Metformin in Li Fraumeni Syndrome (MILI) Trial: A phase II randomised open-label cancer prevention trial of metformin in adults with Li Fraumeni Syndrome

Short title: Metformin in LFS (MILI) Trial

Protocol Version & date:	2.0_01Sep2023_Reformatted_13Sep2023
Protocol Number:	OCTO_104
OCTRU Trial ID:	OCTRU316
EudraCT Number:	2022-000165-41
Ethics Reference number:	23/WS/0051
IRAS project ID:	1006131
Name of Registry	ISRCTN16699730

Sponsor: University of Oxford

Funder: This trial will be coordinated by OCTO (Oncology Clinical Trials Office) which is part of the UKCRC registered Oxford Clinical Trials Research Unit (OCTRU) at the University of Oxford.

This project (NIHR131239) is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care.

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

General queries (Monday to Friday, 09:00-17:00 except Bank holidays)	MILI Trial Team Oncology Clinical Trials Office (OCTO) Department of Oncology, University of Oxford Old Road Campus Research Building Roosevelt Drive, Headington Oxford, OX3 7DQ Email: octo-mili@oncology.ox.ac.uk Tel: 0044 (0)1865 617078 Website: https://www.oncology.ox.ac.uk/clinical-trials/oncology-clinical-trials-office-octo
Clinical queries **During office hours**:	Clinical Queries should be directed to MILI Trial Team (details as above) for action.
Out of office hours:	There is no out of office hours clinical provision
Chief Investigator	Professor Sarah Blagden Department of Oncology Old Road Campus Research Building University of Oxford Oxford, UK. OX3 7DQ Tel.: 01865 617409 Email: sarah.blagden@oncology.ox.ac.uk
Coordinating PI	Dr Helen Hanson Cancer Genetics St George's University Hospital NHS Foundation Trust Blackshaw Road Tooting London, UK. Tel: 0208 672 1255 Email: helen.hanson@stgeorges.nhs.uk
Translational Lead	Dr Simon Lord, Department of Oncology Churchill Hospital Oxford, UK. OX3 7DQ Tel: 01865 227212 Email: simon.lord@oncology.ox.ac.uk
Sponsor	Research Governance, Ethics & Assurance Team (RGEA) University of Oxford, 1st floor, Boundary Brook House Churchill Drive, Headington Oxford OX3 7GB E-mail: rgea.sponsor@admin.ox.ac.uk
Trial Statistician	Maggie Qiao Oxford Clinical Trials Research Unit (OCTRU) Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS) Botnar Research Centre Windmill Road Headington Oxford OX3 7LD

	Email: maggie.qio@ndorms.ox.ac.uk
Senior Trial Statistician	Ioana Marian Oxford Clinical Trials Research Unit (OCTRU) Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences (NDORMS) Botnar Research Centre Windmill Road Headington Oxford OX3 7LD Email: Ioana.marian@ndorms.ox.ac.uk
Lead Radiologist	Dr James Franklin, Institute for Medical Imaging and Visualisation Bournemouth University Gateway Building St Pauls Lane Bournemouth BH8 8GP Tel: 01202 961748 Email: jfranklin@bournemouth.ac.uk

Trial Committees

Trial Steering Committee	Please refer to the TSC charter
MILI Data & Safety Monitoring Committee (DSMC)	Please refer to the DSMC charter
Trial Management Group (TMG):	Please refer to the TMG charter

Protocol Authors and conflict of interest statement

Prof Sarah Blagden	Consultant Medical Oncologist, Dept. Oncology, Oxford University, UK
Linda Collins	Portfolio Lead, OCTO, Dept. Oncology, Oxford University, UK
Rachel Shaw	Trial Manager, OCTO, Dept. Oncology, Oxford University, UK
Maggie Qiao	Trial Statistician, OCTRU, NDORMS, Oxford University, UK
Ioana Marian	Senior Statistician, OCTRU, NDORMS, Oxford University, UK
Dr James Franklin	Consultant Radiologist, Bournemouth University, UK
Claire Brooks	Pharmacovigilance Manager, OCTO, Dept. Oncology, Oxford University, UK

Conflict of Interest
No authors have declared any conflicts of interest

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

Full Title of trial:	Metformin in Li Fraumeni (MILI) Trial: A Phase II Randomised open-label cancer prevention trial of Metformin in adults with Li Fraumeni Syndrome	
Short Title:	Metformin in LFS (MILI) Trial	
Trial Acronym:	MILI	
Clinical Phase:	II	
Trial Design:	Open label, randomised control phase II trial	
Number of centres:	6 centres: Oxford University Hospitals NHS Foundation Trust, The Royal Marsden Hospital NHS Foundation Trust, Guy's & St Thomas' NHS Foundation Trust, Cambridge University Hospitals NHS Foundation Trust, Manchester University Hospitals NHS Foundation Trust, Nottingham University Hospitals NHS Trust.	
	Objectives	**Endpoints**
Primary Endpoint:	To compare cumulative cancer-free survival up to 5 years (60 months) from randomisation between intervention (metformin) and control (no metformin) arms	Cancer free survival – with "cancer" event defined as pathologically confirmed diagnosis of malignant cancer identified during trial participation or death from any cause
Secondary Endpoints:	1. Comparison of cumulative tumour-free survival at 5 years from randomisation between intervention (metformin) and control (no metformin) arms	1. Tumour free survival – with a "tumour" event including pathologically confirmed diagnosis of malignant cancer or clinically/scan detected benign or premalignant lesion (e.g. ductal carcinoma in situ - DCIS) identified during trial participation or death from any cause.
	2. Comparison of overall survival between trial and control arms at 5 years between intervention (metformin) and control (no metformin) arms	2.Time from randomisation to death from any cause.
	3. To compare the impact of taking metformin on the clinical characteristics of emerging cancers.	3. Number and type of emerging cancers, including size, stage and histological grade at diagnosis.
	4. To determine the safety and toxicity of metformin	4. Relevant treatment-emergent adverse events and clinically significant laboratory changes (per NCI CTCAE V5.0) or changes in physical exam and/or vital signs in intervention arm compared to baseline.
	5. To assess the acceptability of metformin	5. MARS-5 questionnaire score
	6. To compare impact of metformin and cancer prevention on quality of life (QOL)	6. Comparison of change in 12-item short form survey (SF12v2), Cancer Worry Scale, Treatment Burden Questionnaire (TBQ) between intervention and control arms and compared to baseline.

	7. To determine the impact of baseline lifestyle risk factors on cancer incidence	7. Correlation of baseline weight, BMI and lifestyle factors (e.g. smoking and diet) with cancer-free survival.
Translational research endpoints	**Objectives**	**Endpoints**
	1. To establish the mechanism of action of metformin as a cancer preventative	i. Whether baseline insulin sensitivity predicts cancer-free survival in LFS
		ii. Whether metformin's chemoprevention effects are *indirect* via insulin sensitivity in LFS using HOMA-IR as surrogate marker.
		iii. Whether metformin's chemoprevention effects are *indirect* via changes in circulating PI3K/AKT/mTOR signalling in LFS.
		iv. Whether metformin's chemoprevention effects are *direct* via alterations to oxidative phosphorylation in LFS using OXPHOS gene signature as surrogate marker.
	2. To identify biomarkers of response/ cancer	i Surrogate genetic markers of cancer-free survival or metformin response.
		ii Proteomic markers of cancer-free survival or metformin response.
		iii Penetrance of germline TP53 mutation in tumour tissue
		iv Retrospective identification or validation of circulating cancer biomarkers
	3. To assess WB-MRI performance as a diagnostic tool for detecting cancers in participants with LFS.	i. Yield and diagnostic accuracy of WB-MRI
Planned enrolment:	224 adults with LFS will be enrolled into MILI with 1:1 randomisation to active (yearly) cancer surveillance with metformin (intervention) versus active (yearly) cancer surveillance without metformin (control).	
Target Population:	**Inclusion:** To be eligible to enter the trial participants must fulfil all of the following: 1. Diagnosis of LFS from confirmed pathogenic TP53 variant (class IV or V by CanVIG-UK criteria (see appendix 2) 2. Aged ≥16 years 3. Capable of understanding the consent process and participating in the trial and according to the investigators' discretion.	

	Exclusion: 1. Currently taking metformin 2. Metformin intake for more than 3 months in total, within the 2 years antecedent to the date of trial enrolment 3. Completion of cancer systemic therapy within the 6 months antecedent to the date of trial enrolment 4. Current type 1 or 2 diabetes mellitus 5. Presence of active ongoing cancer (detected previously or at baseline scanning) 6. Current pregnancy or lactation 7. Gastro-intestinal condition (such as short-bowel syndrome) that could affect absorption of metformin 8. Concurrent medical condition (other than LFS) that could result in life expectancy of <5 years 9. History of the following cardiac conditions: a. Congestive cardiac failure of > Grade II severity according to the New York Heart Association Functional Classification (defined as symptomatic at less than ordinary levels of activity). b. Ischaemic cardiac event including myocardial infarction within 3 months prior to date of enrolment. c. Uncontrolled cardiac disease, including unstable angina pectoris, uncontrolled hypertension (i.e., sustained systolic BP > 160 mmHg or diastolic BP > 90 mm Hg) 10. Evidence of significant renal impairment eGFR < 50ml/minute/1.73m^2 11. Liver cirrhosis and/or alkaline phosphatase, aspartate transaminase or alanine transaminase >2.5 x upper limit of normal (ULN) 12. Elevated risk of lactic acidosis such as current chronic alcoholism, congenital lactic acidosis, concurrent intake of carbonic anhydrase inhibitor (e.g. acetazolamide) 13. Known allergy to metformin 14. Does not fulfil MRI Safety Screening criteria (e.g. implanted cardiac pacemaker, post-surgical metal hardware – plates etc) and/or unable to undergo baseline scan.
	Name of drug	Formulation, dose, route of administration
Investigational Medicinal Product(s)	Metformin (N,N-dimethylbiguanide) hydrochloride	Immediate-release tablets, given initially as 500 mg once daily and dose increased every 14 days by 500 mg increments to up to 2g/daily (1000 mg bd) given orally. Final dose selected for individual participants according to tolerability.
Treatment Duration	Treatment for up to 5 years from randomisation	
Follow-up duration	Participants will be followed-up for 5 years from randomisation.	
End of trial	Defined by the completion of the planned statistical analyses and the completion of the translational research analysis (whichever comes last). This is expected to be Sep2030.	

TABLE 1: SUMMARY SCHEDULE OF EVENTS

Procedure	-28 days to 0 (Screening & baseline)	Week▲ 1 Day 11	Week 3▲ Day 15	Week 5▲ Day 29	Week 7▲ Day 43	Week 9▲ Day 57	Month 6▲	Month 12▲ (Year 1)	Month 18▲	Month 24▲ (Year 2)	Month 30▲	Month 36 (Year 3)	Month 42▲	Month 48 (Year 4)	Month 54▲	Last visit Month 60▲ (Year 5)	Cancer Diagnosis
Informed Consent	x																
Eligibility checklist	x																
Medical History & Concomitant meds	x																
Physical & dermatological Examination	x							x		x		x		x		x	
Demographics	x																
Pregnancy test[a]	x																
Height (in metres)	x																
Weight (in kg)	x							x		x		x		x		x	
Pharmacodynamic samples (PD) 1[b]	x							x									
Pharmacodynamic samples (PD) 2[c]	x							x		x		x		x		x	
Haematology & Biochemistry[d]	x							x		x		x		x		x	
Whole Body & Brain MRI[e]	x[1]							x		x		x		x		x	
Collect participant contact details	x																
QOL questionnaires[f]	x							x		x		x		x		x	
Randomisation		x															
Telemed call Metformin arm[g]		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Telemed call[h] Control arm		x					x	x	x	x	x	x	x	x	x	x	x
Tumour tissue[i]																	x
Metformin administration		x Metformin to be taken daily for the duration of the trial (metformin arm only)															

Key:

	Screening & baseline assessments at investigator (recruiting) site
	Metformin dose titration phase & metformin dosing phase for trial
	Assessments at yearly visit to investigator (recruiting) site

FOOTNOTES

a) Pregnancy test (urine) for participants of child bearing potential (see Appendix 3 for definition) & who are sexually active.
b) Pharmacodynamic samples PD1: Whole blood for PBMCs processed at the investigator (recruiting) site and shipped to Oxford
c) Pharmacodynamic samples PD2: Whole blood collected in Streck tube and shipped to Oxford
d) Fasting biochemistry & haematology bloods: FBC (Hb, WBC, platelets), Biochem (sodium, potassium, urea, creatinine, eGFR, liver function tests, vitamin B12 (baseline and year 5 only) and for the translational research endpoints insulin, glucose and IGF1 (baseline and M12)
e) Whole body and brain MRI (WB-MRI) to be performed at the investigator (recruiting) sites. If a whole body and brain MRI scan has been completed for the participant in the 3 months prior to randomisation, this will be accepted as the baseline and screening scan.
f) Quality of Life questionnaires: 12-item short form survey (SF12V2), Cancer Worry Scale & Treatment Burden Questionnaire (TBQ) (to be completed via email by participants)
g) Telemed calls metformin arm*: Initial screening- explain randomisation outcome & call structure. Management of dose-titration phase, determine adverse events, changes to concomitant meds and review of adherence, (MARS-5 Medication Adherence Questionnaire from month 6 onwards).
h) Telemed calls control arm*: Screening- explain randomisation outcome & call structure. From month 6 to determine adverse events, changes to concomitant meds.
i) Tumour Tissue: Paraffin-embedded diagnostic tumour tissue block or pre-cancerous tissue to be collected and shipped to Oxford
j) Metformin administration: metformin will be sent by post from the recruiting hospital pharmacy

**For both arms, additional telemed calls may be made by the telemed team*

▲Visit window

Call or visit	Window
Telemed call week 1	Within 3 working days of randomisation
Telemed call (dose titration phase)	Metformin start date + week 3/5/7/9 + within 3 working days
Telemed call months 6-60	+/- 1 week
Annual visit to investigator site	+/- 4 weeks

ABBREVIATIONS

ACMG	American College of Medical Genetics
AMPK	5' Adenosine monophosphate-activated protein kinase
b.d	Bi-daily (twice daily)
BP	Blood pressure
C.I.	Confidence interval
CFS	Cancer-free survival
CI	Chief Investigator
CIP	Clinical Investigation Plan
CO_2	Carbon dioxide
COVID-19	Coronavirus 2019
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CTIMP	Clinical Trial of an investigational medicinal product
DBD	DNA binding domain
DCIS	Ductal carcinoma in situ
DMC/DMSC	Data Monitoring Committee / Data Monitoring and Safety Committee
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
ECMC	Experimental Cancer Medicine Centre
eGFR	Estimated glomerular filtration rate
EMA	European Medicines Agency
EME	NIHR Efficacy and Mechanism Evaluation
EudraCT	European Clinical Trials Database
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GPTT	George Pantziarka TP53 Trust
G1-5	Grade 1-5 (CTCAE)
HRA	Health Research Authority
HRQOL	Health-related quality of life
ICF	Informed Consent Form
IDMC	Independent Data Monitoring Committee
IGF1	Insulin-like growth factor
IMP	Investigational Medicinal Product
IOC	International Oversight Committee
IPD	Individual participant data
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trials Number
LFS	Li Fraumeni Syndrome
LPLV	last visit of the last participant undergoing the trial
MA	Marketing Authorisation
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare products Regulatory Authority
MILI	Metformin in Li Fraumeni Syndrome Trial
MILI-Paed	Paediatric version of MILI (Canada)
MRI	Magnetic Resonance Imaging
mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
NCRI	National Cancer Research Institute
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health and Care Research
o.d.	Once daily
OCTO	Oncology Clinical Trials Office
OCTRU	Oxford Clinical Trials Research Unit
OXPHOS	Oxidative phosphorylation
P53	“Protein 53” tumour suppressor

PBMC	Peripheral blood mononuclear cell
PI	Principal Investigator
PIS	Participant information sheet
PPI	Participant and Public Involvement
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
REC	Research Ethics Committee
RNA	Ribonucleic acid
RPPA	Reverse Phase Protein Array
RSI	Reference safety information
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SF12v2	12-item Short-Form Health Survey (version 2)
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Drug Reaction
TMF	Trial Master File
TMG	Trial management group
Tp53	Gene encoding the P53 protein
TSC	Trial Steering Committee
UKCRC	UK Clinical Research collaboration
ULN	Upper limit of normal
WB-MRI	Whole body (and brain) Magnetic Resonance Imaging
WHO	World Health Organization

1 INTRODUCTION

1.1 Background

Li Fraumeni Syndrome (LFS) is a rare autosomal dominant cancer predisposition syndrome caused by germline or *de novo* pathogenic variants in *TP53*. Males and females with LFS have a 70% and 90% lifetime risk of cancer respectively, with around 50% having their first cancer diagnosis before the age of 46 and 31 years respectively. Typical LFS "core" malignancies include bone and soft-tissue sarcomas, breast, brain and adrenocortical cancers but less commonly lung, colon, haematological, skin, stomach and ovarian cancers.

