ATOM Protocol

Full title: Pre-operative window of opportunity study of the effects of atovaquone on hypoxia in non-small cell lung carcinoma

Short title: Atovaquone as Tumour HypOxia Modifier

Protocol Version & date:	V6.0, 18Sep2018
Protocol Number:	OCTO_073
EudraCT Number:	2015-003746-15
ClinicalTrials.gov identifier:	NCT02628080
NIHR UKCRN ID:	30445

Sponsor: University of Oxford

Funders: Howat Foundation
OCIC
Oxford Institute Clinical Core

Conflict of Interest statement None of the protocol authors have declared a potential conflict of interest

Confidentiality Statement This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Trial Office, the Investigator Team, host NHS Trust(s), regulatory authorities, and members of the Research Ethics Committee unless authorised to do so.

Key trial contacts

Chief Investigator	Dr Geoff Higgins Consultant Clinical Oncologist and Clinician Scientist CRUK/MRC Oxford Institute for Radiation Oncology University of Oxford Old Road Campus Research Building Oxford OX3 7DQ Tel.: 01865 617355 Email: geoffrey.higgins@oncology.ox.ac.uk
Co-Investigator	Dr Mark McCole (Consultant Cellular Pathologist) Address: John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust Tel: 01865 220492
Co-Investigator	Miss Elizabeth Belcher (Cardio-thoracic consultant surgeon) Address: John Radcliffe Hospital, Oxford University Hospitals NHS Foundation Trust Tel: 01865 221749
Co-Investigator	Professor Fergus Gleeson (Radiology consultant) Address: Churchill Hospital, Oxford University Hospitals NHS Foundation Trust Tel: 01865 235746
Co-Investigator	Dr Ruth Macpherson (Radiology consultant) Address: Churchill Hospital, Oxford University Hospitals NHS Foundation Trust Tel: 01865 235733
Co-Investigator	Dr Michael Skwarski (Clinical Research Fellow) Address: Churchill Hospital, Oxford University Hospitals NHS Foundation Trust Tel: 01865 741841
Co-Investigator	Dr Daniel McGowan (Senior Clinical Lecturer) Address: Department of Oncology, University of Oxford Tel: 01865 857045
Oncology Clinical Trial Office (OCTO)	ATOM Trial Manager Oncology Clinical Trials Office (OCTO) Department of Oncology, The University of Oxford Old Road Campus Research Building Oxford OX3 7DQ, UK Tel: 01865 617003 Email: octo-atom@oncology.ox.ac.uk Website: http://www.oncology.ox.ac.uk/trial/atom
Sponsor	Ms Heather House University of Oxford Clinical Trials and Research Governance Team First Floor, Boundary Brook House Churchill Hospital Oxford OX3 7LQ E-mail: ctrg@admin.ox.ac.uk
Statistician	Jane Holmes

Medical Statistician
Centre for Statistics in Medicine (CSM)
University of Oxford
Botnar Research Centre
Windmill Road
Oxford OX3 7LD

Tel: 01865 223452
Email: jane.holmes@csm.ox.ac.uk

Trial Management Group

Geoff Higgins (Chief Investigator)
Mark Middleton (Professor of Experimental Cancer Medicine)
Jane Holmes (Statistician)

Helen Flight (Portfolio Lead & Trial Manager)

Michael Skwarski (Clinical Research Fellow)
Daniel McGowan (Senior Clinical Lecturer)
Daniel Bulte (Clinical Imaging Hub Leader)

During office hours: Clinical Queries should be directed to ATOM Trial Office as above for action.

Out of office hours: Patients should call the Oxford University Hospitals Trust switchboard on Tel: 01865 741841.

Patient Registration: To register a patient on the trial:
Please email the scanned Registration Form to the ATOM Trial Office: octo-ATOM@oncology.ox.ac.uk

Protocol Authors and conflict of interest statement

Geoff Higgins*[1]
Daniel McGowan*[1]
Michael Skwarski*[2]
Claire Hamill*[1]
Lang'o Odondi*[1]
Victoria Y Strauss[*3]

*There are no relationships/conditions/circumstances that present an actual or potential conflict of interest
[1]University of Oxford, Department of Oncology
[2]Oxford University Hospitals NHS Foundation Trust
[3]University of Oxford, Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS)

TABLE OF CONTENTS

PROTOCOL SYNOPSIS **7**

SUMMARY SCHEDULE OF EVENTS 8
STUDY FLOW CHART 10

ABBREVIATIONS **12**

1 INTRODUCTION **13**

1.1 BACKGROUND 13
1.2 INVESTIGATIONAL MEDICINAL PRODUCT USED IN THE STUDY 13
1.3 OTHER RESEARCH INTERVENTIONS 13
1.4 RATIONALE FOR THE STUDY 16

2 TRIAL DESIGN **16**

2.1 DURATION OF PATIENT PARTICIPATION 17
2.2 POST-TRIAL CARE AND FOLLOW-UP 17

3 OBJECTIVES AND ENDPOINTS **18**

4 PATIENT SELECTION **19**

4.1 ELIGIBILITY CRITERIA FOR ENTRY INTO THE MAIN STUDY 19
4.2 EXCLUSION CRITERIA: 19
4.3 PROTOCOL DEVIATIONS AND ENTRY CRITERIA 20
4.4 RE-SCREENING IF PATIENT DOES NOT MEET INCLUSION/EXCLUSION CRITERIA FIRST TIME ROUND 20
4.5 PATIENT REGISTRATION PROCEDURE 20

5 TRIAL ASSESSMENTS AND PROCEDURES **20**

5.1 INFORMED CONSENT 20
5.2 EVALUATIONS DURING THE STUDY 21
5.3 END OF STUDY EVALUATIONS 22
5.4 EVALUATIONS ON EARLY WITHDRAWAL 22

6 EARLY PATIENT WITHDRAWAL **22**

6.1 PATIENT EVALUABILITY AND REPLACEMENT 23

7 SAMPLES FOR LABORATORY ANALYSIS **23**

7.1 SAMPLES TO BE ANALYSED IN LOCAL TRUST'S LABORATORIES 23
7.2 SAMPLES TO BE SENT TO AND ANALYSED IN A CENTRAL LABORATORY 23
7.3 PHARMACOKINETIC ASSAYS 24
7.4 IMMUNOHISTOCHEMISTRY OF RESECTED TUMOUR 24
7.5 EXPLORATORY CENTRAL LABORATORY ASSAYS 24
7.6 LABELLING AND CONFIDENTIALITY OF SAMPLES SENT 24
7.7 CLINICAL REPORTING OF EXPLORATORY RESEARCH ASSAY RESULTS 24
7.8 TRIAL SAMPLE RETENTION AT END OF STUDY 24
7.9 WITHDRAWAL OF CONSENT FOR SAMPLE COLLECTION AND/OR RETENTION 25

8 INVESTIGATIONAL MEDICINAL PRODUCT (IMP) **25**

8.1 NAME OF IMPS 25
8.2 TREATMENT DOSE 25
8.3 DURATION OF TREATMENT 25
8.4 MANAGEMENT OF DRUG ADMINISTRATION 25
8.5 TREATMENT BREAK 25
8.6 COMPLIANCE 25
8.7 MANAGEMENT OF OVERDOSE 26

9 OTHER TREATMENTS (NON-IMPS) **26**

9.1 PIMONIDAZOLE (PIMO) ADMINISTRATION 26
9.2 SUPPORT MEDICATION 27
9.3 CONCOMITANT MEDICATION AND NON-DRUG THERAPIES 27

9.4 PROHIBITED THERAPIES 27
9.5 (POTENTIAL) DRUG INTERACTIONS 27

10 DRUG MANAGEMENT 27

10.1 DRUG SUPPLIES 27
10.2 DRUG ORDERING 28
10.3 IMP RECEIPT 28
10.4 HANDLING AND STORAGE 28
10.5 LABELLING 28
10.6 DOSING DISPENSING 28
10.7 DRUG ACCOUNTABILITY 28
10.8 DRUG RETURNS FROM PATIENTS 29
10.9 DRUG DESTRUCTION 29
10.10 OCCUPATIONAL SAFETY 29

11 EVALUATION OF RESPONSE TO ATOVAQUONE 29

11.1 TUMOUR ASSESSMENT 29
11.2 TUMOUR RESPONSE 29

12 SAFETY REPORTING 29

12.1 ADVERSE EVENT DEFINITIONS 30
12.2 CLINICAL LABORATORY ABNORMALITIES AND OTHER ABNORMAL ASSESSMENTS AS AES AND SAES 31
12.3 DETERMINING ADVERSE EVENT CAUSALITY 31
12.4 REFERENCE SAFETY INFORMATION (RSI) FOR ASSESSMENT OF EXPECTEDNESS 32
12.5 SUSPECTED UNEXPECTED SERIOUS ADVERSE DRUG REACTIONS (SUSARS) 32
12.6 UNEXPECTED SERIOUS ADVERSE REACTIONS (SARS) TO NIMPS 32
12.7 EXPEDITED REPORTING OF SAES 32
12.8 FOLLOW-UP OF SERIOUS ADVERSE EVENTS 32
12.9 REPORTING ADVERSE EVENTS ON THE CRF 33
12.10 EVENTS EXEMPT FROM BEING REPORTED AS AE/ SAES 33
12.11 INFORMING INVESTIGATORS OF NEW SAFETY INFORMATION 34
12.12 SUMMARY OF TRIAL SAFETY REPORTING REQUIREMENTS 34

13 PREGNANCY 34

14 DEFINING THE END OF TRIAL 34

15 STATISTICAL CONSIDERATIONS 35

15.1 SAMPLE SIZE DETERMINATION 35
15.2 DEFINITION OF PRIMARY AND SECONDARY ENDPOINTS 35

16 STATISTICAL ANALYSIS PLAN 35

16.1 INCLUSION IN ANALYSIS 36
16.2 PROCEDURES FOR REPORTING ANY DEVIATION(S) FROM THE ORIGINAL STATISTICAL PLAN 36
16.3 INTERIM ANALYSIS 36
16.4 FINAL ANALYSIS 36
16.5 OUTCOME REPORT 36

17 TRIAL COMMITTEES 36

17.1 TRIAL MANAGEMENT GROUP (TMG) 36
17.2 DATA AND SAFETY MONITORING 37
17.3 INDEPENDENT RADIOTHERAPY & IMAGING OVERSIGHT COMMITTEE (RIOC) 37

18 DATA MANAGEMENT 37

18.1 DATABASE CONSIDERATIONS 37
18.2 ELECTRONIC CASE REPORTS FORMS (ECRFS) 37
18.3 ACCOUNTING FOR MISSING, UNUSED, OR SPURIOUS DATA 37

19 CLINICAL STUDY REPORT 37

20 STUDY SITE MANAGEMENT 38

20.1 STUDY SITE RESPONSIBILITIES 38
20.2 STUDY SITE SET UP AND ACTIVATION 38
20.3 STUDY DOCUMENTATION 38

21 REGULATORY AND ETHICAL CONSIDERATIONS 38

21.1 ETHICAL CONDUCT OF THE TRIAL AND ETHICS APPROVAL 38
21.2 REGULATORY AUTHORITY APPROVAL 38
21.3 NHS RESEARCH GOVERNANCE 38
21.4 PROTOCOL AMENDMENTS 38
21.5 URGENT SAFETY MEASURES 39
21.6 TEMPORARY HALT 39
21.7 SERIOUS BREACHES 39
21.8 TRIAL REPORTS 39

22 EXPENSES AND BENEFITS 40

23 QUALITY ASSURANCE 40

23.1 RISK ASSESSMENT 40
23.2 MONITORING 40
23.3 AUDIT AND REGULATORY INSPECTION 40

24 RECORDS RETENTION & ARCHIVING 41

25 PATIENT CONFIDENTIALITY 41

26 STUDY FUNDING 41

27 SPONSORSHIP AND INDEMNITY 41

27.1 SPONSORSHIP 41
27.2 INDEMNITY 41
27.3 CONTRACTS/AGREEMENTS 41

28 PUBLICATION POLICY 42

29 REFERENCES 42

APPENDIX 1: ECOG PERFORMANCE SCALE 43

PROTOCOL SYNOPSIS

Full Title of study:	Pre-operative window of opportunity study of the effects of atovaquone on hypoxia in non-small cell lung carcinoma	
Short Title:	Atovaquone as Tumour HypOxia Modifier	
Trial Acronym:	ATOM	
Clinical Phase:	Phase 0	
Study Design:	Open label window of opportunity study	
	Objectives	Endpoints
Primary Aim:	To investigate whether atovaquone reduces tumour hypoxia.	• Average hypoxic volume reduction (%) in 18F-MISO/18-F-FAZA uptake as detected by hyp-PET-CT scans. • Changes in plasma levels of serological markers of hypoxia (osteopontin, CAIX and VEGF). • Differences in scoring and/or reconstructed hypoxic volume from immunohistochemistry of pimonidazole.
Secondary Aims:	To further investigate whether atovaquone reduces tumour hypoxia.	Changes in plasma levels of miR-210 (serological marker of hypoxia).
	To determine whether atovaquone results in a change in tumour perfusion.	Changes in perfusion will be measured by CT, DWI-MRI, DCE-MRI scans and PET kinetic modelling.
	To determine whether serological markers of hypoxia may replace hyp-PET-CT imaging in future studies of hypoxia modification.	hyp-PET-CT derived hypoxic volumes will be compared to changes in plasma levels of serological markers of hypoxia (the most informative tests available will be used as appropriate).
	To assess changes in hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI over time.	Comparison of hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI derived parameters.
Planned enrolment:	Minimum of 20 and maximum of 30 evaluable patients, up to 15 in cohort 1, and up to 15 in cohort 2.	
Target Population:	Patients with confirmed or suspected resectable non-small cell lung cancer (NSCLC).	
	Name of drug	**Formulation, dose, route of administration**
Investigational Medicinal Product	Atovaquone	Oral suspension There will be 2 potential dose levels: 750mg (5ml) bd. 1000mg (6.5ml) bd.
Non-Investigational Medicinal Product	Pimonidazole	Pimonidazole is supplied in 200mg and 300mg capsules for oral use. Single dose will be 500mg/m^2.
Treatment Duration	Minimum number of days of atovaquone treatment required is 7 days between scan dates, duration will be maximised in view of planned surgery date (expected to be between 10-17 days).	
Follow-up duration	Following the end of study visit, patients will receive standard care.	
End of study	Last Patient Last Visit (last visit is the day before surgery which may not be a clinic visit).	

