). Several therapeutic approaches that aim to improve loco-regional control are currently under investigation (eg, ARTFORCE PET boost: metabolic dose painting based on fluorodeoxyglucose [FDG] PET imaging [NCT01024829] [79], isotoxic dose-escalation [NCT01836692] [80]), and early results are awaited. In this setting, hypoxia-targeted therapy trials have yielded mixed results. Accelerated radiotherapy with carbogen and nicotinamide did not improve loco-regional control and was overly toxic while nitroglycerin and efaproxiral results are encouraging (41,81,82).

Future investigation of hypoxia-targeted therapies should not be limited to patients treated with concurrent chemoradiotherapy. Trials in locally advanced NSCLC patients treated with radiotherapy alone or sequential chemoradiotherapy are important to establish therapeutic efficacy while avoiding overlapping toxicity with concurrent chemoradiotherapy, especially for hypoxia-activated cytotoxic prodrugs. Further, because several hypoxia-targeted therapies have favorable toxicity profiles (eg, nimorazole [83], metformin [84], nitroglycerin [82], and efaproxiral [41]), these trials could accrue patients not otherwise fit for radiotherapy-drug combinations. A randomized NRG-led phase II trial is underway to determine whether metformin can improve progression-free survival in fit stage IIIA–IIIB NSCLC patients treated with concurrent chemoradiotherapy (NCT02186847). Although the underlying study hypothesis was based on metformin's radiosensitizing ability resulting from engagement of the mediator mammalian target of rapamycin pathway, subsequent translational research from collected biospecimens could unravel a tumor hypoxia role in this process (85). An evofosfamide-radiotherapy combination demonstrated promising results in a preclinical study (86), but the safety and efficacy of hypoxia-activated cytotoxic prodrugs is yet to be determined in combination with thoracic radiotherapy in NSCLC patients. Figure 2 highlights potential therapeutic strategies and important trial considerations for targeting hypoxia in this patient population. Most strategies entail the use of hypoxia-targeted therapies at the start of treatment. An alternative approach is to target residual hypoxia, as demonstrated using midtreatment hypoxia measurements (eg, using imaging) (87). With this approach, only refractory hypoxia is targeted, arguably obviating the unnecessary use of hypoxia-targeted therapies in patients with early tumor re-oxygenation, which can occur during fractionated radiotherapy (88,89).

Radiotherapy Dose Guidance Based on Hypoxia Imaging

Radiotherapy dose painting, based on tumor hypoxia, is an attractive therapeutic approach (13). In adaptive dose painting, additional radiotherapy is spatially and dosimetrically adapted based on tumor response maps acquired by repeat imaging during radiotherapy (90). A phase II trial is underway to investigate the efficacy and safety of radiotherapy boost, based on baseline [^{18}F]fluoromisonidazole (FMISO) PET, in stage III NSCLC patients (RETP 5 [NCT01576796]) (91). However, there are conceptual concerns with this approach and essential prerequisites are yet to be defined, as discussed below.

Hypoxia dose painting requires hypoxia spatial mapping. Hypoxia PET is the current leading hypoxia-specific imaging modality, but multiparametric magnetic resonance imaging (MRI) is promising (92). Because hypoxia radiotracer retention is only possible within viable cells that retain a functional electron transport system (93), extremely hypoxic but necrotic tumor subvolumes will arguably not be subject to unnecessary dose escalation. However, hypoxic cells with clonogenic replicative potential, not necessarily all viable cells, are the only cancer cells likely to benefit from a higher radiotherapy dose (13).

