A DUTCH NATIONWIDE INITIATIVE TO OPTIMIZE THE DIAGNOSTIC WORK UP OF PATIENTS WITH CANCER OF UNKNOWN PRIMARY ORIGIN.

DI – CUP protocol

Protocol ID	CUP protocol
Short title	DI-CUP protocol
Version	Version 2.0
Date	25 – January 2022
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1 INTRODUCTION AND RATIONALE

The Dutch health care system of oncology patients is centered around the origin of the primary tumor; patients are referred to medical specialists with a focus on the specific primary tumor of that patient, they are discussed in dedicated multidisciplinary teams and treated according to care pathways developed per primary tumor site. However, of the patients diagnosed with metastasized cancer the 4th most common cancer in the Netherlands with 1300 patients in 2018, consists of patients with cancer of unknown primary (CUP); or "primaire tumor onbekend" (PTO), origin [1]. These patients are most often found to have widespread metastatic disease while no anatomic primary site is identified after comprehensive diagnostic workup. Consequently, for these patients with CUP no specific specialist, multidisciplinary team, nor care pathways exists in the Netherlands.

Patients with CUP in general have a poor prognosis with a median survival ranging from 2 – 10 months [1-3]. Clearly, the lack of dedicated professionals treating CUP is striking and patients are subjected to an extensive, time-consuming but often futile diagnostic work-up. A recent study has shown that patients with CUP on average undergo five imaging studies (range 1 – 17) [4], not taking into account additional biopsies. Decline of performance status during diagnostic work-up often follows given the aggressive nature of this disease limiting treatment options even further. Furthermore, the lack of primary tumor presents a therapeutic dilemma and as a result only one-third of the patients with CUP currently receive any treatment in the Netherlands[1]. Understandably, the diagnostic process with often disappointing results leads to frustration and deterioration of quality of life for and of patients with CUP and their families.

Hence, improving the diagnostic process for patients with (probable) CUP would not only shorten it, it could potentially also lead to more treatment options and ultimately improve quality of life and survival.

1.1 Definition of CUP and current guidelines

In current national and international guidelines regarding CUP a clear definition of CUP is lacking. Additionally, they do not agree on which specific diagnostic tests should be used [5-7]. This lack of definition is probably due to the fact that the diagnosis of CUP is based on exclusion, on not finding the site of origin and consequently, whether a patient fits such definition might potentially change per diagnostic step. This also complicates adequate registration since patients might change diagnosis during work-up.

The NICE (national institute for health and care excellence) guideline of the United Kingdom provides an inventive solution to this problem by creating multiple (sub)definitions for patients with CUP depending on the step of the diagnostic phase. The following definitions are being used: on initial presentation, patients with metastatic malignancy, identified by any form of examination, without an obvious site of origin, are labeled as having a "malignancy of undefined primary origin" (MUO); After initial, but not exhaustive investigations without identifying the primary site, patients with metastatic carcinoma of pathologic proven epithelial, neuro-endocrine or undifferentiated lineage are regarded as having "provisional carcinoma of unknown primary origin" (provisional CUP, pCUP). Consequently, patients who were found to have cancer of non-epithelial origin, i.e. lymphoma, melanoma, sarcoma, germ-cell, are not defined as CUP since treatment would then occur per specific tumor type. Lastly, after the results of all tests are complete and a primary tumor site has not been found, patients will be diagnosed with "confirmed CUP" (cCUP). Using different definitions during the diagnostic work

up takes into account the dynamics of this process and would enable adequate registration and follow-up of these patients. (www.nice.org.uk/guidance/cg104)

Another division that is made in the guidelines and literature is based on favorable or unfavorable risk subgroups[7, 8]. This distinction is based on a potentially subgroup of patients in which clinical and/or pathological features clearly hint toward site of origin even if no actual primary tumor is detected and for which an obvious, even potentially curable, treatment strategy exists (Table 1). Although, strictly speaking these patients could be labeled as CUP, this group is not hampered by the same diagnostic and therapeutic challenges.

Table 1: Favorable risk group CUP

Treatment occurs according to analogous tumor type.

