**CUPID** – Transforming **C**ancer of **U**nknown **P**rimary with **I**ntelligent **D**iagnostics

**Unique Protocol Identification Number:**

**Australian New Zealand Clinical Trials Registry (ANZCTR) Identified Number:**

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**Sponsor: Flinders University**

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**Version: 1.1**

**23 May 2023**

# 1 Protocol Summary

## 1.1 Synopsis

|  |  |
| --- | --- |
| **Short Title:** | CUPID study |
| **Title:** | Transforming **C**ancer of **Un**known **P**rimary with **I**ntelligent **D**iagnostics  |
| **Study Description:** | This study evaluates the benefits of implementing a uniform care pathway along with the use of tissue of origin classifier and comprehensive somatic mutation profiling for patients with suspected or confirmed CUP. This study is a prospective trial, and a retrospective cohort of patients with CUP diagnosis will be used as a comparator. |
| **Objectives and Endpoints:**Specific objectives and corresponding endpoints for the study are outlined in Table 1: |

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| --- | --- |
| **Objectives** | **Corresponding Endpoints** |
| To identify the proportion of patients initially diagnosed with CUP, or suspected to have CUP, who then get assigned a primary cancer using tissue of origin analysis   | Proportion of patients with an initial diagnosis of CUP who then have a primary cancer site identified by tissue of origin analysis  |
| To identify the proportion of patients initially diagnosed with CUP who get a diagnosis other than CUP following somatic molecular profiling | Proportion of patients with initial diagnosis of CUP who then have a primary cancer site identified by somatic molecular profiling |
| To identify the proportion of patients who have an actionable mutation with a recognised matched therapy (or therapies) following somatic molecular profiling | Proportion of patients with CUP with an actionable mutation following somatic molecular profiling  |
| To implement/increase awareness of the nationally recognised Optimal Care Pathway (OCP) for all patients with CUP. | * Proportion of patients discussed at MDT after implementation of OCP compared to a historical comparator cohort
* Completion rate of a standardised diagnostic workup (measured as time from initial consultation to time of completion of diagnostic work up).
 |
| To identify the proportion of patients whose treatment was changed after a diagnosis other than CUP was made using tissue of origin test | Proportion of patients with change of treatment from results following tissue of origin test |
| To identify the proportion of patients who were able to receive optimal therapy based on their somatic mutation profiling results | Proportion of patients that received a therapy guided by somatic mutation profiling |
| To determine the overall survival of patients with a CUP diagnosis following the implementation of the OCP and to compare it with a historical comparator cohort | Overall survival (OS), defined as the time from diagnosis to death from any cause. The date of diagnosis will be taken as the date of histological confirmation of cancer diagnosis, e.g., the date of biopsy)  |
| **Exploratory Objectives** | **Exploratory Endpoint** |
| To characterise metabolomic signatures of CUP | To identify metabolomic signatures |

## 1.2 Schema/Study Design

Figure 1: Workflow of patients with a suspected CUP diagnosis (prospective)

Response assessment

Change in treatment?

* Patients with suspected CUP
* Local diagnosis (clinical/radiological/histological) compatible with CUP
* No prior systemic therapy for CUP
* Available tumour FFPE blocka

Treating team follows diagnostic steps as per OCP

Tissue of origin and somatic mutation profiling results

Molecular tumour board discussion and treatment recommendations

CUP co-ordinator notified

Standard Treatment Commenced

6- 8 weeks

6- 8 weeks

MDT, Multi-disciplinary team; OCP, Optimal care pathway; CUP, Cancer of unknown primary;

a. A tumour tissue sample that is suitable for: 1) the initial diagnosis of CUP at the study site’s local

laboratory, AND 2) generation of a comprehensive genomic profile. If, after local diagnosis of CUP, insufficient tumour tissue (in quantity or quality) remains for the central pathology laboratory to confirm the CUP diagnosis and generate a genomic profile, then a fresh biopsy sample will be collected during the screening period that meets the study’s requirements (refer to the Laboratory Manual for suitability details and specimen collection instructions).