SUMMARY SCHEDULE OF EVENTS

Cohort 1 (Atovaquone group)

		Consent and baseline[i]	Visit 1[i]	Atovaquone Treatment	Visit 2	Day prior to surgery	Surgery
Day	-42 to -1	-41 to 0	1	1-17 [b]	8-17[b]	8-17	9-18
Patient Info sheet	X						
Consent		X					
Pre-dosing evaluations[e]		X					
Vital signs, haematology, biochemistry			X		X		
hyp-PET-CT			X		X		
DWI-MRI[g]			X		X		
DCE-MRI[g]			X		X		
Perfusion CT			X [h]		X [h]		
Research Bloods[a]			X		X		
Atovaquone[b]			X	X (twice daily)	X	X	
Post-dosing evaluations[f]					X		
AE assessment			X		X		
Pimonidazole dispensed					X		
Pimonidazole taken						X	
Surgical resection							X

Cohort 2 (control group)

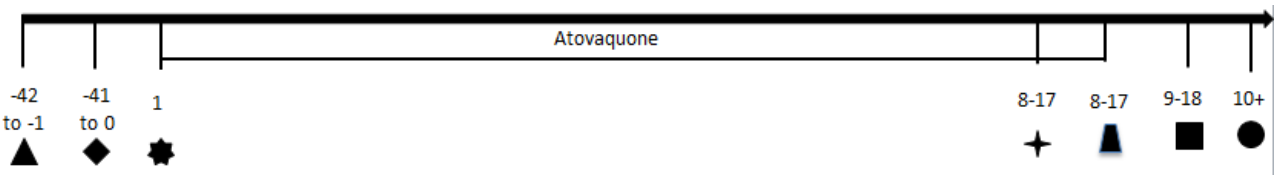
		Consent and baseline[i]	Visit 1[i]	Visit 2	Day prior to surgery	Surgery
Day	-42 to -1	-41 to 0	1[c]	3-17[d]	3-17	4 -18
Patient Info sheet	X					
Consent		X				
Evaluations[e] (as per pre-dosing evaluations for cohort 1)		X				
Vital signs, haematology, biochemistry			X	X		
hyp-PET-CT			X	X		
DWI-MRI[g]			X	X		
DCE-MRI[g]			X	X		
Perfusion CT			X [h]	X [h]		
Research Bloods[a]			X	X		
Evaluations[f] (as per post-dosing evaluations for cohort 1)				X		
AE Assessment			X	X		
Pimonidazole dispensed				X		
Pimonidazole taken					X	
Surgical resection						X

[a] Plasma samples for hypoxia markers (osteopontin, miR-210, VEGF andCAIX)
(Includes PK blood samples for Cohort 1 – no additional draw required)

[b] Number of days of atovaquone treatment to be maximised within constraint of planned surgery date, a minimum of 7 days is required between scan dates. The last dose of atovaquone will be taken the evening before surgery.
[c] Timing of scans in cohort 2 can be flexible. First set of scans to be performed after consent (can be same day).
[d] Timing of scans in cohort 2 can be flexible. Second set of scans to be performed no sooner than 48 hours after first set of scans, and shortly before surgery.
[e] Demographic details, ECOG performance status, haemoglobin, creatinine, ALT, bilirubin, medical history, concomitant medications, pregnancy test, complete physical examination.
[f] ECOG performance status, concomitant medications, complete physical examination.
[g] DWI-MRI and DCE-MRI may be omitted if contraindicated (e.g. if patient is claustrophobic or has a pacemaker)
[h] Perfusion CT may be omitted if contraindicated (e.g. contrast allergy)
[i] The consent and baseline visit and visit 1 can be combined into a single visit if convenient for the patient and deemed appropriate by the trial team.

Study Flow Chart

Cohort 1 (atovaquone group)



▲ Surgical outpatient visit. Cohort 1 Patient Information Sheet given.

◆ Consent obtained. Medical history.

✹ Baseline hyp-PET-CT, pCT, DWI-MRI and DCE-MRI. Baseline plasma osteopontin, miR-210, VEGF and CAIX. Start atovaquone (a minimum number of 7 days is required between scan dates and total duration should be maximised within constraint of planned surgery date). This visit can be performed on the same day as the consent visit, if convenient for the patient and deemed appropriate by trial team.

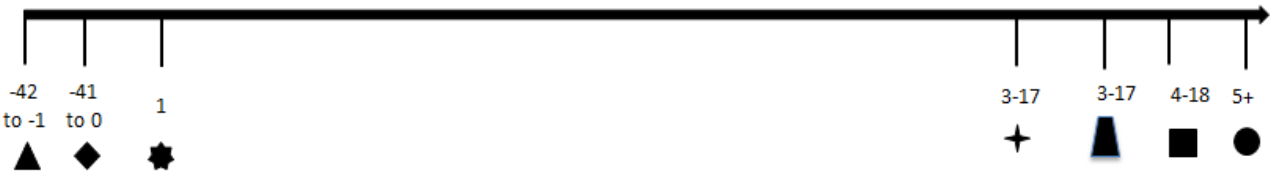
✦ Repeat hyp-PET-CT, pCT, DWI-MRI and DCE-MRI. Repeat plasma osteopontin, miR-210, VEGF, CAIX and plasma atovaquone PK measurement.

Pimonidazole administered 16-24 hours before surgery and atovaquone stopped (last dose of atovaquone evening before surgery).

■ Scheduled surgery. Resected specimen containing tumour inflated and fixed with formalin.

● Tumour orientated to correlate with hyp-PET-CT prior to the specimen cut-up. If specimen orientation is difficult, *ex-vivo* MRI may be performed. Paraffin embedded tumour blocks generated. Tumour IHC for CAIX, pimonidazole, CD31 and CD146. Tumour gene expression and mutational analysis. HPLC analysis of tumour atovaquone concentration.

Cohort 2 (control group)



▲ Surgical outpatient visit. Cohort 2 Patient information sheet given

◆ Consent obtained. Medical history

✹ Baseline hyp-PET-CT, pCT, DWI-MRI and DCE-MRI. Baseline plasma osteopontin, miR-210, VEGF and CAIX. This visit can be performed on the same day as the consent visit, if convenient for the patient and deemed appropriate by the trial team.

✦ Repeat hyp-PET-CT, pCT, DWI-MRI and DCE-MRI. Repeat plasma osteopontin, miR-210, VEGFand CAIX.

Pimonidazole administered 16-24 hours before surgery

■ Scheduled surgery. Resected specimen containing tumour inflated and fixed with formalin.

- Tumour orientated to correlate with hyp-PET-CT prior to the specimen cut-up. If specimen orientation is difficult, *ex-vivo* MRI may be performed. Paraffin embedded tumour blocks generated. Tumour IHC for CAIX, pimonidazole, CD31 and CD146. Tumour gene expression and mutational analysis.

ABBREVIATIONS

ADC	Apparent diffusion coefficient
AE	Adverse Event
ALT	Alanine transaminase
BD	Twice daily
CAIX	Carbonic Anhydrase IX (9)
CSM	Centre for statistics in medicine
CT	Computed Tomography
DCE	Dynamic contrast enhanced
DWI	Diffusion Weighted Imaging
eCRF	Electronic Case Report Form
ECOG	Eastern Cooperative Oncology Group
eGFR	Estimated Glomerular Filtration Rate, usually based on serum Creatinine level, age, sex, and race
^{18}F-MISO	^{18}F-fluoromisonidazole
^{18}F-FAZA	^{18}F-fluoroazomycin arabinoside
HPLC	High Performance Liquid Chromatography
hyp-PET	hypoxia positron emission tomography with either ^{18}F-MISO or ^{18}F-FAZA
IB	Investigator Brochure
IHC	Immunohistochemistry
IR	Ionising radiation
IMP	Investigational Medicinal Product
MA	Marketing Authorisation
MDT	Multidisciplinary team
miR-210	A MicroRNA with altered expression in tumour tissue which may function as an oncogene or tumour-suppressor gene
MRI	Magnetic Resonance Imaging
NIMP	Non-Investigational Medicinal Product
NSCLC	Non-small cell lung cancer
OCIC	Oxford Cancer Imaging Centre
OCTO	Oncology Clinical Trials Office
OS	Overall survival
PK	Pharmacokinetic
PCP	Pneumocystis pneumonia
PET	Positron Emission Tomography
PFS	Progression free survival
PI	Principal Investigator
PIMO	Pimonidazole
REC	Research Ethics Committee
RIOC	Radiotherapy & Imaging Oversight Committee
RSI	Reference safety information
RT	Radiotherapy
SAR	Serious Adverse Reaction
SmPC/SPC	Summary of Product Characteristics
SUV	Standardised uptake value
TBR	Tumour to blood ratio
TMG	Trial Management Group
LPLV	Last visit of the last patient undergoing the trial
NSCLC	Non-small-cell lung carcinoma
SUSAR	Suspected Unexpected Serious Adverse Drug Reaction
VEGF	Vascular endothelial growth factor

1 INTRODUCTION

1.1 Background

Solid tumours often have a highly disorganised tumour microvasculature that results in poor perfusion and oxygen delivery. This combined with their high metabolic rates leads to oxygen demand outstripping oxygen supply causing tumour hypoxia. Tumour hypoxia is integral in the pathophysiology of tumour biology driving multiple cellular processes involved in the hallmarks of cancer and thus malignant progression. In addition, tumour hypoxia decreases the effectiveness of anticancer treatments resulting in poor clinical outcomes. This is especially true for patients treated with radiotherapy since it has been recognised for over 60 years that hypoxic tumour cells require approximately 3 times the dose of radiation to cause the same amount of cell death as cells irradiated under normoxic conditions (1).

To date, the majority of attempts at overcoming tumour hypoxia have focused on increasing oxygen supply. Such treatments include hyperbaric oxygen, ARCON therapy and the use of oxygen mimetics such as nimorazole. However, such techniques have produced modest benefits at best and subsequently have not been adopted into current clinical practice.

An interesting alternative approach to tackling tumour hypoxia is to decrease oxygen 'demand' by reducing tumour oxygen consumption. This strategy has been suggested to be more effective in reducing hypoxia than previous methods aimed at increasing oxygen delivery (2).

Pre-clinical data from our group has demonstrated that the commonly prescribed anti-protozoal drug atovaquone significantly reduces oxygen consumption at clinically relevant concentrations in a variety of tumour cell lines *in vitro*. This reduction in oxygen consumption leads to a profound reduction in tumour hypoxia in subcutaneous mouse xenograft experiments. It is anticipated that if these effects on tumour hypoxia could be reproduced in human patients, then tumours could be rendered markedly more sensitive to radiotherapy.

This window of opportunity trial will assess whether atovaquone significantly reduces tumour hypoxia in adult patients with suspected non-small cell lung cancer. This will be assessed using a combination of functional imaging and plasma markers of hypoxia. If atovaquone is demonstrated to result in a reduction in tumour hypoxia, larger clinical trials will be conducted to determine whether this well-tolerated and inexpensive agent improves radiotherapy efficacy and ultimately clinical outcomes.

1.2 Investigational Medicinal Product used in the study

Atovaquone has an EU marketing authorisation (held by Glaxo Wellcome UK Ltd) and is indicated for acute treatment of mild to moderate Pneumocystis pneumonia (PCP). It is also used in combination with proguanil for malaria prophylaxis. The recommended oral dose for PCP is 750mg (5ml) twice a day for 21 days. Higher dosing regimens [750 mg three times daily (n=8) and 1500 mg twice daily (n=8)] have been given safely in HIV infected volunteers with severity criteria comparable to patients with PCP. Atovaquone will be given for a shorter duration than the recommended standard 21 days in this study (7-17 days). In this study, the first 5 patients in cohort 1 will receive atovaquone at 750mg (5ml) bd. If a drug effect is shown the dose will remain the same. If no drug effect is seen on tumour hypoxia the dose will be increased to 1000mg (6.5ml) bd for the remainder (n=10) of the patients in cohort 1. In this study, atovaquone is being used as a potential modifier of tumour hypoxia.

Mechanism of action

In vitro studies by our group have demonstrated that atovaquone at clinically relevant concentrations significantly reduces oxygen consumption in a number of tumour cell lines. Subcutaneous murine tumour models have been used to confirm that oral atovaquone causes a significant reduction in tumour hypoxia. Our data suggests that atovaquone causes these effects by reducing oxidative phosphorylation in mitochondria.

Pimonidazole is being used in this study. This is a Non-Investigational Medicinal Product (NIMP) (See Section 9).

1.3 Other research interventions

Methods for measuring changes in tumour hypoxia

This trial will use both functional imaging and serological investigations to study the effect of atovaquone on tumour oxygenation. Hyp-PET-CT scans will be used to measure changes in tumour hypoxia and perfusion CT, DWI-MRI and

DCE-MRI scans will be used to measure changes in tumour perfusion providing data on diffusivity and vascular permeability. In addition, changes in plasma levels of known markers of tumour hypoxia (osteopontin, CAIX, VEGF and miR-210 RNA) will also be measured.

These measures of tumour hypoxia will be conducted prior to commencing atovaquone and after 7 to 17 days of twice daily atovaquone treatment. It is expected that the duration of atovaquone treatment will usually range between 10-17 days, but may be as few as 7 days. 17 days is the maximum treatment length. The minimum length of treatment is based on the number of days required to reach steady plasma concentration. Treatment duration will be defined by the constraints of the surgery date and surgery will not be delayed. The methods of measuring tumour hypoxia are discussed briefly below:

Functional imaging to measure changes in tumour oxygen physiology

PET-CT

Positron emission tomography (PET) has become the principle imaging modality for imaging tumour hypoxia and a wide range of PET radiopharmaceuticals have been developed for this purpose. This technique enables non-invasive measurement of tumour oxygenation, provides 3-dimensional (3-D) tumour representation and permits quantification of levels of tumour hypoxia. Of the numerous PET radiotracers available to study hypoxia, ^{18}F-MISO and ^{18}F-FAZA remain the most recognised and studied to date, and will be used in this study.