CUP characteristics	Analogous tumor type
Poorly differentiated neuroendocrine carcinoma of unknown primary	Poorly differentiated neuroendocrine carcinoma
Well differentiated neuroendocrine tumor of unknown primary	Well differentiated neuroendocrine tumor
Peritoneal adenocarcinomatosis of serous papillary histology in women	Stage III-IV ovarian cancer, primary peritoneal cancer
Isolated axillary nodal metastases in women	Stage II-III breast cancer
Squamous cell carcinoma involving cervical lymph nodes	Head and neck squamous cell cancer
Squamous cell carcinoma involving inguinal lymph nodes with unknown primary	Urogenital squamous cell carcinoma
Poorly differentiated carcinoma involving mediastinum or retroperitoneum in young males, especially with elevated β-hCG or α-fetoprotein	Extragonadal germ cell tumor
CUP with colorectal IHC (CK20+, CDX2+, CK7–)	Colorectal cancer
Single metastatic lesion of unknown primary	Oligometastatic disease (radical local treatment)
Bone metastases with IHC/serum PSA expression in males	Prostate cancer
Adjusted from Table 1, Lee et al. [33288500]; CUP: carcinoma of unknown primary; IHC: immunohistochemistry;	

1.2 Molecular based diagnostic tests

The increasing knowledge of the molecular basis of cancer with extensive analyses of the genomics, transcriptomics, epigenetics and proteomics of a specific cancer type has also been applied to the identification of the site of origin for CUP. The earliest ventures in this regard consisted of gene expression assays in which the gene expression profile of a CUP is compared for level of similarity with gene expression profiles of cancers of known origin. The most well-known is the 92 gene set (commercialized: CancerType ID, Biotheranostics, San Diego, CA, USA) that has been tested for clinical utility in two studies. The first study suggested a site of origin in 247 CUP patients out of 252 patients in whom the assay was successfully performed out of a total of 289 patients.

In total 223 patients received treatment of whom 194 according to assay results. The median overall survival of this group was 12.5 months, which was deemed to be an improvement compared to historical control[9]. The second study, a prospective observational study measured the changes in final diagnosis and treatment after testing with the 92 gene set. In the study 444 patients were enrolled of which 271 were provided by medical oncologist. In 80/271 patients a differential diagnosis of 2 or more sites existed, in 66% of these cases the diagnosis was narrowed to one and in 27% a previously unsuspected site was identified. In 97% of the 112/ 271 patients labeled as CUP, a diagnosis based on the assay was made. This led to changes in treatment recommendations in 49% and respectively 42% of both patient groups[10] .

Other tests developed to predict the primary tumor consist of gene expression microarray, microRNA or RT-PCR based techniques[8]. Subsequently, prospective randomized controlled trials have been performed in which patients with CUP were randomized between empiric chemotherapy or specific therapy based on the result of the molecular test for identification of the primary tumor. A Japanese study, used a self-developed microarray classifier and patients were randomized to carboplatin/paclitaxel or primary tumor based therapy. No difference in overall survival was seen (median OS 12.5 and 9.8 months, respectively, p=0.90). The GEFCAPI-04 trial used the well-known 92-gene set classifier and randomized patients to cisplatin/gemcitabine and site specific therapy as determined by the classifier. Results were presented at the ESMO 2019, in total 243 patients were enrolled and 91/123 of the patients included in the classifier arm received site specific therapy. No improvement in progression free or overall survival was seen (median 5.3 vs 4.7 months and 10 vs 10.7 months, respectively) [11].

Next to gene expression tests, DNA aberrations itself have been tested for their ability to predict the site of origin of CUP by using next generation sequencing (NGS). Patterns of mutations specific for certain tumor types were developed into genomic classifiers and tested for their ability to predict a site of origin in patients with CUP[8]. Although promising results have been described, prediction of the site of potential origin was not substantially different when compared to gene expression tests when using NGS with limited gene panels. Therefore there is an urgent need to expand the possibility for comprehensive gene tests. Additionally, another promising result using such tests could be to identify the presence of clinically meaningful targetable mutations leading to potentially new treatment options. This strategy is being explored in multiple studies in which CUP patients also can be enrolled, such as the NCI-MATCH [12], TAPUR [13] and the DRUP trial[14]. More specific for patients with CUP, a phase II randomizing between platinum-based chemotherapy and targeted therapy based on genomic alterations is currently enrolling patients (NCT03498521). Interim feasibility analyses were presented at the ESMO 2019 showing retrospectively that 96/303 could have been matched to targeted therapy based on mutations and fusions[15].

In most studies using NGS a limited number of genes were investigated and although these platforms are also expanding they only address a one part of the diagnostic spectrum. For example to test for potential targetable fusions another diagnostic platform (mostly Archer DX, Inc., Boulder, CO) needs to be used. In other tumor types this is usually performed in a step-wise fashion to reduce cost. However, this sequence is not only time consuming but also tissue consuming, both of which are in general limited in patients with CUP. Furthermore, investigating more than a select gene panel with NGS by adding an extensive molecular workup (including whole exome and RNA sequencing) was shown to be of additional value in a recent study. In 13/55 patients with CUP sequencing-directed therapy based on found genomic alterations could be started and in 8/55 patients a

pathogenic germline variant was found of which 4 were therapeutically relevant[16]. However, no survival data has been reported so far.