# 2 Introduction

## 2.1 Background on Cancer of unknown primary

Cancer of Unknown Primary (CUP) is defined as histologically confirmed metastatic cancer for which there is no histologically or clinically confirmed primary site. It is a diagnosis of exclusion for which a standardized diagnostic work-up including comprehensive clinical examination, imaging, tumour markers, morphological and panel of immunohistochemical (IHC) assessment of tumour sample fails to identify the site of tumour origin at the time of diagnosis1–3.

CUP accounts for 3-5% of all malignancies worldwide3 and prognosis remains poor4–6. Patients with CUP have a median overall survival (OS) of 8-11 months2. In Australia, CUP constitutes 1.6% of all cancers and its incidence has significantly decreased between 1982 and 2019 (from 16 to 8.1 per 100,000 persons), however, like the global statistics, the prognosis is still poor. The 5-year survival of a patient with CUP remains low at 13%7.

Research indicates that significant disparities in the incidence and survival of people with CUP diagnosis exist within Australia. In addition, **the health service delivery and diagnostic pathways of people with CUP differs markedly compared to people with other cancers**. Although the diagnosis of CUP should only be made in people with a metastatic cancer after thorough investigations have failed to identify the site of primary tumour, analysis of population-based cancer registry data demonstrates that many people with a CUP diagnosis failed to receive adequate investigations 8–11. A retrospective US study reported that only 35% of CUP patients reported to the cancer registry received a timely and complete diagnostic evaluation12. These findings were similar in Australia 11. Cancer registry based studies showed that people with CUP were more likely to be elderly and living in aged care facilities, attend an emergency department prior to diagnosis, and undergo less invasive diagnostic tests, leading to missed opportunities for diagnosis and management8, 11,13. Thus, people with suspected CUP may experience inappropriate or underutilisation of recommended tests, leading to delayed or inaccurate diagnosis and missed opportunities to identify a primary site, thus leading to poorer health outcomes. It is also likely that in a proportion of such cases with widely disseminated metastatic disease, additional diagnostic tests were not warranted given the limited ability to change the course of the disease.

Guidelines indicate that if the primary can be identified based on presentation, site-specific therapies are initiated3. However, if a primary site cannot be identified, the CUP is classified into one of two clinico-pathological sub-groups (**favourable and unfavourable risk**). Favourable risk CUP (15-20% of all CUPs) have survival similar to those with advanced cancer with a known primary site. However, the large majority (80-85%) of CUP belong to the unfavourable risk group with no effective treatment options and poor survival from broad spectrum empirical chemotherapy14. There is a need to develop novel ways to identify the primary site so that effective therapies can be applied and to develop new therapeutic strategies for those without an identifiable primary site.

**2.1.1 Management of Cancer of Unknown Primary**

**2.1.1.1 Cancer of Unknown Primary Optimal Care Pathway**

It is important that the diagnosis of CUP is only made after a comprehensive and standardised diagnostic workup based on the OCP has been done. To further evaluate the potential tissue of origin of CUP, as well as to exclude chemo-sensitive and potentially curable tumours (e.g. germ-cell tumours), one of the most important steps is the ascertainment of histopathological subtypes based on the cell of origin using morphology and IHC techniques by an experienced pathologist.

It is anticipated that having a uniform diagnostic pathway will identify those with a true CUP accurately and aid in avoiding incorrect classification arising from inadequate investigations as well reduce disparities that exist within CUP population. The Australian optimal care pathways (OCPs) were developed with the goal of reducing variations in cancer care and benchmark against quality indicators. OCPs set out a standardised approach to delivering consistent and best cancer care. In the year 2020, the Australian OCP for CUP was released 15. Elsewhere, the US National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology (ESMO) have CUP-specific guidelines3. The OCPs were developed in collaboration with a wide range of clinicians, epidemiologists, consumers and carers by the Cancer Council Victoria, and the National Cancer Expert Reference Group and supported by the Australian Health Minsters’ Advisory Council. The Australian OCP for CUPmaps the whole patient journey from diagnosis of suspected CUP to death. However, the OCP for CUP has not yet been implemented in most states and territories. This study will support the implementation of the OCP for CUP in SA.