^{18}F-MISO PET-CT

^{18}F-fluoromisonidazole (^{18}F-MISO) is a radiotracer which belongs to the nitroimidazole family of compounds which passively diffuses across cellular membranes. In low oxygen tensions this tracer is irreversibly trapped intracellularly and therefore accumulates in hypoxic tissues, such as tumours. The amount of tracer uptake within tumours correlates with oxygen levels and this has been verified using invasive hypoxia measuring techniques (3). ^{18}F-MISO PET-CT scans are therefore able to non-invasively image and quantify tumour hypoxia and have been shown to be an independent prognostic measure in head and neck cancer (4, 5, 6).

^{18}F-FAZA PET-CT

^{18}F-fluoroazomycin arabinoside (^{18}F-FAZA) is a second generation 2-nitroimidazole tracer which has been developed to have faster tumour-specific accumulation and reduced washout due to its clearance and hydrophilicity characteristics.

We define “hyp-PET-CT” as PET-CT using either ^{18}F-MISO or ^{18}F-FAZA. ^{18}F-MISO will be the principally used tracer; ^{18}F-FAZA will be used in the situation that ^{18}F-MISO is unavailable.

Perfusion CT

Dynamic contrast-enhanced perfusion CT is a technique used to study the vasculature within tumours and other tissues (7). It is non-invasive and readily incorporated into existing CT protocols using conventional contrast agents and provides information on tumour blood flow, blood volume and mean transit time. Used alongside hyp-PET-CT, perfusion CT will provide important information on the effect of treatment with atovaquone on tumour vasculature.

DWI-MRI

High-resolution MRI at 3T will be used to investigate tumour properties. Diffusion-weighted imaging (DWI-MRI) provides image contrast based on the small random motion of water. In highly restricted regions such as the tumour core there is low diffusion, whereas if a tumour shrinks and the volume is replaced by fluid the diffusion dramatically increases. DWI will be performed with b values of 0 and 1000 s/mm2, and an apparent diffusion coefficient (ADC) map will be calculated. The ADC is affected by cellularity, extracellular space, and macromolecules in tissue. MRI scans may be contraindicated in some patients (e.g. due to claustrophobia or a pacemaker). Where this is the case the MRI scans may be omitted and relevant trial endpoints will be assessed only by the other imaging methods.

DCE-MRI

This study will also use T1 weighted dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) as a non-invasive method of assessing the perfusion and permeability of tumour vasculature. Tumour vessel permeability is an important measure which, at least in part, reflects important tumour processes such as angiogenesis. Using the two compartment Tofts model, transendothelial permeability, capillary surface area, and lesion leakage space can be measured using quantitative parameters. In addition, to measuring tumour hypoxia and perfusion, determining the effect of treatment with atovaquone on tumour vascular permeability by this method is important to fully investigate and appreciate the effect of this treatment on tumour oxygen physiology as a whole. MRI scans may be contraindicated in some patients (e.g. due to claustrophobia or a pacemaker). Where this is the case the MRI scans may be omitted and relevant trial endpoints will be assessed only by the other imaging methods.

Serological markers to measure changes in tumour hypoxia

A number of circulating plasma markers of tumour hypoxia have been discovered including osteopontin, CAIX, VEGF and miR210 RNA. The expression of these markers correlates with tumour hypoxia, and plasma levels have been shown to correlate with other measures of tumour hypoxia as outlined below. In this study the effects of atovaquone on the levels of these plasma hypoxia markers will also be evaluated. Furthermore, this trial will investigate whether such markers correlate with hypoxia functional imaging results and therefore whether these circulating markers can be used alone, or in combination, as a potential 'plasma hypoxia signature' in future tumour hypoxia trials.

Osteopontin

Osteopontin is a secreted phosphoglycoprotein (SSP1) expressed by tumour cells as well as a range of human tissues. Tumour expression of this protein can be measured in plasma, and has been shown to increase under conditions of tumour hypoxia. Several important cellular signalling pathways including the PI3 kinase, MAPK and NF-kB/IkBq/IKK pathways are activated by osteopontin and therefore this protein influences multiple tumour processes responsible for tumour progression and metastasis. The level of osteopontin is often viewed as a biomarker of tumour aggressiveness and elevated levels of osteopontin have been associated with poor clinical outcomes in a number of tumour types. Furthermore, higher levels of this protein has been linked to a significant reduction in the effectiveness of radiotherapy in a number of tumour types including prostate cancer and head and neck cancers.

Carbonic anhydrase IX

Carbonic anhydrase isoform IX (CAIX) is a metalloenzyme overexpressed in tumour cells and is significantly over-expressed in the presence of tumour hypoxia. The expression of CAIX is important in mediating the hypoxia-induced stress response in cancer cells. In particular, CAIX is responsible for mediating the intracellular and extracellular pH under hypoxic conditions enabling cancer cell survival under such conditions. High circulating levels of CAIX have been shown to be an independent biomarker of prognosis in patients with a number of tumour types.

MiR-210 RNA

MicroRNAs (miRNAs) are non-coding oligonucleotides, which bind to target genes inhibiting translation or resulting in mRNA degradation and constitute an important post-translational gene regulation mechanism. Under hypoxic conditions HIF-1 is one of the main transcription factors responsible for expression of miRNAs. MiRNA-210 (miR-210) is one of the commonly upregulated miRNAs under hypoxic conditions and its expression has been shown to correlate with other measures of tumour hypoxia and patient outcome measures in a number of tumour types. In addition to expression in tumour tissue, expression levels in other bodily fluids, including circulating levels of miR-210 have been shown to be stable and easily quantifiable using RT-PCR techniques and also have been linked with patient outcomes. This suggests that plasma miR-210 may be an important hypoxia-related prognostic biomarker.

VEGF

Since its isolation nearly half a century ago, vascular endothelial growth factor (VEGF) has become the most well characterised cytokine governing tumour angiogenesis. The VEGF family is comprised of seven glycoproteins that share a common VEGF homology domain (VGF-A, -B, -C, -D, -E, and PlGF-1 and -2) which stimulate VEGF receptors expressed on endothelial cells of arteries, veins, lymphatics and also on tumour cells. VEGF is one of the most potent mitogens for endothelial cells and a potent promoter of vascular permeability. Subsequent release of plasma proteins into the interstitial space results in a further pro-angiogenic environment. In addition, VEGF regulates the function of matrix-degrading metalloprotinases in basement membrane degradation and endothelial cell migration thus formation of new vessel networks.

Under hypoxic conditions, HIF-1 translocates to the nucleus, binds to the VEGF promoter, and leads to increased VEGF transcription. High plasma levels of VEGF have been demonstrated to be associated with inferior clinical outcomes in a number of tumour types including NSCLC.

PK studies

Pre-clinical studies suggest that the observed effects of atovaquone on reducing tumour hypoxia *in vivo* occur at plasma levels likely to be observed in this study. Plasma atovaquone levels will be measured after a minimum of 7 days of atovaquone (at visit 2) and also in the resected tumour specimen to assess whether variations in atovaquone concentrations correlate with atovaquone induced changes in tumour hypoxia.

Tumour Immunohistochemistry for hypoxia

The 'gold standard' method for measuring tumour hypoxia is pO2 polarography. However, this direct method is invasive, resulting in pain, tissue damage and is associated with a risk of bleeding and infection. Sampling is limited to anatomically convenient sites and an additional imaging modality needs to be employed to distinguish between hypoxic but viable tumour and radiobiologically insignificant necrotic areas. An additional method of characterising tumour hypoxia is immunohistochemistry for exogenous hypoxia markers – predominantly nitroimidazole based compounds such as pimonidazole.

Pimonidazole (PIMO) immunohistochemistry will be used to characterise hypoxic areas. PIMO is a 2-nitroimidazole derived compound which is ingested as an encapsulated solid 16-24 hours prior to surgical excision. PIMO uptake in cells offers qualitative information with respect to cellular oxygen levels.

In this study immunohistochemical staining will also be performed for endogenous hypoxia and vasculature markers to further characterise tumour hypoxia. The endogenous markers investigated in this study are CAIX, CD31 and CD146.

1.4 Rationale for the study

Non-clinical

Using the Seahorse XF analyzer, our group has recently undertaken a screen of 1600 FDA approved compounds to identify drugs which decrease oxygen consumption of FaDu hypopharangeal squamous cell carcinoma cells. This screen found that atovaquone significantly reduces oxygen consumption at clinically relevant concentrations within 30 minutes of administration in a large panel of tumour cell lines (including NSCLC lines). The observed reduction in oxygen consumption is postulated to occur through inhibition of the electron transport chain reducing oxidative phosphorylation.

Subsequent 3-D tumour spheroid experiments using FaDu and HCT116 cells have shown that atovaquone administration reduces hypoxia, as assessed by EF5 immunohistochemical staining after 24 hours of treatment. Subcutaneous murine experiments have subsequently confirmed that atovaquone exerts similar effects *in vivo*. Seven days of oral atovaquone at clinically relevant doses reduced tumour hypoxia by approximately 90%.

If atovaquone was shown to reduce tumour hypoxia in patients, it is likely that combining this treatment with radiotherapy would be a safe and inexpensive method of markedly improving the outcomes for patients treated with radiotherapy. This window of opportunity study will investigate whether atovaquone reduces tumour hypoxia in patients prior to embarking on subsequent efficacy trials combining this drug with radiotherapy.

Clinical

Although there is much data on the use of atovaquone as an anti-protozoal treatment, there are no published studies of atovaquone in modifying tumour hypoxia *in vitro* or *in vivo*. Consequently, no clinical studies have previously investigated the possible uses of this drug in this context.

2 TRIAL DESIGN

This is a phase 0, open label, 'window of opportunity' study investigating whether a short course of twice-daily atovaquone reduces tumour hypoxia in patients with suspected non-small cell lung cancer (NSCLC) prior to surgical resection.

Two cohorts of patients will be recruited. Patients who are considered likely to have hypoxic tumours will be recruited from Oxford University Hospitals NHS Foundation Trust and other referring local NHS trusts.

A maximum 30 evaluable patients will be recruited into the 2 cohorts with a minimum of 10 patients per cohort. Initially a minimum of 10 and maximum of 15 evaluable patients will be recruited to Cohort 1 then Cohort 2 will be opened. Timing of opening Cohort 2 will be decided by the TMG on review of recruitment rate and to ensure adequate time is available to recruit to Cohort 2. Recruitment to Cohort 2 will then continue until an equal number of evaluable participants are recruited. Once this is achieved the TMG will meet and dependent on the available time remaining to recruit to the trial will either:

a) Close recruitment or

b) Continue recruitment by first filling any remaining slots in Cohort 1 (up to a maximum of 15 evaluable participants) then filling any remaining slots in Cohort 2.

The investigators will endeavour to ensure that, as far as possible, patients in Cohort 2 are broadly balanced with Cohort 1 on key features such as tumour size and duration between F-MISO scans. However this may be limited by recruitment rate and other operational issues (e.g. available scan & surgery dates). Patients in Cohort 2 will not be formally matched with patients in Cohort 1. Any variation in key features between the cohorts will be described in the study report.

For both cohorts, baseline tumour imaging will be performed and plasma hypoxia markers will be measured and then repeated prior to surgical resection.

The same tracer for the hyp-PET-CT must be used for both sets of scans, per individual patient.

Oral pimonidazole (NIMP) will be given to all patients 16-24 hours prior to surgical resection and used to assess the degree of hypoxia present in the resected tumour by immunohistochemistry.

In cohort 1, between the two imaging time points, patients will receive a minimum of 7 days of oral atovaquone (IMP), 10-17 days is preferable. The first 5 patients will receive the standard clinical dose 750mg (5ml) bd. If a drug effect is shown the dose will remain the same. If no drug effect is shown, the dose will be increased to 1000mg (6.5ml) bd for the remaining Patients (n=10) in Cohort 1.

In Cohort 2 the patients will receive no intervention between the two imaging points.
Cohort 2 will:

- Allow for comparison with Cohort 1 in terms of levels of hypoxia by comparing the amount of pimonidazole staining in resected tumours, thus utilising another measure for whether atovaquone causes a reduction in tumour hypoxia.
- Allow for comparison with Cohort 1 in terms of tumour perfusion
- Combine with the data from Cohort 1 to provide greater numbers to the secondary objective considering replacing hyp-PET-CT by assessment of serological plasma hypoxia markers.
- To assess changes in hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI over time.
- Provide test-retest of serology correlated with immunohistochemistry on pimonidazole staining
- Provide comparison with Cohort 1 of hypoxia metagene signature in resected tumours
- Allow correlation of hypoxia metagene signature with pimonidazole staining in resected tumours
- Provide comparison with Cohort 1 of PFS and OS

Refer to the schedule of events and flow chart for details of the study visits and procedures.

2.1 Duration of patient participation

Participants will typically be on the study for approximately 3 weeks from consent to last protocol visit. The last dose of atovaquone is the evening prior to surgery. For both cohorts the day before surgery is the last trial visit (although the patient does not attend clinic on this day).

2.2 Post-trial care and follow-up

Following the end of study visit, patients will receive standard care.