In the UK there are over 600 people with a genetic diagnosis of LFS and, with increasing use of genetic sequencing in diagnostic practice, this number will rise (1). In view of the high penetrance for cancers, published guidelines require *TP53* mutation-carriers to undergo intensive surveillance comprised of annual whole body + brain (WB) magnetic resonance imaging (MRI) alongside annual breast MRI (for women who have not undergone prophylactic mastectomy) and skin examination (2-4). Tragically, without this surveillance, average life expectancy is under 40 years (5,6). Although imaging surveillance is considered standard-of-care, it is conducted haphazardly across the UK and many LFS participants receive only yearly physical examinations without scans. Anticipatory loss is a common emotion amongst affected families who describe "waiting for the shoe to drop" and cancers to inevitably appear followed by grief as they experience the impact of cancer diagnosis, treatments and death (7). Evidence from surveillance studies such as UK SIGNIFY have shown LFS participants have measurably higher levels of worry and intrusive thoughts about cancer (8-10) and have poorer mental and physical functioning compared to others. Hence, LFS is a condition of high physical and psychological morbidity (8).

There are many unanswered questions around the biology of LFS: what cellular events induce cancer and can these be measured and averted, and is cancer risk the same for all *TP53* mutations? These knowledge gaps are amplified by the lack of a central registry of LFS participants in the UK. There are no medications licenced to prevent cancer in LFS and, so far, no chemoprevention studies have been conducted in LFS. In recognition of this, the UK's LFS advocacy organisation, the George Pantziarka *TP53* Trust (GPTT), has repeatedly called for more research into LFS along with better coordination of surveillance scanning and the introduction of cancer prevention trials: www.tp53.co.uk . However, the rarity of LFS makes such studies challenging to conduct and statistically power.

The protein encoded by *TP53* is P53. P53 is described as the "guardian of the genome" for its protective roles in multiple cellular programmes such as DNA repair, proliferation, senescence, apoptosis, autophagy, metabolism and angiogenesis. P53 mainly functions as a transcription factor, binding around 200 protein-coding genes and non-coding RNAs to activate their expression. Interestingly, P53 also directly binds other proteins, such as those in the mitochondria of cells to control cell metabolism, apoptosis and autophagy (11). It is now known that most LFS participants carry a mutation within exons 4-9 of *TP53*, which encode its DNA binding domain (DBD) **(Figure 1).** The majority of these mutations are missense - in which a single nucleotide is substituted for another – creating a more stable form of P53 but one that is less able to bind its target genes but more able to bind others. In *in vitro* studies, mutated P53 (mutp53) can repress normal P53 protein and has additional capabilities, such as binding and inhibiting 5' adenosine monophosphate-activated protein kinase (AMPK) (12). By this "gain-of-function" mechanism, mutp53 transforms into an oncogene (13). Interestingly, a trial reported repeated cancers in one (but not both) of a pair of monozygotic LFS twins indicating that emergence of cancer (also known as penetrance) is not entirely genetically predetermined and is dependent not only on the type and site of *TP53* mutation but other factors including their age, sex (14,15) and environment.

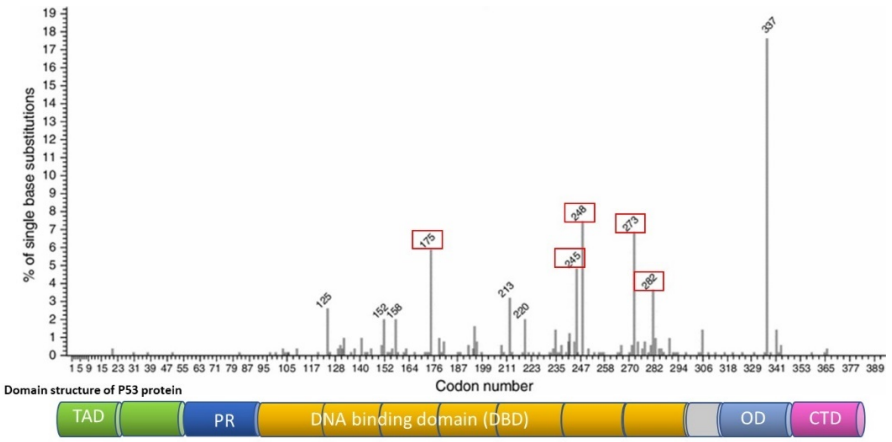


Figure 1: *LFS mutation "hotspots" in TP53 and their corresponding location within p53 protein domains. Red squares highlight mutations frequently observed in sporadic cancers. TAD: transactivation domain, PR: proline rich domain, DBD: DNA binding domain, OD: oligomerisation domain, CTD: C-terminal domain. Figure derived from (1).*

To understand why *TP53* mutations cause cancer, a *tp53*^{515A} knock-in "LFS mouse" was made in 2004. This carried a genetic alteration equivalent to a missense mutation at the R175 hotspot within the DBD (16). As well as forming spontaneous tumours, mice that were homozygous or heterozygous for this mutation showed increased oxidative metabolism synonymous with heightened mitochondrial activity and this finding was replicated in myoblasts obtained from the skeletal muscle of LFS participants after exercise (11). Cells from LFS models also revealed enhanced lipolysis and fatty acid synthesis caused by inhibition of AMPK (17). Paul Hwang's team at NCI then showed that tumour development in LFS mice was a direct result of their altered mitochondrial function (18). When crossed with mice bearing a heterozygous or homozygous mutation in the mitochondrial gene DNA polymerase gamma, rates of metabolism were lowered and cancer-free survival was increased by 40% and 79% respectively (18). These findings indicate that the mitochondrial activity of mutP53 is key to its tumorigenic activity as it activates oxidative phosphorylation (OXPHOS) and cellular metabolism (19) and, via this mechanism, mutp53 drives cell proliferation and cancer formation (tumorigenesis). As the anti-diabetic agent metformin is known to inhibit mitochondrial respiration, mice were given metformin from 4 weeks of age and this caused a reduction in oxidative metabolism markers and the time to mice developing cancer was delayed by 27% (18).

Although these findings indicate metformin's main anticancer activity is "**direct**" by repressing mitochondrial respiration, others believe its effects are **indirect** by reducing hepatic gluconeogenesis and hence circulating glucose, insulin and IGF1 levels (19-23). It is possible that metformin regulates other molecular, immunological or epigenetic factors may determine how and when cancer develops. The purpose of MILI is to assess the impact of metformin on the incidence and survival from cancer in participants with a confirmed diagnosis of LFS and to simultaneously explore the biology underpinning its activity as a cancer preventative. Participants will be randomised to: intervention arm (metformin daily for up to 5 years) versus control (no metformin).

1.2 Medicinal Product(s) used in the trial

Metformin:

Metformin (1,1-dimethylbiguanide) is a synthetic derivative of a natural compound found in French lilac. It is an oral glucose-lowering agent that has been used to treat type 2 diabetes for over 60 years. It is cheap, well-tolerated and has been prescribed to more than 120 million people since it was first licenced in the 1950s. Although an old drug, metformin has multiple mechanisms of action; its central function is activation of AMPK in the liver which suppresses fatty acid synthesis and gluconeogenesis, increasing glycolysis and enhancing glucose uptake. This reduces circulating levels of insulin and glucose and reverses diabetic hyperglycaemia (24). In addition, through its activation of AMPK, metformin suppresses mammalian target of rapamycin (mTOR) which reduces cell growth and increases apoptosis (23). Despite its positive attributes, metformin can cause gastrointestinal side effects in up to 30% participants, including diarrhoea, nausea, vomiting, abdominal bloating and anorexia. These symptoms usually occur at the onset of therapy, rarely persist and can be mitigated by slowing dose-escalation or dose-reduction (24). Because of its repression of aerobic mitochondrial respiration, metformin causes the accumulation of lactic acid. Overdose or failure to excrete metformin (90% is renally cleared) alongside difficulties in compensating for raised lactate can cause a fall in body pH and lactic acidosis. This is a rare but serious side effect of metformin with an estimated incidence of 6 cases per 100,000 participant-years (25). For this reason, contraindications to metformin use include chronic kidney disease, liver disease and heart failure. It is also standard practice to withhold metformin for up to 48 hours prior to administering renally-cleared intravenous contrast agents. It is standard practice to assess the renal function of any participant starting metformin and to carefully dose-escalate to reach the standard dose of 2g/day (26). Pharmacodynamic studies have previously shown (27) that the maximal efficacy of metformin occurs at 2mg (its recommended dose) as, above this level, drug absorption decreases and incidence of gastro-intestinal toxicity increases.

Metformin as a Cancer Preventative in sporadic cancers

Whilst its benefit in reducing established cancers is controversial (28,29) the cancer preventative activities of metformin are more compelling. A meta-analysis of 10 million diabetic participants showed those taking metformin were at significantly lower risk of developing cancer with an odds ratio of 0.7 (0.65-0.76 95% CI) (27) although risk reduction was not uniform across all cancer types (28). The strongest association between metformin and reduced cancer risk was shown amongst the commonest malignancies such as colorectal cancer (30). These findings have resulted in studies evaluating metformin as a cancer preventative (**Table 2**) in non-diabetic participants. One of the earliest to complete was a prospective prevention trial evaluating the

impact of a 12-month course of metformin on reducing recurrent colorectal adenomas or polyps and showed that metformin reduced recurrence by 21% (31). The dose of metformin given in these prevention studies ranged from 250 mg/day to 2g/day.

Cancer Type	Name of Trial/Description	Dose of Metformin	Duration of Metformin	Status/outcome
Colorectal	NCT03047837, ASAMET (tertiary prevention)	850 mg bd = 1500mg/day	12 months	Ongoing
Breast	NCT01905046; impact of metformin on atypical hyperplasia or in situ breast ca	850 mg od or bd = up to 1500mg/day	24 months	Ongoing
Breast	NCT02028221; impact of metformin on obese women with high breast density	850 mg bd = 1500mg/day	12 months	Ongoing
Oesophagus	NCT01447927; impact of short course of metformin on proliferation markers in Barrett's oesophagus	500-2000mg/day dose escalated	12 weeks	Completed - negative (32)
Colon	UMIN000006254 metformin after prior polyps or adenomas resected	250mg/day	1 year	Complete – 21% reduction in recurrent lesion (31)

Table 2: Metformin cancer prevention studies

Proof of Concept Trial: Metformin alters metabolism in LFS participants:

The proof of concept work behind this proposal is a pilot trial conducted by Christina Annunziata and team at NCI (33). The background to this trial was Paul Hwang's data showing that metformin reversed aberrant mitochondrial activity in LFS mice and, with that, delayed the emergence of cancers (9,16). To explore whether metformin was as effective at reversing metabolism in LFS participants with a broad spectrum of *TP53* mutations, Annunziata and team recruited 26 participants (20 females and 6 males) to receive daily metformin for 14 weeks. Blood samples were obtained for measurement of serum IGF1, insulin and IGFBP3 and hepatic mitochondrial function was assessed by measuring fasting exhaled CO_2 after ingestion of ^{13}C-labelled methionine. Metformin was given at a starting dose of 500mg once daily and dose-escalated to 2g/day in 500mg increments every two weeks. Even though participants were non-diabetic, metformin lowered circulating IGF-1 and IGFBP3 levels, increased markers of fatty acid beta-oxidation and lowered levels of exhaled ^{13}C-methionine at weeks 8 and 14 compared with baseline. Metformin was generally well-tolerated with only low grade (grade 1 or 2) side effects such as diarrhoea (affecting 50%), nausea (46%), dyspepsia (19%) and headache (30%). The majority reported complete resolution of these symptoms by week 14 at the maximal dose of metformin (2g/day). No episodes of lactic acidosis were observed. This confirmed that metformin was effective at reversing oxidative metabolism in LFS participants although the short duration and the design of the trial did not allow assessment of its impact on cancer emergence. The purpose of MILI is therefore to proceed to exploring the impact of metformin on cancer.

1.3 Rationale for the trial

The overarching purpose of MILI is to assess whether daily intake of metformin for up to 5 years reduces incidence and death from cancers.

Measure the impact of Metformin on incidence and deaths from cancer over 5 years:

We have formed an International LFS Consortium with investigators from National Cancer Institute (NCI, USA), Hannover (Germany) and SickKids (Canada), to conduct international randomised studies to evaluate metformin as a cancer preventative. Trial centres in each nation are seeking independent funding to conduct MILI in adults and/or children using a locally-adapted core protocol. Each trial is sufficiently powered to address the primary objective as a standalone trial. Results from all studies will be pooled in an individual participant data (IPD) meta-analysis to definitively determine the benefit of metformin at preventing cancer in this high-risk participant group and address secondary and translational endpoints.

The majority of invasive cancer is preceded by a latency phase characterised by the presence of a pre-invasive or an intra-epithelial lesion which is histologically non-malignant (34). In the LFS mouse, metformin was demonstrated to delay the emergence of invasive cancers, either because it (i) prevents pre-invasive and, later, invasive lesions from forming or (ii) because metformin extends the pre-invasive latency period. To explore this, we will compare the number and type of non-malignant "tumours" that are detected in each arm of MILI as well as the number of invasive cancers diagnosed during the 5 years of the trial. To assess the side effect profile of metformin in LFS, we will be collecting safety and toxicity information about 'reportable' events (those *a priori* deemed important and relevant) during MILI, every two weeks during the titration phase and every 6 months thereafter. Acceptability will be assessed by monitoring the number of participants who remain

on metformin and the total dose of metformin prescribed per participant for the 5-year duration of the trial. As prevention studies have not been conducted in LFS participants before, MILI presents a unique opportunity to evaluate the impact of metformin, and of cancer prevention as a whole, on wellbeing before and during the trial. We will be assessing HRQOL using questionnaires validated during UK SIGNIFY (QOL) trial (35).

As part of MILI, we will be collecting this information at baseline and correlating with outcome within the UK trial set and the IPD meta-analysis. As part of MILI, we will test whether the direct or indirect effects of metformin are most responsible for its chemopreventative activities by measuring change from baseline of circulating metabolic (insulin and IGF1) and PI3K activation markers, as well as a transcriptional mitochondrial OXPHOS gene signature, after metformin treatment and which most closely corresponds to cancer-free survival. We will also be collecting plasma and cell-free DNA (cfDNA) for identification or validation of biomarkers that could be used to predict the emergence of cancer in patients with LFS.

2 COVID-19 CONSIDERATIONS

Cancer remains a fatal disease that continues to cause more daily deaths than COVID-19. The need to identify better treatments through clinical research is not affected by the presence of the pandemic. Pausing such research for months, if not years, until the pandemic subsides will likely result in more deaths as improvements in care are delayed.

The COVID-19 vaccination programme has been implemented across the UK. There is no evidence to suggest that non-live (inactivated) vaccines, including COVID-19 vaccines, are best avoided during metformin treatment. There is no evidence to suggest that metformin treatment should be discontinued if a participant is diagnosed with COVID-19.

3 TRIAL DESIGN

MILI is a randomised, open-label, Phase II trial evaluating the impact of metformin on cancer incidence in adults with Li Fraumeni Syndrome. MILI will be conducted in UK investigator (recruiting) sites.