**2.1.1.2 Intelligent diagnostics - Assessment of Tissue of Origin and somatic mutations**

Given the poor survival for those with a CUP diagnosis, newer approaches are urgently required. In other cancers, precision medicine approaches with somatic mutation profiling have been increasingly studied. Moreover, tissue of origin studies incorporating molecular changes within tumour tissues were also explored.

*2.1.1.2.1 Precision Medicine*

The National Institutes of Health define precision medicine as "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person"16.

Given the therapeutic challenges imposed when the tissue of origin is unknown, patients with CUP would be well suited for a targeted therapy approach. Patients with unfavourable risk CUP have poor prognosis with currently available chemotherapy. Like in other cancers, somatic mutation molecular profiling techniques have been employed with varying success to identify matched therapies for people with CUP 17–22.

We also have evidence to suggest that molecular diagnostic testing methods can assist in identifying the primary site of origin (**tissue of origin**) for patients with CUP. Some methods appear to identify the primary site of origin accurately in more than 90% of patients tested23. However, none of these tests are routinely used in the current clinical practice in Australia either due to lack of proven benefits in clinical trials, difficulty in accessing these tests, lack of national funding or lack of external validation24. There is an urgent need to establish locally available testing methods that are validated and approved by the Therapeutic Goods Administration. With widespread use and access to somatic mutation profiling in Australia, it is anticipated that such testing in patients with CUP may provide clues towards the primary site of origin as well as improve their survival outcomes through the availability of matched therapies through future clinical trials.

# 3.0 Objectives and Endpoints

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| --- | --- |
| **Objectives** | **Corresponding Endpoints** |
| To identify the proportion of patients initially diagnosed with CUP, or suspected to have CUP, who then get assigned a primary cancer using tissue of origin analysis   | Proportion of patients with an initial diagnosis of CUP who then have a primary cancer site identified by tissue of origin analysis  |
| To identify the proportion of patients initially diagnosed with CUP who get a diagnosis other than CUP following somatic molecular profiling | Proportion of patients with initial diagnosis of CUP who then have a primary cancer site identified by somatic molecular profiling |
| To identify the proportion of patients who have an actionable mutation with a recognised matched therapy (or therapies) following somatic molecular profiling | Proportion of patients with CUP with an actionable mutation following somatic molecular profiling  |
| To implement/increase awareness of the nationally recognised Optimal Care Pathway (OCP) for all patients with CUP. | * Proportion of patients discussed at MDT after implementation of OCP compared to a historical comparator cohort
* Completion rate of a standardised diagnostic workup (measured as time from initial consultation to time of completion of diagnostic work up).
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| To identify the proportion of patients whose treatment was changed after a diagnosis other than CUP was made using tissue of origin test | Proportion of patients with change of treatment from results following tissue of origin test |
| To identify the proportion of patients who were able to receive optimal therapy based on their somatic mutation profiling results | Proportion of patients that received a therapy guided by somatic mutation profiling |
| To determine the overall survival of patients with a CUP diagnosis following the implementation of the OCP and to compare it with a historical comparator cohort | Overall survival (OS), defined as the time from diagnosis to death from any cause. The date of diagnosis will be taken as the date of histological confirmation of cancer diagnosis, e.g., the date of biopsy)  |
| **Exploratory Objectives** | **Exploratory Endpoint** |
| To characterise metabolomic signatures of CUP | To identify metabolomic signatures |

# 4.0 Study design

## 4.1 Description of the Study

This is a multicentre trial evaluating the benefits of implementing a uniform care pathway along with the use of tissue of origin classifier and comprehensive somatic mutation profiling for people with suspected or confirmed CUP. No treatment is being provided to the participants through this study however, the results from the molecular tests may guide treatment. The study will include a prospective and retrospective component.