3 OBJECTIVES AND ENDPOINTS

Primary Objective	Endpoints/ Outcome measures	Time point(s) of evaluation of this end point	Groups for comparison
• To investigate whether atovaquone reduces tumour hypoxia.	• Average hypoxic volume reduction (%) in ^{18}F-MISO/^{18}-F-FAZA uptake as detected by hyp-PET-CT scans. • Changes in levels of plasma markers of tumour hypoxia (osteopontin, CAIX, and VEGF) • Differences in scoring and/or reconstructed hypoxic volume from the immunohistochemistry of PIMO	• baseline and pre-surgery • baseline and pre-surgery • post-surgery	• Within Cohort 1 (pre and post-treatment) AND • Between Cohort 1 and Cohort 2
Secondary Objectives	**Endpoints/Outcome measures**	**Time point(s) of evaluation of this end point**	
• To further investigate whether atovaquone reduces tumour hypoxia.	• Changes in plasma levels of miR-210 (plasma marker of hypoxia)	• baseline and pre-surgery	• Within Cohort 1 (pre and post-treatment) AND • Between Cohort 1 and Cohort 2
• To determine whether atovaquone results in a change in tumour perfusion.	• Changes in perfusion will be measured by CT, DWI-MRI, DCE-MRI scans and PET kinetic modelling	• Tumour perfusion will be measured at baseline and pre-surgery	• Within cohort 1 (pre and post--treatment) AND • Between Cohort 1 and Cohort 2
• To determine whether plasma markers of hypoxia may replace hyp-PET-CT imaging in future studies of hypoxia modification.	• hyp-PET-CT derived hypoxic volumes will be compared to changes in levels of plasma markers of hypoxia (the most informative tests available will be used as appropriate)	• Correlate levels of plasma markers of hypoxia with ^{18}F-MISO/^{18}-F-FAZA uptake at baseline and pre-surgery	• Cohort 1 & Cohort 2 combined: baseline vs. pre-surgery
• To assess changes in hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI over time.	• Comparison of hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI derived parameters	• baseline and pre-surgery	• Within Cohort 2
Tertiary/Exploratory Objectives	**Endpoints**		
• To assess whether PK levels of atovaquone in plasma and in the resected tumour correlate with hypoxia modification	• HPLC based measurement of plasma level of atovaquone • HPLC based measurement of tumour level of atovaquone	• measured following 7-17 days treatment • measured post-resection	• Cohort 1
• Correlations between imaging and histology	• Comparison of histological hypoxia and vasculature parameters with imaging measuring hypoxia, perfusion and glycolysis	• baseline and pre-surgery • post-surgery (histological parameters) • pre-study (routine staging FDG-PET)	• Cohort 1 & Cohort 2 combined
• Correlations between plasma hypoxia markers and histology	• Comparison of plasma hypoxia parameters with immunohistochemistry on pimonidazole staining	• Pre-surgery and • Post-resection	• Between Cohort 1 and Cohort 2
• To assess whether underlying genetic changes in the tumour act as markers of hypoxia level • To assess whether atovaquone results in a lower level of hypoxia metagene signature expression	• Comparison of hypoxia metagene signature expression in resected tumour with hypoxia levels assessed by other means • Individual tests to measure gene expression/mutation as required	• Post-resection vs. baseline and pre-surgery	• Cohort 1 & Cohort 2 combined • Between Cohort 1 and Cohort 2
• To assess whether a reduction in hypoxic volume improves survival	• Progression free survival • Overall survival	• Surgical co-investigators will be asked to provide data collected at routine surgical follow-up clinic at 12 & 24 months from Day1	• Within Cohort 1 (pre and post-treatment) AND • Between Cohort 1 and Cohort 2
• To assess immune system interaction with tumour	• Immunohistochemistry staining of resected tumour tissue for relevant	• post-surgery	• Cohort 1 and Cohort 2

hypoxia	markers of leukocyte subtypes/ immune response • Comparison with pimonidazole immunohistochemistry staining		
• To assess the effect of atovaquone treatment on peripheral blood mononuclear cells (PBMCs)	• Comparison of PBMC immunopopulations	• baseline and pre-surgery	• Cohort 1 and Cohort 2
• To study metabolite levels in hypoxic tumour tissue	• Metabolite levels in resected tumour tissue • Comparison with pimonidazole immunohistochemistry staining	• post-surgery	• Cohort 1 and Cohort 2

4 PATIENT SELECTION

Written informed consent must be obtained before any study specific procedures are performed. The Investigator will determine patient eligibility based on the following criteria.

4.1 Eligibility criteria for entry into the main study

Inclusion criteria:

A patient will be eligible for inclusion in this study if all of the following criteria apply.

1. Confirmed or suspected NSCLC considered suitable for surgical resection by the lung MDT.
2. At least one measurable lesion (greater than 2cm maximal length in any direction) that the investigators consider on routine imaging (CT or PET-CT scan performed in the 60 days prior to consent (older scans may be accepted at the discretion of the CI providing the results remain clinically significant)) likely to contain regions of hypoxia.
3. Male or female, Age $\geq$ 18 years.
4. ECOG performance score of 0-2
5. The patient is willing and able to comply with the protocol, scheduled follow-up visits and examinations for the duration of the study.
6. Written (signed and dated) informed consent.
7. Haematological and biochemical indices within the ranges shown below:

Lab Test	Value required
Haemoglobin (Hb)	≥ 9.0 g/dL
Creatinine	Below 2 x upper limit of normal
ALT and bilirubin	Below 2.5 x upper limit of normal range

4.2 Exclusion criteria:

A patient will not be eligible for the trial if any of the following apply:

1. Previous systemic chemotherapy or biological therapy within 21 days of commencing atovaquone treatment.
2. Treatment with any other investigational agent, or participation in another interventional clinical trial within 28 days prior to enrolment.
3. Known previous adverse reaction to atovaquone or its excipients.
4. Active hepatitis, gallbladder disease or pancreatitis
5. Patients with impaired gastrointestinal (GI) function or GI disease that may significantly alter absorption of atovaquone.
6. Administration of contraindicated agents in the 14 days prior to starting atovaquone as outlined in section 9.4 and the current atovaquone SmPC.
7. Concurrent administration of warfarin in the 14 days prior to starting atovaquone.
8. Concurrent administration of known electron transport chain inhibitors, such as metformin, is prohibited. A wash-out period prior to administration of atovaquone is required (e.g. 4 days for metformin). Refer to section 9.4 for further detail. .
9. Other psychological, social or medical condition, physical examination finding or a laboratory abnormality that the Investigator considers would make the patient a poor trial candidate or could interfere with protocol compliance or the interpretation of trial results.

10. Patients who are known to be serologically positive for Hepatitis B, Hepatitis C or HIV (Hepatitis and HIV testing specifically for confirming eligibility for this trial are not required).
11. Pregnant or breast-feeding women or women of childbearing potential unless highly effective methods of contraception are used.

4.3 Protocol deviations and entry criteria

Protocol adherence is a fundamental part of the conduct of a clinical study. Changes to the approved protocol need prior approval unless for urgent safety reasons.

Investigators must contact OCTO to obtain guidance and/or clarification as necessary if unsure whether the patient satisfies all the entry criteria and to clarify matters of clinical discretion. OCTO will contact the chief investigator or clinical coordinators as necessary.

Investigators should not request a protocol waiver to enter a patient who does not satisfy the selection criteria.

The investigator must document and explain any deviations/violations from the approved protocol. The investigator should promptly report any important violations that might impact patient safety, data integrity or be a possible serious breach (section 21.7) to the trial office.

4.4 Re-screening if patient does not meet inclusion/exclusion criteria first time round

Screen failures are ineligible and will not be rescreened.

4.5 Patient registration procedure

Participants will be recruited from the lung MDT and via referral from Oxford Cancer Centre services for the surgical management of suspected NSCLC. Eligible patients with suspected NSCLC will include candidates for surgical resection in the period between staging and resection.

A screening log must be kept of all patients considered for the study including any that are subsequently excluded; the reason for exclusion must be recorded on this form. A copy of the screening log should be sent to the trial office on request, but without patient identifiers. The original must be retained on site.

Before entering a patient onto the study the Principal Investigator or designee will confirm eligibility. If in any doubt the Chief Investigator must be consulted before entering the patient. Details of the query and outcome of the decision must be documented on the registration/ eligibility checklist.

After completing suitability checks, Informed Consent Form for trial participation and blood and tissue sample collection and the Registration Form for the patient, site staff will complete the trial registration form(s) and email the scanned form to the trial office to confirm the patient's eligibility. The patient will then be registered, where applicable.

5 TRIAL ASSESSMENTS AND PROCEDURES

Please refer to the Schedule of Investigations given at the front of this protocol. Details of all protocol evaluations and investigations must be recorded in the patient's medical record for extraction onto the CRF.

5.1 Informed consent

Potential participants will be given a current, approved version of the patient information sheet and consent form. They will also receive clear verbal information about the study detailing no less than: the nature of the study; the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be explained that they will be free to withdraw from the study at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal. They will have at least 24 hours to consider the information provided and the opportunity to question the Investigator, their GP or other independent parties before deciding whether to participate.

The Investigator who obtains consent must be suitably qualified and experienced. All delegates must be authorised by the Chief/Principal Investigator to obtain consent. The Investigator is responsible for ensuring that the trial consent procedures comply with current applicable GCP Regulatory and ethical requirements. Informed consent discussions and outcomes must be well documented in the medical record. The Investigator must be satisfied that the patient has

made an informed decision before taking consent. The patient and the Investigator must personally sign and date the current approved version of the informed consent form in each other's presence. A copy of the information and signed consent form will be given to the participant. The original signed form will be retained at the trial site, with copies held in both the medical record and Investigator Site File (ideally the original if local policy permits).

Contraceptive/ Pregnancy counselling

All participants must be advised on the need to use highly effective methods of contraception during the study. The advice should include:

(1) The acceptable methods, including: combined (oestrogen and progesterone containing oral, intravaginal or transdermal) or progesterone only (oral, injectable or implantable) hormonal contraception associated with inhibition of ovulation, intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomised partner (provided that partner is the sole sexual partner of the WOCBP and that the vasectomised partner has received medical assessment of the surgical success) and sexual abstinence (however, this is only considered effective if defined as refraining from heterosexual intercourse from registration until 30 days post last atovaquone administration).
(2) Males should continue to take these precautions for a minimum of 1 month after the last dose of study drug.
(3) Females should continue to take these precautions a minimum of 1 month after the last dose of study drug.
(4) That any pregnancy (also applies to females partners of male trial subjects) occurring within 16 weeks of the last administration of study drug / other trial intervention should be notified by the trial participant to the study team. The pregnancy will be followed up and the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) will be reported and followed up even if participant is discontinued from the study.

Medical History and concomitant medications

Medical history includes clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 4 weeks prior to Day 1. A history of pleural or pericardial effusion or of ascites requiring intervention should be entered in the medical history. Cancer history will include an assessment of prior treatment with BCG for early bladder cancer.

Demographic details

Demographic data will include age, ECOG performance status, sex, and self-reported race/ethnicity.

Physical examination

A complete physical examination should include an evaluation of the head, eyes, ears, nose, and throat and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. Any abnormality identified at baseline should be recorded on the General Medical History and Baseline Conditions eCRF. Height and weight should be measured and recorded in the eCRF. At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations should be performed. Changes from baseline abnormalities should be recorded in patient notes. New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

5.2 Evaluations during the study

A breakdown of the investigations performed at each visit are summarised below:

Assessment of eligibility

The following assessments must be performed/obtained between days -41 and day 0

- Demographic details
- ECOG performance status
- Haemoglobin (must be ≥ 9.0g/dL)
- Creatinine (must be below 2 x upper limit of normal range)
- ALT and bilirubin (must be below 2.5 x upper limit of normal range)
- Medical History
- Concomitant medications
- Pregnancy test: serum or urine Human Chorionic Gonadotropin (HCG) test to rule out pregnancy at study entry; results must be obtained and reviewed before the first dose of atovaquone is administered or any ionising imaging is performed.
- Complete physical examination as described above

Evaluations on day 1 (visit 1) (pre-dosing evaluations (or equivalent day for Cohort 2))

On the day the first dose of atovaquone is given (or the equivalent day for Cohort 2):

- Vital signs: systolic / diastolic blood pressure (BP), pulse rate, temperature and body surface area (BSA).
- Haematology – white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets.
- Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, alk phos, and INR.
- Plasma samples for hypoxia markers (osteopontin, VEGF, CAIX and miR-210 RNA) will be collected.
- hyp-PET-CT, perfusion CT. The same tracer for the hyp-PET-CT must be used for both sets of scans, per individual patient. The perfusion CT may be omitted if contraindicated (e.g. contrast allergy)
- DWI-MRI and DCE-MRI. The MRI scans may be omitted if contraindicated (e.g. if patient is claustrophobic or has a pacemaker).
- Cohort 1 patients will be supplied with atovaquone and will commence daily treatment on day 1 for 7-17 days.

Where convenient for the patient and the trial site, visit 1 may be organised to take place on the same day as the consent and screening visit, to reduce the number of additional visits for the patient.

Evaluations on visit 2(or the equivalent day for Cohort 2) (day 8-17; pre-surgery)

- ECOG performance status.
- Vital signs: systolic /diastolic blood pressure (BP), pulse rate and temperature.
- Haematology – Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets
- Biochemistry – sodium, potassium, calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alk phos, ALT and INR.
- Concomitant medications
- Complete physical examination as described above.
- Toxicity screen
- Plasma samples for hypoxia markers (osteopontin, VEGF, CAIX and miR-210 RNA) will be collected.
- Hyp-PET-CT, perfusion CT. The same tracer for the hyp-PET-CT must be used for both sets of scans, per individual patient.
- DWI-MRI and DCE-MRI. The MRI scans may be omitted if contraindicated (e.g. if patient is claustrophobic or has a pacemaker).
- Atovaquone PK blood sample (Cohort 1 only)
- Pimonidazole administration (both cohorts)

5.3 End of study evaluations

The last day of study participation is the day before surgery. If a patient is subsequently unable to have surgery, their imaging and plasma investigations will still be suitable for evaluation and the patient will not be replaced.

Follow-up evaluations

The patient will return to follow-up as per standard care in the NHS. Patients will be followed for progression free survival and overall survival via routine surgical follow-up clinics. The surgical co-investigators will be asked to provide these data at approximately 12 months and 24 months from Day 1. This data will be requested via the notes and a clinic visit is not required.

5.4 Evaluations on early withdrawal

Where possible, patients who withdraw early from the study will be evaluated as described in the 'end of study evaluation' section above.

6 EARLY PATIENT WITHDRAWAL

Treatment Withdrawal

During the course of the trial, a patient may withdraw early from treatment. This may happen for a number of reasons, including:

- AE/SAEs requiring discontinuation

- Significant protocol deviation or inability to comply with trial procedures
- Clinical decision (other than disease progression)
- Disease Progression
- Patient decision
- Consent withdrawn
- Pregnancy
- Deceased

When the patient stops treatment early, the 'End of Treatment' Form needs to be completed, and any other relevant CRFs (example SAE Form). The reason for withdrawing from treatment early should be clearly documented in the medical records. Patients who withdraw from the study prior to the second hyp-PET-CT scan will not be considered evaluable for the primary endpoint and will be replaced.

