Investigation of Somatic alterations in Tumours of the Eye

STUDY PROTOCOL

IRAS Number: 242945

KEY STUDY CONTACTS

Chief Investigator **Pui Ying Chan** (email:
Wellcome Sanger Institute
Hinxton CB10 1SA

Sponsor **Genome Research Limited**
Wellcome Genome Campus
Hinxton CB10 1SA
Sponsor representative: Dr Martin Dougherty (email: md21@sanger.ac.uk)
Sponsor contact: Dr Joanne Doleman (email: researchgovernance@sanger.ac.uk)
Phone: 01223 495399

Funders **The Wellcome Trust**
The Wellcome Sanger Institute

ROLE OF STUDY SPONSOR AND FUNDER

The roles and responsibilities of the sponsor and funder do not extend to the study design or conduct. Data analysis and interpretation will be carried at the Wellcome Sanger Institute (SANGER). The dissemination of results will be in accordance with the SANGER Data Sharing Policy and the SANGER Publication Policy.

STUDY SUMMARY

Study Title	Investigation of Somatic alterations in Tumours of the Eye
Short Title	iSITE
Study Design	An observational cohort study of patients with ocular melanoma.
Study Participants	Patients > 18 years of age diagnosed with ocular melanoma.
Planned Sample Size	Target accrual: • Cohort A: 104 uveal melanoma patients • Cohort B1: 16 uveal melanoma patients • Cohort B2: 50 uveal melanoma patients • Cohort C: 10 conjunctival melanoma patients
Follow Up Duration	Up to 3 years
Planned Study Period	July 2018 – December 2024
Research Objectives	▪ Define the order of somatic mutations leading to cancer development, metastases and resistance to treatment. ▪ Explore how somatic mutations compare across different regions within the same tumour. ▪ Determine whether mutations detected in circulating tumour DNA reflect the mutational landscape of tumours from the same patient.

GLOSSARY

Buffy coat	Layer of concentrated white blood cells and platelets in centrifuged blood.
Enucleation	Surgical removal of the eye.
FFPE	Formalin-fixed paraffin-embedded.
Germline	Inherited genetic material from eggs or sperm that are passed on to offspring.
Malignant	Term used to describe cells which have become cancerous.
Metastasis	Spread of the original cancer to a distant site.
Uveal melanoma	A disease where cancer cells form in the uveal tract of the eye.
Local recurrence	Re-growth of the original eye tumour in the eye, following treatment.
Observational cohort study	Following a group of individuals over time to determine certain outcomes, where no attempt is made to affect the outcome.
Ocular melanoma	Collective term for cancers of the eye, including uveal melanoma and conjunctival melanoma.
Plaque brachytherapy	Small metal disc placed on the surface of the eyeball, emitting radiation over a number of days before being removed from the eyeball.
Somatic mutation	Non-inherited genetic alteration acquired by a cell.
Subclone	Population of cells with mutations different from the major population of cells in the tumour.
Tumour	Abnormal growth of tissue.
Vitreous fluid	Fluid that fills the space between the lens and the back of the eye.

TITLE

Investigation of somatic alterations in tumours of the eye (iSITE).

SUMMARY

Modern DNA sequencing technologies enable researchers to identify mutations that have been acquired during the lifetime of patients (somatic mutations). Some of these somatic mutations occur in cancer genes and increase the risk of developing cancer. This study will apply such sequencing technologies to cancers of the eye (ocular melanoma) in order to identify mutations associated with these cancers. Sequencing patients at different stages of their disease will allows us to build a timeline of the order of mutations that occur at each stage. We can use this information to understand how these cancers develop, spread (metastasise) and respond to treatment. Furthermore, we will look at which of these somatic mutations are present in the blood, by collecting blood samples and sequencing fragments of DNA which have been released by tumours into the bloodstream (circulating tumour DNA, ctDNA). This will determine whether ctDNA can be used as a way of monitoring mutations present in the tumour. This study will provide much needed insight into a rare and understudied cancer type, with the long-term aim of improving the survival of patients by identifying key mutations to develop novel therapies against.