The following essential prerequisites should be defined to allow the sound development of dose painting strategies based on hypoxia imaging. First is the investigation of image repeatability for hypoxia radiotracers. In NSCLC, this was established for 3-[^{18}F]fluoro-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (HX4) (94) and FMISO (95). Unfortunately, spatial comparisons (eg, DICE overlap) relevant

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to radiotherapy dose painting studies were not reported in either study (96). Lack of hypoxia imaging repeatability could be explained by imperfect image registration, patient motion, and inconsistencies in image acquisition, analysis, or target volume definition between scans as well as true biological factors (eg, emergence of acute hypoxia) (95). Second, spatiotemporal stability of intratumor radiotracer distribution must be investigated throughout radiotherapy to ensure that higher radiotherapy doses, guided by baseline hypoxia imaging, are delivered to the intended hypoxic subvolume throughout treatment. Repeat hypoxia imaging, using current approaches, is unlikely to be adopted in clinical settings. Further, it is not clear how to best account for cyclical hypoxia and whether this negatively impacts image repeatability. Third, it is prudent to spatially map hypoxic tumor subvolumes to sites of loco-regional recurrence to confirm clinical validity. This was established for FDG, but not for hypoxia radiotracers (79). A multimodality imaging study in NSCLC patients showed that boosting FDG avid subvolume(s) will invariably encompass hypoxic subvolume(s) in most patients, albeit to a lower dose (97). However, nonoverlapping tumor subvolumes also exist in NSCLC (36,98).

There is evidence to support early tumor re-oxygenation during standard therapy in head and neck cancer patients (87,88,99–102), with less consistent results in NSCLC patients (88,89,103). Although the clinical implications of dynamic changes in tumor oxygenation remain to be defined (104–106), early re-oxygenation of hypoxic radioresistant cancer cells during standard therapy could arguably invalidate the need for delivery of additional radiotherapy dose, guided by baseline hypoxia imaging. Further, it is not clear whether radiotherapy dose escalation above the currently accepted standard (60–66 Gray) is safe in NSCLC patients, particularly for centrally located tumors (78). Finally, a recently published preclinical study supports the hypothesis that tumor response is dependent on minimal, not maximal, radiotherapy dose (107).

In summary, evidence to support hypoxia-based radiotherapy dose guidance is still lacking to allow valid clinical investigation (108).

Targeting Hypoxia in Metastatic NSCLC

To date, hypoxia-targeted therapy trial results in the firstline setting in metastatic NSCLC patients have been disappointing (42–44,46,109). Evofosfamide, a hypoxia-activated cytotoxic prodrug, possesses single-agent activity (consistent with a bystander effect), acceptable tolerability (consistent with bioreductive activation at a low oxygen concentration), and a relatively long half-life (to target cyclical hypoxia), making it a promising therapeutic agent (6,55,110,111). However, a randomized phase II trial of pemetrexed with or without evofosfamide as second-line treatment in metastatic nonsquamous NSCLC was recently terminated because of futility, although interim analysis revealed a 30% progression-free survival improvement with combination treatment (11). Arguably, this is likely due to lack of patient selection, rather than lack of therapeutic efficacy, resulting in the dilution of clinical benefit. Work is underway to unravel whether specialized liquid biopsy technology (ADAPT Biotargeting System, Caris Life Sciences, Irving, TX) could identify patients who benefited from evofosfamide in a phase III trial that barely missed statistical significance in advanced pancreatic cancer patients (112) and could guide future evofosfamide development in NSCLC. Efaproxiral reduces the risk of death in select NSCLC patients with brain metastases treated with whole-brain radiotherapy according to a subgroup analysis of a randomized phase III trial (113). However, development of this drug was subsequently stopped.