Patients in cohort 1 who do not take at least 75% of the total minimum required dose of atovaquone between scan days will be considered to be unevaluable. This will mark the end of their involvement in the trial and no further trial procedures will be performed with that patient.

Consent Withdrawal
Consent withdrawal means that a patient has expressed a wish to withdraw from the study altogether. Under these circumstances, the site needs to document all relevant discussions in the patient notes and notify the Trial Office, which will allow the office to mark all future CRFs as not applicable.

Under these conditions, investigators are still responsible to follow up any SAEs till resolution.

6.1 Patient evaluability and replacement

Data from the trial will be analysed by a modified intention-to-treat approach.
Patients who are not evaluable for the primary endpoint may be replaced by recruitment of a further participant into the same cohort. This includes:

- patients without a NSCLC diagnosis (e.g. if the lesion was a metastasis from a different primary cancer) on post-surgical pathology
- patients who did not have the second hyp-PET-CT scan
- patients who do not have two sets of technically interpretable hyp-PET-CT scans
- patients who do not have sufficient baseline hypoxia on first hyp-PET_CT scan
- patients in cohort 1 who took less than 75% of total minimum required atovaquone dose between scan days

Situations where patients will <u>not</u> be replaced are:

- Patients who do not go on to have surgery
- Patients who do not take pimonidazole
- Patients with missing blood sample collection or unsatisfactory blood test results (non-analysable)

7 SAMPLES FOR LABORATORY ANALYSIS

7.1 Samples to be analysed in local Trust's laboratories

Diagnostic Laboratories
Samples for haematology and biochemistry analysis will be labelled with standard patient identifiers and sent to the local hospital diagnostic laboratory. Results will be processed in the standard way and entered into the routine hospital reporting system. Samples will be stored, held, reported and subsequently destroyed in accordance with standard local laboratory practice.

7.2 Samples to be sent to and analysed in a Central Laboratory

All research samples will be collected, processed, stored and forwarded to the relevant laboratory by the clinical study site in accordance with written instructions that will be provided in a separate Sample Handling Manual. In the presence of limited material, prioritisation of the analyses will be made by the Chief Investigator.

Genetic analysis will be performed to provide gene expression and mutational characterisation of the resected tumour. Particular attention will be paid to genes that are thought to be associated with hypoxia and reoxygenation.

This work will be conducted through a third party central laboratory, the details of which are to be decided. This is to perform gene expression analysis of a validated hypoxia metagene profile. This metagene analysis will be correlated with the hyp-PET and immunohistochemistry derived hypoxia data.

For plasma markers of hypoxia, blood samples will be collected at time points indicated in section 5. These will be forwarded to the University of Oxford GCP lab by the clinical study site.

7.3 Pharmacokinetic assays

For atovaquone PK studies plasma will be collected from cohort 1 patients on the day of the second set of scans. Samples will be retained and dispatched to the University of Oxford Bioanalysis Core Laboratories for HPLC plasma atovaquone concentration measurement.

Atovaquone concentration will also be measured in resected tumour specimens. Tumour samples will be retained and dispatched to the University of Oxford Bioanalysis Core Laboratories for HPLC tumour atovaquone concentration measurement.

7.4 Immunohistochemistry of resected tumour

The resected lung specimen will follow the usual route of processing and be fixed with formalin and embedded in wax blocks as per standard clinical practice. The specimen will be orientated using the preoperative hyp-PET-CT scan taken as part of this study. To ensure that sectioning of the tumour correlates with the hyp-PET-CT scan images the study representative, radiologist and pathologist will all be present during this step. If orientation is proving difficult, an MRI scan of the resected lung specimen will be performed to help guide sectioning.

From the tumour blocks generated, slides will be produced and forwarded to the University of Oxford GCP lab for immunohistochemistry staining. Immunocytochemistry for pimonidazole, CAIX, CD31 and CD146 will be performed to investigate hypoxia and vascularity. This data will be correlated with preoperative research imaging and serological hypoxia markers and also with results from tumour gene expression and mutational analysis.

7.5 Exploratory central laboratory assays

In collaboration with other researchers within the University of Oxford and/or in specialist laboratories located elsewhere a number of exploratory assays are also planned:

- Immunohistochemistry staining of resected tumour tissue for relevant markers of leukocyte subtypes/ immune response, in order to assess immune system interaction with hypoxic tumour tissue.
- Comparison of peripheral blood mononuclear cell immunopopulations, to assess the effect of atovaquone treatment on these.
- Study of metabolite levels in resected tumour tissue.

7.6 Labelling and confidentiality of samples sent

All samples sent to analytical Laboratories will be labelled with the trial code, trial patient number, and date taken. Should a laboratory receive any samples carrying unique patient identifiers the recipient must immediately obliterate this information and re-label. The study site will be informed of their error.

7.7 Clinical reporting of exploratory research assay results

The results of the research assays undertaken within the trial are exploratory and are not intended to influence individual patient medical care. Findings will not be reported routinely to the responsible clinician except in the unlikely event that the result might be beneficial to the patient's clinical management.

7.8 Trial sample retention at end of study

The Chief Investigator has overall responsibility for custodianship of the trial samples. Laboratories are instructed to retain any surplus samples pending instruction from the Chief Investigator on use, storage or destruction. It is possible that new or alternative assays may be of future scientific interest. At the end of the research study any surplus samples will be retained for possible use in other projects that have received ethical approval. During the consent process for this study, consent for the use of unused or surplus samples in future research that is approved by a research ethics committee will be requested. Hence, any surplus study samples may be transferred to a licensed tissue bank where they will be managed in accordance with applicable host institution policies and the Human Tissue Act (HTA) requirements.

7.9 Withdrawal of consent for sample collection and/or retention

A patient may withdraw consent to provide samples for research at any time without giving a reason. The Investigator must ensure that their wishes are recorded in the medical record and will inform the Trial Office accordingly. The investigator should discuss with patients the valuable use of samples that have already been provided and under circumstances where these samples have already been processed and anonymised; it would not be possible to destroy such samples.

8 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

8.1 Name of IMPs

Atovaquone suspension (750mg/5ml)

8.2 Treatment dose

Atovaquone will be supplied as a suspension (750mg/5ml) and administered by the patient twice daily commencing on the evening of day 1. Patients will be provided with a syringe rather than a 5ml spoon to accurately measure each dose of suspension. The dose will not be adjusted to body weight or surface area. The first 5 patients in cohort 1 will receive atovaquone at 750mg (5ml) bd. If activity is shown the dose will remain the same. If no activity is shown the dose will be increased to 1000mg (6.5ml) bd for the remainder of the patients in cohort 1 (n=10).

8.3 Duration of treatment

Patients in cohort 1 should take atovaquone twice daily for a minimum of 7 consecutive days between scan dates. This is based on the number of days required to reach a steady state. Treatment should continue until the day before surgery with the last dose administered the evening prior to surgery. The maximum atovaquone treatment duration is 17 days. It is anticipated that most patients will receive between 10-17 days of atovaquone treatment. The aim is for cohort 1 patients to receive the maximum number of days of treatment possible prior to surgery.

8.4 Management of drug administration

Patients will be instructed to take atovaquone twice daily preferably in the morning and evening at approximately the same time each day. The bioavailability of atovaquone is increased up to three fold when administered with food. Patients will therefore be asked to take atovaquone with a meal, if possible containing a high fat content (e.g. butter, whole milk, cheese, ice cream, eggs etc.)

If a patient misses a dose, the dose should be taken later provided the patient remembers within 4 hours. If the patient does not remember within 4 hours, the missed dose should be omitted. Doses should NOT be doubled to make up for missed doses.

If a patient vomits after taking the suspension, the dose should not be replaced.

8.5 Treatment Break

Side effects associated with atovaquone such as nausea or diarrhoea may be managed by supportive treatment for example with antiemetics and antimotility medications.

If a patient experiences unacceptable toxicities not controlled by supportive medication, the atovaquone may be stopped until the toxicity has resolved to ≤ grade 1 at which point the atovaquone could be restarted. A minimum of 75% of total minimum required atovaquone dose is required between scans for the patient to be evaluable. The atovaquone dose will not be reduced.

8.6 Compliance

Patients will be instructed to keep a record of compliance with treatment, by means of using a “patient diary” that will be provided to them.

Patients will be telephoned by the study team the day prior to surgery to confirm compliance with atovaquone and pimonidazole, and check for adverse events.

Patients should be asked to bring completed diaries and all their unused/remaining trial medicines (empty, open or unopened) with them to each clinic visit.

8.7 Management of overdose

There is no specific antidote for atovaquone. Patients experiencing toxicities upon mis-dosing or overdosing will be treated at the discretion of the investigator with adequate supportive care and followed until recovery.

9 OTHER TREATMENTS (NON-IMPS)

9.1 Pimonidazole (PIMO) administration

Pimonidazole is a marker for regions of hypoxia in normal and tumour tissue when ingested as an encapsulated solid. Following oral administration, PIMO distributes throughout the body where it covalently binds to normal and tumour tissues that have regions of dissolved oxygen concentrations ≤ 14 micromolar corresponding to a pO_2 of ≤ 10mmHg at 37^0C.

Constituents

Pimonidazole: 1-[(2-hydroxy-3-piperdinyl)propyl]-2-nitroimidazole hydrochloride

Administration

Pimonidazole should be taken 16-24 hours prior to surgical excision. The dose of pimonidazole should be calculated as 500mg/m^2. Pimonidazole is supplied in 200mg and 300mg capsules for oral use which should be dose banded according to the following table:

Total amount of PIMO	200mg capsules	300mg capsules	Total number of capsules
600mg	0	2	2
700mg	2	1	3
800mg	1	2	3
900mg	0	3	3
1000mg	2	2	4
1100mg	1	3	4
1200mg	0	4	4

For the purpose of determining the correct number of capsules, the total amount of PIMO in milligrams is rounded to the nearest whole number. For example, if the total amount calculated per m^2 was calculated to 649mg, this would be rounded down to 600mg. If the total amount calculated per m^2 was 650mg or more, the dose would be rounded up to 700mg.

Patients should be advised to take the pimonidazole capsules 16-24 hours prior to surgery. The capsules should be swallowed whole with a glass of water. The capsules should NOT be dismantled and cannot be dissolved in water before ingestion.

The ideal window for taking the PIMO is between 16 and 24 hours prior to surgical excision, so the exact timing may vary from patient to patient. Examples of appropriate timings can be found in the table below. A member of the study team will contact the patient within the recommended window to remind them to take the capsules.

Time of surgery	Window for taking PIMO (day prior to surgery)	
09:00	09:00	17:00
10:00	10:00	18:00
11:00	11:00	19:00
12:00	12:00	20:00
13:00	13:00	21:00
14:00	14:00	22:00
15:00	15:00	23:00
16:00	16:00	00:00
17:00	17:00	01:00
18:00	18:00	02:00

Toxicity

A single dose of 500mg/m^2 of pimonidazole hydrochloride used for tumour hypoxia measurement in humans is equivalent to approximately 13 mg/kg and, therefore, far below the dose of 300 mg/kg/day for 10 consecutive days that was the threshold for liver damage in primates[64]. Furthermore, 500mg/m^2 each day for 20 consecutive days produced no symptoms of central nervous system toxicity that is the limiting toxicity for pimonidazole HCl[65 66]. Pimonidazole hydrochloride, like many 2-nitroimidazole drugs, is toxic to hypoxic cells; however, not at the concentrations used for hypoxia marking in animals or humans.

First Aid Measures

In the unlikely event that one of the capsules breaks and the pimonidazole powder comes into contact with the skin or eyes of either the patient or a member of the study team it should be rinsed off immediately with water.

9.2 Support medication

Side-effects associated with atovaquone will be managed as per local practice. Nausea will be managed with anti-emetic therapy (other than metoclopramide which may alter plasma levels of atovaquone). Concomitant medication to treat diarrhoea such as loperamide given at a standard dose will be considered for grade 1-2 diarrhoea along with oral hydration and dietetic measures. More severe diarrhoea should be treated appropriately at the investigators discretion and may include IV fluid administration. Skin rash will be managed at the investigators discretion and may include the use of anti-histamines and corticosteroids.

9.3 Concomitant medication and non-drug therapies

Concomitant medication may be given as medically indicated. All patients will be asked to provide a complete list of prescription and over-the-counter medications that have been taken within the previous 4 weeks prior to the first treatment visit. They must also inform the Investigator about any new medication started while in the trial.

Details (including indication, doses, frequency and start / stop dates) of concomitant medication taken during the trial until the completion of the off-study visit must be recorded in the medical record and the appropriate CRF.

9.4 Prohibited therapies

Patients should not be prescribed any other anti-cancer or investigational therapies while participating in this study, except for long-standing hormone therapy with evidence of stable disease. Concurrent administration of warfarin in the 14 days prior to starting atovaquone is prohibited. Concurrent administration of known electron transport chain inhibitors, such as metformin, is prohibited (due to possible alteration of hypoxia interpretation). Metformin should not be administered within 4 days of starting atovaquone, for other electron transport chain inhibitors the length of wash-out period will be discussed and agreed by the trial team with reference to the available drug reference data.

9.5 (Potential) Drug Interactions

The main known interactions are given for ease of reference. Refer to the SmPC for atovaquone for further details.

Concomitant administration with the following drugs is not recommended because they are known to reduce plasma concentrations of atovaquone:

- Rifampicin or rifabutin
- Metoclopramide (give an alternative antiemetic if required)
- Tetracycline
- Efavirenz or boosted protease-inhibitors

Atovaquone can increase the levels of etoposide and its metabolite. Caution should be advised in patients receiving concomitant therapy with etoposide.

Atovaquone has high plasma protein binding and should therefore be used in caution with other drugs with high protein binding and narrow therapeutic windows such as warfarin.

10 DRUG MANAGEMENT

10.1 Drug supplies

Atovaquone (IMP) will be purchased by the site pharmacy as commercial stock manufactured by GSK and supplied as 750mg (5ml) oral suspension in 226ml bottles. The arrangements for reimbursing the participating pharmacy will be detailed in an agreement.