**Retrospective component**

Data for all patients diagnosed with CUP at the study sites from the 1 January 2000 to 31 December 2022 will be retrospectively collected under a waiver of individual consent. Once patients are identified from medical records, information such as demographics (age of diagnosis, gender and ethnicity), dates and types of investigations done, dates and types of treatment received, number of lines of treatment, progression free survival and overall survival will be extracted. Information will be obtained from case notes, electronic medical records and pharmacy records.

**Prospective Component**

The study design for the prospective component of the trial is presented in Figure 1.

Figure 1: Workflow of patients with a suspected CUP diagnosis

Response assessment

Change in treatment?

* Patient with suspected CUP
* Local histological diagnosis compatible with CUP
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MDT, Multi disciplinary team; OCP, Optimal care pathway; CUP, Cancer of unknown primary;

a. A tumour tissue sample that is suitable for: 1) the initial diagnosis of CUP at the study site’s local

laboratory, AND 2) generation of a comprehensive genomic profile. If, after local diagnosis of CUP, insufficient tumour tissue (in quantity or quality) remains for the central pathology laboratory to confirm the CUP diagnosis and generate a genomic profile, then a fresh biopsy sample must be collected during the screening period that meets the study’s requirements (refer to the Laboratory Manual for suitability details and specimen collection instructions).

**4.1.1 Screening Period for Prospective Study**

The population to be included in this study corresponds to patients first diagnosed with CUP for whom a likely tissue of origin cannot be posited from routine diagnostic investigations.

To be eligible, patients must have a histological diagnosis of cancer without pathological findings that confirm a definite primary site, as determined by the study site’s local laboratory on a contemporaneous tissue sample.

The diagnosis of CUP must be ascertained prior to entering the screening period of the study.

4.1.1.1 During Screening

As this study aims to direct treatment according to genomic profiles, participants will be asked to agree to the following:

1. A tumour tissue sample to be analysed for generation of a comprehensive genomic profile
2. Archival tumour FFPE block prior to screening will be acceptable for these central analyses. However, if an acceptable archival tumour FFPE block is not available or is not suitable (in quantity and quality) at screening, an FFPE block from a freshly obtained biopsy sample will be obtained.

**4.1.2 Implementation of the Optimal Care Pathway (OCP)**

1. A survey will be sent out to medical professionals to determine the level of awareness about the OCP at the start and the end of the study
2. Participants fulfilling eligibility criteria will undergo investigations coordinated by their treating oncologist/treating team as per the OCP while the molecular tests are performed.
3. The assessment based on the CUP OCP include:
	* Minimal basic work-up which will need to be conducted **within 2 weeks** of a medical oncologist/specialist review:
		+ Thorough medical history and physical examination (including, e.g., head and neck and breast examination)
		+ Basic blood and biochemical analyses
		+ Contrast-enhanced computed tomography (CT) scans of chest, abdomen and pelvis
	* Further work-up (including, but not limited to):
		+ Mammography in women
		+ Breast MRI in women with axillary disease
		+ Endoscopies guided by sign-, symptom- or laboratory abnormalities
		+ Tumour markers:
			- Serum assessment of α-fetoprotein, human chorionic gonadotropin, plasma chromogranin A and PSA in male patients to exclude potentially curable extragonadal germ-cell tumors, neuroendocrine tumors and prostate cancers amenable to hormonal treatment
		+ Whole-body FDG–PET/CT suggested for patients with cervical adenopathies from CUP and those with a single CUP metastasis
	* In situations where clinical and histopathological pictures are suggestive of a specific primary origin for the cancer, an appropriate work-up to exclude this specific cancer should also be performed. These situations include (but are not limited to) the following:
		+ Breast MRI in women with lymph nodes in the breast drainage areas in the context of IHC results suggestive of breast cancer
		+ MRI liver/MRCP in patients with one or few liver lesions presenting with no extra-hepatic disease or with extra-hepatic disease limited to lung metastases and/or lesions in the upper abdomen, in the context of IHC suggestive of cholangiocarcinoma or pancreatobiliary or upper gastrointestinal disease
		+ Adequate abdominal and pelvic imaging in the presence of high-grade serous carcinoma
		+ Bosniak classification of any kidney lesion in the context of IHC suggestive of renal cell carcinoma or other kidney malignancy
		+ ENT (Ear-Nose-Throat) examination and MRI and/or PET imaging in patients with IHC and/or a clinical picture suggestive of salivary gland carcinoma (this includes poorly defined masses in the neck or predominance of neck lymph nodes in the clinical presentation)