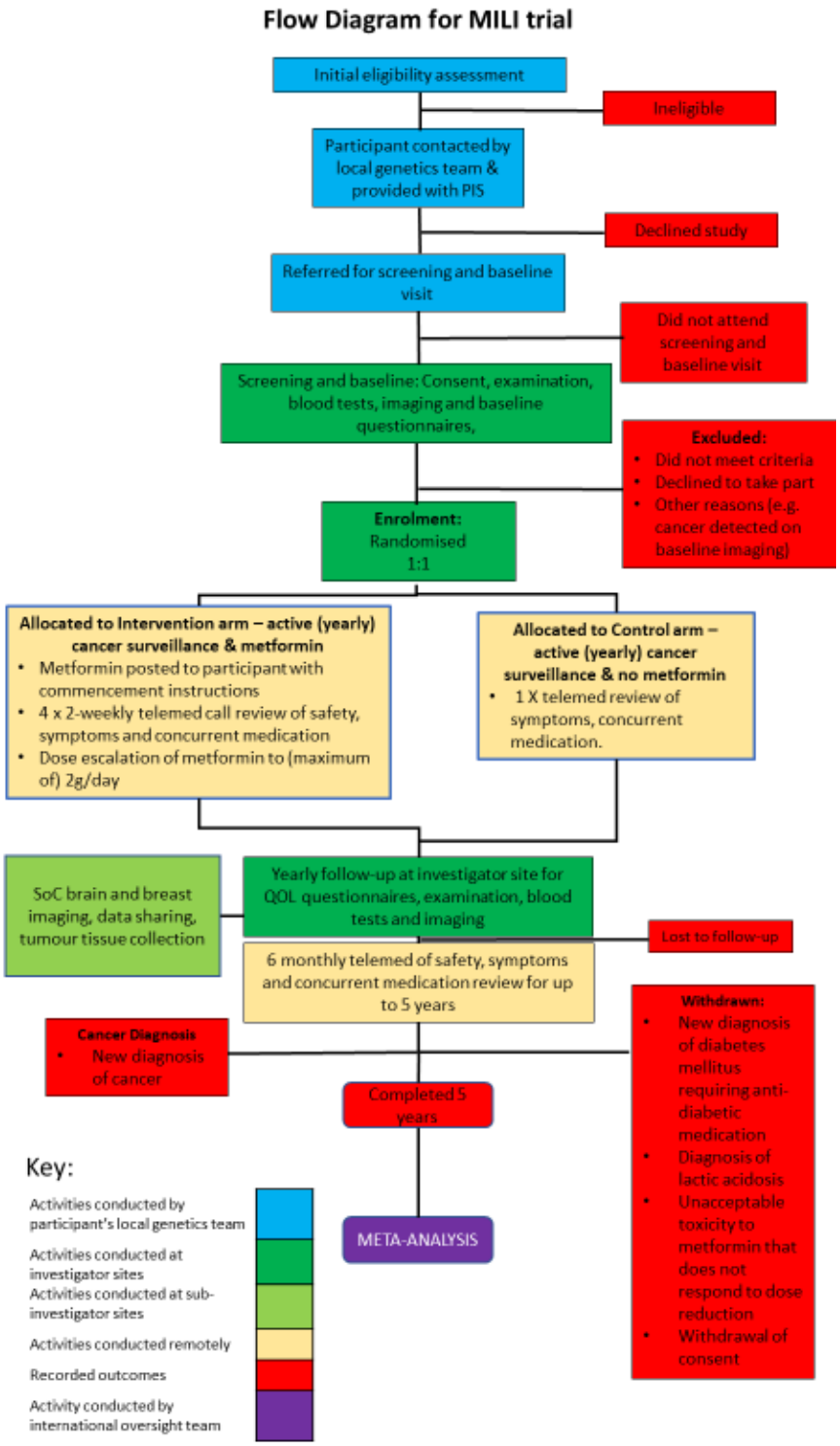
Participants will be referred to the investigator (recruiting) sites by their local genetics team. The local genetics team may be based at an sub-investigator site or at an investigator site.

The trial has adopted the hub and spoke model for the Principal Investigator oversight of trial activities. Trial activities conducted at the investigator (recruiting) sites require a Principal Investigator employed by the site to oversee the activities (consent, eligibility assessment, translational sample collection). Activities conducted at the sub-investigator sites do not require a Principal Investigator to be based at the site because they are conducting Standard of Care activities but for this trial do require a Principal Investigator from one of the investigator sites to provide oversight because of the data sharing with the investigator site.

It is estimated that 224 adults with LFS will be recruited and randomised 1:1 to metformin (intervention) versus no metformin (control). Participants randomised to metformin will receive up to 2g (1000mg bd) taken daily for up to 5 years. Those in the control arm will receive no metformin. Those in both trial arms will undergo yearly cancer surveillance. The trial has adopted an open-label trial design as the question can be answered through this design as there is no likelihood that the participant can influence the outcome of the trial.

See flow chart for details of the trial visits and procedures (figure 2).

3.1 Figure 2: MILI FLOW DIAGRAM



3.2 Duration of participation

Participants will be in the trial for up to 5 years from randomisation to last protocol visit.

3.3 Participant evaluability and replacement

To ensure that we have adequate power to assess the difference between metformin and treatment-as-usual conditions outlined in section 17.1, we will recruit additional participants for those lost during the initial 6-month titration period to ensure that we have the planned total number of 112 participants that have commenced a fixed-dose schedule of metformin.

The primary analysis for MILI follows the intention-to-treat principle, and therefore the trial's primary objective is to assess the effect of offering metformin to participants, irrespective of level of adherence to their prescribed amount (a 'treatment policy' objective, see section 18). Participants who end active trial participation (due to withdrawal/cancer event) within 6 months of enrolment will still be included in the final primary analysis.

This analysis negates the risk of immortal time bias (36) in the between-groups treatment estimate that may occur due to the exclusion of participants that drop out during the 6-month titration phase.

All participants in the metformin arm who receive at least one dose of metformin will be evaluable for the safety analysis.

3.4 Post-trial care and follow-up

Following a participant's final protocol visit, they will continue with usual NHS care.

4 OBJECTIVES, ENDPOINTS, AND ESTIMANDS

4.1 Trial Objectives

1) The primary aim of MILI is determine the impact of taking metformin on the time to diagnosis of cancer in participants with LFS. This corresponds to **the primary objective** of comparing cumulative cancer-free survival up to 5 years from randomisation between trial (metformin) and control (no metformin) arms.

2) **Secondary objectives** of MILI are:

   i) to determine the impact of taking metformin on the time to diagnosis of non-invasive/pre-cancerous lesions. This corresponds to the secondary objective of comparing tumour-free survival at 5 years from randomisation between trial (metformin) and control (no metformin) arms.

   ii) to determine the impact of taking metformin on the overall survival of LFS participants. This corresponds to the objective of comparing overall 5-year survival between the two trial arms.

   iii) to compare the impact of taking metformin on the clinical characteristics of emerging cancers. This will be assessed by the comparison of site, histological type, stage and grade of cancers diagnosed within 5 years of randomisation between trial arms.

   iv) to determine the safety and toxicity of metformin by comparing the incidence of relevant adverse events and/or laboratory changes within 5 years of randomisation in the metformin arm.

   v) to assess acceptability of metformin by measuring compliance with medication for those in the metformin arm of the trial.

   vi) to compare quality of life, with particular attention to the trade-off between cancer worry and the impact of chronic metformin use.

   vii) to determine the impact of lifestyle factors such as BMI and alcohol intake on survival outcomes.

4.2 Trial Endpoints and Relation to Objectives

Table 3

Primary Objective	Endpoints/ Outcome measures	Time point(s) of evaluation of this end point
To compare cumulative cancer-free survival up to 5 years (60 months) from randomisation between intervention (metformin) and control (no metformin) arms	Cancer free survival – with "cancer" event defined as pathologically confirmed diagnosis of malignant cancer identified during trial participation or death from any cause.	Comparison of time to development of new cancer in each trial arm within 60 months of randomisation and % events
Secondary Objectives		
1. Comparison of cumulative tumour-free survival at 5 years from randomisation between intervention (metformin) and control (no metformin) arms	1. Tumour free survival – with a "tumour" event including pathologically confirmed diagnosis of malignant cancer or clinically/scan detected benign or premalignant lesion (e.g. ductal carcinoma in situ - DCIS) identified during trial participation or death from any cause.	1. Comparison of time to development of new cancer and/or benign lesion in each trial arm within 60 months of randomisation and % events.
2. Comparison of overall survival between trial and control arms at 5 years between intervention (metformin) and control (no metformin) arms	2. Time from randomisation to death from any cause.	2. Comparison of time to death in each trial arm within 60 months post-group allocation and % events
3. To compare the impact of taking metformin on the clinical characteristics of emerging cancers.	3. Number and type of emerging cancers, including size, stage and histological grade at diagnosis.	3. Comparison of number, type and stage of cancer diagnosed within each trial arm within 60 months post-group allocation
4. To determine the safety and toxicity of metformin	4. Relevant treatment-emergent adverse events and clinically significant laboratory changes (per NCI CTCAE v5.0) or changes in physical exam and/or vital signs in investigation arm compared to baseline.	4. Comparison of number of and severity of relevant AEs in the intervention (metformin) trial arm within 60 months post-group allocation
5. To assess the acceptability of metformin	5. MARS-5 questionnaire score	5. Change in MARS-5 score in the intervention (metformin) trial arm from group allocation to 60 months
6. To compare impact of metformin and cancer prevention on quality of life (QOL)	6. Comparison of change in 12-item short form survey (SF12v2), Cancer Worry Scale, Treatment Burden Questionnaire (TBQ) between intervention and control arms and compared to baseline.	6. Comparison of scores between group over 60 months post-group allocation
7. To determine the impact of baseline lifestyle risk factors on cancer incidence	7. Correlation of baseline weight, BMI and lifestyle factors (e.g. smoking and diet) with cancer-free survival.	7. Until cancer event or 60 months post-group allocation has elapsed
Translational research objectives	**Endpoints/ Outcome measures**	**Time point(s) of evaluation of this end point**

1. To establish mechanism of action of metformin as a cancer preventative	i. Whether baseline insulin sensitivity predicts cancer-free survival in LFS ii. Whether metformin's chemoprevention effects are *indirect* via insulin sensitivity in LFS using HOMA-IR as surrogate marker. iii. Whether metformin's chemoprevention effects are *indirect* via changes in circulating PI3K/AKT/mTOR signalling in LFS. iv. Whether metformin's chemoprevention effects are *direct* via alterations to oxidative phosphorylation in LFS using OXPHOS gene signature as surrogate marker.	i. Correlation between HOMA-IR score at baseline with cancer-free survival at 5 years in metformin arm ii. Correlation between change in HOMA-IR score with cancer-free survival at 5 years in metformin arm iii. Comparison between PI3K Activity Score in PBMCs at baseline and after 1 year in interventional (metformin) and control arms and correlation with cancer-free survival at 5 years in metformin arm iv. Change in OXPHOS gene signature in PBMCs at I year and correlation with cancer-free survival at 5 years in metformin arm
2. To identify biomarkers of response/ cancer	i. Surrogate genetic markers of cancer-free survival or metformin response. ii. Proteomic markers of cancer-free survival or metformin response. iii. Penetrance of germline TP53 mutation in tumour tissue. iv. Retrospective identification or validation of circulating cancer biomarkers	i. RNA-seq of PBMCs (baseline and dynamic change at 1 year) for changes in wider metabolic pathway markers correlated with cancer free survival. ii. Mass spec analysis of PBMCs for metabolic pathway expression iii. RNA-seq of TP53 in tumour tissue (if cancer diagnosis during trial) compared to known germ-line mutation iv. Evaluation of novel cancer biomarkers using longitudinal plasma samples and longitudinal cfDNA in patients who develop cancers during trial
3. To assess WB-MRI performance as a diagnostic tool for detecting cancers in participants with LFS.	i) Yield (including relevant and incidental findings), and diagnostic accuracy of WB-MRI.	i) Description of yield and diagnostic accuracy at each MRI timepoint (screening, years 1,2,3,4, and 5) against final cancer outcome.

4.3 Estimands

An *estimand* is a precise description of the treatment effect to be estimated by the analysis. Taking the objectives and endpoints outlined in section 4.2, we define the primary estimand deeming the success of this trial as follows:

Primary estimand. This trial seeks to estimate, in all individuals with a confirmed diagnosis of Li-Fraumeni Syndrome, the hazard ratio / absolute risk difference of developing a cancer event during a 60-month observation window, for those offered metformin, relative to those on usual care, and irrespective of concomitant medication use, and the occurrence of other concurrent illness (e.g. a change in diabetes status), and treatment discontinuation/switching.

A 'cancer event' is defined as either a pathologically confirmed diagnosis of malignant cancer identified during trial participation, or death from any cause.

5 PARTICIPANT ELIGIBILITY

Written informed consent must be obtained before any trial specific procedures are performed. The Investigators at the investigator (recruiting) sites will determine participant eligibility based on the following criteria. Eligibility must be confirmed by a medically qualified doctor.

5.1 Inclusion criteria:

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

1. Diagnosis of LFS from confirmed pathogenic *TP53* variant (class IV or V by CanVIG-UK criteria (see Appendix 2))
2. Aged ≥16 years
3. Capable of understanding the consent process and participating in the trial according to the investigators' discretion.

5.2 Exclusion criteria:

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

1. Currently taking metformin
2. Metformin intake for more than 3 months in total, within the 2 years antecedent to the date of trial enrolment
3. Completion of cancer systemic therapy within the 6 months antecedent to the date of trial enrolment
4. Current type 1 or 2 diabetes mellitus
5. Presence of active ongoing cancer (detected previously or at baseline scanning)
6. Current pregnancy or lactation
7. Gastro-intestinal condition (such as short-bowel syndrome) that could affect absorption of metformin
8. Concurrent medical condition (other than LFS) that could result in life expectancy of <5 years
9. History of the following cardiac conditions:
   a. Congestive cardiac failure of > Grade II severity according to the New York Heart Association Functional Classification (defined as symptomatic at less than ordinary levels of activity).
   b. Ischaemic cardiac event including myocardial infarction within 3 months prior to date of enrolment.
   c. Uncontrolled cardiac disease, including unstable angina pectoris, uncontrolled hypertension (i.e., sustained systolic BP > 160 mmHg or diastolic BP > 90 mm Hg)
10. Evidence of significant renal impairment eGFR <50ml/minute/1.73m^2
11. Liver cirrhosis and/or alkaline phosphatase, aspartate transaminase or alanine transaminase >2.5 x upper limit of normal (ULN)
12. Elevated risk of lactic acidosis such as current chronic alcoholism, congenital lactic acidosis, concurrent intake of carbonic anhydrase inhibitor (e.g. acetazolamide)
13. Known allergy to metformin
14. Does not fulfil MRI Safety Screening criteria (e.g. implanted cardiac pacemaker, post-surgical metal hardware – plates etc.) and/or unable to undergo baseline scan.

5.3 Protocol waivers to entry criteria

Protocol adherence is a fundamental part of the conduct of a clinical trial. Before entering a participant onto MILI the Principal Investigator or designee will confirm eligibility. If unsure whether the participant satisfies all the entry criteria, and to clarify matters of clinical discretion, investigators must contact the Trial office, who will contact the Chief Investigator or designated clinicians as necessary. If in any doubt, the Chief Investigator must be consulted before entering the participant. Details of the query and outcome of the decision must be documented in the TMF and the patient medical records at the investigator (recruiting) site.

5.4 Clinical queries and protocol clarifications

Every care has been taken in drafting this protocol. Trial teams should contact the trial office for clarification if any instructions seem ambiguous, contradictory or impractical. Clinical queries must also be directed to the trial office. All clinical queries and clarification requests will be logged, assessed and a written response provided. Minor administrative corrections or clarifications will be communicated to all trial investigators for

information as necessary. For urgent safety measures or changes that require protocol amendment see section 22.5.

5.5 Participant referral, screening and randomisation procedure

Participants that are potentially eligible for MILI will initially be identified through information provided by their local genetics team. The local genetics team may be based at what the trial refers to as a "sub-investigator site" or they may be based at one of the "investigator (recruiting) sites". Information about the trial will also be available on the George Pantziarka TP53 Trust website however, potential participants must first consult with their local genetics team if they wish to take part in the trial.

The local genetics team will provide potential participants with a trial invitation letter and a copy of the Participant Information Sheet. Potential participants will be asked to contact their local genetics team if they are interested in taking part.

Those who have contacted the local genetics team will be referred (if applicable) and as per local policy, to one of the investigator (recruiting) sites for an initial visit for consent, screening and baseline assessment. The investigator (recruiting) site must have a confirmation of the diagnosis of LFS from confirmed pathogenic TP53 variant (class IV or V by CanVIG-UK criteria) at the referral stage.

5.5.1 Screening number and screening logs

Screening numbers will be generated by the REDCap clinical trial database. The screening number will be used as the trial number throughout the trial.

A screening log must be kept by the investigator (recruiting) sites of all participants screened for MILI including any that are eligible but decide not to take part, or any that are subsequently excluded; the reason for exclusion must be recorded on this form.

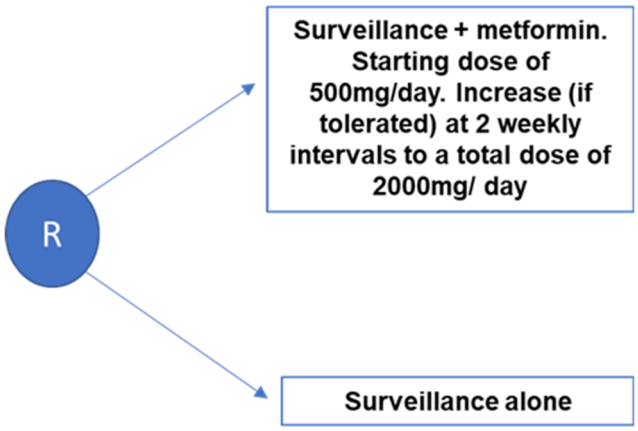
As per local hospital standard practice, those who are found to be ineligible for the trial during the screening assessments will be phoned by the PI at the investigator site and arrangements made regarding their on-going care.