Pimonidazole (NIMP) will be supplied in bottles of 7 capsules of 200mg or 300mg strength capsules for oral use by Natural Pharmacia International, Incorporated (NPI, Inc.), USA. The company will provide certificate of analysis per batch, material safety data sheets and an Investigator Brochure. As the PIMO does not have a marketing authorisation, it will be imported under a 'specials' licence notified to the MHRA as per the part 2 and 3 of Schedule 4 of The Human Medicines Regulations 2012 (SI 2012/1916). Pimonidazole will be delivered directly to the participating pharmacy

10.2 Drug ordering

The local investigator and Pharmacy are responsible for liaising to ensure that commercial supplies of atovaquone are held in stock as necessary to supply the recruited patients.

Supply of pimonidazole will be sent out to the participating hospital pharmacy by Catalent after they have been informed by the Trial Office (OCTO) that all approvals are in place. One batch will be sent which be enough for all patients.

10.3 IMP Receipt

If pimonidazole supplies are damaged on arrival contact the Trial Office. Damaged supplies should be destroyed on site and a drug destruction form completed.

10.4 Handling and storage

Unopened bottles of atovaquone may be stored at controlled room temperature up to 25^{o}C. Unopened containers have a shelf life of 2 years. After first opening, the suspension may be stored for up to 21 days.

Pimonidazole should be stored in a locked facility in a dry place at room temperature (15-30^{o}C) and under conditions of subdued light. Pimonidazole capsules have a shelf life of 3 years.

10.5 Labelling

The responsible Pharmacy will ensure that IMP supplies dispensed for trial use are appropriately labelled in accordance with all applicable regulatory requirements. Packs that are given to patients should be also be labelled with full information regarding the trial. These labels are included in the Pharmacy File provided by OCTO upon site activation.

Emergency contact details will be supplied to the participant separately.

NIMP (pimonidazole) supplies will be over labelled and managed as for atovaquone.

NB Local labels can be used as long as they contain exactly the same information as in the labels provided.

10.6 Dosing dispensing

Atovaquone will be dispensed as one 240 ml high density polyethylene bottle with child resistant polypropylene closure, containing 226 ml of atovaquone suspension. A measuring syringe will be included. One bottle will contain sufficient drug for the duration of the trial for one patient. The atovaquone suspension will not be diluted.

Pimonidazole will be dispensed in HDPE bottles (1 bottle per capsule strength) containing the correct number of capsules as per the dose calculated in the table in section 9.1.

10.7 Drug accountability

Drug accountability is the responsibility of the PI at each site but can be undertaken by the site pharmacist listed on the trial delegation log. Full drug accountability records must be maintained. Accountability Logs for atovaquone and pimonidazole are provided in the trial Pharmacy File provided by OCTO upon site activation.

NB Local Accountability Logs can be used **as long as they contain exactly the same information as in the logs provided.** The drug dispensing logs should be kept up to date and must be available for inspection by the monitor in the case of a triggered monitoring visit.

At the conclusion of the study the overall volume of drug purchased, the volume dispensed and the volume destroyed or returned will be provided by the pharmacy. An account must be given of any discrepancy.

10.8 Drug returns from patients

Patient returns of atovaquone should be returned to pharmacy. Pharmacy staff will collect and measure patient returns using the weight of the returned bottle (minus bottle standard weight), converted to fluid in ml. Returns must be recorded in the patient's Accountability Log. Returns should be reconciled against the patient diary and the reason for any discrepancy documented.

10.9 Drug destruction

Any patient returns should be disposed of at site according to local hospital policy and a drug destruction form completed. At the end of the study, once authorised to do so, any unused drug should be disposed of at site according to local hospital policy and a drug destruction form completed. The original drug destruction form should be placed in the Pharmacy File and a copy scanned and emailed to the Trial Office.

10.10 Occupational safety

The product is not expected to pose an occupational safety risk to site staff under normal conditions of use and administration.

11 EVALUATION OF RESPONSE TO ATOVAQUONE

11.1 Tumour assessment

Response to atovaquone treatment will be based upon changes in tumour hypoxia and perfusion. Imaging investigations will be performed prior to commencing atovaquone treatment and repeated prior to the patient having surgery. The rationale for this timing is to observe whether atovaquone can reduce tumour hypoxia and increase tumour perfusion.

The same methods of assessment and the same techniques will be used throughout the trial.

Changes to hypoxia will be detected by changes in ^{18}F-MISO/^{18}F-FAZA uptake on hyp-PET-CT imaging. Some of the imaging data obtained during this trial (from cohort 2) will provide information regarding test/retest fluctuations associated with hyp-PET-CT imaging and may be used to provide information regarding the significance of any changes observed pre- and post-atovaquone in cohort 1. The same tracer for the hyp-PET-CT must be used for both sets of scans, per individual patient.

Changes to perfusion will be detected by changes in perfusion parameters including blood flow (BF), blood volume (BV), mean transit time (MTT), and permeability surface area product (PS) identified on the basis of perfusion CT imaging.

MRI will be used to measure changes in diffusion as measured by DWI-MRI and calculating an ADC (apparent diffusion coefficient) image from these data. Changes in diffusion measures have been found to correlate with treatment response in lung cancer. Diffusion tends to increase with responders and remain unchanged or decrease in non-responders (9). DCE-MRI will directly measure changes in tumour perfusion and permeability using a 2-compartment Tofts model. The K-trans and k-ep parameters have been shown to both predict, and change in correlation with, treatment response (10). These images can be assessed against perfusion CT data to determine the pros and cons of the different modalities. Perfusion CT, dynamic hyp-PET-CT, DWI-MRI and DCE-MRI scans will be performed and analysed as documented in the imaging manual and imaging analysis plan. MRI scans may be omitted if contraindicated.

11.2 Tumour response

Imaging investigations performed in this study will aim to identify changes in tumour physiology rather than tumour measurements. Tumour responses will therefore not be expected or formally recorded.

12 SAFETY REPORTING

The Investigator will monitor each patient for clinical and laboratory evidence of adverse events on a routine basis throughout the study. Should an Investigator become aware of any study drug related SAEs following this period these must also be reported as stated below. Adverse event monitoring starts from the time the patient consents to the study until they complete the trial. All reportable AEs except those considered to be definitely unrelated to the trial intervention will be followed to a satisfactory conclusion. Any reportable drug-related AEs that are unresolved at the end of treatment visit are to be followed up by the Investigator until resolution or stabilisation.

All AEs reported to the trial office will be processed according to internal SOPs. The trial office may request additional information for any AE as judged necessary.

12.1 Adverse Event Definitions

For the purpose of this study we will be collecting Grade 3 and 4 (CTCAE v.4.0) AEs, unless the event is deemed to be related to any aspect of study participation, in which case Grades ≥ 2 will be reported.

An Adverse Event or experience (AE) is any untoward medical occurrence in a study subject temporally associated with the administration of an investigational medicinal product (IMP) or a comparator product, whether or not considered related to the IMP or a comparator product. An AE can therefore be any unfavourable and unintended sign, symptom, disease (new or exacerbated) and/or significant abnormal laboratory or physiological observation temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

A Serious Adverse Event (SAE) is any AE, regardless of dose, causality or expectedness, that:

• **Results in death**	
• **Is life-threatening**	This refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
• **Requires in-patient hospitalisation or prolongs existing inpatient hospitalisation**	In general, hospitalisation signifies that the subject has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event is serious. When in doubt as to whether hospitalisation occurred or was necessary, the AE should be considered serious.
• **Results in persistent or significant incapacity or disability**	This means a substantial disruption of a person's ability to conduct normal life functions. It does not include experiences of relatively minor medical significance or accidental trauma (e.g. sprained ankle), which do not constitute a substantial disruption.
• **Is a congenital anomaly or birth defect**	
• **Is any other medically other medically important event**	Defined as an event that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above. Any new primary cancer must be reported as an SAE.

An Adverse Drug Reaction (ADR) is an AE which is considered to be causally related to any dose of the IMP. This means that a causal relationship between the IMP and the AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. **For the purpose of this study we will be collecting ≥Grade 2 (CTCAE v.4.0) ADRs**

An Unexpected Drug Reaction is an adverse drug reaction, the nature or severity of which, is not consistent with applicable product information (referring to information in SPC or IB).

A Suspected Unexpected Serious Adverse Drug Reaction (SUSAR) is a serious adverse drug reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational product or SPC for an approved product).

12.2 Clinical laboratory abnormalities and other abnormal assessments as AEs and SAEs

Abnormal laboratory findings (e.g., clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g., ECGs, X-rays and scans) that are judged by the investigator as clinically significant will be recorded as AEs or SAEs if they meet the definitions given above. By definition, **all Grade 3 and or 4 laboratory abnormalities should usually be reported as SAEs**. However, if a lab result is categorised as CTCAE Grade 4 but did not fulfil the safety reporting criteria for an SAE (asymptomatic, not life threatening, no intervention) it is the clinician's decision whether to report the event. If the event is not reported the reason why should be documented in the patient notes.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the patient's condition, or that are present or detected at the start of the study and do not worsen, will not be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

12.3 Determining adverse event causality

The assessment of "relatedness" must be determined by a medically qualified individual and is primarily the responsibility of the PI at site or agreed designee. AEs that will be considered related will include any AE that is documented as possibly, probably or definitely related to protocol treatment. The assessment of relatedness is made using the following:

Classification	Relationship	Definition
drug-related	**Definitely related**	• Starts within a time related to the study drug administration *and* • No obvious alternative medical explanation.
	Probably related	• Starts within a time related to the study drug administration *and* • Cannot be reasonably explained by known characteristics of the patient's clinical state.
	Possibly related	• Starts within a time related to the study drug administration *and* • A causal relationship between the study drug and the adverse event is at least a reasonable possibility.
not drug related	**Probably not related**	• The time association or the patient's clinical state is such that the study drug is not likely to have had an association with the observed effect.
	Definitely not related	• The AE is definitely not associated with the study drug administered.

The Investigator must endeavour to obtain sufficient information to confirm the causality of the adverse event (i.e. relation to surgery, study drug, background treatment, other illness, progressive malignancy etc.) and give their opinion of the causal relationship between each AE and study drug. This may require instituting supplementary investigations of significant AEs based on their clinical judgement of the likely causative factors and/or include seeking a further specialist opinion.

12.4 Reference safety information (RSI) for assessment of expectedness

The reference safety information (RSI) for the trial is section 4.8 of the SPC for atovaquone which lists all the expected side effects associated with the use of atovaquone. The RSI for pimonidazole is page 9 of the IB for pimonidazole.

A copy of the current approved version of the RSI documents must be held in the Site File for reference. Any change or update to the RSI during the trial will be made via substantial amendment. Please note that the list of expected side effects in the SPC are those listed for treatment of mild to moderate Pneumocystis pneumonia and in patients who are intolerant of co-trimoxazole therapy. It is therefore possible that in this study population other side effects may occur, or the patient might suffer a more severe reaction.

12.5 Suspected Unexpected Serious Adverse Drug Reactions (SUSARs)

All SUSARS must be reported to the responsible Authority and main REC by the Trial Office within the required timelines:

- Fatal or life threatening SUSARs will be reported within 7 days of the Trial Office receiving the initial report. Any additional information will be reported within eight days of sending the first report.
- All other SUSARs will be reported within 15 days of the Trial Office receiving the initial report

In addition, other safety issues qualify for expedited reporting where they might materially alter the current risk assessment of an IMP or be sufficient to change IMP administration or the overall conduct of the trial.

12.6 Unexpected Serious Adverse Reactions (SARs) to NIMPs

Serious suspected unexpected adverse drug reactions (ADRs) relating to pimonidazole must be reported by the prescriber or pharmacist supplying the pimonidazole to the MHRA through the Yellow Card Scheme stating the manufacturer and indicating that the product is unlicensed.

12.7 Expedited reporting of SAEs

The following SAE reporting requirements apply regardless of the Investigator's assessment of the causality or expectedness of the SAE. All SAEs should be reported on the trial SAE report form (see SAE report form and completion guidelines).

If a Serious Adverse Event occurs that requires reporting, a Serious Adverse Event reporting form should be completed and a scanned copy emailed within 24 hours of becoming aware of the event to:

Pharmacovigilance Office, OCTO
octo-safety@oncology.ox.ac.uk

In the event a scanned document via email is not possible the form may be faxed however an email should be sent to the safety team at the time of send, to enable tracking of SAEs sent by fax.
Fax no: 01865 617010
Tel no: 01865 227181

If the SAE has not been reported within the specified timeframe, a reason for lateness must be provided when sending the SAE Report Form.

Investigators should also adhere to their local Trust policy for incident and SAE reporting in research.

12.8 Follow-up of Serious Adverse Events

A follow-up report must be completed when the SAE resolves, is unlikely to change, or when additional information becomes available. If the SAE is a suspected SUSAR then follow up information must be provided as requested by the trial office.

If new or amended information on a reported SAE becomes available, the Investigator should report this on a new SAE form using the completion guidelines. If using the original form to notify further information, you must initial and date all new or amended information so that all changes are clearly identified.

SAEs that are considered to be definitely unrelated to the trial intervention will not be followed up and monitored.

12.9 Reporting Adverse Events on the CRF

Reportable AEs, including Serious AEs must be recorded on the case report forms (CRF) for that patient (unless otherwise specified in section 12.10. Please note that AEs are recorded on eCRFs within OpenClinica and SAEs are additionally recorded on the paper SAE report form and a scanned copy emailed to OCTO. The information provided will include date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome and relationship of the AE to study drug. Any concomitant medications or any therapy used to treat the event must be listed. The Investigator will provide an "other" cause for serious AEs considered to be unrelated to the study drug. Sites should ensure data entered into the CRF is consistent with the SAE report information where applicable.

Each separate AE episode must be recorded. For example, if an AE resolves completely or resolves to baseline and then recurs or worsens again, this must be recorded as a separate AE. For AEs to be considered intermittent, the events must be of similar nature and severity.