RATIONALE OF THE STUDY

Uveal Melanoma

Uveal melanomas (cancer of the eye involving the iris, ciliary body, or choroid - collectively referred to as the uvea) are rare cancers that arise from pigments cells (melanocytes) located in the eye. Like most rare cancers, there is limited interest in developing new therapies and a lack of clinical trials. This strongly contributes towards the relatively worse survival rates compared with common cancers. Following treatment of uveal melanoma with either surgical removal of the eye (enucleation) or local radiation (plaque brachytherapy), approximately half of the patients will go on to develop metastases. Most patients will die within a few months despite current therapies[1]. Many of the treatments currently available to patients with metastatic uveal melanoma were initially developed for skin cancer (cutaneous melanoma), which arise from pigment cells of the skin. Patients with uveal melanoma were underrepresented and often excluded from the very clinical trials that led to the approval of the drugs they are now treated with. Importantly, despite both cancers arising from melanocytes, uveal melanoma and cutaneous melanoma are completely distinct in their mutational profile, and indeed their clinical behaviour. It is therefore not surprising that the treatments currently being used are not effective in uveal melanoma.

Conjunctival Melanoma

Conjunctival melanomas (cancer of the surface of the eye which lines the inside of the eyelids) are an extremely rare subset of ocular melanomas. They share more similarities to cutaneous melanoma in their clinical behaviour. Whilst there are similarities in the mutational profiles of conjunctival and cutaneous melanoma, the order of mutational changes acquired during development, growth and recurrence of conjunctival melanoma remain poorly understood. As such, few advances have been made in the treatments offered to the metastatic patient population, resulting in poor survival outcomes.

This study will address the following unique questions:

(1) What somatic mutations are acquired during development and metastases?
(Cohorts A, B1, B2 and C: Uveal melanoma and conjunctival melanoma)

Different mutations can lead to cancer development in the eye, the spread of cancer to other parts of the body and resistance to treatment. Tracking the mutational changes of a tumour in the same patient over time during each of these stages will allow us to determine the order in which mutations are acquired and therefore provide insight into their roles in disease biology. Most studies have sequenced primary tumours and metastatic tumours from different patients. This makes comparison between the two groups of tumours difficult due to the presence of mutations specific to patients, but not necessarily important for cancer development. To determine the relevance of these commonly altered genes, a larger number of paired tumours will be sequenced. To date only one small study of paired primary and metastatic uveal

melanoma samples has been performed, revealing a group of 135 mutations across 5 paired samples[2]. The mutational profiles of paired primary and metastatic conjunctival melanomas have not been comprehensively studied.

(2) Is intratumoural heterogeneity associated with poor clinical outcome?
(Cohorts A, B1, B2 and C: Uveal melanoma and conjunctival melanoma)

There is emerging evidence that diverse populations of cells exist in different regions within a single tumour (intratumoural heterogeneity) in uveal melanoma. This is a result of the development of different groups of mutations (subclones) which evolve - a reflection of the adaptive nature of cancer over time. Intratumoural heterogeneity has been linked with poor clinical outcomes in other cancer types. This study will determine what subclones exist, and to what extent they are associated with poor clinical outcomes in uveal and conjunctival melanoma.

(3) Do mutations detected in ctDNA reflect the somatic mutations found within the tumour?
(Cohorts A and B1: Uveal melanoma only)

ctDNA carry tumour-specific mutations which allow identification and differentiation from non-tumour DNA. ctDNA is detectable in many different cancer types including uveal melanoma, and ctDNA levels have been shown to correlate with tumour burden and survival outcomes[3]. This study will include longitudinal blood sampling for the detection of mutations in ctDNA. If these mutations are found to be a real-time reflection of the mutations present within the tumour, this would be a valuable minimally-invasive approach to studying tumour evolution and monitoring disease.

RESEARCH QUESTIONS

(1) What is the order of somatic mutations leading to cancer development, metastases and resistance to treatment in ocular melanomas?

(2) How do somatic mutations compare across different regions within the same ocular tumour?

(3) Do the mutations detected in circulating tumour DNA reflect the mutational landscape of uveal tumours?

EXPERIMENTAL DESIGN AND METHODS

Typical diagnostic and treatment schedules for primary and metastatic uveal melanoma are summarised below.