5.5.2 Summary of the Randomisation procedure

1. Randomisation should take place within 28 days of screening & baseline assessments done by the investigator (recruiting) site.
2. PI or designee (medically qualified doctor) will confirm eligibility once the results of the screening assessments and laboratory tests (as listed in sections 7. 1 & 7.7) have been reviewed. If radiological procedures need to be repeated, these repeat scans need to be reviewed and reported to the PI trial team with PI review, before eligibility can be confirmed and the randomisation form completed.
3. Investigator (recruiting) site staff will confirm diagnosis and will confirm the participant's eligibility by completing the eligibility checklist within the trial randomisation form and randomising the participant in the REDCap clinical trial database.

4. The participant will be allocated to one of the two arms as shown below:

Figure 3: 1:1 Randomisation



5. Confirmation of the randomised allocation will be sent automatically from the trial database to the Principal Investigator, Main Contact (at investigator (recruiting) site), Data Manager (at investigator (recruiting) site) and to the OCTO MILI trial office. The investigator (recruiting) site pharmacy team will be notified if the participant is allocated to the metformin arm.
6. The investigator (recruitment) site staff will be responsible for:

- Saving the randomisation result in the ISF.
- Writing the prescription for the initial supply of metformin (metformin arm only)
- Sending the letter confirming the randomisation outcome and providing the participant with a trial participation card which lists the investigator (recruiting) site contact details in case of emergencies.
- Sending the letter outlining the trial to the participant's GP and to the local genetics team at the sub-investigator site. File/scan (if notes are electronic) a copy into participant notes and/or annotate participant notes stating the version sent.

Please refer to the MILI investigator (recruiting) site screening and treatment allocation procedure located in the ISF for further guidance.

5.6 Re-screening

Re-screening may be required for the following reasons:

If a participant does not meet the inclusion/exclusion criteria first time round, he/she can be re-screened.

5.7 Group Allocation Procedure

Group allocation will be conducted using the REDCap randomisation system, a centralised validated computer randomisation programme provided by Oxford Clinical Trials Research Unit (OCTRU), accessed within the MILI REDCap trial database to assign participants to one of the two trial arms.

Participants will be allocated in a 1:1 ratio to surveillance plus metformin (intervention) or surveillance alone (control) arms. The randomisation will be undertaken using a minimisation algorithm, which will balance participants according to the following factors deemed prognostically relevant to the development of cancer following a diagnosis of Li-Fraumeni Syndrome: sex, age (16-25/26-35/36-45/46+ years), prior cancer, prior bilateral mastectomy, *de novo* versus familial mut*TP53*. The minimisation algorithm will be seeded with the first few participants randomised using simple randomisation and a probabilistic element included to prevent predictability of the treatment allocation.

Emergency randomisation procedure:
In the event that the randomisation service is unavailable in the REDCap clinical trial database, an emergency back-up randomisation schedule will be available. Investigator sites should first report the problem to the OCTO MILI team. Please refer to the MILI emergency randomisation procedure for further guidance.

6 TRIAL CONSENT AND CONTRACEPTION COUNSELLING

6.1 Provision of Participant Information Sheet and Consent Forms

Potential participants will be given a current, approved version of the participant information sheet and consent forms either in paper or electronic format by the sub-investigator sites and/or investigator (recruiting) sites. Consent should be taken on a paper version of the form as detailed below.

For potential participants who do not speak English, local site translation services will be used in line with local Trust policy; this also applies to the telemed calls.

Potential participants 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.

6.2 Informed Consent

Participants will receive clear verbal information about the trial in person at the investigator (recruiting) site detailing no less than: the nature of the trial; 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 trial at any time, for any reason, without prejudice to future care, and with no obligation to give a reason for withdrawal.

The Investigator who obtains consent must be suitably qualified and experienced. All delegates must be authorised by the 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 records. The Investigator must be satisfied that the participant has made an informed decision before taking consent.

The participant and the Investigator must personally sign and date the current approved version of the informed consent form in each other's presence which will be a paper document. A copy of the information and signed consent form will be given to the participant.

The original signed form and a further copy will be retained at the investigator (recruiting) site, to file in the medical record and Investigator Site File (ideally the original in the ISF if local policy permits). A copy of the signed consent form should be sent to the biobank upon request to comply with HTA requirements. This is to be done at the end of the trial by each investigator (recruiting) site.

6.2.1 Informed consent for translational research

Participants who agree to participate in MILI will be required to contribute samples for the translational research.

6.2.2 Contraception

A limited amount of data from the use of metformin in pregnancy does not indicate an increased risk of congenital abnormalities, and therefore contraception is not mandatory during trial participation. However, participants are required to inform the trial team at the investigator (recruiting) site if they become pregnant or intend to breastfeed during the trial (see Section 14.2)

7 TRIAL PROCEDURES AND ASSESSMENTS

7.1 Screening Evaluations (at Investigator (recruiting) sites)

- **Written informed consent** see section 6.2 for details. If participant consent falls out of the allowed screening window for example due to a delay in imaging, the participant can be re-consented at the next opportunity and necessary screening assessments restarted.
- **Evaluation against inclusion and exclusion criteria.**
- **Medical and surgical history** to include cancer history, prior cancer therapies and procedures, and clinically significant disease,
- **Concomitant diseases and medications**
- **Physical Examination**: Blood pressure, respiratory, cardiovascular, abdominal examination, lymph nodes, breast exam

- **Dermatological assessment (visual only).** Any abnormalities identified at baseline should be recorded.
- **Whole Body MRI** to be conducted and reported within 28 days of consent (WB-MRIs conducted at the local sub-investigator site will be accepted for baseline if they were done within 3 months prior to randomisation)
- **Brain scan**: to be conducted if no previous brain scan within the last 3 months prior to the visit.
- **Urine pregnancy test** (participants of childbearing potential, defined as WOCBP (APPENDIX 3)) and who are sexually active.
- **Fasting blood samples for baseline haematology, biochemistry, glucose, insulin, and IGF1 (see section 7.7)**

7.1.2 Baseline evaluations (at Investigator (recruiting) sites)

- **Demographic details** include age, sex, and self-reported race/ethnicity, childbirth status, family cancer history, educational background, geographical location, alcohol intake, smoking history.
- **Height (in metres) and weight (in kg)** (Body Mass Index to be calculated by central trial team)
- **Blood samples** for pharmacodynamic testing
- **Collect contact details:** Name, postal address, phone number, email address

To be completed during screening period by participants via email

- **Quality of Life assessment** using 12-item short form survey (SF12v2) & Cancer Worry Scale.

7.2 On Trial Evaluations

Trial participation will be for up to 5 years. Participants in both trial arms will undergo yearly Whole Body and Brain MRI as well as regular dermatological assessments. Those randomised to the metformin arm will have continuous daily metformin from as close to Day 1 as possible. (D1-D57 comprise the titration phase. Please refer to section 9.6).

Telemed calls will be managed by the research team at the investigator (recruitment) site in Oxford. Following the telemed call, participants will be sent an email message to confirm any dose change to the metformin.

The Principal Investigator will be responsible for assessing the causality of any adverse events determined to meet the criteria of an SAE for both arms and for grade 2 or above events during the dose titration phase (metformin arm).

During the first six months of the trial, those in the control arm will have a telemed call during week 1 and at month (M) 6.

Cancer diagnosis management is the responsibility of the Principal Investigator (see section 7.6). Should the participant inform the telemed team of a new cancer diagnosis, the telemed team should immediately inform the Principal Investigator's team.

7.2.1. Information to be collected at telemed calls: screening & baseline to M6:

Week 1 - metformin arm (must be conducted within 3 working days of randomisation)

Randomisation outcome, initial metformin dose & outline aims and structure of calls:

- Confirm randomisation outcome with participant
- Collect changes to concomitant medications.
- Collect adverse events
- Provide starting dose of metformin (if starting criteria met)
- Explain metformin dosing schedule for titration period & method used by telemed team for recording adherence during trial.

Week 1 - control arm (must be conducted within 3 working days of randomisation)

- **Randomisation outcome & outline aims and structure of calls:**
- Confirm randomisation outcome**.**
- Collect changes to concomitant medications.
- Collect adverse events

7.2.2 Dose Titration Phase – Metformin arm

Weeks 3/ 5 /7 / 9 (must be conducted from start date of metformin + week 3/5/7/9 + within 3 working days)

- **Adverse event assessment:** Record and grade (using NCI CTCAE criteria) of reportable adverse events with date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome. Grade 2 or above events to be shared with PI for review and causality assessment and reporting if they meet the criteria for an SAE. (Please refer to dose titration section 9.6)
- **Collect changes to concomitant medications**.
- **Additional investigations:** check if participant has undergone any additional medical investigations.
- **Dose-titration decision**: depending on the grade of the Adverse Reactions (ARs), participants will be advised to increase, continue on same dose, or reduce metformin dose (Please refer to dose titration table in section 9.6)
- **Contact details check (D57):** check for any updates to participant contact details (for sending next batch of metformin)

7.2.3 Month 6 – both arms

(To be conducted within a window of +/- 1 week from due date of call)

- **Adverse event assessment (medical complications):** Record and grade (using NCI CTCAE criteria) of reportable adverse events with date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome. Events that meet the criteria for seriousness will be shared with the PI for reporting.
- **Collect changes to concomitant medications**
- **Additional investigations:** Check if participant has undergone any additional investigations
- **Pregnancy status check (WOCBP)**

Metformin arm only:

- **Contact details check**: check for any updates to participant contact details (for sending next batch of metformin)
- **Medication adherence** using 5 item Medicine Adherence Rating Scale (MARS-5) questionnaire

7.3 Main Trial Phase from years 1- 5

Annual Visits to investigator (recruiting) sites (M12, M24, M36, M48, M60) – both arms

(To be conducted within a window of +/- 4 weeks from due date of annual visit)

The following will be conducted

- **Physical and dermatological examination:** Respiratory, cardiovascular, abdominal examination, lymph nodes, breast exam, dermatological assessment (visual only). Any abnormalities identified should be recorded.
- **Weight** (in kg) (Body Mass Index to be calculated by central trial team)
- **Fasting blood samples** for haematology, biochemistry (vitamin B12 at year 5 only), and glucose, insulin and IGF1 levels (M12 only) (see section 7.7)
- **Blood samples** for pharmacodynamic testing
- **WB & brain MRI (**to be reported within 4 weeks of the scan)
- **Record dates and outcomes of SOC surveillance**: Dates and results (where known) of breast scans

To be completed annually by participants via email

- **Quality of Life assessment** using 12-item short form survey (SF12v2), Cancer Worry Scale, Treatment Burden Questionnaire (TBQ)

7.3.1 Telemed Calls (M12, M18, M24, M30, M36, M42, M48, M54) – both arms

(To be conducted within a window of +/- 1 week from due date of call)

- **Collect changes to concomitant medications**:
- **Adverse event assessment:** Record and grade (using NCI CTCAE criteria) of reportable adverse events with date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome and causality.
- **Additional investigations:** Check if participant has undergone any additional investigations
- **Contact details check:** check for any updates to participant contact details
  Pregnancy status check (WOCBP)

Metformin Arm Only:

- **Medication Adherence** using 5-Item Medicine Adherence Rating Scale (MARS-5) questionnaire

7.4 End of Trial telemed call (M60) – both arms

- **Changes to concomitant medications**: collect generic name where applicable, dose, frequency, route, start date and indication
- **Adverse event assessment:** Record and grade (using NCI CTCAE criteria) of serious adverse events with date of onset, event diagnosis (if known) or sign/symptom, severity, time course, duration and outcome
- **Contact details check**: check for any updates to participant contact details (for informing of results)

7.5 Unscheduled telemed calls

- Unscheduled telemed calls may occur should a participant wish to speak to a member of the research team at the Oxford investigator (recruiting) site concerning their participation in the trial. These calls will not be scripted due to the unscheduled nature of the call but will be recorded in the relevant CRFs in the clinical trial database. Appropriate action taken by the trial team (for example recording of any reportable AEs/SAEs that are identified during the call), changes to contact details and notification of pregnancy.

7.6 Cancer Diagnosis – both arms

In the event of a cancer diagnosis, the decision to continue in the trial will be managed by the Principal Investigator's team (further information on withdrawal is in section 7.10):

Participants will be invited to either:

If treatment predicted to last ≥12 months:

- Permanently withdraw from active trial participation – mandatory

If treatment predicted to last <12 months:

- Temporarily pause active trial participation and restart after <12 months. No cross-over allowed.
- Permanently withdraw from active trial participation

Data collection:

In addition, the investigator (recruiting) site will be responsible for:

- Collecting the date and location of surgery/biopsy and treatment plan [Cancer Event CRF].
- Arranging for the tumour samples to be retrieved and sent to Oxford (please refer to Sample Handling Manual).

7.7 Blood testing

See Summary schedule of events table 1

- **Haematology**: Hb, white blood cells (WBC) with differential count (neutrophils and lymphocytes) and platelets

- **Biochemistry**: sodium, potassium, calcium, urea, creatinine, eGFR, total protein, albumin, bilirubin, alkaline phosphatase (alk phos), AST and/or ALT and LDH, fasting Insulin, glucose, vitamin B12 (baseline and M60) and IGF1 (Baseline and M12 visits only)
- **PD1**: PBMCs spun, frozen, and sent to Oxford (see Sample Handling Manual)
- **PD 2:** Whole blood in Streck tube, stored at room temperature and sent to Oxford (see Sample Handling Manual)

The amount of blood taken at each visit will be up to 60mls, which is the equivalent of 4 tablespoons of blood which would be 360mls for the whole trial.

7.8 Imaging (Surveillance), M12, M24, M36, M42, M48, M60)

- Participants with LFS are recommended to undergo annual breast, brain, and whole-body MRI.
- **Annual brain MRI and breast surveillance** should be arranged through the participants local imaging services (if performed locally). If brain scans are not performed locally, these will be conducted by the investigator (recruiting) site either as part of the whole body MRI or separately (depending on site procedure). Investigator (recruiting) site to request image data from local sub-investigator sites as required.
- **Annual Whole Body MRI** will be arranged at one of the investigator (recruiting) sites. There will be local variation in WB-MRI implementation but this will include T1-weighted imaging, STIR and DWI imaging, ideally with coverage from skull base to feet (some centres may exclude skull). Imaging will be reviewed and reported at the investigator (recruiting) sites by trial radiologists, using a predefined proforma. Depending on local expertise and experience, studies may be single or double read.
- **Imaging reports** prior to trial entry will be requested by the investigator (recruiting) sites for clinical purposes.
- **Secondary image review and analysis:** Whole Body MRI imaging data from the trial will be sent, pseudo anonymised, to the nominated imaging platform for secondary review and analysis.
- **Secondary imaging review** will be performed for 10% of studies throughout the trial. Retrospective secondary image review will be conducted for participants who develop cancer during MILI.

7.9 Follow-up evaluations post-treatment

For those participants who have reached their final visit for the trial (year 5 /month 60 visit) no further follow-up is required.

7.10 Temporary pause from active trial participation (all participants)

Participants may wish to temporarily pause active trial participation (e.g. due to concurrent illness or reasons other than toxicity). Reasons for and the duration of the pause will be recorded in the 'End of Trial Participation CRF'.

Investigator (recruiting) sites will be responsible for keeping in contact with the local genetics team to ascertain when they will be returning to active trial participation and to ensure other relevant CRFs are completed such as the SAE form.

On returning to active trial participation, timings for trial investigations will continue as if uninterrupted. For example, a person wishing to stop for 12 months during year 2 will resume year 3 investigations on re-entering the trial.

If a participant has been allocated to the investigation arm, there will not be a re-titration period. The participant will re-enter the trial on last tolerable dose of metformin taken.

If a participant chooses to recommence trial participation after a temporary pause, the trial will team will review what assessments are required depending on when the participant paused active trial participation.

7.11 Permanent withdrawal from active trial participation (all participants)

During the course of the trial, a participant may permanently withdraw early from active trial participation. This may happen for a number of reasons, including:

- New diagnosis of diabetes mellitus requiring anti-diabetic medication
- Unacceptable toxicity/AE/SAEs of metformin requiring discontinuation

- Loss to follow-up
- Significant protocol deviation or inability to comply with trial procedures
- Clinical decision
- Participant decision (it is important this is distinguished from consent withdrawal in the participant's medical notes)

When the participant permanently withdraws from active trial participation, an 'End of Trial Participation Form should be completed, and any other relevant CRFs (example SAE Form, Pregnancy Notification Form). The reason for withdrawing from active trial participation should be clearly documented in the medical records. For participants who are allocated to the metformin arm, an End of Treatment Form should also be completed.