**4.1.3 Treatment Phase**

4.1.3.1 Initiating treatment

1. As per the CUP OCP, treatment should start **within 2 weeks** of the decision to treat.
2. While the molecular tests are performed, the standard care for patients with suspected CUP will proceed as per the OCP. The treating clinician will discuss all cases of suspected CUP in the state-level multi-disciplinary team (MDT) meeting.
3. The treating clinician will proceed with treatment planning according to the OCP guide.

4.1.3.2 Molecular Tumour Board (MTB)

1. Results from somatic mutation profiling will be discussed at the already established state-wide molecular MDT meetings held monthly.
2. A recommendation on the possible primary site and potentially identified matched therapies including standard therapies and available clinical trials will be provided to the treating clinician/team.
3. The results from the MTB will support the treating team to adopt/modify treatment after initial response assessment with the previously chosen therapies. If there is disease progression identified on first-line therapy, the treating clinician and the patient may or may not choose to adopt therapies based on the molecular analysis for primary site and matched targeted therapies.

## 4.2 Scientific Rationale for Study Design

**4.2.1 Rationale for Population Selection**

The inclusion and exclusion criteria for the study are aimed at selecting patients with confirmed or suspected CUP, for whom a tissue of origin cannot be definitively posited with the following rationale:

1. These patients have similar outcomes and constitute a rather homogeneous population, as opposed to favourable prognosis patients.
2. As the tissue of origin is unknown and the disease is inherently heterogeneous, a therapeutic approach based on molecular profiling may change the outcome for this group of patients.

 **4.2.2 Rationale for Suggested Time Frames**

1. The approximate turnaround time for tissue of origin and somatic mutation profiling results

is between 4 and 8 weeks. Results from these tests will then be discussed at the state-wide molecular MDT meetings held monthly. A recommendation on the possible primary site and potentially identified matched therapies including standard therapies and available clinical trials will be provided to the treating clinician/team. Novel treatments may also be recommended to the treating physician. As image assessment for response is usually performed approximately 6-8 weeks after the start of first-line therapy, this timeline for the release of molecular results is appropriate. These results will support the treating team to adopt/modify treatment after initial response assessment with the previously chosen therapies. If there is disease progression identified on first-line therapy, the treating clinician and the patient may or may not chose to adopt therapies based on the molecular analysis for primary site and matched targeted therapies.

## 4.4 End of Study Definition

All participants will be followed for survival outcomes until voluntary withdrawal from the study or death.

# 5 Study Population

## 5.1 Inclusion Criteria

Eligible participants must fulfill all the following criteria:

1. Age 18 years or more
2. Suspected or confirmed CUP diagnosis
3. Willing to provide informed consent
4. Able to understand English or understand study involvement through interpreter services
5. No prior systemic therapy for the treatment of CUP
	* Patients who have received prior surgery and/or radiotherapy (including radioembolization of tumour) are eligible upon evidence of cancer recurrence or progression.