Primary uveal melanoma
Moorfields Eye Hospital: **Clinical diagnosis** ↓ Intraocular biopsy not routinely performed (unless specific reason e.g. patient choice, diagnostic uncertainty) ↓ **Routine blood sampling** ↓ **Plaque brachytherapy** (excess tumour tissue and vitreous fluid not typically available following surgery) **OR** **Enucleation** (excess tumour tissue and vitreous fluid available following surgery)
St Bartholomew's Hospital: ↓ First **metastatic surveillance** appointment (**routine blood sampling** for all patients) ↓ Lifelong surveillance for metastatic disease: 6 monthly radiological scans of the liver (Blood sampling is not routinely performed as part of metastatic surveillance)

Metastatic uveal melanoma
St Bartholomew's Hospital: **Radiological diagnosis** ↓ **Biopsy** of metastatic disease is routinely performed for histological confirmation ↓ **Routine blood sampling** ↓ **Surgery** for minority of patients who have low burden of metastatic disease (excess tumour tissue available) **OR** **Systemic therapy** for majority of patients (**routine blood sampling** during treatment) ↓ Surveillance post-treatment (**routine blood sampling**) ↓ Metastatic biopsies may be performed e.g. upon disease progression (not routine)

Typical systemic therapy options:	
Ipilimumab	3 weekly ipilimumab for a total of 4 treatments.
Pembrolizumab	3 weekly pembrolizumab until intolerance or disease progression.
Ipilimumab with nivolumab	3 weekly combination ipilimumab and nivolumab for 4 treatments, followed by 2 weekly nivolumab until intolerance or disease progression.

This study will be divided into several cohorts: Samples will be prospectively collected from patients with both primary uveal melanoma (cohort A) and metastatic uveal melanoma patients (cohort B1). If the patients in cohorts A and B1 have stored samples which have been previously collected, consent will be requested from these patients to obtain these samples.

Previously collected and stored tissue samples will be retrospectively collected from patients with metastatic uveal melanoma patients (cohort B2) and primary and/or metastatic conjunctival melanoma (cohort C). There will be no prospective sampling from cohorts B2 and C.

Pre-collected blood samples will be purchased from commercial providers to enable us to test our experiments with less precious samples before we utilise the samples collected as part of this study.

		Prospective sample collection	Retrospective sample collection
Primary uveal melanoma	Cohort A	✓	✓
Metastatic uveal melanoma	Cohort B1	✓	✓
	Cohort B2		✓
Conjunctival melanoma	Cohort C		✓

Eligibility criteria

Patients will be eligible for the study, as defined below.

Inclusion criteria

- Patients ≥ 18 years of age (no upper age limit).
- Histological diagnosis of primary or recurrent/metastatic ocular melanoma*.
- Healthy eye, blood and liver samples stored in biobanks with consent for use in research.
  *With the exception of primary uveal melanoma where a clinical diagnosis is sufficient. A diagnostic accuracy of >99% is achieved using the combination of ophthalmoscopy (examination of the back of the eye), fundus photography (photograph of the back of the eye) and an eye ultrasound.

Exclusion criteria

- Patients < 18 years of age.

Recruitment and Consent

Cohorts A and B1 (prospective and retrospective sampling)

For cohorts A and B1, a member of the patient's existing clinical care team, a research nurse or member of the Clinical Research Network (CRN) will have access to patient records to identify potential participants and check whether they meet the eligibility criteria. If so they will be introduced to the study and offered a participant information sheet (PIS) during an outpatient consultation.

If a patient is interested in the study and wishes to find out more and/or participate in the study, a further appointment will be made in a specialist clinic or dedicated study-specific clinic, in addition to the normal routine care appointments, for recruitment onto this study. At this visit, a designated and trained NHS or CRN staff member will inform patients of the nature and objectives of the study, go through the participant information sheet, highlighting possible risks associated with their participation and reiterate the voluntary nature of this study. Patients will be given the opportunity to ask questions pertaining to the study. If the potential participant is still interested in taking part, written informed consent for the study will then be received, and a copy of the informed consent will be given to the participant to keep.