7.12 Follow-up post permanent withdrawal form active trial participation (all participants)

For those who have permanently withdrawn from active trial participation early (not due to a cancer diagnosis), the investigator site will follow-up on the participant's health status with the local clinical team annually where possible for up to 5 years or until the trial ends (whichever comes first) in accordance with Table 4.

Assessments will be conducted to:

- Ascertain health status: Cancer diagnosis check/date of death

7.13 Treatment withdrawal only (metformin arm)

During the course of the trial, a participant may withdraw from the trial treatment metformin for the reasons given above but continue with active participation in the trial.

When the participant stops metformin treatment early, an '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.

These participants will remain in active trial participation until they have completed 5 years of participation and will continue to have trial assessments at the investigator (recruiting) sites. Should the participant not agree to follow-up, there should be a discussion about consent withdrawal.

7.14 Compassionate administration of Metformin

Metformin is not currently provided as standard of care for people with LFS. It will not be provided compassionately after completion of MILI. There is no cross-over option.

7.15 Consent Withdrawal (all participants)

Consent withdrawal means that a participant has expressed a wish to withdraw from active trial participation and follow-up via their local genetics team. Under these circumstances, the investigator (recruiting) site needs to document all relevant discussions in the participant's medical notes. It is important to clearly document that the participant understands how their data/samples collected prior to consent withdrawal are to be used as part of the trial.

No subsequent data follow-up data should be captured in the CRF. The site should notify the Trial Office, which will allow the office to mark all future CRFs as not applicable. When the participant withdraws consent, a 'Consent Withdrawal' Form needs to be completed. The participants must have the use of their samples and data explained to them.

Use of data and samples collected prior to withdrawal: The trial would like to keep any data and samples collected prior to withdrawal. However, the participant is free to request that their blood or tissue samples are destroyed at any time during and after the trial, unless they have already been analysed at the time of the request, in which case it will not be possible to destroy them. No further data or samples will be collected after withdrawal.

SAE follow-up:

In cases of consent withdrawal, investigators are still responsible for follow up of IMP-related SAEs which are ongoing at the time of withdrawal, and continue to report the SAE to resolution (or until the end of the trial) in the CRF and to the investigator (recruiting) site.

Table 4 Temporary Pause and Withdrawal options

Length of pause or withdrawal	Reason for withdrawal	Type of withdrawal	CRF completion (Arm: T= treatment , C= control)	Safety follow-up required?	Post-withdrawal collection of information from medical notes (includes tumour tissue) until Last Participant Last Visit (LPLV)
Temporary pause	• Illness • Toxicity • Cancer diagnosis & not receiving anti-cancer treatment predicted to last ≥12 months (see section 7.6)	**Mandatory/ investigator decision**	T: End of treatment CRF T & C: End of Trial Participation CRF	Yes (as per safety section)	Investigator site will keep in contact with participant during agreed period of temporary pause.
Permanent withdrawal	• New diagnosis of diabetes mellitus • Toxicity • Pregnancy • Significant protocol deviation or inability to comply with trial procedures • Clinical decision • Participant decision	**Treatment** - Metformin treatment only	T: End of treatment CRF C: N/A	Yes (as per safety section)	N/A
Permanent withdrawal	• Cancer diagnosis & receiving anti-cancer treatment predicted to last ≥12 months • Significant protocol deviation or inability to comply with trial procedures • Loss to follow-up	**Mandatory/ investigator decision**	T: End of treatment CRF T & C: End of Trial Participation CRF	Yes (as per safety section)	Investigator site will follow-up on participant health status via local genetics team.
Permanent withdrawal	• Illness • Toxicity • Significant protocol deviation or inability to comply with trial procedures • Participant decision	**Consent withdrawal**	T: End of treatment CRF T & C: Consent withdrawal CRF	Yes (as per safety section)	No subsequent data (including routine care data) should be captured in the CRFs.

8 SAMPLES FOR LABORATORY ANALYSIS

The MILI trial will be collecting and analysing samples for the purpose of monitoring the safety and toxicity of metformin. In addition to this, the trial will be collecting samples for translational endpoints (please refer to Sample Handling Manual and the Sample Analysis Plan).

8.1 Samples to be analysed in local Trust's laboratories

Diagnostic Laboratories
Samples for haematology, biochemistry, fasting glucose, insulin and IGF1 analysis will be collected and labelled with standard participant 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. Results from these analyses will be entered by the investigator site team into the appropriate CRFs.

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

Pharmacodynamic samples for translational research
PD1: Whole blood for PBMCs will be collected spun and frozen at the investigator (recruiting) sites. These will be sent to a central lab in based at Oxford University. From here, samples will be sent to other labs based at Oxford University for analysis. A portion of the samples will also be sent to a lab in the United States for analysis. At the end of the trial, any remaining samples not used in the analysis by Oxford labs will be stored in an Oxford University biobank. Remaining samples that were sent outside of the UK will not be sent to the biobank.

PD2: Whole blood for plasma and cfDNA will be collected in Streck tubes at the investigator (recruiting) sites, stored at room temperature and sent to a lab based at Oxford University for processing . From here some of the samples will be sent to another lab based at Oxford University for DNA-based biomarker detection work (e.g. using next generation sequencing). Any remaining samples will be analysed in specialist or commercial labs based at Oxford University, elsewhere in the UK or worldwide (within or outside the European Union) for protein-based biomarker detection and development. trial,

See Sample Handling Manual for details.

Pathology
Routine diagnostic pathology samples taken at cancer diagnosis or pre-cancerous tissue which have been labelled, processed and reported according to local hospital protocols will be collected by the trial. The diagnostic block/slices should be sent to a central lab based at Oxford University for analysis. Remaining tissue blocks will be returned to the hospital. At the end of the trial, any remaining tissue will be stored in a biobank based at Oxford University. Any used tissue slides will be destroyed.

For details on sample handling and analysis, please refer to the Sample Handling Manual.

8.3 Summary of samples/assays to be taken during the trial

Please see the Sample Handling Manual for the management of the assay/sample handling and storage for the translational research.

8.4 Samples for Biobanking

Participants in this trial will be asked to provide consent to the long-term retention of samples for use in possible other future research linked to trial data.

See Lab Manual for sample collection and processing instructions;
biobank contact details and shipping arrangements.

8.5 Labelling and confidentiality of samples sent

All samples sent to the Translational Support Unit (TSU) at Oxford will be labelled with the trial code, participant initials, trial participant number, and date/time taken. Should a laboratory receive any samples

carrying unique participant identifiers, other than those specified, the recipient must immediately obliterate this information and re-label with the correct details following confirmation from the Trial Office, and report a data breach to the Trial Office.

8.6 Clinical reporting of exploratory research assay results

The results of the research assays are exploratory and are not intended to influence the individual participant's 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 participant's clinical management.

8.7 Trial sample retention at end of trial

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 trial any surplus samples may be retained for use in other projects that have received ethical approval. Hence, any surplus trial 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.

8.8 Withdrawal of consent for sample collection and/or retention

A participant 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 participants 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.

9 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

9.1 Metformin

Any UK licenced commercial brand of Metformin hydrochloride immediate-release tablets may be used in the trial.

9.2 Treatment dose

Metformin immediate-release tablets, given initially as 500 mg once daily and dose increased every 14 days by 500 mg increments to 2g/daily (1000 mg b.d.) given orally.

9.3 Duration of treatment

Metformin will be given daily and continuously for up to 5 years.

9.4 Management of drug administration

Metformin tablets should be swallowed whole with a full glass of water and not crushed, broken, or chewed. Tablets should be taken at approximately the same time each day with meals to help reduce stomach or bowel side effects.

If a participant misses their morning or evening dose of metformin, they should take it as soon as possible after that time. However, if there has been a delay of more than 6 hours since the missed dose, the dose should be skipped and the next dose of metformin taken at its regular time.

If a participant vomits after taking a metformin dose, the dose should be replaced only if the vomited tablets can actually be seen and counted. If the vomited tablet cannot be seen, the dose should be omitted and the next dose of metformin taken at its regular time.

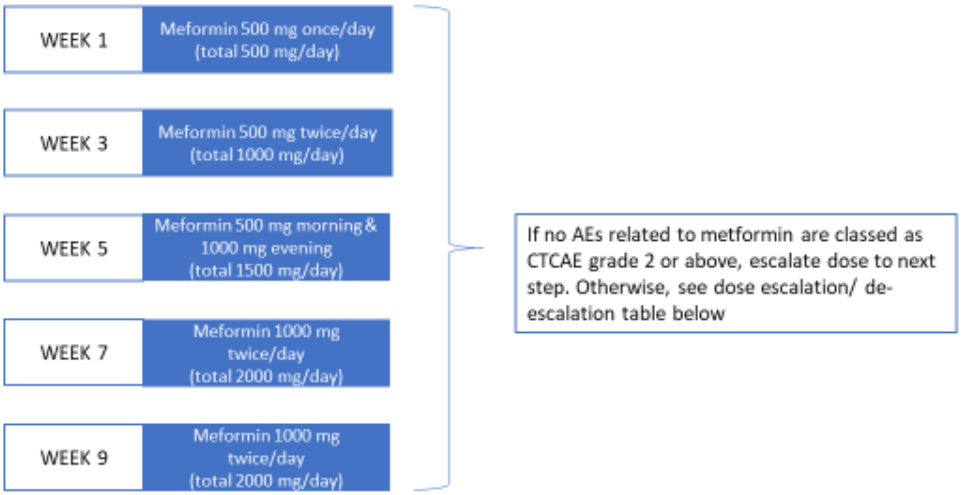
Hypersensitivity to metformin is very rare. Signs of an allergic reaction could include difficulty breathing, swelling of the face or hands, and a skin rash. Local policy should be followed.

9.5 Special precautions

Please refer to SmPC for any precautions.

9.6 Dose titration

Figure 4: Dose titration during MILI



9.6.1 Dose-titration decision

The research nurse team at the Oxford investigator (recruiting) site will collect adverse events as reported by the participant and will assign a CTCAE grading. This data will be entered into the clinical trial database for the Principal Investigator to assign causality where events are determined to be grade 2 (CTCAE) or above.

If during the telemed call, the research team at the Oxford investigator (recruiting) site ascertain that there have not been any grade 2 (CTCAE) or above events or any other queries or concerns from the information collected during the telemed call, the participant will be informed in the telemed call by the research team that they can increase the dose of metformin as per the dose-titration plan above (figure 4).

9.6.2 Role of Principal Investigator

If during the telemed call the research team ascertain that there have been grade 2 or above events or other queries or concerns during the telemed call, the Principal Investigator will be asked to review the safety and toxicity information collected during the telemed call in order to assign causality and to make the dose-titration decision.

The dose decision should take place within 2 days of being contacted by the telemed team. Depending on the grade and causality of the AE or other information collected, the participant will then be advised to increase, continue at same dose, reduce or stop the metformin dose (Please refer to dose titration table 6 in section 9.6). Adverse events unrelated to metformin will not influence dose decisions.

Any deviations to the amount of metformin taken by the participants will recorded and followed by the telemed team in the appropriate CRF.

Table 5: Dosing by Adverse Reaction (AR) CTCAE criteria

CTCAE grade (G)	Metformin Dosing Decision	2-week review
≤G1	Increase dose as per dose-titration plan, review in 2 weeks	Increase dose as per dose-titration plan
G2	Dosing decision to be made following review by Principal Investigator.	If resolved to ≤G1 increase dose as per dose titration plan. If G2 ongoing, continue metformin at current level and do not follow titration plan
G3	Temporarily stop metformin Review in 2 weeks.	If resolved to ≤G1 /G2, restart metformin titration plan at lower dose of metformin. If on lowest dose (500mg od) metformin when experienced toxicity, stop metformin permanently.
G4	Discontinue metformin and repeat symptom check/institute medical management as appropriate.	

9.7 Metformin adherence

Participants will be asked to complete the MARS-5 questionnaire to assess metformin adherence. If adherence is below equivalent of 6 months over 5 years (10%) the participant will be non-evaluable.

During the titration phase, metformin tolerance will be checked every 2 weeks via a telemed call by the research team based at the Oxford investigator (recruiting) site. Thereafter, adherence will be checked every 6 months via a telemed call using the MARS-5 questionnaire for the duration of the trial.

9.8 Management of overdose

Metformin overdose associated with lactic acidosis presents with nonspecific symptoms and includes severe nausea, vomiting, diarrhoea, epigastric pain, thirstiness, lost appetite, lethargy and hyperpnoea. Hypotension, hypothermia, acute renal failure, coma and cardiac arrest also represent significant clinical features. All trial participants will be provided with safety information cards outlining these symptoms with emergency contact information.

Overdose is medically managed and participants are advised to seek emergency medical support at their nearest hospital.

10 CONCOMITANT MEDICATION AND NON-DRUG THERAPIES

10.1 Concomitant medication

Concomitant medication may be given as medically indicated. All participants 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 trial 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-trial visit must be recorded in the medical record and the appropriate CRF.

10.2 Support medication

There is no requirement for support medications to be instituted. Any AEs found to be related to metformin will be managed with modification/discontinuation of metformin.

10.3 Prohibited therapies

Participants should not take metformin or phenformin obtained from other sources while participating in this trial.

10.4 (Potential) Drug Interactions

Medicinal products that can acutely impair renal function are associated with increased risk of lactic acidosis from metformin and should be initiated with caution for participants in the metformin arm of the trial

- Diuretics such as acetazolamide and methazolamide.
- Anti-epileptic medications such as topiramate (Topamax) and zonisamide (Zonegran).
- Regular NSAIDs given for pain relief such as aspirin, ibuprofen, naproxen and indomethacin

(note: low-dose aspirin (75mg) is safe and is not considered an NSAID https://www.nhs.uk/conditions/nsaids/)

10.5 Interaction with other medicinal products and other forms of interaction

Alcohol-intoxication is associated with an increased risk of lactic acidosis, particularly in case of fasting, malnutrition or hepatic impairment.

- Participants having surgery, including dental surgery, or any major medical procedure, or undergoing contrast enhanced radiological procedure may need to discontinue metformin before the procedure. Metformin should not be re-started until at least 48 hours after, provided that renal function has been re-evaluated and found to be stable.

11 DRUG MANAGEMENT

11.1 Drug supplies

The IMP Metformin will be sourced from the hospital stock within the pharmacy at the investigator (recruiting) site. The Metformin to be used should be in blister packs of immediate-release 500mg film-coated tablets.

11.2 Drug ordering

The hospital pharmacy will be responsible for ensuring that commercial supplies are held in stock as necessary to supply the recruited participants.

The hospital pharmacy will monitor the Metformin stock and reorder as required.

Once the hospital pharmacy has received the notice of randomisation and a prescription form from the Principal Investigator (or designee), the hospital pharmacy will post an initial supply of Metformin to the participant. The hospital pharmacy will be responsible for the supply of the Metformin tablets to the trial participants randomised to this arm for the duration of the trial.

Metformin tablets will be posted in batches to the trial participants. Trial participants will be supplied with extra boxes of Metformin tablets to ensure they have a continued supply in case there are delays in receiving the tablets.

The pharmacy staff will keep a log of the number of Metformin tablets supplied to each participant which will be shared with the MILI trial office for monitoring purposes.

11.3 Reordering

The MILI trial office will monitor the Metformin supplies the participants have remaining using supply information shared by the hospital pharmacy and from the 6 monthly telemed call with the participants (refer to section 7.2).

Subsequent batches of Metformin will be posted by the hospital pharmacy on receipt of a prescription from the Principal Investigator (or designee). The metformin tablets will be supplied on a quarterly to six-monthly basis for the duration of the trial unless it is communicated otherwise.

Further information regarding drug management is contained in the Pharmacy Manual and the Pharmacy File.

11.4 IMP Receipt

The participant will be asked to confirm receipt via a link that will be sent electronically via email message. Reminder email messages will be sent out if there is no response. It will be the responsibility of the MILI trial office to ensure that there is a receipt for all deliveries of metformin.

Please refer to the Pharmacy manual for further details.

11.5 Handling and storage

Unopened Metformin tablets do not require any special storage conditions. Stability studies indicate that unopened blister packs are stable for 5 years.

11.6 Labelling

The hospital pharmacy will ensure that IMP supplies dispensed for trial use are appropriately labelled in accordance with all applicable regulatory requirements. Please refer to the Pharmacy manual for further details.

11.7 Dosing dispensing

Metformin tablets will be supplied to participants via post. Participants will be supplied with immediate release 500mg metformin tablets.

11.8 Drug accountability

Drug traceability will be maintained during the trial. The hospital pharmacy will keep a participant specific dispensing log of the batches of Metformin tablets supplied to each of the participants including how many immediate release 500mg tablets were supplied and the date of supply.

The hospital pharmacy will manage the stock of metformin as per local policy.

The hospital pharmacy will be able to use its own dispensing logs and stock control logs.