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

AEs which are serious must be reported to OCTO from the first dose of study medication up to and including 30 days after administration of the last dose of study medication. Any SAE that occurs at any time after completion of medication treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator consider to be related to any study drug must be reported to OCTO.

Terms and Grading of Events

All adverse events and toxicities must be graded according to the NCI Common Terminology Criteria for adverse events (NCI-CTCAE) Version 4.0 (currently up to Version 4.03).

12.10 Events exempt from being reported as AE/ SAEs

This section specifies adverse events that do not require reporting providing the Investigator is certain that they are as expected given the natural course of the disease under study and /or expected outcomes of any background routine standard of care. The event must be reported if the Investigator cannot exclude the possibility that any trial intervention (including tests and other procedures) might be causally implicated or if the frequency, severity or pattern of events is not as expected for the patient's condition.

Grade 1 and 2 (CTCAE v.4.0) AEs will not be reported; unless the event is deemed to be related to any aspect of study participation therefore all ≥Grade 2 (CTCAE v.4.0) ADRs grades will be reported.

Progression of underlying disease

Disease progression and resultant death will be captured on the eCRF. Adverse events including hospitalisation that are clearly consistent with disease progression will not be reported as individual AE/SAEs. Clinical symptoms of progression will only be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

Every effort should be made to document the objective progression of underlying malignancy. In some cases, the determination of clinical progression may be based on symptomatic deterioration. For example, progression may be evident from clinical symptoms, but is not supported by tumour measurements. Or, the disease progression is so evident that the investigator may elect not to perform further disease assessments.

Death on study

Death due to disease under study is to be recorded on the Death CRF form providing the death is not unexpected or if a causal relationship suspected. The investigator must clearly state whether the death was expected or unexpected and whether a causal relationship to the study IMP or other protocol treatment intervention is suspected. Lung resections can be associated with mortality as high as 6% - this is unrelated to atovaquone and it would be inappropriate for these to be considered 'deaths on study'.

Elective admissions and supportive care

Elective admissions to hospital for patient convenience or for planned procedures or investigations or treatment as specified in this protocol and standard supportive care are not SAEs, and do not require SAE reporting.

12.11 Informing Investigators of new safety information

The Trial Office or the Chief Investigator will ensure that all investigators are kept informed in a timely manner, as new safety profile information becomes available. Investigators are responsible for briefing their study team and onward transmission to their R&D office as appropriate.

12.12 Summary of trial safety reporting requirements

Event	SAE	AE	AE/SAE
Medically important events in the context of this trial (as per SAE definition)	**Submit SAE Form within 24 hrs.**	**Report in AE eCRF**	**Non reportable**
Any AE ≥ grade 3	& defined as "Serious"	X	
Any AE ≥ grade 2 and related to Atovaquone	& defined as "Serious"	X	
Any AE ≤ grade 2			X
AE/SAE considered more severe than expected	& defined as "Serious"	X	
AE/SAE that is unexpected and related to Atovaquone	X (Unexpected SAR)	X	
AE/SAE resulting in withdrawal of Atovaquone	X	X	
Expected toxicities of surgery			X
All other AEs, abnormal assessments or laboratory results	If ≥ grade 3	If ≥ grade 3	If ≤ grade 2
Disease progression and resultant death[1]			
Hospitalisation (for AE or elective or supportive care)			X
Clinical symptoms of progression			X
Death[2]	Possibly Atovaquone related		

[1] Adverse events including hospitalisation that are clearly consistent with disease progression will not be reported as individual AE/SAEs. Clinical symptoms of progression will only be reported as adverse events if the symptom cannot be determined as exclusively due to the progression of the underlying malignancy, or does not fit the expected pattern of progression for the disease under study.

[2] Death due to disease under study is to be recorded on the Death eCRF providing the death is not unexpected or if a causal relationship is not suspected. The investigator must clearly state whether a causal relationship to Atovaquone is suspected, and if so, whether the death was expected or unexpected.

13 PREGNANCY

Pregnancies (in a participant or partner) occurring while participating in this trial require expedited reporting. A pregnancy notification form should be completed and submitted to the trial office within the same timelines as an SAE. Women who become pregnant should be withdrawn from atovaquone treatment immediately.

Pregnancy is only considered an AE/SAE if there is reason to believe it may be the result of an interaction between the study IMP and the contraceptive used.

All reported pregnancies should be followed and the outcome reported using the same form. If the outcome of the pregnancy meets any of the criteria for seriousness, it must also be reported as an SAE. Examples of pregnancy outcomes that are SAEs include reports of:

- congenital anomalies or developmental delay, in the foetus or the child.
- foetal death and spontaneous abortion.
- suspected adverse reactions in the neonate that are classified as serious

14 DEFINING THE END OF TRIAL

For this study the end of the trial is defined as the day before surgery for the last patient undergoing the trial (LPLV).

The study will be stopped when:

- The stated number of evaluable patients to be recruited is reached.

The sponsor and the Chief Investigator reserve the right to terminate the study earlier at any time. In terminating the study, they must ensure that adequate consideration is given to the protection of the participants' best interests.

The Clinical Study Report will not include the analysis of the routine survival data collected at 12 and 24 months (exploratory endpoint). This will be reported in a second report which will be presented after 2 years has passed since all patients completed treatment.

15 STATISTICAL CONSIDERATIONS

15.1 Sample size determination

There will be no formal sample size calculation done for this study and there will be no randomisation of patients into either cohort (this will lessen burden on patients, and promote faster recruitment). Although we anticipate a huge number of variations in the size of tumours that are resected, potential bias in terms of selecting patients based on resection date will be mitigated by the fact that patients all typically have to wait approximately 2-3 weeks from clinic assessment to surgical resection. The trial anticipates recruiting a total of 30 patients with equal allocation: 15 patients in cohort 1 under atovaquone treatment and 15 patients control in Cohort 2. The investigators will endeavour to ensure that, as far as possible, patients in Cohort 2 are broadly balanced with Cohort 1 on key features such as tumour size and duration between F-MISO scans. However this may be limited by recruitment rate and other operational issues (e.g. available scan & surgery dates). Patients in Cohort 2 will not be formally matched with patients in Cohort 1. Any variation in key features between the cohorts will be described in the study report.

Patients who are not evaluable for the primary endpoint may be replaced as recommended by the Chief Investigator and ratified by the independent Radiotherapy and Imaging Oversight Committee. Detailed definition of evaluability is listed in Section 6.1.

15.2 Definition of primary and secondary endpoints

Two sets of hypoxic volume measurements in ^{18}F-MISO/^{18}F-FAZA uptake as detected by hyp-PET-CT scans for each patient will be taken prior to and after administration of atovaquone for Cohort 1 (i.e. to evaluate treatment efficacy). For cohort 2, patients' hypoxic volume measurements will be taken at baseline and pre-surgery.

Pre-surgery measurements will be taken to help calculate percentage change in hypoxic volume from baseline per patient. The difference in hypoxic volume for Cohort 1 will be calculated from values measured prior to and post atovaquone administration while for cohort 2 the difference will be calculated from baseline measurement and pre-surgery values.

Primary outcomes will be expressed as patient average percentage change relative to the baseline per cohort (between cohort analysis) or per time point (within cohort analysis). The analysis will also measure changes in baseline and pre-surgery levels of plasma markers of hypoxia (osteopontin, CAIX, VEGF and miR210 RNA) which will be evaluated as measurements of tumour hypoxia pre-and post-atovaquone.

In addition, the analysis will also evaluate differences in scoring and/or reconstructed hypoxic volume from the immunohistochemistry of pimonidazole.

The secondary endpoints for the study include changes in tumour perfusion and diffusion parameters; measured by perfusion CT, DWI-MRI, DCE-MRI scans, and PET kinetic modelling (see section 11.1) . In addition, hyp-PET-CT derived hypoxic volumes will be compared to changes in levels of plasma markers of hypoxia and comparison made of hyp-PET-CT, perfusion CT, serological tests, DWI-MRI and DCE-MRI derived parameters. Tumour perfusion for cohort 1 will be measured prior to treatment with atovaquone and following 7-17 days of daily treatment.

16 STATISTICAL ANALYSIS PLAN

A detailed statistical analysis plan will be available from the time the first patient is recruited and will be finalised before any analysis is undertaken. The analysis plan will be written in accordance with current Standard Operating Procedures and will be finalised and agreed by the trial statistician, the CI and TMG. Sites must report any protocol deviations (intended or not) to OCTO.

Baseline characteristics will be summarised using numbers (with percentages) for binary and categorical variables and means (and standard deviations), or medians (with lower and upper quartiles) for continuous variables separately for all registered patients in each of the two cohorts. All analyses will be based on modified intention-to-treat on evaluable patients as defined in Section 6.1.

Hypoxic volume measurements taken at baseline and pre-surgery will be summarised by descriptive statistics of patients grouped by cohort. In Cohort 1, for each endpoint, a pairwise comparison (pre and post) will be assessed using appropriate statistical tests. Potential tests include paired t-test and Wilcoxon signed rank test. For Cohort 1, we will also consider analysis of covariance to provide the effect of atovaquone on hypoxic volumes after controlling for covariates. For each primary endpoint, a between cohort comparison will be assessed using appropriate tests. These include two group t-test and Wilcoxon rank-sum test.

The main secondary objective is to determine whether atovaquone results in a change in tumour perfusion measured as changes in perfusion at baseline and pre-surgery for patients within Cohort 1 and between patients in Cohort 1 and cohort 2. To evaluate treatment efficacy for cohort 1, an appropriate test (paired t-test) will be used to compare hypoxic volume values prior to and post-treatment while average cohort hypoxic values will be used to provide a comparison between cohort 1 and cohort 2.

Another secondary aim is to determine whether serological plasma markers of hypoxia may replace hyp-PET-CT imaging in future studies of hypoxia modification by atovaquone and will be described and presented with numbers measured at baseline and pre-surgery. An appropriate test (t-test) will be used to explore correlation of levels of serological plasma markers of hypoxia with ^{18}F-MISO/^{18}F-FAZA uptake at baseline and pre-surgery. Within cohort 2, an appropriate test (e.g. intraclass correlation coefficient) will be used to assess change over time and therefore possibly the reproducibility/reliability of imaging derived parameters evaluated at baseline and pre-surgery.

16.1 Inclusion in analysis

Data from evaluable patients who have given informed consent and with a confirmed trial registration number will be included for primary/secondary analysis. Detailed evaluability definition is listed in Section 6.1. Baseline characteristics will be summarised for all enrolled patients.

16.2 Procedures for reporting any deviation(s) from the original statistical plan

Any deviation(s) from the original statistical plan will be documented and described and justified in the final study report.

16.3 Interim analysis

An interim analysis of hypoxic response will be performed after 5 patients in cohort 1 have completed both hyp-PET-CT scans at the starting dose level of atovaquone 750mg (5ml) bd. The results of this analysis will inform a TMG decision to continue atovaquone at the same dose, or treat the remainder of the cohort 1 patients (n=10) at the escalated dose of 1000mg (6.5ml) bd. Further details will be described in the protocol decision point plan.

16.4 Final analysis

Based upon projected accrual rates, this trial is expected to complete recruitment within 30 months of opening to recruitment. Final analysis of primary and secondary objectives will begin 2 months post LPLV.

Analyses will be based on modified intention-to-treat on evaluable patients as defined in Section 6.1.

16.5 Outcome report

A report of primary, secondary and exploratory objectives will be presented 4 months after LPLV.
A second report on PFS and OS at 12 and 24 months will be presented after 2 years has passed since all patients completed treatment.

17 TRIAL COMMITTEES

17.1 Trial Management Group (TMG)

The Chief Investigator will chair a TMG responsible for overseeing the successful conduct and publication of the trial. Members of the TMG will include the Chief Investigator, Co-Investigators, Clinical Trial Coordinator, Trial Statistician and others as required. The TMG will meet as necessary to discuss trial data and progress.

17.2 Data and Safety Monitoring

An independent Data & Safety Monitoring Committee (DSMC) will not be established for this trial. Trial Oversight will be provided by the Independent Radiotherapy & Imaging Trial Oversight Committee which will monitor the primary outcome measures including safety and efficacy, and monitor data quality and completeness. In addition, safety data will be reviewed by a clinician independent to the trial.

17.3 Independent Radiotherapy & Imaging Oversight Committee (RIOC)

The RIOC is a committee of independent members that provides overall supervision of the trial and fulfils the role of both a DSMC and a Trial Steering Committee. The role of the RIOC is to act on behalf of the Sponsor, to provide overall supervision for the trial, to ensure that it is conducted in accordance with GCP, and to provide advice through its independent chairman. The RIOC will review the data from the trial and will decide on continuing or stopping the trial, or modifying the protocol. It will meet at least annually when it will consider a trial report, as well as results of other trials and new information which has arisen, and recommend appropriate action.

18 DATA MANAGEMENT

18.1 Database considerations

Data management will be performed via a web-based, bespoke trial database (OpenClinica). OpenClinica is a dedicated and validated clinical trials database designed for electronic data capture. See: http://www.openclinica.org. The trial office will provide sites with instructions and a video link for training purposes.

The participants will be identified by a unique trial specific number and/or code in any database. The name and any other identifying detail will NOT be included in any trial data electronic file.

18.2 Electronic Case reports forms (eCRFs)

The Investigator and study site staff will ensure that data collected on each subject is recorded in the CRF as accurately and completely as possible. All appropriate laboratory data, summary reports and Investigator observations will be transcribed into the CRFs from the relevant source data held in the site medical record(s). CRFs entries will not contain any source data (unless otherwise specified in the completion instructions provided by the trial office). It is important to ensure that:

- the relevant CRFs are completed.
- all CRF data are verifiable in the source documentation or the discrepancies must be explained.
- CRF sections are completed in a timely fashion, as close to the visit or event being recorded as possible.
- Data queries are resolved and documented by authorised study staff in a timely fashion. The reason for the change or correction should be given where appropriate.
- As much data as possible is entered and cleaned in preparation for each study database lock point.

Note: 'in a timely fashion' means within no more than 10 working days of the initial event and within 28 days of receipt of a data query unless otherwise specified.

The above considerations also apply to patients who are withdrawn early. If a patient withdraws from the study, the reason must be noted on the appropriate form and the patient must be followed-up as per protocol.