Tumor hypoxia could predict therapeutic resistance to a wide array of oncological treatments, with emerging evidence supporting that hypoxia biomarkers could identify patients more likely to benefit from additional therapies in a number of cancers (114). A preclinical study demonstrated the potential for tumor hypoxia to identify patients more likely to benefit from an olaparib/radiotherapy combination (115). Another promising research topic is the combination of EGFR-TKIs with hypoxia-targeted therapies. EGFR signaling is linked to tumor hypoxia while EGFR blockade (eg, using erotinib or gefitinib) was shown to improve tumor blood flow and reduce hypoxia (116,117). Conversely, hypoxia promotes gefitinib resistance in EGFR-mutant NSCLC cell lines through activation of insulin-like growth factor–1 (118) and wild-type EGFR, which is reversed by HIF-1α knockdown (26). EGFR overexpression under hypoxic conditions was also shown to promote metastases in NSCLC cell lines (119). A "proof of concept" study demonstrated that early tumor hypoxia changes (detected by repeat [^{18}F]FMISO PET performed 10–12 days after initiation of therapy) predicted response to erlotinib in two EGFR-mutant NSCLC patients (120). These observations support preclinical and pilot clinical investigation of hypoxia-targeted therapy/EGFR-TKIs combinations in EGFR-mutant NSCLC. An alternative approach to counteract EGFR-TKI resistance is to deliver an irreversible EGFR inhibitor to hypoxic tumor subvolumes. Tarloxotinib bromide is a hypoxia-activated membrane-anchored prodrug designed to release an irreversible EGFR-TKI under hypoxic conditions. Unfortunately, poor response rates led to discontinuation of a phase II trial of tarloxotinib bromide in EGFR-mutant, T790M-negative stage IV NSCLC patients who failed treatment with EGFR-TKIs (NCT02454842) (12). This study highlights the importance of choosing an appropriate trial end point. The trial end point was tumor response. Arguably, hypoxia-targeted therapies have limited single-agent efficacy against well-oxygenated, rapidly proliferating tumor cells and, in principle, are unlikely to induce evident tumor regression. Early tirapazamine preclinical studies support this hypothesis (121,122).

A hypoxic tumor microenvironment can form an immunotherapy barrier, with emerging preclinical data supporting that normalization of tumor oxygenation, using hypoxia-targeted therapies, could improve immunotherapy efficacy and counter therapeutic resistance (123). Future research should investigate these potentially synergistic, clinically deliverable drug combinations. A schematic representation of this approach is summarized in the Hypoximmuno trial (hypoxia-targeted therapy/immunotherapy/radiotherapy combination trial) video (https://www.youtube.com/watch?v=vFZvCF-N18w). Predictive biomarker validation within these trials may allow renewed investigation of promising but unsuccessful hypoxia-targeted therapies (eg, evofosfamide) in enriched populations.

NSCLC Hypoxia Biomarkers

The National Institutes of Health has recently stated that a biomarker is a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention, including therapeutic interventions" (124). Biomarkers can be molecular, histologic, radiologic, or physiologic in nature. The ideal hypoxia

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biomarker should quantify cellular hypoxia in viable (not necrotic) tumor cells and should be hypoxia-specific rather than measuring a hypoxia-related process (eg, perfusion). Different hypoxia severity levels may be relevant for radiobiology or hypoxia-targeted therapies (eg, oxygen levels to activate cytotoxic hypoxia prodrugs) (125). Further, spatial mapping (for radiotherapy dose guidance) and repeated measurements (for adaptive therapy) may or may not be required. Therefore, the choice of hypoxia biomarker will depend on the intended clinical application, as well as practical considerations (eg, difficulty in obtaining tumor material in NSCLC patients) and cost-effectiveness. In vivo oximetry approaches (eg, electron paramagnetic resonance [EPR] oximetry) have provided useful insight into tumor biology through direct and repeatable readouts of tissue pO_2. However, the necessity of implanting a paramagnetic oxygen reporter in the tumor is likely to limit the implementation of this approach in NSCLC patients (126,127). Imaging biomarkers are useful for "proof of concept" studies to establish therapeutic efficacy because they obviate the need for repeated biopsies (65). Imaging biomarkers could also highlight intertumor hypoxia heterogeneity (different hypoxia levels in primary tumor vs metastatic sites), which exists in NSCLC (103). This is only possible with tissue-based hypoxia biomarkers if each metastatic site is biopsied separately, which is generally impractical. Selected hypoxia measurement strategies are listed in Supplementary Box 1 (available online). The advantages and limitations of each approach should to be carefully appreciated to allow optimal integration within clinical trials.