## 5.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

1. Metastatic disease from a known primary site
2. Patients with histology and immunohistology profiles (per 2015 ESMO guidelines) that are compatible with extragonadal germ-cell tumours, neuroendocrine tumours, sarcoma, melanoma, mesothelioma, haematological malignancies (this list is not limitative)

## 5.4 Screen Failures

Participants who do not meet all the inclusion criteria, or do meet any one of the exclusion criteria, will not be eligible for study participation.

## 5.5 Strategies for Recruitment and Retention

Patients attending cancer clinics with a new diagnosis of CUP will be informed of the study by their treating oncologists. If interested, the CUP project coordinator/manager will engage with the potential participant. The participant will be fully informed of study requirements, procedures, and any associated risks. There are no plans to publicly advertise the study but there will be communication across oncology centres to facilitate and promote patient recruitment.

# 6 Study Intervention

## 6.1 Study Intervention(s) Administration

### 6.1.1 Study Intervention Description

After informed consent, participants with CUP will be offered genomic studies incorporating both the tissue of origin assay and comprehensive genomic profiling testing.

At the time of initial diagnostic work-up, either image guided or open biopsy from one or more metastatic site(s) which is regularly obtained as part of standard care will be sent for tissue of origin and somatic gene panel analyses. Samples from fresh or archived tissue (paraffin blocks obtained from repeat biopsy, or where not available, from stored samples) will be transported to the sequencing site in Adelaide and undergo RNA/DNA extraction and Illumina TruSight ™ Oncology 500 NGS testing. This is an integrated DNA/RNA assay which targets 523 genes for assessment of small variants, tumour mutational burden, microsatellite instability, splice variants, gene fusions and CNVs. RNA aliquots will be shipped to QIMR Berghofer and undergo the nanostring CUP assay (archived samples) or RNASeq (fresh frozen derived RNA). The CUP classifier based on Bayesian deep learning models will be used to provide a prediction of primary tissue and an estimate of uncertainty, and how uncertain that prediction is for clinical decisions. Metabolomic profiling using small volumes of peripheral blood analysed on high resolution mass spectrometers will also be performed to identify novel mutation-specific or site-of-origin specific biomarkers. This data will be integrated with the other molecular analyses.

While the molecular tests are performed, the standard care for patients with CUP will proceed as per the OCP. The treating clinician will be encouraged to discuss all cases of suspected or confirmed CUP in the state-level multi-disciplinary team (MDT) meeting. The treating clinician will proceed with treatment planning according to the OCP guide and specific/nonspecific CUP subsets with favourable or unfavourable risk.

The approximate turn-round time for these analyses is between 4 and 8 weeks. Results from these tests will be discussed at the already established state-wide molecular MDT meetings held monthly. A recommendation on the possible primary site and potentially identified matched therapies including standard therapies and available clinical trials will be provided to the treating clinician/team. Novel treatments may also be recommended using the metabolomic platform. As image assessment for response is usually performed approximately 6-8 weeks after the start of first-line therapy, this timeline for the release of molecular results is appropriate. These results will support the treating team to adopt/modify treatment after initial response assessment with the previously chosen therapies. If there is disease progression identified on first-line therapy, the treating clinician and the patient may or may not choose to adopt therapies based on the molecular analysis for primary site and matched targeted therapies.

### 6.1.2 Dosing and Administration

Not applicable.

## 6.2 Measures to Minimize Bias: Randomization and Blinding

There is no randomization or blinding

## 6.4 Study Intervention Compliance

Participant's involvement is minimal as standard of care biopsies will be utilised for genomic studies.

# 7 Study Intervention Discontinuation and Participant Discontinuation/Withdrawal

## 7.1 Discontinuation of Study Intervention

The genomic studies and blood sample for metabolomic studies are one-off testing.