Recently, new broad NGS panels have been developed to include over 500 genes. An example of which is the TSO500 NGS platform and in combination with Archer Dx. most if not all targetable mutations for which treatment in the Netherlands is available, are covered. Furthermore, a recent study showed that with this method the genomic alterations (over all possible mutation classes including single and multiple nucleotide variants (SNV's/ MNV's), insertion and deletion (indels), copy number alterations (CNA's) fusion genes and microsatellite instability) could be identified[17]. Importantly both these assays can be applied on fresh frozen paraffin embedded (FFPE) tissue that is routinely available from all cancer patients for primary diagnoses and only a small amount of DNA is needed for analyses. However, so far for patients with CUP no classifier to predict the site of origin has been developed with this platform.

1.3 Whole genome sequencing

WGS is a next generation NGS technology that covers the complete genomic landscape, including fusions and relevant pathogenic germline mutations. WGS therefore provides the potential means to perform one test not only in a timely manner but also a tissue efficient manner with the broadest insight in the molecular aberrations for cancer patients. Furthermore, analysis of the center for personalized cancer treatment (CPCT) study has shown that a pan-cancer WGS analysis on metastatic tumor and normal DNA resulted in identification of an actionable alteration with a predicted sensitivity to an approved anti-cancer drug in up to 31% of the patients (in a subset of 2405 patients). Of these, a registered indication of therapy was present in 18% of the patients, while 13% was outside the labeled indication. In an additional 31% of the patients an alteration with a predicted sensitivity to experimental therapy was found. These genomic alterations were distributed over all possible mutation classes (including single and multiple nucleotide variants (SNV's/ MNV's), insertion and deletion (indels), copy number alterations (CNA's) fusion genes and microsatellite instability) underlining the importance of extensive comprehensive tumor genomic profiling[18].

Moreover, with the WGS data of the CPCT study and recent WIDE (WGS Implementation in standard cancer Diagnostics for Every cancer patient) study[19] a classifier has been developed based on the corresponding DNA aberrations of known cancer types to predict site of origin in case of CUP (CUPPA algorithm, article in preparation). Therefore using WGS would not only identify targetable mutations but could potentially lead to identification of the primary tumor in patients diagnosed with CUP and thereby offer regular treatment options. This CUPPA analysis is a learning dynamic algorithm which will be enhanced by adding new cases over time.

Most qualities of all molecular diagnostics seem to come together in WGS, however costs of WGS are still higher albeit decreasing over time. Another downside of WGS is the necessity of fresh frozen tumor tissue, which is not always available.

1.4 In summary

For patients with CUP, the above-mentioned qualities of WGS seem to fill the great clinical need in this group, namely: i) timely manner of getting a full comprehensive genomic analyses; ii) identifying a potential site of origin by comparing the genomic landscape to known cancer types; and iii) to maximize the likelihood of identifying the most of potentially actionable genomic targets and thereby identifying potential therapeutic options. Given this potential the Dutch Healthcare Authority already approved WGS under specific circumstances for patients with CUP. However, in case no fresh frozen tissue is available the use of broad NGS panels (>500 genes) in combination with the Archer Dx. analysis could also improve care of patients with CUP by providing potentially treatment options or identifying a specific mutation completing the diagnostic puzzle.

Therefore, this protocol aims to ensure standardization of the diagnostic workup, nation-wide structured access to new diagnostic broad molecular testing entities and prospectively collecting data on this patient group. Thereby collecting data on the added value of new diagnostic techniques compared to current standard practice and ultimately optimize patient care.

2 OBJECTIVES

2.1 Primary objectives

- To standardize the diagnostic workup of patients with carcinoma of unknown primary (CUP) by creating a well-defined diagnostic structure including WGS and broad NGS panels when appropriate.
- To collect prospective clinicopathological, and when applicable genomic data on patients initially diagnosed with CUP.

2.2 Secondary objectives

- To shorten the diagnostic process of patients with CUP to 4-6 weeks by using a predefined time-frame and structure compared to the recent IKNL analysis[1].
- To compare the incidence of cCUP during the study with historical control (2016 – 2019)
- To determine the number of patients with an identified primary tumor by broad molecular testing (WGS or broad panel NGS techniques when applicable) compared to standard diagnostics
    - comparing a theoretical model of current molecular diagnostics (compilation of 50 most often tested genes by current NGS panels used in the Netherlands) and WGS/ broad molecular testing, taking into account the diagnostic time-frame of 4-6 weeks of this protocol
- To determine the number of patients with CUP who could potentially be treated with (targeted) therapy based on the outcomes of broad molecular testing such as WGS
- To measure the Quality of Life by means of questionnaires during the diagnostic process
- To expand the genomic knowledge of CUP and improve the CUPPA algorithm by expanding the cases
- To collect liquid biopsies for potential non-invasive biomarker development

3 DESIGN

This protocol describes two parts: a) the diagnostic process including access to WGS/ broad NGS panels; b) a prospective cohort data registry process. This protocol aims to include patients with CUP in the Netherlands and will run in a time frame of 36 months.