Hypoxia is a complex biological process. Different biomarkers quantify different aspects of hypoxia, and each will provide a slightly varied representation of true tumor hypoxia. For this reason, a single all-purpose gold-standard hypoxia measurement technique is difficult to establish. Further, an approach focused entirely on tumor hypoxia assessment (with no integration of other relevant molecular and genetic tumor characteristics) could be overly simplistic. In prostate cancer, the prognostic capacity of three RNA hypoxia signatures were upheld only when patients were separated according to degree of tumor genome alteration (128). It is not clear whether this is also true in NSCLC or other cancer sites. Hypoxia biomarkers are yet to be validated in NSCLC clinical trials.

Potential hypoxia biomarker applications include patient selection, identification of optimal hypoxia-targeted therapy dose or duration, therapy monitoring and early response prediction, and providing measurable surrogate trial end points (Supplementary Box 1, available online). (129). Hypoxia biomarker cutoffs (thresholds) are likely to be tumor-specific. In NSCLC, different hypoxia biomarker cutoffs might be required according to tumor histology (eg, squamous cell carcinoma vs adenocarcinoma). Further, biomarkers that are predictive in one therapeutic setting (eg, radiotherapy alone) might not retain this predictive capacity in different settings (eg, chemoradiotherapy) within the same tumor. The ultimate test of hypoxia biomarker cutoff significance should be based on their performance as predictive therapeutic biomarkers within hypoxia-targeted therapy trials. In NSCLC, hypoxia biomarker repeatability has only been established for two hypoxia PET tracers (HX4 and FMISO) (94,95) while biomarker kinetics during therapy were assessed in only a few hypoxia PET imaging studies (88,103,130). Hypoxia biomarker technical validation (eg, circulating and imaging biomarker stability and kinetics) and biological validation/clinical utility (eg, predictive capacity) should be evaluated within carefully designed, quality-controlled studies (131,132). Unfortunately, efforts to prospectively validate hypoxia biomarkers are fragmented across a vast array of hypoxia measurement techniques, often overlooked even in modern hypoxia-targeted therapy trials (eg, NCT02093962). The pharmaceutical industry should not be discouraged from companion biomarker development. Enhanced therapeutic efficacy in enriched patients will increase drug registration success, partially offsetting financial loss from market segmentation (133). We identified only six ongoing NSCLC trials that have well-defined hypoxia biomarker validation components (61,82,134–137). There are also a handful of pilot hypoxia biomarker validation studies (Table 3). It is noteworthy that in all studies an imaging modality was chosen as one of the investigatory biomarkers. Imaging biomarkers should be validated according to consensus recommendations (65), and global data sharing could be enhanced by adopting standardized image acquisition and analysis parameters (138).

A comprehensive review of hypoxia biomarkers is beyond the scope of this review. We address selected invasive (gene expression signatures) and noninvasive biomarkers (hypoxia PET imaging) as well as other promising methods (circulating biomarkers and multiparametric MRI) at various developmental stages that are likely to be integrated in future NSCLC trials.

Hypoxia Response Gene Expression Signatures

Numerous downstream molecular alterations occur in neoplastic and stromal tissue in response to hypoxia (139). Some genes are also expressed as a result of other processes, for example, oncogenic activation. For this reason, an assay of a single molecule is inadequate to assess hypoxia status (140). Gene expression signatures have emerged as robust tissue-based hypoxia biomarkers (141). Currently available signatures were not specifically developed in NSCLC patients. However, a common hypoxia "metagene" was prognostic in two independent data sets of 216 NSCLC patients (142). In this study, genes were selected according to their level of connectivity with a set of widely studied hypoxia-inducible seed genes in head and neck and breast cancer data sets rather than association with prognosis, reinforcing a hypoxic rather than mere prognostic significance. Two separate hypoxia gene expression signatures were shown to predict response from hypoxia-targeted therapies in head and neck cancer, retaining prognostic (but not predictive) capacity in other tumor sites (142–145). It is unclear whether a hypoxia gene expression signature derived from head and neck squamous cell carcinoma is more likely to retain prognostic/predictive capacity in squamous cell lung cancer (as opposed to adenocarcinoma or large cell). An NSCLC-specific hypoxia gene expression signature is currently under development, with evidence suggesting different prognostic capability according to tumor histology (squamous cell vs adenocarcinoma) (Catharine M. L. West, unpublished data).