Surveys will not be sent to participants but survival outcomes may still be collected if they withdraw all consent through public access. (eg death notices)

## 7.2 Participant Discontinuation/Withdrawal from the Study

Participants can withdraw from the study at any time without any impact on their care from the treating team.

## 7.3 Lost to Follow-Up

All consented participants will be included for analysis.

# 8 Study Assessments and Procedures

## 8.1 Efficacy Assessments

**8.1.1 Schedule of assessments**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Screening period | Investigation Period- Implementing OCP by treating team | Treatment Period (pre-MTB) | Molecular tumour board meeting | Response assessment | Treatment Period(Treatment as per MTB suggestion) |
| Informed consent | X |  |  |  |  |  |
| Eligibility criteria | X |  |  |  |  |  |
| Complete physical examination  | X | X |  |  | X |  |
| Limited physical examination |  |  | X |  |  | X |
| ECOG performance status | X | X | X |  |  |  |
| Blood tests (Haematology, Biochemistry) | X | X | X |  |  | X |
| Tumour assessment- medical imaging | X | X | X |  | X | X |
| Tumour assessment- tumour markers | X | X | X |  | X | X |
| Notify CUP co-ordinator if eligibility confirmed | X |  |  |  |  |  |
| Archival tumour tissue | After eligibility confirmation |  |  |  |  |  |
| Tumour tissue biopsy (if insufficient or not suitable archival tissue is available) | After eligibility confirmation |  |  |  |  |  |
| Initiate treatment  |  | X |  |  |  |  |
| Discuss tissue of origin and somatic mutation profiling results |  |  |  | X |  |  |
| Molecularly-guided therapy in patients with a CR, PR or SD |  |  |  |  |  | X |

Once potential the participants are identified and they have shown interest in the study, a consent and screening visit will be organised.

Screening visit activities -

Consent for the study including genomic studies will be first obtained. A baseline clinical and demographic data will be obtained; cancer information including site of biopsy and imaging; past medical history will all be collected.

Either archival or fresh tumour samples (if available) will be sent for genomic studies (tissue of origin classifier and comprehensive somatic mutation profiling). The participant will then return to the usual treating team for standard of care treatment. If they are started on systemic therapy (with or without local therapy) as per the CUP standards, results of the treatment will be collected on cancer outcomes using imaging and cancer markers (if any elevated) as routinely done by the treating teams. In the interim, a molecular MDT meeting will occur to discuss the results from the genomic studies and provide recommendations based on the Mi-ONCOSEQ genomic alteration tiers25. Tier 1 and 2 alterations are considered potentially clinically actionable. The recommendations based on sequencing studies will be provided to the treating team who may choose to use the results for a sequencing directed therapy through other clinical trials, off-label or on-label medications to be provided to the participants. This information along with the type of treatment and cancer outcomes from the new second-line treatment will be collected.

## 8.2 Safety Assessments

As the study does not provide any intervention to the treating physician/treating team, safety assessments are not needed.

# 9 Statistical Considerations

## 9.1 Sample Size Determination

The expectation is that the prospective component of this study will recruit 50 patients per year over a 4-year period (total 200 patients). The study will determine the overall survival of patients with a CUP diagnosis following the implementation of the OCP and to compare it with a historical comparator cohort. The expected median survival in the historical cohort is 9 months. Our sample size will have an 80% power of detecting a 33% improvement in survival, from a median of 9 to a median of 12 months, with a sample size of 200.

The study aims to recruit as many participants as possible over a 4-year period. Many of the study endpoints are defined as proportions of the total study population. Specifically, these proportions are defined as follows;

1. Proportion of patients with initial diagnosis of CUP who then have a primary cancer site identified by tissue of origin analysis
2. Proportion of patients with initial diagnosis of CUP who then have a primary cancer site identified by somatic molecular profiling
3. Proportion of patients with suspected CUP with an actionable mutation following somatic molecular profiling
4. Proportion of patients discussed at MDT after implementation of OCP compared to a historical comparator cohort
5. Completion rate of a standardised diagnostic workup (measured as time from initial consultation to time of completion of diagnostic work up).
6. Proportion of patients with change of treatment from results following tissue of origin test

As no formal comparative tests are planned for the above proportional assessments, a pre-specified sample size is not applicable to these endpoints.