Written informed consent will be obtained prior to the participant undergoing any activities that are specifically for the purposes of the study. This will include the collection of patient clinical information, which will be linked anonymised. Upon entry into the study participants will be asked to complete a questionnaire on personal characteristics and family history of related cancers. All patients recruited onto the study will be suitable for treatment in accordance with NICE guidelines and standard of care. Consent will also be received from participants for use of their previously obtained blood and/or tissue samples, which may be stored within the local pathology diagnostic archive. Local sites are responsible for:

- Assessing patient capacity to give informed consent;
- Ensuring patients receive latest REC approved version of the participant information sheet and consent form;
- Receiving and storing written informed consent and providing the participants with a copy of this consent.

Cohorts B2 and C (retrospective sampling only)

For cohorts B2 and C, a member of the patient's existing clinical care team, a research nurse or member of the Clinical Research Network (CRN) will have access to patient records to retrospectively identify patients and available samples. Patients who have, and those who have not, previously given written consent for their samples that were obtained during standard procedures of treatment, to be used in research, will be eligible.

For the majority of samples in these two cohorts consent for the use of the samples in research would have been received at the time of sample collection using the hospitals standard consent from. For some of the samples in

cohort B2 and cohort C consent for use of the samples for research may not have obtained when these samples were collected. Given the poor survival outcomes in these populations of patients, it is anticipated that most patients within these cohorts will be deceased by the time of study set up, as such it would not be possible to re-consent the patients for inclusion in this study. However, given that the samples were collected from the living at the time of collection, they will be anonymous to the Sanger research team and they are a recognised rare tumour type, we are seeking REC approval for the use of these samples in this study.

Sample Collection

Tissue collection (all cohorts)

Primary and locally recurrent tumour and healthy samples will be collected from the UCL Institute of Ophthalmology and /orMoorfields Biobank. Metastatic tumour samples will be collected from St Bartholomew's Hospital, Cancer Tissue Bank @ Barts Health. All tissue samples will be taken from surplus material following biopsies or surgery. Specimens used in the study will be surplus to diagnostic and pathological requirements, and comprise FFPE blocks or sections. Where possible, fresh tissue will be obtained at the time of surgery for sequencing, and in a subset of patients to generate cell lines. If samples have been collected from ocular melanoma patients as part of other REC approved studies, we will receive consent from the patient to use any available surplus samples in the iSITE study.

Blood samples will be purchased from commercial providers active in the UK whom provide access to samples collected with fully informed consent for use in research.

Typical tissue storage sites	**Eye Tissue**	**Metastatic Tissue**
Diagnostic archive	UCL Institute of Ophthalmology	St Bartholomew's Hospital
Research tissue bank	Moorfields Biobank	Cancer Tissue Bank @ Barts Health

Vitreous Fluid collection (cohort A)

Vitreous fluid is not required for diagnostic purposes and is removed with the eye during enucleation. In patients undergoing enucleation who have consented for their primary tumours to be used for research, up to 2mls of vitreous fluid will be aspirated in theatres by the operating team, immediately after the eye has been enucleated. This will take place in the operating theatres at Moorfields Eye Hospital.

Whole blood collection (cohorts A and B1)

Blood sampling for cohort A will initially take place at Moorfields Eye Hospital (2 time points) where the primary treatment is being carried out. The remaining blood samples for cohort A will be collected at St Bartholomew's Hospital, where patients are routinely commenced on surveillance. Blood sampling for cohort B1 will take place in St Bartholomew's Hospital, where patients are treated for metastatic disease. As much as possible, additional research blood sampling all study participants will coincide with their routine treatment visits.

30mls of whole blood will be collected from patients at all time points. Whole blood samples taken at baseline and at first recurrence will be processed for buffy coat and plasma analysis. At all other time points, 30mls of whole blood will be collected and processed for plasma analysis. Samples will be stored at -80°C locally prior to being transferred to SANGER. The maximum total blood draw for each patient per annum is anticipated to be 150mls for cohort A (5 time points, 30mls per time point) and 540mls for cohort B1 (18 time points for the majority of patients, 30mls per time point).

Retrospective sample collection (all cohorts)

In addition, local site research teams will request surplus healthy and diseased tissue, vitreous fluid and blood samples which have been previously collected from UCL Institute of Ophthalmology, St Bartholomew's Hospital, Moorfields biobank, Cancer Tissue Bank @ Barts Health and local hospitals (including samples that have been processed for extraction of nucleic acids) and stored in:

(a) Local Pathology Diagnostic Archives by liaising with their local pathology department;
(b) Research Tissue Banks by liaising with their local Research Tissue Bank Manager.