Gene expression signatures can be obtained using formalin-fixed paraffin-embedded samples, making this technique deliverable in the clinic (collected biopsies could be easily transferred across centers). In NSCLC patients planned for nonsurgical treatment, tissue-based hypoxia measurement will be based on limited tumor and/or lymph node biopsies, questioning the representative nature of these techniques due to sampling bias (141). This is likely to be less of a problem for hypoxia gene signatures, which are associated with lower intratumor heterogeneity, compared with individual genes or pimonidazole (146).

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Table 3. Ongoing NSCLC clinical trials with hypoxia biomarker validation components and pilot hypoxia biomarker clinical validation studies

Modality	Design	Stage	Treatment	Status	Major aim(s)
[^{18}F]FMISO PET (202)	Pilot	n/a	Neoadjuvant chemotherapy, sequential/concurrent CTRT	Open and recruiting (NCT02016872)	Assess prognostic value of [^{18}F]FMISO PET
[^{18}F]FAZA PET (203)	Pilot	I–IIIB	RT or CTRT	Open and recruiting (NCT00765986)	Examine relationship between hypoxia changes and early tumor response, correlate [^{18}F]FAZA PET with local failure
[^{18}F]FMISO, [^{18}F]FAZA PET (204)*	Pilot	n/a	Surgery	Open and recruiting (NCT02490696)	Compare [^{18}F]FAZA and [^{18}F]FMISO PET, correlate findings to tissue-based hypoxia biomarkers
Multi-parametric MRI (OE and DCE), [^{18}F]FAZA PET (205)†	Pilot	I–III	Surgery, RT, or CTRT	Open and recruiting/n/a	Validate multiparametric MRI as hypoxia imaging, define optimal scanning parameters and repeatability for [^{18}F]FAZA PET
[^{18}F]FMISO/ [^{18}F]FAZA PET (206)	Window of opportunity	n/a	Neoadjuvant atovaquone followed by surgery	Open and recruiting (NCT02628080)	Investigate whether atovaquone reduces tumor hypoxia, repeatability of hypoxia imaging
[^{18}F]FHX4 PET (82)	Window of opportunity/ phase II	IB–IV	Nitroglycerin, sequential/ concurrent CTRT	Completed recruitment (NCT01210378)	OS at 2 y, evaluate effect of nitroglycerin on [^{18}F]HX4 uptake/ tumor perfusion, correlate with survival and local tumor control
[^{18}F]FMISO PET, perfusion CT (135)	Phase I	Any	BKM120 with concurrent RT	Open and recruiting (NCT02128724)	Determine MTD of BKM120 and determine whether it affects [^{18}F]FMISO PET
[^{18}F]FHX4 PET (207)	Phase II	I–III	Radical RT or CTRT	Open and recruiting (NCT02976883)	Determine whether [^{18}F]FHX4 PET could predict RT response
[^{18}F]FMISO PET (136)	Phase II	IIIA–IIIB	CTRT‡	Open and recruiting (NCT01507428)	Correlate [^{18}F]FMISO PET with loco-regional control
[^{18}F]FMISO PET (134)	Phase II	III	CTRT§	Open but not recruiting (NCT01576796)	Evaluate local control following RT dose painting based on baseline [^{18}F]FMISO PET
Multi-parametric MRI (BOLD and DCE) (137)	Phase II	IIA–IIIB	Hypofractionated radiotherapy boost followed by CTRT	Open and recruiting (NCT02262325)	Primary tumor control, treatment response on multiparametric MRI
[^{18}F]FDG & [^{18}F]HX4 PET	Phase III	II–IIB	Randomized boost of high FDG (highly correlated to hypoxic areas)	Open and recruiting/n/a	Evaluate local PFS, correlate tumor relapse with [^{18}F]HX4 PET uptake, evaluate overlap between [^{18}F]FDG & [^{18}F]HX4 PET