Metformin receipt information from the participants will be stored in the clinical trial database.

The participant dispensing information will be added to the CRFs in the clinical trial database in order to monitor participant supplies and participant compliance.

There is no end-of-trial drug reconciliation.

11.9 Drug returns from participants

There is no requirement to collect participant returns. Participants will be instructed to take any unused or expired Metformin tablets to their local community pharmacy for destruction.

11.10 Drug destruction

Table 6: Unused drug/ returns

Drug left unused / expired drug at hospital pharmacy	Disposal at investigator (recruiting) site according to local hospital policy. A dated certificate of disposal should be completed and retained in the Pharmacy File.
Participant returns	Participants to take unused or expired Metformin tablets to their local community pharmacy for destruction.

11.11 Occupational safety

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

12 IMAGING AUDIT

12.1 Whole-body MRI

Data from whole -body-MRI (WB-MRI) will be collected during the trial for the purposes of an imaging audit. Anonymised data from the audit will be used for future teaching and further research.

WB-MRI should be reviewed and reported as per local radiological practice, which may include single or double reporting. Imaging reports will be published to the radiological information systems at the investigator (recruiting) sites as per normal clinical practice. Reports should be actioned by the participant's responsible clinician as per normal clinical practice. This may include recommendations for further tests at local sites.

For the purpose of the audit, 10% of baseline and on-trial WB-MRI for secondary review (in line with national audit standards for whole body imaging with PET-CT) will be selected for review from the scans uploaded onto the imaging-sharing platform. These images will be made accessible to trial radiologists in a pseudo anonymised format via the platform. This will support ongoing QA of trial quality and reporting. Trial radiologists will also complete a pseudo anonymised proforma report, which will be requested by the trials team at University of Oxford. Further guidance is available in the imaging data capture manual.

13 SAFETY REPORTING

The Investigator team will monitor each participant for clinical and laboratory evidence of adverse events on a routine basis throughout the trial. Should an Investigator become aware of any trial drug related SAEs following this period these must also be reported as stated below.

Adverse event monitoring starts from the time the participant consents to the trial until **30 days** after the last dose of metformin (for participants on the metformin arm) or for those in the control arm, their last trial visit.

All reportable AEs 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. The final status of the AE will be recorded at the end of the trial.

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

13.1 Adverse Event Definitions

An Adverse Event (AE) is any untoward medical occurrence in a clinical trial participant.

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 trial procedures or the administration of an investigational medicinal product (IMP).

AEs may be spontaneously reported by the participant and/or in response to an open question from trial personnel or revealed by observation, physical examination, or other diagnostic procedures.

For the purposes of this trial this definition is being extended to any untoward medical occurrence observed while the participant is on trial, i.e. from consent until the 30 days after the last dose of metformin. For the control arm, until the last trial visit is complete.

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

Table 7

• **Results in death**	
• **Is life-threatening**	This refers to an event in which the participant 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-participant hospitalisation or prolongs existing in-patient hospitalisation**	In general, hospitalisation signifies that the participant 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-participant 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. If hospitalisation is due to diagnosis of cancer, or cancer treatment complications, this is not defined as an SAE
• **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**	Pregnancy is not, in itself an SAE. In the event that a participant or his/her partner becomes pregnant whilst taking part in a clinical trial or during a stage where the foetus could have been exposed to the medicinal product (in the case of the active substance or one of its metabolites having a long half-life) the pregnancy should be followed up by the investigator until delivery for congenital abnormality or birth defect, at which point it would fall within the definition of "serious"
• **Is any other medically important event**	Defined as an event that may jeopardise the participant or may require intervention to prevent one of the outcomes listed above. A new primary cancer is not defined 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.

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).

A reportable event is any serious adverse event which must be reported to the REC and Competent Authority. To fulfil this requirement, Investigator (recruiting) sites must immediately (within 24 hours of the telemed team or investigator site team's knowledge of the event), report these SAEs to the OCTO Pharmacovigilance office using the SAE CRF.

13.2 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 as follows.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the trial or are present at baseline and significantly worsen following the start of the trial will be recorded as AEs and reported as SAEs if they meet the definitions above.

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.

13.3 Determining adverse event causality

An Adverse Reaction (AR) is an AE that may be related to trial treatment. The assessment of "relatedness" must be determined by a medically qualified individual (doctor) 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:

Table 8

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

The Investigator must endeavour to obtain sufficient information to confirm the causality of the adverse event and give their opinion of the causal relationship between each AE and trial 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.

13.4 Reference safety information (RSI) for assessment of expectedness

The reference safety information (RSI) for the trial is section **4.8** of the current approved version of **SPC Glucophage metformin hydrochloride 500mg film coated tablets** which lists all the expected side effects associated with the use of metformin.

A copy of the current approved version of the RSI document must be held in the Investigator 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 the management of type II diabetes in people who do not have LFS. It is therefore possible that in this LFS trial population other side effects may occur, or the participant might suffer a more severe reaction.

13.5 Updates to the Reference safety information (RSI)

The sponsor or delegate will manage the regulatory approvals required for updates to the RSI for the trial. The trial team will set annual reminders to check www.medicines.org.uk for any SmPC updates. The trial team will inform site teams when a new version of the RSI has been approved for use in the trial. Only approved versions of the RSI should be used for the IMP in this trial.

13.6 Suspected Unexpected Serious Adverse Drug Reactions (SUSARs)

All SUSARS must be reported to the Competent Authority and main REC by the OCTO Pharmacovigilance office within the required timelines:

- Fatal or life threatening SUSARs will be reported within 7 days of the OCTO Pharmacovigilance 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 OCTO Pharmacovigilance 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.

13.7 Immediate reporting of SAEs

The following SAE reporting requirements apply regardless of the Investigator's assessment of the causality of the SAE. All SAEs that require immediate reporting (as defined in 13.1) should be reported on the trial SAE report form in the REDCap clinical trial database (see SAE completion guidelines) and digitally submitted to the OCTO Pharmacovigilance office within 24 hours of becoming aware of the event.

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

Participants will be asked about serious adverse events during the telemed calls.

Any SAE that occurs at any time after completion of medication treatment that the investigator and/or sub-investigator consider to be related to the trial drug must also be reported (digitally submitted) to the OCTO Pharmacovigilance office via the REDCap clinical trial database.

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

The email address for the OCTO Pharmacovigilance office is: octo-safety@oncology.ox.ac.uk

13.8 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 OCTO Pharmacovigilance office (see SAE completion guidelines).

If new or amended information on a reported SAE becomes available, the Investigator must submit the updated information on a follow up SAE CRF in REDCap, superseding the previous form.

SAEs that are considered to be definitely unrelated to the trial intervention may be followed up and monitored as for a related SAE at the discretion of the independent Data & Safety Monitoring Committee (DSMC).

13.9 Events exempt from being immediately reported as SAEs

Diagnosis or Progression of cancer

Adverse events including hospitalisation that are clearly consistent with cancer diagnosis or progression will not be reported as individual SAEs.

Events reported as SAEs which on investigation are confirmed as cancer diagnosis or progression will not be reported further as SAEs.

Clinical symptoms of cancer diagnosis or progression will only be reported as AEs if the symptoms cannot be determined as exclusively due to the underlying malignancy, or does not fit the expected pattern.

Every effort should be made to document the diagnosis or progression of cancer on the Cancer Diagnosis CRF.

Death on trial

Death due to cancer is to be recorded on the Death CRF form and is exempt from SAE reporting providing the death is not unexpected or suspected related. The investigator must clearly state whether the death was expected or unexpected and whether a causal relationship to metformin or other protocol treatment intervention is suspected.

Elective admissions and supportive care

Elective admissions to hospital for participant 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.

Laboratory abnormalities and other abnormal assessments

Laboratory abnormalities and other abnormal assessments which are determined to be grade 2 or above will only be recorded during dose-titration. Outside of the titration phase, abnormalities such as the examples given, will be reviewed and determined whether clinically significant by the PI at site:

- lowered insulin or glucose levels,

- lowered vitamin B12 levels.

Adverse Events

Up to the initial telemed call, AEs that are determined to be grade 2 or above will be recorded in the Adverse Event CRF for all participants.

At the initial telemed call and thereafter, AEs that are determined to be grade 2 or above will only be recorded in the Adverse Event CRF for participants randomized to the metformin arm, and only during the titration phase. Grade 1 AEs are not required to be recorded in the CRF, as the safety profile of metformin is well established.

Adverse events which meet the criteria for seriousness and occur in patients randomized to the control arm are not defined as reportable SAEs. These will be recorded elsewhere in the Medical Complications eCRF.

13.10 Informing Investigators of new safety information

The MILI 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 at the investigator (recruiting) sites are responsible for briefing their trial team and onward transmission to their R&D office as appropriate.

13.11 Reporting Serious Adverse Events on the SAE CRF

The following events are considered reportable events (as defined in13.1) and must be immediately reported by the investigator to the OCTO Pharmacovigilance office on the SAE case report form (eCRF) for that participant (unless otherwise exempt from reporting as specified in section 13.9above).

- Any SAE occurring in patients randomised to the metformin arm.

The information provided will include:

- event diagnosis (if known) or sign/symptom - terms should be specific medical terms. Abbreviations, combined terms e.g. nausea and vomiting and ambiguous terms e.g. deranged, abnormal, should be avoided.
- date of onset and of resolution
- severity - adverse events and toxicities must be graded according to the NCI Common Terminology Criteria for adverse events (NCI-CTCAE) Version 5.0.
- relationship of the AE to trial drug. The Investigator will provide an “other” cause for serious AEs considered to be unrelated to the trial drug
- seriousness
- outcome concomitant medications or other therapy used to treat the event

13.12 Recording Adverse Events on the AE CRF

The following events must be recorded on the AE CRF:

- Any AE ≥ CTCAE Grade 2 occurring in a participant on the metformin arm during the titration phase only.

The information provided will include:

- event diagnosis (if known) or sign/symptom - terms should be specific medical terms. Abbreviations, combined terms e.g. nausea and vomiting and ambiguous terms e.g. deranged, abnormal, should be avoided.
- date of onset and of resolution
- severity - adverse events and toxicities must be graded according to the NCI Common Terminology Criteria for adverse events (NCI-CTCAE) Version 5.0.
- relationship of the AE to trial drug. The Investigator will provide an “other” cause for serious AEs considered to be unrelated to the trial drug.
- whether or not the AE meets the criteria for seriousness

- outcome

Any concomitant medications or other therapy used to treat the event must be listed on the ConMed CRF.

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.

13.13 Adverse Event Coding

All adverse event terms will be coded by the OCTO pharmacovigilance office. The version of MedDRA that will be used for the coding of AEs and SAEs will be the version that is current on the date of MHRA approval and will remain the same version throughout the trial.

14 PREGNANCY

14.1 Reporting pregnancies

Pregnancies in a participant which occur while participating in this trial, require expedited reporting. Pregnancies should be reported in the Pregnancy Notification Form in the REDCap clinical trial database for review by the OCTO Pharmacovigilance office.

The completed pregnancy form should be submitted digitally to the OCTO Pharmacovigilance office within the same timelines as an SAE. 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

Any pregnancy occurring within 28 days of the last administration of metformin (or up to the final study visit in the control arm) 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 the participant withdraws from the trial early.

For this trial, it is not necessary to report or follow up the pregnancies in the partner of male trial participants in the metformin arm.

14.2 Active trial participation during pregnancy and breast-feeding

Pregnancy (both arms):

- Participants who become pregnant during the trial will be advised not to have annual whole-body MRI scans and blood tests for translational research during their pregnancy. They may continue to actively participate in the trial and receive the six-monthly telemed calls but they will not be required to attend annual visits to the recruitment centre. Visits to the recruitment centre will resume following pregnancy.

Metformin and pregnancy:

- Participants allocated to the metformin arm who become pregnant during the trial will have the choice to continue to take metformin or omit metformin during pregnancy. Although metformin crosses the placenta into the baby's bloodstream, there is no evidence that metformin is harmful to the mother or baby during pregnancy; metformin is commonly used to treat women with diabetes or those who develop gestational diabetes. Studies have shown that children up to four years of age born from mothers who were taking metformin whilst pregnant had normal motor and social development(37). However, longer term studies looking at the effects of metformin in children over four years of age have not yet been conducted.

- Once participants have given birth, they will be recommended to restart metformin within 4 weeks unless there are medical reasons contraindicating it, or they wish to continue to omit metformin while breastfeeding.

Metformin and breastfeeding:

- Metformin passes into breast milk in tiny amounts and has not been linked to side-effects in healthy breastfed babies. However, mothers on the metformin arm of the trial who are breastfeeding premature babies or babies with kidney problems should be advised to stop taking metformin and seek advice from their GP or specialist as to when it is safe to restart it.
- Once participants have completed breastfeeding, they will be recommended to restart metformin within 4 weeks unless there are medical reasons contraindicating metformin requiring a delay to restarting or study discontinuation.

15 DEFINING THE END OF TRIAL

For this trial, the end of the trial is defined as:

- By the completion of the *a priori* planned statistical analysis and the completion of the translational research analysis (whichever comes last).
- The sponsor and the Chief Investigator reserve the right to terminate the trial earlier at any time. In terminating the trial, they must ensure that adequate consideration is given to the protection of the participants' best interests.

16 STATISTICAL CONSIDERATIONS

16.1 Sample size and power

Data collected between 2013-2017 from 1000 adult participants by the international Li-Fraumeni Exploration (LiFE) Research Consortium revealed an average yearly cancer incidence of 5%. Therefore, we assume a 5% per year cancer incidence in our control arm. We predict that the addition of metformin would reduce new cancer events by 50% at 5 years corresponding with a reduction in all-cause mortality by 20%. This assumes that 77% participants will be cancer free at 5 years in the control arm and if the treatment reduces the incidence of cancer by 50% then 88.5% in the treatment arm will be cancer free at 5 years. Therefore, the median survival across the cohorts will be 28 years (from recruitment) in the metformin arm versus 13 years in the control arm, 15 years difference. In order to detect an effect of this size with 80% power and one-sided log rank test with 5% error, we need to observe 44 events. A sample size of 224 participants (112 in each arm) recruited over 2 years with a further 5 years of follow-up is therefore needed in order to observe this number of events with these assumptions. We have also assumed that 1% of participants will be lost in each arm and that there is no crossover of treatments.

17 STATISTICAL ANALYSIS PLAN

All analyses will be on an intention-to-treat basis. This means that participants will be analysed as they are randomised irrespective of the treatment actually received. The intention-to-treat population will include all participants who have given their informed consent and for whom there is confirmation of successful treatment allocation via randomisation .

It is therefore important that every effort is made to encourage participants, including those participants who do not receive/complete their allocated treatment, to attend for follow-up clinic visits and complete the questionnaires to avoid bias in the analysis of the results.

Baseline Characteristics

Means and standard deviations of baseline characteristics of participants will be reported in the case of normally distributed, continuous variables; medians, ranges, and interquartile ranges in the case of continuous variables with distributions that deviate from normality; and frequencies and proportions in the case of categorical variables (ordered and non-ordered). Baseline characteristics will be split out by treatment group. No formal statistical comparisons of baseline data will be undertaken.

Other Descriptive Statistics:

We will produce Kaplan-Meier curves for each intervention group separately throughout the course of the trial, describing all time-to-event outcomes (time-to-cancer event; time-to-tumour event; overall survival).

Primary Outcome Analysis:

To compare the hazard rates of developing a cancer event (as defined in section 4.3, the primary outcome) between the two arms, we will use a Cox proportional hazards regression model, with time-to-cancer event as defined in section 4.3 as the outcome, allocated group [metformin/control] as a predictor, plus the minimisation factors as covariates (sex, age in years, prior cancer, prior bilateral mastectomy, *de novo* versus familial mut*TP53).* The specific estimate of interest is the coefficient for the allocated group taken from this model.

The Cox Proportional Hazards models will be assessed via inspection of Kaplan-Meier curves to ensure that the proportional hazards assumption is valid before commencing the analysis. If this is not appropriate, we will instead report restricted mean survival times.