Sample Processing

Multi-region sampling will be performed using techniques such as microscopy-guided dissection. DNA and/or RNA will be extracted from these and other patient-derived samples including blood. Libraries will be prepared and sequenced on the Illumina HiSeq 4000 or other platforms. Non-tumour regions within tissue specimens and buffy coats from blood samples will undergo similar methods for germline analysis. Sequencing may be whole genome, whole exome or to targeted sets of cancer genes.

Primary sequence data will be entered into the SANGER variant calling pipelines, to identify somatic base changes and structural variants. This information will be correlated with linked anonymised patient clinical data (e.g. pathology reports, scan reports) in all cohorts, to determine their association with clinical outcomes.

Where feasible, fresh tumour samples will be collected from patients during standard procedures such as biopsy or enucleation, for (i) sequencing to allow for more accurate identification of mutations and (ii) to develop uveal melanoma cancer cell models such as cell lines and organoids. These will be used to study disease biology in vitro and predict response and resistance to therapies. Consent will be received from patients to deposit any anonymised samples and cancer cell line models, remaining at the end of the study, into tissue and/or cell banks for future ethically approved research.

Statistical Plan

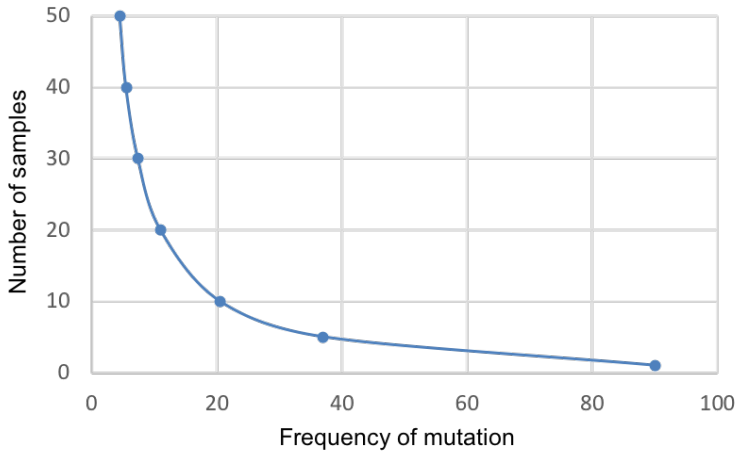
The target accrual for each cohort is listed below:

- Cohort A: 104 uveal melanoma patients
- Cohort B1: 16 uveal melanoma patients
- Cohort B2: 50 uveal melanoma patients
- Cohort C: 10 conjunctival melanoma patients

<u>Cohorts A and B1</u>

The sample size required for ctDNA analysis is heavily influenced by the sensitivity of the methods used to detect ctDNA. This is determined by both the error rate of the methods used (to be established as part of the study) and the number of mutations detected (to be established from the number of mutations present in tumour samples). Due to the exploratory nature of the analyses, this study will aim for a conservative target accrual of 20% over 2.5 years (104 patients) for cohort A and 75% accrual over 2.5 years (16 patients) for cohort B1.