*Immunohistochemistry for tissue-based hypoxia biomarkers is also performed. BOLD = blood oxygen-level dependent; CT = computed tomography; CTRT = chemoradiotherapy; DCE = dynamic contrast-enhanced; FAZA = fluoroazomycin arabinoside; FMISO = fluoromisonidazole; HX4 = 3-[^{18}F]fluoro-2-(4-((2-nitro-1H-imidazol-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propan-1-ol; MTD = maximum tolerable dose; NSCLC = non–small cell lung cancer; OE = oxygen-enhanced; OS = overall survival; PFS = progression-free survival; RT = radiotherapy.

†Multigene signatures, endogenous (HIF1α and CA-IX) and exogenous (pimonidazole) tissue-based and circulating hypoxia biomarkers also performed in a subset of patients.

‡Compares standard radiotherapy (30 fractions) vs individualized adaptive image-guided radiotherapy (30 fractions) based on [^{18}F]FDG PET performed between fractions 18 and 19.

§Compares standard radiotherapy (patients with negative baseline [^{18}F]FMISO PET) with individualized radiotherapy dose painting until the maximum tolerable radiation dose of the lung is reached (patients with positive baseline [^{18}F]FMISO PET).

However, gene expression signatures are not easily quantifiable using cytological samples acquired via endobronchial ultrasound guidance due to low tumor cellularity of these samples, limiting the application of this method.

Circulating Hypoxia Biomarkers

Tumor and circulating osteopontin (147–150) and carbonic anhydrase-IX (151) are prognostic in NSCLC, with conflicting

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Table 4. Strength and weaknesses of commonly applied image analysis approaches for hypoxia PET imaging

Analysis approach*	Description	Main advantage(s)	Main limitation(s)
SUV_{max}	Quantification of maximum tumor SUV	Simple tumor segmentation does not affect result	Wide variability, sensitive to image noise, unlikely to be biologically relevant
TBR	Ratio of tumor uptake to background uptake/blood radioactivity	Likely to be biologically relevant	Complex, depends on accurate tumor segmentation, blood sampling may be required, variability in reference tissue definition
Hypoxic volume	Tumor volume with TBR above a certain threshold	Likely to be biologically relevant, can form basis of radiotherapy dose escalation	Same limitations as TBR, variability in setting threshold
Hypoxic fraction	Fraction of hypoxic volume to tumor volume	Likely to be biologically relevant	Same limitations as hypoxic volume
Semiqualitative	Tumor uptake scored by readers	Simple image analysis not required†	Consensus interpretation advised‡

*It is not clear whether analysis should be restricted to primary, nodal, and/or metastatic lesions in singularity or in combination. PET = positron emission tomography; TBR = tumor to blood ratio.
†[^{18}F]FMISO PET analyzed using this approach shown to predict benefit from tirapazamine in head and neck cancer trial (208).
‡Interobserver visual analysis agreement for [^{18}F]FMISO is low (209). Interobserver visual analysis agreement yet to be determined for other hypoxia radiotracers.

evidence on the ability of circulating osteopontin to predict hypoxia-targeted therapy response in head and neck cancer (152,153). A number of plasma microRNA (miRs) are induced in response to tumor hypoxia, with select members (miR-26, -107, and -210) also possessing downstream effects (eg, proapoptotic signaling) (154). There is growing evidence of miR-210 involvement in hypoxic cell radioresistance (155), with conflicting evidence on the prognostic impact of tumor expression of miR-210 in NSCLC patients (156,157). miRNAs are remarkably stable in the circulation, and high serum miR-210 was linked with advanced stage (III–IV) and poor chemotherapy response in NSCLC patients (158). Methodological approaches for circulating biomarkers (sample collection, storage, and analysis) need to be validated and standardized across laboratories (159).