18.3 Accounting for missing, unused, or spurious data.

The statistical analysis plan will describe the procedure for accounting for missing, unused or spurious data.

19 CLINICAL STUDY REPORT

All clinical data will be presented at the end of the study as data listings. These will be checked to confirm the lists accurately represents the data collected during the course of the study. The trial data will then be locked and a final data listing produced. The clinical study report will be based on the final data listings. The locked trial data may then be used for analysis and publication. The Clinical Study Report will not include the analysis of the routine survival data collected at 12 and 24 months (exploratory endpoint). This will be reported in a second report which will be presented after 2 years has passed since all patients completed treatment.

20 STUDY SITE MANAGEMENT

20.1 Study site responsibilities

The Principal Investigator (the PI or lead clinician for the study site) has overall responsibility for conduct of the study, but may delegate responsibility where appropriate to suitably experienced and trained members of the study site team. All members of the study site team must complete the Staff Contact Responsibility Sheet provided prior to undertaking any study duties. The PI must counter sign and date each Staff Contact Responsibility Sheet, authorising staff to take on the delegated responsibilities.

20.2 Study site set up and activation

The Principal Investigator leading the investigational study site is responsible for providing all required core documentation. Mandatory Site Training organised by the trial office must be completed before the site can be activated. The Trial Office will check to confirm that the site has all the required study information/documentation and is ready to recruit. The site will then be notified once they are activated on the trial database and able to enter patients.

20.3 Study documentation

The trial office will provide an Investigator File and Pharmacy File to each investigational site containing the documents needed to initiate and conduct the study. The trial office must review and approve any local changes made to any study documentation including patient information and consent forms prior to use. Additional documentation generated during the course of the trial, including relevant communications must be retained in the site files as necessary to reconstruct the conduct of the trial.

21 REGULATORY AND ETHICAL CONSIDERATIONS

The Sponsor and Investigators will ensure that this protocol will be conducted in compliance with the UK Clinical Trials Regulations[1] and the applicable policies of the sponsoring organisation. Together, these implement the ethical principles of the Declaration of Helsinki (1996) and the regulatory requirements for clinical trials of an investigational medicinal product under the European Union Clinical Trials Directive.

21.1 Ethical conduct of the trial and ethics approval

The protocol, patient information sheet, consent form and any other information that will be presented to potential trial patients (e.g. advertisements or information that supports or supplements the informed consent) will be reviewed and approved by an appropriately constituted, independent Research Ethics Committee (REC). Principal Investigators will be approved by the REC.

21.2 Regulatory Authority approval

This study will be conducted under a UK Medicines and Healthcare Products Regulatory Agency (MHRA) Clinical Trials Authorisation (CTA). Approval to conduct the study will be obtained from the Responsible Authority prior to initiating the study.

21.3 NHS Research Governance

Investigators are responsible for ensuring they obtain local Trust management agreement to conduct the trial in accordance with local arrangements and policies.

21.4 Protocol amendments

Amendments are changes made to the research following initial approval. A 'substantial amendment' is an amendment to the terms of the Responsible Authority application (if applicable), the REC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of the investigational medicinal product(s) used in the trial.

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

Non-substantial amendments are those where the change(s) involve only minor logistical or administrative aspects of the study.

All amendments will be generated and managed according to the trial office standard operating procedures to ensure compliance with applicable regulation and other requirements. Written confirmation of all applicable REC, regulatory and local approvals must be in place prior to implementation by Investigators. The only exceptions are for changes necessary to eliminate an immediate hazard to study patients (see below).

It is the Investigator's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The Investigator must ensure this is documented in the patient's medical notes and the patient is re-consented if appropriate.

21.5 Urgent safety measures

The sponsor or Investigator may take appropriate urgent safety measures to protect trial participants from any immediate hazard to their health or safety. Urgent safety measures may be taken without prior authorisation. The trial may continue with the urgent safety measures in place. **The Investigator must inform the trial office IMMEDIATELY if the study site initiates an urgent safety measure:**

The notification must include:

- Date of the urgent safety measure;
- Who took the decision; and
- Why the action was taken.

The Investigator will provide any other information that may be required to enable the trial office to report and manage the urgent safety measure in accordance with the current regulatory and ethical requirements for expedited reporting and close out. The Trial office will follow written procedures to implement the changes accordingly.

21.6 Temporary halt

The sponsor and Investigators reserve the right to place recruitment to this protocol on hold for short periods for administrative reasons **or** to declare a temporary halt. A temporary halt is defined a formal decision to:

- interrupt the treatment of subjects already in the trial for safety reasons;
- stop recruitment on safety grounds; or
- stop recruitment for any other reason(s) considered to meet the substantial amendment criteria, including possible impact on the feasibility of completing the trial in a timely manner.

The trial office will report the temporary halt via an expedited substantial amendment procedure. The trial may not restart after a temporary halt until a further substantial amendment to re-open is in place. If it is decided not to restart the trial this will be reported as an early termination.

21.7 Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations require the Sponsor to notify any "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach. A serious breach is defined as "A breach of GCP or the trial protocol which is likely to effect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial; or
- the scientific value of the trial"

Investigators must notify the Trial Office within one working day if any serious breach of GCP is suspected. The Trial Office will review the event and, if appropriate, a report will be submitted by the Sponsor to the REC, Regulatory Authority and the NHS host organisation within 7 days of the Trial Office becoming aware of the breach as per Trials Office SOPs.

21.8 Trial Reports

This protocol will comply with all current applicable Regulatory Authority, Research Ethics Committee and Sponsor reporting requirements.

The trial office will determine which reports need to be circulated to Principal Investigators and other interested parties. Study sites are responsible for forwarding trial reports they receive to their local Trust as required.

22 EXPENSES AND BENEFITS

The participating study site will provide reasonable travel expenses incurred for attending additional research visits in excess of standard of care as per local practice. The local arrangements will be explained to the patient during the informed consent discussions prior to trial entry. However, there is no direct study funding to reimburse patient expenses

23 QUALITY ASSURANCE

23.1 Risk assessment

A risk assessment and a monitoring plan will be prepared before the study opens and will be reviewed throughout the study if necessary in the light of significant changes while the study is ongoing or in response to outcomes from monitoring activities. Monitoring plans will be amended as appropriate.

Potential risks to trial participants:

- Exposure to investigational agent atovaquone however this is a well-tolerated drug with excellent toxicity and safety data.
- Additional ionising radiation dose due to research scans.
- Discomfort and bruising due to additional research blood tests.

23.2 Monitoring

The Study site will be monitored centrally by checking incoming data for compliance with the protocol, consistency, completeness and timing. The case report data will be validated using appropriate set criteria, range and verification checks. The study site must resolve all data queries in a timely manner. All queries relating to key outcome and safety data and any requiring further clarification will be referred back to the study site for resolution. For other non-critical data items, OCTO staff may resolve data queries centrally providing the correct answer is clear. Such changes will be clearly identified in the CRF and the study site informed.

The Study site will also be monitored remotely and/or by triggered site visit as necessary to ensure their proper conduct of the trial. OCTO staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have. The investigator and institution involved in the study will permit study-related monitoring and provide direct on-site access to all study records and facilities if required. They will provide adequate time and space for the completion of monitoring activities. Monitoring reports will be sent to the site in a timely fashion. The Investigator is expected to action any points highlighted through monitoring and must ensure that corrective and preventative measures are put into place as necessary to achieve satisfactory compliance.

The study site will provide copies of the following participant information to the trial office on request for remote monitoring purposes. All patient personal identifiers must be obliterated from the information except where explicit consent for release of personal information has been obtained from the patient:

- Participant screening log

23.3 Audit and Regulatory Inspection

All aspects of the study conduct may be subject to internal or external quality assurance audit to ensure compliance with the protocol, GCP requirements and other applicable regulation or standards. It may also be subject to a regulatory inspection. Such audits or inspections may occur at any time during or after the completion of the study. Investigators and their host Institution(s) should understand that it is necessary to allow auditors/inspectors direct access to all relevant documents, study facilities and to allocate their time and the time of their staff to facilitate the audit or inspection visit. Anyone receiving notification of a Regulatory Inspection that will (or is likely to) involve this trial must inform the Trial Office without delay.

24 RECORDS RETENTION & ARCHIVING

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable the conduct of a clinical trial and the quality of the research data to be evaluated and verified. All essential documents must be stored in such a way that ensures that they are readily available, upon request for the minimum period required by national legislation or for longer if needed. The medical files of trial subjects must be retained in accordance with applicable national legislation and the host institution policy.

Retention and storage of laboratory records for clinical trial samples must also follow these guidelines.
Retention and storage of central laboratory records supporting PK or PD endpoints and the disposition of samples donated via the trial must also comply with applicable legislation and Sponsor requirements.

It is the University of Oxford's policy to store data for a minimum of 5 years. Investigators may not archive or destroy study essential documents or samples without written instruction from the trial office.

25 PATIENT CONFIDENTIALITY

Personal data recorded on all documents will be regarded as confidential, and to preserve each patient's anonymity, only their initials and year of birth will be recorded on the CRFs.

Medical source data or scans acquired and exported for research purposes will be linked anonymised as per Departmental Protocol so that the patient cannot be identified from them.

The Investigator site must maintain the patient's anonymity in all communications and reports related to the research. The Investigator site team must keep a separate log of enrolled patients' personal identification details as necessary to enable them to be tracked. These documents must be retained securely, in strict confidence. They form part of the Investigator Site File and are not to be released externally.

26 STUDY FUNDING

This study is undertaken via the University of Oxford core clinical and research infrastructure underpinned by strategic research programme grant funds. The research imaging is enabled by funding provided by the Oxford Cancer Imaging Centre (OCIC) and the Howat Foundation. OCIC is funded by a strategic initiative from Cancer Research UK jointly with the Engineering & Physical Sciences Research Council, the Medical Research Council and the Department of Health. We also acknowledge the support provided by: The Oxford National Institute for Health Research Biomedical Research Centre (BRC Cancer Theme) and the Oxford Experimental Cancer Medicine Centre (ECMC).

27 SPONSORSHIP AND INDEMNITY

27.1 Sponsorship

The Sponsor will provide written confirmation of Sponsorship and authorise the trial commencement once satisfied that all arrangements and approvals for the proper conduct of the trial are in place. A separate study delegation agreement, setting out the responsibilities of the Chief Investigator and Sponsor will be put in place between the parties.

27.2 Indemnity

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment which is provided.

27.3 Contracts/Agreements

This trial is subject to the Sponsor's policy requiring that written agreements are agreed formally by the participating bodies as appropriate. An Academic Collaboration Agreement will be placed between the Sponsor and participating organisation prior to site activation.
The Sponsor will also set up written agreements with any other external third parties involved in the conduct of the trial as appropriate.

28 PUBLICATION POLICY

The Trial Management Group will retain ownership of all data arising from the trial. The intention is to publish this research in a specialist peer reviewed scientific journal on completion of the study. The results may also be presented at scientific meetings and/or used for a thesis. The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the trial and retain final editorial control.

Authors will acknowledge that the study was sponsored by and performed with the support of the Sponsor, and with funding support from the University of Oxford Department of Oncology, the Howat Foundation, Oxford Cancer Imaging Centre and the Oxford ECMC.

29 REFERENCES

1. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC. The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. The British journal of radiology. 1953;26(312):638-48.
2. Secomb TW, Hsu R, Ong ET, Gross JF, Dewhirst MW. Analysis of the effects of oxygen supply and demand on hypoxic fraction in tumors. Acta oncologica. 1995;34(3):313-6
3. Krohn, K A, Link, J M & Mason, RP. Molecular imaging of hypoxia. J Nucl Med. 2008 Jun;49 Suppl 2:129S-48S.
4. Rajendran JG, Schwartz DL, O'Sullivan J, Peterson LM, Ng P, Scharnhorst J, Grierson JR, Krohn KA. Tumor hypoxia imaging with [F-18] fluoromisonidazole positron emission tomography in head and neck cancer. Clin Cancer Res. 2006 Sep 15;12(18):5435-41.
5. Kikuchi M, Yamane T, Shinohara S, Fujiwara K et al. 18F-fluoromisonidazole positron emission tomography before treatment is a predictor of radiotherapy outcome and survival prognosis in patients with head and neck squamous cell carcinoma. Ann Nucl Med. 2011 Nov;25(9):625-33.
6. Rischin D, Hicks RJ, Fisher R, Binns D, Corry J, Porceddu S, Peters LJ; Trans-Tasman Radiation Oncology Group Study 98.02. Prognostic significance of [18F]-misonidazole positron emission tomography-detected tumor hypoxia in patients with advanced head and neck cancer randomly assigned to chemoradiation with or without tirapazamine: a substudy of Trans-Tasman Radiation Oncology Group Study 98.02. J Clin Oncol. 2006 May 1;24(13):2098-104.
7. Fraioli F, Anzidei M, Zaccagna F, Mennini ML, Serra G, Gori B, et al. Whole-tumor perfusion CT in patients with advanced lung adenocarcinoma treated with conventional and antiangiogenetic chemotherapy: initial experience. Radiology. 2011;259:574-82.
8. Wahl RL, Jacene H, Kasamon Y, Lodge MA. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med. 2009;50 Suppl 1:122S-50S.
9. Chang Q, Wu N, Ouyang H, Huang Y. Diffusion-weighted magnetic resonance imaging of lung cancer at 3.0 T: a preliminary study on monitoring diffusion changes during chemoradiation therapy. Clin Imaging. 2012 Mar-Apr;36(2):98-103.
10. Chang YC, Yu CJ, Chen CM, Hu FC, Hsu HH, Tseng WY, Ting-Fang Shih T, Yang PC, Chih-Hsin Yang J. Dynamic contrast-enhanced MRI in advanced nonsmall-cell lung cancer patients treated with first-line bevacizumab, gemcitabine, and cisplatin. nJ Magn Reson Imaging. 2012 Aug;36(2):387-96.

APPENDIX 1: ECOG PERFORMANCE SCALE

Activity Performance Description	Score
Fully active, able to carry out all on all pre-disease performance without restriction.	0
Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light housework, office work.	1
Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care. Confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry out any self-care. Totally confined to bed or chair.	4