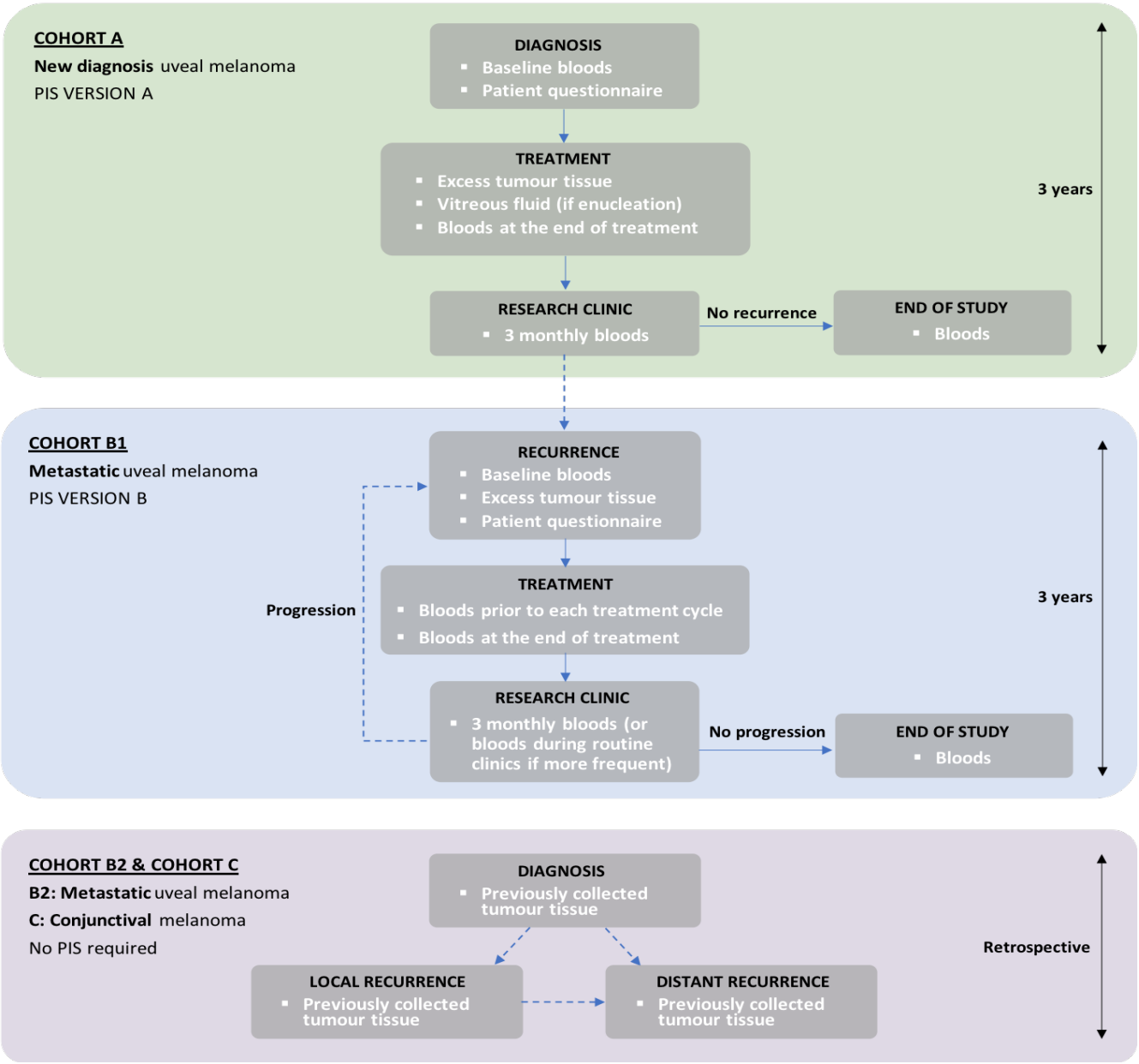
<u>Cohorts B2 and C</u>



The graph on the left represents the number of samples required to detect a mutation at a given frequency, with 90% power (i.e. a cumulative binomial probability of 90%). It forms the basis of the target accrual for cohort B2. In this study, with a cohort B2 sample size of 50 patients, and a frequency of mutation of 4.5%, we estimate that the power to detect at least one patient with a mutation is 90%. The maximum accrual for cohort C is predicted to be 10 patients due to the extremely low incidence of metastatic conjunctival melanoma.

STUDY FLOW CHART

Newly diagnosed uveal melanoma patients (cohort A, in green on study flow chart) will be invited to take part in the study and offered Version A of the PIS. Patients with metastatic uveal melanoma (cohort B1, in blue on study flow chart) will be invited to take part in the study and offered Version B of the PIS. Patients in cohort A who go on to develop metastatic disease can be recruited into cohort B1. Metastatic uveal melanoma tissue samples (cohort B2,) and conjunctival melanoma tissue samples (cohort C, both B2 and C in purple on study flow chart) will be retrospectively identified and collected from diagnostic archives and research tissue banks. Patients will not be prospectively recruited into cohort B2 and cohort C.



The timing and type of samples to be collected is outlined below.