Hypoxia PET Imaging

Hypoxia radiotracers are loosely classified into nitroimidazole and dithiosemicarbazone derivatives. All current hypoxia radiotracers, with the exception of Cu-diacetyl-bis(N(4)-methylthiosemicarbazone (Cu-ATSM), fall in the former category (93). ATSM has been radiolabeled with four isotopes, with half-lives varying from a few minutes (^{60}Cu and ^{62}Cu) to several hours (^{61}Cu and ^{64}Cu) (14,33,160,161). The mechanism of Cu-ATSM retention in hypoxic cells is not clear. The overriding principle of nitroimidazoles is enzymatic stabilization under hypoxic conditions resulting in cellular retention. These are radiolabeled with ^{18}F (half-life, 110 minutes). Among the different nitroimidazole radiotracers, [^{18}F]FMISO and, to a lesser extent, [^{18}F] fluoroazomycin arabinoside (FAZA) are the most widely established (14). [^{18}F]FMISO is an older compound (first reported in 1989), which allowed accumulation of more clinical data compared with newer radiotracers (162). Standardized and relatively simple radiosynthesis explains sustained research utilization (93). The latest contender [^{18}F]HX4 is proprietary to Threshold Pharmaceuticals, Inc. (San Francisco, CA). Nitroimidazole radiotracers vary in lipophilicity, resulting in differences in cellular entry and plasma clearance and, thus, time to reach equilibrium. Lipophilic compounds are cleared more slowly but distribute more uniformly, leading to a higher tumor to blood ratio (TBR) at equilibrium (163). Very few studies compared hypoxia radiotracers (163,164).

High [^{18}F]FMISO, [^{62}Cu]ATSM, [^{18}F]fluoroerythronitroimidazole (FETNIM), and [^{18}F]FAZA tumor uptake were consistently linked with poor prognosis in NSCLC patients (13,164–166). Often there is a disconnect between preclinical and clinical hypoxia radiotracer imaging studies. For example, immediate scanning following ^{18}F-labeled nitroimidazole radiotracer injection is occasionally employed in spite of overwhelming evidence supporting that such acquisition will reflect radiotracer delivery, not hypoxia (167,168). Knowledge gained from carefully conducted preclinical studies should guide clinical investigation. Time to plateau TBR can be determined through conduct of dynamic PET scans and will inform an optimal scanning window that will achieve the highest and most stable TBR (Supplementary Table 1, available online). However, this is not automatically synonymous with a biologically optimal scanning window, which should be based on validation against tissue-based hypoxia biomarkers or achievement of optimal predictive capacity in hypoxia-targeted therapy trials. Hypoxia PET images have low contrast to background. For this reason, qualitative image analysis is less robust as compared with FDG PET. Longer acquisitions improve image contrast but are associated with reduced signal to noise (169), and long scans can reduce patient compliance and scan acceptability, both of which are important if hypoxia PET is to be successfully integrated within hypoxia-targeted therapy trials. Image analysis techniques vary according to scanning acquisition complexity and the requirement for accurate tumor segmentation. The merits of the various approaches need to be carefully considered (Table 4).

Integration of hypoxia PET in NSCLC hypoxia-targeted therapy trials has been slow. This is mainly explained by high imaging costs, limited radiotracer availability, lack of technical validation, and logistical and quality assurance complexities associated with radiotracer synthesis and delivery. Many challenges need to be resolved within multicenter feasibility studies prior to implementation in large phase II/III trials (Box 1). As shown, hypoxia PET research is resource demanding. For this reason, efforts should focus on validating one or two leading hypoxia radiotracers based on technical characteristics, such as [^{18}F]FAZA and [^{18}F]HX4.