The retrospective component of the study will include a review of 20 years of experience of managing patients with CUP from each centre. The sample size of the retrospective component will be determined by retrospective record capture.

# 10 Supporting Documentation and Operational Considerations

## 10.1 Regulatory, Ethical, and Study Oversight Considerations

### 10.1.1 Ethics and regulatory compliance

This study will be conducted according to the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) annotated with TGA comments (Therapeutic Goods Administration DSEB July 2000) and in compliance with applicable laws and regulations. The study will be performed in accordance with the NHMRC Statement on Ethical Conduct in Research Involving Humans (© Commonwealth of Australia 2007), and the NHMRC Australian Code for the Responsible Conduct of Research (©Australian Government 2007), and the principles laid down by the World Medical Assembly in the Declaration of Helsinki 2008.

### 10.1.2 Informed consent

An Informed Consent Form will be used at each site. If applicable, it will be provided in a certified translation of the local language. Patients will be told that they are free to refuse to participate and may withdraw their consent at any time for any reason. A separate, specific signature will be required to document a patient's agreement to participate in optional procedures. Patients who decline to participate will not provide a separate signature.

The Consent Forms must be signed and dated by the patient before his or her participation in the study. The case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained prior to participation in the study.

The Consent Forms will be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate.

If the Consent Forms are revised (through an amendment or an addendum) to communicate information that might affect a patient's willingness to continue in the study, the patient or a legally authorized representative must re-consent by signing the most current version of the Consent Forms or the addendum in accordance with applicable laws and IRB/EC policy. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient. All signed and dated Consent Forms must remain in each patient’s study file or in the site file and must be available for verification by study monitors at any time.

### 10.1.3 Confidentiality and Privacy

Confidentiality standards will be maintained by coding each patient enrolled in the study through assignment of a unique patient identification number (including comprehensive genomic profiling services provided by the central laboratory).

Patient medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law. Medical information may be given to a patient’s personal physician or other appropriate medical personnel responsible for the patient’s welfare, for treatment purposes.

Given the complexity and exploratory nature of exploratory biomarker analyses, data derived from these analyses will generally not be provided to patients unless required by law.

Data generated by this study must be available for inspection upon request by representatives of national and local health authorities, representatives, and collaborators, and the IRB/EC for each study site, as appropriate. Study data, which may include imaging data and data on genomic variants, may be submitted to government or other health research databases or shared with researchers, government agencies, companies, or other groups that are not participating in this study. These data may be combined with or linked to other data and used for research purposes, to advance science and public health, or for analysis, development, and commercialization of products to treat and diagnose disease. In addition, redacted Clinical Study Reports and other summary reports will be provided upon request.

#### 10.1.4 Study Records Retention

Records and documents pertaining to the conduct of this study, including electronic and paper medical records. Informed consent forms, laboratory test results, medication records, and images, will be retained by the Principal Investigator for 15 years after completion or discontinuation of the study or for the length of time required by relevant national or local health authorities, whichever is longer. After that period, the documents may be destroyed, subject to local regulations.

### 10.1.5Publication and Data Sharing Policy

Regardless of the outcome of a trial, the researchers are dedicated to openly providing information on the trial to healthcare professionals and to the public, at scientific congresses, in clinical trial registries, and in peer-reviewed journals.

The researchers will comply with all requirements for publication of study results. Study data may be shared with others who are not participating in this study (see Section 10.1.3 for details), and redacted Clinical Study Reports and other summary reports will be made available upon request

The results of this study may be published or presented at scientific congresses.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

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