Secondary planned analyses:

To address our planned secondary objectives, the following analyses are planned:

- To compare the hazard rates of developing a tumour event (as defined below) between the two arms, we will use a Cox proportional hazards regression model, with time-to-tumour event as defined below as the outcome, allocated group [metformin/control] as a predictor, plus the minimisation factors as covariates (sex, age in years, prior cancer, prior bilateral mastectomy, *de novo* versus familial mut*TP53).* The specific estimate of interest is the coefficient for the allocated group taken from this model.
    - A 'tumour (pre-cancer) event' is defined as either a pathologically confirmed diagnosis of non-invasive/pre-cancerous lesion identified during trial participation.
- We will describe the adverse event rates at the trial time points, split by each treatment arm.
- Metformin acceptability will be assessed by describing the number of participants who remain on metformin at trial end, and the total dose metformin prescribed per participant for the 5-year duration of the trial
- To examine the impact of adherence to treatment, we will construct a Cox proportional hazard regression model with time to cancer event as the outcome, allocated group [met/control] as a predictor plus minimisation factors as covariates (sex, age in years, prior cancer, prior bilateral mastectomy, de novo versus familial mutTP53). Also includes interaction term between adherence level to metformin (operationalised as the MARS-5 score) and allocated group, allowing the hazard ratio to be moderated by the level of adherence.
- We will also model MARS-5 score across the trial timepoints in the metformin effects model to characterise change in adherence level over time.
- To determine yield of the WB-MRIs, we will report the overall frequencies of WB-MRI findings according to the following yield categories:
    - Normal
    - Benign finding (irrelevant non-cancer finding)
    - Equivocal (indeterminate finding)
    - Malignant finding (relevant cancer finding)
- We will perform retrospective analyses determining the sensitivity and specificity of WB-MRI to detect cancer events found in the trial, comparing correspondence of findings (benign, equivocal, and malignant) with subsequent cancer events at the relevant body site. A randomly-selected 10% of WB-MRI images at each site will be read twice by a (blinded) additional radiologist, allowing a inter-observer reliability statistics to be generated for these images. An additional proforma assessing subjective agreement with findings will also be collected, and the frequencies of the agreement categories will be reported.
- We will also report the number of cancer and incidental further investigations triggered by WB-MRIs and relate them to the number of subsequent cancer events.

As with the primary outcome analysis, all Cox Proportional Hazards models will be assessed via inspection of Kaplan-Meier curves to ensure that the proportional hazards assumption is valid before commencing the analysis. If this is not appropriate, we will change the models to report restricted mean survival times instead, as this method does not rely on this assumption.

17.1 Inclusion in analysis

For the primary outcome analysis, we will include all participants randomised to both arms, irrespective of drop out, and according to the intervention they were allocated, irrespective of intervention use/discontinuation/switching (an 'intention-to-treat' population). This includes those participants that discontinued metformin during the titration period.

17.2 Subgroup analysis

Subgroup analysis of participants with de novo versus familial mutTP53 will be conducted as part of MILI. Pooled international analyses will be conducted after completion of MILI as part of a separate trial.

17.3 Accounting for missing, unused, or spurious data.

The primary outcome analysis will be analysed using multiple imputation by chained equations (MICE) to account for missing observations in the trial outcomes and covariates, under the assumption that data observed are able to predict missing observations ('missing at random' assumption). A sensitivity analysis which uses only available observations will also be used to assess the robustness of the treatment estimates in the absence of missing data, assuming that the missing observations are 'missing completely at random'.

17.4 Final analysis

Based upon projected accrual rates, this trial is expected to complete recruitment within 24 months of opening to recruitment. Final analysis will be after all participants have been followed up for at least 60 months.

18 TRIAL COMMITTEES

18.1 Trial Management Group (TMG)

The Trial Management Group (TMG) will be responsible for the day-to-day running of the trial and will meet at least once every 6 months or more regularly as required. The Chief Investigator will chair the TMG which is responsible for overseeing the successful conduct and publication of the trial. The CI will take responsibility and delegate discussions as needed. The TMG will include the investigator site PIs, the co-ordinating PI, a member of the OCTO Pharmacovigilance team, OCTO MILI team, trial statistician, radiology lead, a member of the telemed team and patient representatives from the George Pantziarka TP53 Trust.

18.2 Trial Steering Committee

A Trial Steering Committee (TSC) will act as the oversight committee for the MILI trial. It will be a committee of independent members (75%) that provide overall supervision of the trial and fulfils the role of a TSC according to its terms of reference. The role of the TSC 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 chairperson.

The TSC will review the recommendations from the DSMC and will decide on continuing or stopping the trial, or modifying the protocol. It will meet at least annually though the exact frequency will depend on the rate of accrual and event rates, and may be increased upon request by the committee. The TSC will review trial progress against agreed milestones, adherence to the protocol compliance as well as the results of other trials and new information which has arisen, and recommend appropriate action. The Trial Steering Committee will be chaired by an independent clinician and will comprise of other independent clinicians, a statistician, patient and public representatives and a member of the OCTO Pharmacovigilance team.

18.3 Data & Safety Monitoring Committee (DSMC)

An independent Data & Safety Monitoring Committee (DSMC) will be established for this trial. This committee will meet to assess the trial data, on at least an annual basis. The exact frequency will depend on the rate of accrual and event rates, and may be increased upon request by the committee. The DSMC will monitor recruitment to the trial and protocol compliance as well as toxicity and serious adverse events taking into account relevant worldwide information.

The main outcomes will be analysed as stated above in the analysis plan. There will also be an interim analysis to review metformin adherence levels. The DSMC will make confidential recommendations to the Independent Trial Steering Committee.

19 DATA MANAGEMENT

The data management aspects of the trial are summarised here with details fully described in the Data Management Plan. See section in DMP on participant confidentiality for information on management of personal data.

19.1 Source data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records which could be electronic or paper (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs and correspondence.

Source Data Table

Table 9 Source Data location and details relating to Primary Objective

Primary Objective	Endpoint	Data required	CRF data[1]	Non-CRF[2]	Source data	Source data location
1. Cumulative cancer free survival up to 5 years (60 months) following randomisation	Cancer free survival – with "cancer" event defined as pathologically confirmed diagnosis of malignant cancer identified during trial participation or death from any cause.	Comparison of time to development of new cancer in each trial arm within 60 months of randomisation and % events Diagnostic pathology report Radiological Diagnostic Information Clinic report	Date and outcome of trial treatment allocation The date and location of surgery/biopsy and treatment plan [Cancer proforma]. Date & cause of death	Radiological Diagnostic Information-scan	Medical notes	Investigator (recruiting) site or sub-investigator (local) site (if diagnosed here)

Table 10 Source Data location and details relating to Secondary Objectives

Secondary Objectives	Endpoints	Data required	CRF data1	Non-CRF2	Source data	Source data location
1. Cumulative tumour free survival at 5 years	Tumour free survival – with a "tumour" event including pathologically confirmed diagnosis of malignant cancer or clinically/scan detected benign or premalignant lesion (e.g. ductal carcinoma in situ - DCIS) identified during trial participation or death from any cause.	Pathologically confirmed diagnosis of malignant cancer or clinically/scan detected benign or premalignant lesion Diagnostic pathology report Radiological Diagnostic Information Clinic report	Date and outcome of trial treatment allocation The date and location of surgery/biopsy and treatment plan [Cancer proforma].	Radiological Diagnostic Information-scan	Medical notes	Investigator (recruiting site) or sub-investigator (local) site (if diagnosed here)
2. Overall 5-year survival	Time from randomisation to death from any cause.	Death notification	Date and outcome of trial treatment allocation Date & cause of death	N/A	Medical notes	Investigator (recruiting) site or sub-investigator (local) site (if recorded first here)
3. Incidence and type of	Number and type of emerging cancers, including size, stage	Diagnostic pathology report	The date and location of surgery/biopsy and	Radiological Diagnostic	Medical notes	Investigator (recruiting) site or sub-

Secondary Objectives	Endpoints	Data required	CRF data1	Non-CRF2	Source data	Source data location
cancers at 5 years	and histological grade at diagnosis.	Radiological Diagnostic Information	treatment plan [Cancer proforma]. Scan results- date of scan, staging etc.	Information-scan		investigator (local) site (if diagnosed here)
4. Safety and toxicity of metformin	Relevant treatment-emergent adverse events and clinically significant laboratory changes (per NCI CTCAE v5.0) in investigation arm compared to baseline.	Participant reported symptoms	Medical Complications, AE, SAE data Annual Biochemistry and haematology results	N/A	Medical notes (annual visit) & Notes from telemed calls (six-monthly)	Investigator (recruiting) site Notes taken by Oxford telemed team
5. Acceptability of metformin	MARS-5 questionnaire score	Data from questionnaires	Data from questionnaires	N/A	Notes from telemed calls (six-monthly)	Notes taken by Oxford telemed team
6. Impact of metformin and cancer prevention on quality of life (QOL)	Comparison of change in 12-item short form survey (SF12v2), Cancer Worry Scale & Treatment Burden Questionnaire (TBQ) between intervention and control arms and compared to baseline	Data from questionnaires	Data from questionnaires	N/A	Electronic questionnaire	REDCap clinical database
7. Impact of baseline lifestyle risk factors on cancer incidence	Correlation of baseline weight, BMI and lifestyle factors (e.g. smoking and diet) with outcome	BMI Weight Medical history / dietary and life style questions	BMI Weight Medical history / dietary and life style questions	N/A	Medical notes (annual visit)	Investigator (recruiting site)

Table 11 Source Data location and details relating to Exploratory Objectives

Exploratory Objectives	Endpoints	Data required	CRF data[1]	Non-CRF[2]	Source data	Source data location
1. Mechanism of action of metformin as a cancer preventative	i. Whether baseline insulin sensitivity predicts cancer-free survival in LFS ii. Whether metformin's chemoprevention effects are indirect via insulin sensitivity in LFS using HOMA-IR as surrogate marker. iii. Whether metformin's chemoprevention effects are indirect via changes in circulating PI3K/AKT/mTOR signalling in LFS. iv. Whether metformin's chemoprevention effects are direct via alterations to oxidative phosphorylation in LFS using OXPHOS gene signature as surrogate marker.	PBMC blood – insulin, Glucose, IGF-1	Date sample collected Results of insulin, glucose, IGF-1 blood samples	Data collected in clinical trial database (REDCap) to be integrated with the database managed by the analysing laboratory.	MILI sample collection	Medical or lab records at recruiting site
2. Identify biomarkers of response/ cancer	v. Surrogate genetic or vi. Proteomic markers of cancer-free survival or metformin response. vii. Penetrance of germline TP53 mutation in tumour tissue viii. Retrospective identification or validation of circulating cancer biomarkers	Tumour tissue blocks	Date sample collected Results of insulin, glucose, IGF-1 blood samples	Data collected in clinical trial database (REDCap) to be integrated with the database managed by the analysing laboratory.	MILI sample collection	Medical or lab records at recruiting site
3. Investigate the reliability of WB-MRI	Yield and diagnostic accuracy of WB-MRI	Whole-body MRI scan	Date of Whole-body MRI scan	Radiological Diagnostic Information- scan To be stored in the nominated imaging platform.	Radiological Diagnostic scan	Investigator (recruiting) site

1 CRF data: captured in REDCap clinical trial database eCRF.
2 Non-CRF data: MRI Images will be held in the nominated imaging platform, data from translational samples will be held in a separate database managed by the analysing laboratory

19.1 Case reports forms (CRFs)

The Investigator (recruiting) site staff will ensure that data collected on each participant is recorded in the CRF as accurately and completely as possible. Any data for the trial that is provided by the sub-investigator sites will be recorded into the CRFs by the investigator (recruiting) site staff. 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 trial 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 trial database lock point.

Note: 'in a timely fashion' means within no more than 14 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 participants who are withdrawn early. If a participant withdraws from the trial, the reason must be noted on the appropriate form and the participant must be followed-up as per protocol.

19.2 Non-CRF data

Diagnostic radiological data:

Copies of radiological scans will be transferred from the Investigator (recruiting) sites for the radiological exploratory endpoint (see source data above) and stored on the nominated imaging platform. Please refer to the trial specific Data Management Plan (DMP).

Pharmacodynamic samples data:

Data from pharmacodynamic blood samples and tumour tissues blocks and pre-cancerous tissue collected will be stored in a separate database at a central lab in Oxford. Please refer to lab manual and sample analysis plan.

19.3 Electronic Data Capture

Electronic data capture (EDC) and data management will be performed via a web-based, bespoke trial database (REDCap). REDCap is a dedicated and validated clinical trials database designed for electronic data capture. The trial office will provide sites with instructions and a link to online training.

19.4 Clinical trial report

All clinical data will be presented at the end of the trial as data listings. These will be checked to confirm the lists accurately represents the data collected during the course of the trial. The trial data will then be locked and a final data listing produced. The clinical trial report will be based on the final data listings. The locked trial data may then be used for analysis and publication.

20 TRIAL SITE MANAGEMENT

20.1 Trial site responsibilities

MILI is adopting the "Hub and Spoke" approach towards the oversight of research activities. Trial sites conducting research activities for the trial that are not within the usual care competence require oversight by a PI employed by that legal entity is to ensure effective oversight. These sites are known as investigator sites. at the Trial sites conducting activities within their usual care competence will only require PI oversight from one of the investigator sites. For the purpose of this trial, these sites will be known as "sub-investigator " sites.

Investigator sites where PI oversight is required

The Principal Investigator (the PI)has overall responsibility for conduct of the trial, but may delegate responsibility where appropriate to suitably experienced and trained members of the site team. All members of the site team must complete

the delegation log and trial contacts log provided prior to undertaking any trial duties. The PI must counter sign and date each entry in a timely manner, authorising staff to take on the delegated responsibilities.

Sub-investigator Sites where no PI is required (PI oversight provided elsewhere in Investigator site)

Activities that require only some level of PI oversight can be undertaken at a sub-investigator site that does not employ (substantively or via honorary contract) the PI, under the delegation of a PI employed elsewhere. In such a circumstance, the Sub-investigator Site being overseen from a distance is part of the Investigator Site, as oversight of the activities is still required from a PI. A PI may oversee a number of Sub-investigator Sites without being based in any of those locations.

20.2 Trial site set up and activation

Investigator (recruiting) sites where PI oversight is required:

Mandatory Site Training which is organised by the trial office (usually carried out as a telephone conference call or personal visit) must be completed before the site can be activated. The Trial Office will check to confirm that the site has all the required trial information/documentation and is ready to recruit. The site will then be notified once they are activated on the trial database and are able to begin recruiting participants.

Sub-investigator sites where no PI is required (PI oversight provided by Investigator site):

Sub-investigator sites will be provided with relevant instructions and documents required to carry out the tasks delegated to them. This will be organized by the trial office. There will be no mandatory site training. The Trial Office will check to confirm that the site has all the required trial information/documentation prior to referring participants.

20.3 Arrangements for sites outside the UK

It is not anticipated that this trial will open in non-UK sites.

20.4 Trial documentation

Investigator sites where PI oversight is required

The trial office will provide an Investigator Site File to the Investigator (recruiting) sites and a Pharmacy File to the pharmacies at the investigator (recruiting) sites containing the documents needed to initiate and conduct the trial. The trial office must review and approve any local changes made to any trial documentation including participant 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.

Trial Sites where no PI is required (PI oversight provided elsewhere in Investigator site)

Trial sites will be provided with relevant instructions and documents required to carry out the tasks delegated to them (image sharing and diagnostic biopsy collection information).

20.5 Protocol deviations

Protocol compliance is fundamental to GCP. Changes to the approved protocol need prior approval unless for urgent safety reasons. The investigator must document and explain any deviations/violations from the current approved protocol. The investigator must promptly report any important deviation from Good Clinical Practice or protocol to the trial office by email. Examples of important deviations are those that might impact on participant safety, primary/ secondary endpoint data integrity, or be a possible serious breach of GCP (see serious breach 21.7 below).

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.

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

21.1 Ethical conduct of the trial and ethics approval

The protocol, participant information sheet, consent form and any other information that will be presented to potential trial participants (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), HRA and host institution.

21.2 Regulatory Authority approval

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

21.3 NHS Research Governance

Once HRA & HCRW approval is in place for the trial, sites will confirm capability and capacity to participate in the trial.

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 sub-investigator documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the participants 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.

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

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 Sponsorship, 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 trial participants (see below).

It is the Investigator's responsibility to update participants (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the participant's willingness to continue in the trial. The Investigator must ensure this is documented in the participant's medical notes and the participant 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 trial 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 participants 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 participants of the trial; or
- the scientific value of the trial”

Investigators must notify the Trials Office immediately if any serious breach is suspected. In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the CI the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the relevant NHS host organisation within seven calendar days.

21.8 Trial Reports

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

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee, HRA (where required), Host NHS Trust and Sponsor.

For assessment of SARs in the DSUR, the RSI that was approved at **the start of the safety reporting period** will be used. Where changes to the RSI have been approved by substantial amendment during the reporting period, the RSI used for the next DSUR will differ from the RSI used to assess expectedness for SARs on or after the date the amendment is approved.

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

21.9 Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database.

Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration.

Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and results will be uploaded to all those public registries within 12 months of the end of the trial declaration.

22 EXPENSES AND BENEFITS

Reasonable travel expenses for visits to investigator (recruiting) sites will be reimbursed for all participants on production of receipts or a mileage claim.