COHORT A	Baseline	Treatment	Surveillance	End of study
New diagnosis uveal melanoma	At diagnosis / prior to treatment	During surgery	3 monthly research clinics	3 years after entry into study
Tumour tissue	Nil	Fresh tissue (if available) FFPE blocks	Nil	Nil

		H&E slides*		
Vitreous fluid	Nil	0.5 to 2.0mls (if enucleation)	Nil	Nil
Whole blood	30mls blood	30mls blood	30mls blood (first sample to be taken prior to discharge, following surgery)	30mls blood
Archived samples (if available)	Previously collected samples (inc. tissue & blood)	Nil	Nil	Nil

COHORT B1 Metastatic uveal melanoma	**Metastasis** Prior to systemic treatment	**Systemic treatment** Prior to each cycle & at completion of therapy (typical treatment schedule shown on next page)	**Surveillance** 3 monthly research clinics / routine clinic visits (whichever more frequent)	**Progression** At failure of treatment	**End of study** 3 years after entry into study
Tumour tissue	Fresh tissue (if available) FFPE blocks H&E slides*	Nil	Nil	Fresh tissue (if available) FFPE blocks H&E slides*	Nil
Whole blood	30mls blood	30mls blood	30mls blood	30mls blood	30mls blood
Archived samples (if available)	Previously collected samples (inc. tissue & blood)	Nil	Nil	Nil	Nil

COHORT B2 Metastatic uveal melanoma & **COHORT C** Conjunctival melanoma	**Primary Tumour** Taken at time of diagnosis	**Local Recurrence** Recurrence within the eye	**Distant Recurrence** Recurrence outside of the eye
Archived samples (Healthy and diseased) (if available)	FFPE blocks H&E slides*	FFPE blocks H&E slides*	FFPE blocks H&E slides*

*Should FFPE blocks be unavailable, H&E slides will be collected instead.

Patient & Public Involvement

This study is a follow up of a pilot study of 18 patients who were successfully enrolled over a period of 8 months between July 2016 and February 2017. Patients and their relatives were consulted during clinic consultations on the design and acceptability of the research being carried out. This contributed towards the design of the research with respect to decisions regarding volume of blood sampling and frequency of both blood sampling and clinic visits. It is not anticipated that participation within the study will be associated with significant risks outside of those pertaining to phlebotomy.

ETHICAL CONSIDERATIONS

Research Ethics Committee (REC) and other Regulatory Review

Health Research Authority (HRA) approval and NHS REC approval for the study will be sought. Substantial amendments will not be implemented until approval had been received. In accordance with the Human Tissue Act 2004 (Ethical Approval, Exceptions from Licensing and Supply of Information about Transplants) Regulations 2006, human tissue held for a REC approved research project can be stored on premises without a HTA licence.

Data Protection and Patient Confidentiality

No personal identifiable information will be shared with the sponsor organisation or the research team at Sanger. As all participant data in this study will not allow for participant identification, both using the data on its own, or in combination with other accessible information, it is not classed as personal data, and the GDPR transparency requirements do not apply. All investigators and study site staff will comply with the requirements of the Data Protection Act 1998 with regards to the collection, storage, processing and disclosure of personal information. To maintain patient confidentiality, all participants in the study will be assigned a unique study identifier code. The link between the identifiable participant information and the unique study code will be maintained on password protected computers at each recruiting study site. The research team at SANGER will not have access to this linkage information. Any information or documentation (e.g. pathology reports, scan reports) being transferred between participating sites and researchers will labelled only with this unique identification code. Individual patients will be referred to only by their unique study identifier, and any other patient identifiers will be removed/redacted.

Human genetics and/or genomics data will be processed in accordance with the SANGER Human Data Security Policy. The raw genetic information that is produced will be deposited and stored indefinitely in a central electronic data archive such as the European Genome-Phenome Archive (EGA), which is a secure database established by the European Bioinformatics Institute, Hinxton, Cambridge, UK, for the sharing of data with the research community. Access to the anonymised information stored in this archive will only be accepted via applications from appropriately qualified researchers who sign a legally-binding Data Access Agreement in which they commit to:

- use the data only for research purposes;
- protect the data confidentiality;
- provide appropriate data security;
- not attempt to identify individual participants from whom data were obtained;
- not redistribute the data or any subset or derivative that could be used to identify the research participant.

REFERENCES

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[3] Bidard, F.-C., Madic, J., Mariani, P., et al. (2014) ‘Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma’, International Journal of Cancer, 134(5): 1207–1213.