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Box 1. Logistical and technical challenges of hypoxia PET imaging*

Radiotracer synthesis and transportation
- Radiotracer synthesis is currently only possible in highly specialized research centers
- Substantial costs are associated with radiosynthesis, quality assurance, and transportation of radiotracer across centers (in the UK, this can amount to £2000–£3000/scan)
- Radiotracer transport can be logistically complex (eg, if airborne)
- Production failures and variability in radiotracer specifications can cause a nuisance to the patient and scanning staff

Image analysis
- Patient positioning should be standardized to allow optimal image registration with radiotherapy planning or FDG-PET scans (if tumor definition required for analysis)
- Images acquired using different scanners/software should take variability in image reconstruction and smoothing (among other factors) into account by:
  - Stringent standardization of the entire image acquisition and analysis pathway
  - Introduction of correction parameters to account for previously determined differences in image acquisition and analysis pathway across centers
- Variability in scanning time window post–radiotracer injection could impact image interpretation and quantification and should be avoided, if possible
- Radiotracer dose should ideally be based on body weight (not fixed dose), particularly for lipophilic radiotracers (more uniformly distributed) to limit variability; radiotracer dose should also be adjusted to scanner sensitivity (eg, TOF) vs non-TOF scanners) and scanning time window (higher dose for later scans)
- Additional variability will be introduced if radiotracer delivery is delayed, more than one patient is injected from same radiotracer production to reduce study costs, or due to scanner delays

* FDG = fludeoxyglucose; PET = positron emission tomography; TOF = time of flight.

Multiparametric MRI

A number of MRI methods have been investigated to estimate tumor hypoxia. Various capillary permeability kinetic parameters (eg, K^{trans}), quantifiable using dynamic contrast-enhanced MRI, provide information about tumor blood flow and permeability and offer an indirect tumor hypoxia assessment (170,171). However, the association between poor blood flow and hypoxia is imperfect because hypoxia exists in tumor regions with measurable blood supply (hypoxia is partially determined by oxygen consumption, not only supply) (172). Blood oxygenation level–dependent MRI was also investigated for this purpose, but clinical implementation is hindered due to artefact from intrinsic susceptibility, particularly at air-tissue interfaces, and is confounded by the presence of blood degradation products (172).

An alternative method that has gained recent interest is oxygen-enhanced (OE) MRI, where inhaled molecular oxygen acts as a paramagnetic contrast agent and increases proton longitudinal relaxation rate in well-oxygenated tissue, but shows a lack of this effect in hypoxic tissue (9). The feasibility of OE-MRI measurements of tumor oxygenation as a potential hypoxia biomarker was established in solid tumors in two preliminary clinical studies (173,174) and around a dozen preclinical studies (14). More recently, segmenting tumors into perfused and nonperfused subregions and then detecting oxygen-refractory tumor was validated against pimonidazole as providing a robust method to identify, map, and quantify tumor hypoxia (92). Further work is needed to compare this method with other imaging (eg, PET-based) and nonimaging (eg, gene and circulating) biomarkers to establish biological and clinical validation.

Conclusions

To date, hypoxia-targeted therapies have not been adopted as part of standard treatment in NSCLC (9). There is an unmet need to develop more effective and less toxic hypoxia-targeted therapies. In the meantime, promising therapies should be investigated in selected NSCLC patients in combination with radiotherapy, chemotherapy, and biological therapies. Negative trial results of effective therapies are likely due to lack of patient selection, resulting in the dilution of clinical benefit.

Hypoxia biomarker validation should become a research priority. Noninvasive biomarkers (eg, imaging) are more suitable in NSCLC due to tissue access challenges. Successful biomarker development requires close collaboration between basic and clinical researchers. Several technical (eg, multicenter reproducibility/image analysis) and logistical (eg, radiotracer synthesis and transportation) challenges need to be overcome prior to biomarker investigation within large clinical trials.

Attention should be paid to the design and execution of future hypoxia-targeted therapy trials to avoid the pitfalls of previous experiences and increase the chances of clinical translation. A strong partnership with the pharmaceutical industry is vital for drug development, design of novel clinical trials, and rollout of successful agents. "Window of opportunity" trials provide an ideal platform for expedited investigation of promising therapeutic agents together with hypoxia biomarkers (61). Potentially synergistic therapeutic combinations (eg, hypoxia-targeted therapy/immunotherapy) should be pursued in carefully designed multidisciplinary studies. Finally, the development of companion biomarkers will increase the chances of patient benefit from these therapeutic approaches (133).

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manuscript; or the decision to submit the manuscript for publication.

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