23 QUALITY ASSURANCE

23.2 Assessment and management of risk

This protocol is designed to deliver a risk-adapted approach to conducting the research. A risk assessment has been conducted and a monitoring plan will be prepared before the trial opens. The known and potential risks and benefits to participants have been assessed in comparison to those of standard of care. A risk management strategy is in place and will be reviewed and updated as necessary throughout the trial or in response to outcomes from monitoring activities. Monitoring plans will be amended as appropriate.

23.3 Monitoring

Regular monitoring will be performed according to the monitoring plan. Data will be evaluated for compliance with the

protocol, completeness and accuracy. The investigator and institutions involved in the trial will permit trial-related monitoring and provide direct on-site access to all trial records and facilities if required. They will provide adequate time and space for the completion of monitoring activities.

Trial sites 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 trial 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 trial site for resolution. For other non-critical data tems, Trial Office staff may resolve data queries centrally providing the correct answer is clear. Such changes will be clearly identified in the CRF and the trial site informed.

Note: 'in a timely manner' means within no more than 10 working days of the data query and within 28 days of receipt of a data query unless otherwise specified. The Trial Office will decide the maximum CRF lag time based on protocol requirements and feasibility. In general, a 2 week turn around is an acceptable default for all non-urgent data.

Trial sites will also be monitored remotely and/or by site visit, as necessary, to ensure their proper conduct of the trial. Trial Office staff will be in regular contact with site personnel to check on progress and deal with any queries that they may have. 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, within 28 days as a minimum, or sooner if the monitoring report requests.

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

- Participant screening log
- Location and type of TP53 mutation
- Histology report

OCTO requirements for remote monitoring will be kept to the minimum. Priority should be given to items required for central review.

23.4 Audit and Regulatory Inspection

All aspects of the trial 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 trial. Investigators and their host Institution(s) should understand that it is necessary to allow auditors/inspectors direct access to all relevant documents, trial 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 & DATA SHARING

24.2 Records retention

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 participants 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 sub-investigator PK or PD endpoints and the disposition of samples donated via the trial must also comply with applicable legislation and Sponsor requirements.

24.3 Archiving

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

Trial data and associated metadata will be retained electronically in a suitable format in a secure server area maintained and backed up to the required standard. Access will be restricted to the responsible Archivist and will be controlled by a formal access request. On completion of the mandatory archiving period the TMF and associated archived data sets will be destroyed or transferred as appropriate, according to any data sharing requirements and the relevant SOPs.

24.4 Data sharing

Detailed plans for sharing data generated by this trial will be listed in the Data Management Plan. Requests for access to trial data will be considered according to the OCTO and OCTRU data sharing policies, considering terms and conditions defined in contracts with third parties, e.g. funders and collaborators.

25 PARTICIPANT CONFIDENTIALITY

The trial will comply with the General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. Personal data recorded on all documents will be regarded as confidential.

The processing of the personal data of participants will be minimised by making use of a unique participant trial number only on all trial documents and any electronic database(s), with the exception of the CRF, where date/year of birth may be added. Original consent forms will be archived at the investigator sites but a copy will be sent upon request to the biobank by the investigator sites in order to meet HTA requirements.

For the purpose of running the trial, the trial office will collect the participants name and contact details. This personal data will be stored in an area of the database which is separate to the clinical trial database. Access to the participant contact details will be limited to members of the trial team who need to contact the participant during the trial.

All documents will be stored securely and only accessible by trial staff and authorised personnel. The trial staff will safeguard the privacy of participants' personal data.

The Investigator site will maintain the participant's anonymity in all communications and reports related to the research. The Investigator site team will keep a separate log of enrolled participants' 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. Data Breaches will be highlighted to the relevant site staff and reported as required by the GDPR and Data Protection Act 2018. This will also be deemed a protocol deviation.

26 TRIAL FUNDING

This trial is funded by the National Institute for Health and Care Research (NIHR) EME Project (NIHR131239).

The translational research work is funded by Cancer Research UK (CRUK) PPRC Project

The George Pantziarka TP53 Trust is supporting travel expenses for participants through its fundraising activities.

27 SPONSORSHIP AND INDEMNITY

27.2 Sponsorship

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

27.3 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 that is provided.

27.4 Contracts/Agreements

This trial is subject to the Sponsor's policy requiring that written contracts/agreements are agreed formally by the participating bodies as appropriate. A Clinical Trial Agreement (CTA) will be placed between the Sponsor and participating organisations 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. Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

28 PUBLICATION POLICY

The sponsor will retain ownership of all data arising from the trial. 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 trial was Sponsored by and performed with the support of the Sponsor and other funding bodies as appropriate.

29 REFERENCES

1. Malkin D. Li-fraumeni syndrome. Genes Cancer. 2011;2(4):475-84.
2. Kratz CP, Achatz MI, Brugières L, et al. Cancer Screening Recommendations for Individuals with Li-Fraumeni Syndrome. Clin Cancer Res. 2017;23(11):e38-e45.
3. Hanson H, Brady AF, Crawford G, et al. UKCGG Consensus Group guidelines for the management of patients with constitutional TP53 pathogenic variants [published online ahead of print, 2020 Jun 22]. J Med Genet. 2020. Bojadzieva J, Amini B, Day SF, Jackson TL, et al. Whole body magnetic resonance imaging (WB-MRI) and brain MRI baseline surveillance in TP53 germline mutation carriers: experience from the Li-Fraumeni Syndrome Education and Early Detection (LEAD) clinic. Fam Cancer. 2018;17(2):287-294
4. Chompret A, Brugières L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer. 2000;82(12):1932-7.
5. Evans DG, Ingham SL. Reduced life expectancy seen in hereditary diseases which predispose to early-onset tumors. Appl Clin Genet. 2013;6:53-61.
6. Werner-Lin A, Young JL, Wilsnack C, et al. Waiting and "weighted down": the challenge of anticipatory loss for individuals and families with Li-Fraumeni Syndrome. Fam Cancer. 2020;19(3):259-268. Bancroft EK, Saya S, Brown E, et al. Psychosocial effects of whole-body MRI screening in adult high-risk pathogenic TP53 mutation carriers: a case-controlled trial (SIGNIFY). J Med Genet. 2020;57:226-236.
7. Custers JAE, Kwakkenbos L, van de Wal M, Prins JB, Thewes B. Re-validation and screening capacity of the 6-item version of the Cancer Worry Scale. Psychooncology. 2018;27(11):2609-2615.
8. Salsman JM, Schalet BD, Andrykowski MA, Cella D. The impact of events scale: a comparison of frequency versus severity approaches to measuring cancer-specific distress. Psychooncology. 2015;24(12):1738-45.
9. Wang PY, Ma W, Park JY, et al. Increased oxidative metabolism in the Li-Fraumeni syndrome. N Engl J Med. 2013; 368:1027-1032.
10. Beckerman R, Prives C. Transcriptional regulation by p53. Cold Spring Harb Perspect Biol. 2010;2(8):a000935.
11. Zhou G, Wang J, Zhao M, et al. Gain-of-function mutant p53 promotes cell growth and cancer cell metabolism via inhibition of AMPK activation. Mol Cell. 2014;54(6):960-74.
12. Giavedoni P, Ririe M, Carrera C, Puig S, Malvehy J. Familial Melanoma Associated with Li-Fraumeni Syndrome and Atypical Mole Syndrome: Total-body Digital Photography, Dermoscopy and Confocal Microscopy. Acta Derm Venereol. 2017 Jun 9;97(6):720-723.
13. Malkin D, Li FP, Strong LC. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science. 1990; 250:1233-1238.
14. Lang GA, Iwakuma T, Suh YA, et al. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell. 2004;119(6):861-72.
15. Parrales A, Iwakuma T. p53 as a Regulator of Lipid Metabolism in Cancer. Int J Mol Sci. 2016;17(12):2074.
16. Wang PY, Li J, Walcott FL, et al. Inhibiting mitochondrial respiration prevents cancer in a mouse model of Li-Fraumeni syndrome. J Clin Invest. 2017;127(1):132-136.
17. Park JH, Zhuang J, Li J, Hwang PM. p53 as guardian of the mitochondrial genome. FEBS Lett. 2016;590(7):924-34.
18. Klil-Drori AJ, Azoulay L, Pollak MN. Cancer, obesity, diabetes, and antidiabetic drugs: is the fog clearing? Nat Rev Clin Oncol. 2017;14(2):85-99.
19. Pernicova I, Korbonits M. Metformin--mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014;10(3):143-56.
20. Lord SR, Cheng WC, Liu D et al. Integrated Pharmacodynamic Analysis Identifies Two Metabolic Adaption Pathways to Metformin in Breast Cancer. Cell Metab. 2018;28(5):679-88 e4.
21. Liu X, Romero IL, Litchfield LM, Lengyel E, Locasale JW. Metformin Targets Central Carbon Metabolism and Reveals Mitochondrial Requirements in Human Cancers. Cell Metab. 2016;24(5):728-39.
22. Li M, Li X, Zhang H, Lu Y. Molecular Mechanisms of Metformin for Diabetes and Cancer Treatment. Front Physiol. 2018; 9:1039.

23. Kaneto H, Kimura T, Obata A, Shimoda M, Kaku K. Multifaceted Mechanisms of Action of Metformin Which Have Been Unraveled One after Another in the Long History. Int J Mol Sci. 2021;22(5):2596.
24. Hermann LS. Metformin: a review of its pharmacological properties and therapeutic use. Diabete & Metabolisme. 1979;5(3):233-245
25. Blough B, Moreland A, Mora A Jr. Metformin-induced lactic acidosis with emphasis on the anion gap. Proc (Bayl Univ Med Cent). 2015;28(1):31-3.
26. Diabetes -type 2: Metformin. NICE guidelines Jan 2021. https://cks.nice.org.uk/topics/diabetes-type-2/prescribing-information/metformin
27. Garber AJ, Duncan TG, Goodman AM, Mills DJ, Rohlf JL. Efficacy of metformin in type II diabetes: results of a double-blind, placebo-controlled, dose-response trial. Am J Med. 1997;103(6):491-7.
28. Seo HS, Jung YJ, Kim JH, Lee HH, Park CH. The Effect of Metformin on Prognosis in Patients With Locally Advanced Gastric Cancer Associated With Type 2 Diabetes Mellitus. Am J Clin Oncol. 2019;42(12):909-917.
29. Pimentel I, Lohmann AE, Ennis M, et al. A phase II randomized clinical trial of the effect of metformin versus placebo on progression-free survival in women with metastatic breast cancer receiving standard chemotherapy. Breast. 2019;48:17-23. DOI: 10.1016/j.breast.2019.08.003
30. Zhang K, Bai P, Dai H, Deng Z. Metformin and risk of cancer among patients with type 2 diabetes mellitus: A systematic review and meta-analysis. Prim Care Diabetes. 2020; S1751-9918: 30213-8.
31. Higurashi T, Hosono K, Takahashi H, et al. Metformin for chemoprevention of metachronous colorectal adenoma or polyps in post-polypectomy patients without diabetes: a multicentre double-blind, placebo-controlled, randomised phase 3 trial. Lancet Oncol. 2016;17: 475-483. DOI: 10.1016/S1470-2045(15)00565-3
32. Chak A, Buttar NS, Foster NR et al; Cancer Prevention Network. Metformin does not reduce markers of cell proliferation in esophageal tissues of patients with Barrett's esophagus. Clin Gastroenterol Hepatol. 2015;13(4):665-72.e1-4.
33. Walcott FL, Wang PY, Bryla CM, et al. Pilot Trial Assessing Tolerability and Metabolic Effects of Metformin in Patients With Li-Fraumeni Syndrome. JNCI Cancer Spectr. 2020;4(6):pkaa063. DOI: 10.1093/jncics/pkaa063
34. Song M, Vogelstein B, Giovannucci EL, Willett WC, Tomasetti C. Cancer prevention: Molecular and epidemiologic consensus. Science. 2018;361(6409):1317-1318. DOI: 10.1126/science.aau3830
35. Saya S, Killick E, Thomas S, et al. Baseline results from the UK SIGNIFY trial: a whole-body MRI screening trial in TP53 mutation carriers and matched controls. Fam Cancer. 2017;16:433-440.
36. Lévesque LE, Hanley JA, Kezouh A, Suissa S. Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. BMJ. 2010 Mar 12;340:b5087.
37. Nguyen L, Chan SY, Teo AKK. Metformin from mother to unborn child - Are there unwarranted effects? EBioMedicine. 2018 Sep;35:394-404. doi: 10.1016/j.ebiom.2018.08.047.

APPENDIX 1: SUMMARY OF PROTOCOL AMENDMENTS

Protocol Version & Date	Protocol Section	Details of Changes made <consider whether this should be a summary or tracked changes text>
1.0_28Feb2023	N/A	N/A first version

APPENDIX 2: TP53: CANVIG-UK GENE-SPECIFIC GUIDANCE

Table 12

TP53: CanVIG-UK Gene-Specific Guidance

Date: 04/03/2022 Version: 1.5

CanVIG-UK review of TP53 Jan 2020. Consensus to use TP53 ClinGen Expert Group guidance with additional points of specification as below.
Relevant documents:

(i) ClinGen *TP53* Expert Panel Specifications v1 2.1
(ii) Corresponding HMG publication from ClinGen Expert group (Fortuno et al 2020)
(iii) New surveillance guidelines for Li-Fraumeni and hereditary *TP53* related cancer syndrome: implications for germline *TP53* testing in breast cancer (Evans and Woodward 2020).

For use in conjunction with CanVIG-UK Consensus Specification for Cancer susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification. Evidence lines for which there are no gene-specific recommendations should be reviewed in context of CanVIG-U K Consensus Specification for Cancer Susceptibility Genes.

Evidence towards Pathogenicity

Evidence element and evidence strengths allowed		Thresholds/data-sources/applications specifically relevant to TP53
PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	_VSTR _STR _MOD _SUP	**Exclusion of CHIP should be considered where P/LP variant is detected** in cases for which (i) there is no familial transmission evident AND (ii) VAF<40% AND (iii) phenotype is equivocal. Testing of normal tumour tissue is recommended if possible; otherwise testing of fibroblasts from skin biopsy should be considered). See flowchart in Evans and Woodward 2020
PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_MOD _SUP	
PVS1: **Predicted null variant** (in a gene where LOF is a known mechanism of disease)	VSTR _STR _MOD _SUP	
PS1: Same amino acid change as an established variant	_STR	For PM1_mod: the ≥10 occurrences on cancerhotspots.org, must be ***of the exact same amino acid substitution.***
PM4:Protein-length-changing variant	_MOD _SUP	
PM5:Novel missense change at an amino acid residue where a different missense change determined to be pathogenic seen before	_STR _MOD _SUP	For PM1_sup: ≥5 occurrences on cancerhotspots.org, where the exact same amino acid substitution counts as 1 occurrence and substitution of a different amino acid at the same residue counts as 0.5 of an occurrence.
PP3: In silico: Multiple lines of computational evidence support a deleterious effect on the gene or gene product	_SUP	
PM1, PP2: Enrichment/constraint: **PP2**: Missense variant in a gene that has a low rate of benign missense variation and in which missense	_STR _MOD _SUP	For PS1/PM5, reference variants should be classified as P/LP by ClinGen Expert Group. Until

variants are a common mechanism of disease **PM1:** Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation		such a list exists, we suggest using 'or equivalent' to define a reference P/LP variant
PS3: Functional: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	_VSTR _STR _MOD _SUP	
PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	_VSTR _STR _MOD _SUP	
PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history	_STR _MOD _SUP	
PM3: in trans with a pathogenic variant (**recessive disorders)**	_STR _MOD _SUP	
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	_SUP	
PP4: Phenotypic specificity (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)	_STR _MOD _SUP	
Evidence towards Benigni		
BA1/BS1: Allele frequency is "too high" in ExAC or gnomAD for disorder	_SA _STR	
BS2: Observation in controls inconsistent with disease penetrance. Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age	_STR _SUP	
BP4: In silico: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	_SUP	
BP1:Missense variant in a gene for which primarily truncating variants are known to cause disease	_SUP	
BP7: Synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence	_SUP	
BP3: In-frame deletions/insertions in a repetitive region	_SUP	
BS3: Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	_STR _MOD _SUP _STR	

BS4: Non segregation with disease	_SUP	
BP2:Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR _SUP	
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	_STR _SUP	
BP5: Alternate molecular basis for disease	_SUP	

Revised version	Date	Section	Update	Amended by	Approved by
1.5	***04/03/22***	PM1	Recommendations for application at sub-investigator level of evidence. Clarification that PM 1_mod to be applied where ≥10 occurrences of exactly the same amino acid substitution	Garrett	CStAG

APPENDIX 3: WOMEN OF CHILD BEARING POTENTIAL (WOCBP)

Women of child-bearing potential (WOCBP) are defined as: fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A post-menopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the post-menopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormone replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is sufficient.