A standardized minimal diagnostic work-up has been defined in this protocol for patients with a malignancy of epithelial or undifferentiated lineage in whom no primary tumor could be identified after the protocol-defined diagnostic workup. These patients will be offered a new diagnostic test in the form of WGS. WGS can only be performed after digital (on paper only) review of the indication by a CUP expert panel of an expertise center with a specific molecular tumor board. Following approval from the expert panel, centers with the ability to store fresh frozen samples will send the sample for WGS themselves; centers who do not have these requirements, can refer patients to another center with these requirements for an extra biopsy for WGS. In case WGS is not possible or has failed, patients are offered a broad NGS gene test on paraffin embedded material if available (e.g. TSO 500 and Archer DX.).

Simultaneously with performing WGS, referral of the patient to the above-mentioned expertise center will take place to the medical oncologist and revision of biopsies by the pathologist of the CUP expert panel. Results from revision of patient data and WGS will be discussed with the molecular tumor board and CUP expert panel of the expertise center. Outcome of this discussion will be summarized, if possible and in accordance with patient's whish, in a video consultation with the patient in the presence of his/her treating medical oncologist by the medical oncologist of the CUP expert panel; a (video) consultation with just the patient is also possible. An advisory document including interpretation of WGS will be provided to the treating medical oncologist.

As mentioned above, in case WGS has failed a broad NGS gene test will be performed on FFPE tissue after which the same route of discussion and consultation will be followed as described above with WGS.

Patients will be asked for informed consent to collect clinicopathological and when applicable genomic data as soon as suspected to have CUP on at least a (PET)CT-scan (further requirements see below). A blood sample for potential future biomarker analyses will be stored. Additionally, QoL will be measured by means of standardized questionnaires.

This protocol does not describe treatment suggestions. The CUP expert panel might suggest treatment options based on results of broad molecular testing and possible available trials at that time if applicable. Treatment decisions will be made by the treating medical oncologist in consultation with the patient. Consequently, no experimental intervention will be made in this protocol and the workflow will closely parallel the current diagnostic process. Information on treatment decisions will be collected for those patients that consented to data registration.

All research analyses not mentioned in the objectives will be considered new research questions for which separate approval is required, as outlined in chapter 11.

This is a Dutch nation-wide protocol and will be executed by Dutch expertise centers with pre-defined specific molecular tumor board and other participating hospitals.

4 POPULATION

4.1 Population (base)

Patients with suspected malignancy on CT-scan and/or PET-CT in whom from the start or during the diagnostic workup no clear primary tumor could be detected and thus CUP is part of the differential diagnosis.

Patients can be included in this protocol if a subject meets all of the following criteria:

4.2 Inclusion criteria

1) ECOG performance status 0-2
2) Patients must have acceptable organ function as defined below, according to local standard lab practice:
    a) Absolute neutrophil count ≥ 1.5 x 109/l
    b) Hemoglobin ≥5.0 mmol/l
    c) Platelets ≥75 x 109/l
    d) Total bilirubin ≤3 x ULN, or > 3 in case of obstructive cholestatic hyperbillirubinemia with expected decrease by intervention
    e) AST (SGOT) and ALT (SGPT) ≤5 x institutional ULN (or ≤10 x ULN in patients with known hepatic metastases)
    f) Serum creatinine clearance calculated or measured ≥ 30 mL/min/1.73 m2
3) Presence of malignant lesion(s) of which a histological biopsy can be safely obtained.
4) Patients age > 18 years, willing and able to comply with the protocol as judged by the investigator with a signed informed consent.

4.3 Exclusion criteria

1. Prior successful WGS analysis performed on biopsy of current metastastic disease
2. Patients with any clinically significant medical condition which, in the opinion of the treating physician, would make giving systemic therapy unsafe/undesirable.

5 INVESTIGATION

In case patients already have had multiple diagnostic examinations because of initially suspicion of specific primary tumor, but on workup are suspected of CUP, many diagnostic procedures will already have taken place. These should not be repeated and only missing, relevant diagnostics should be performed. No additional biopsies should be taken before consultation with the CUP expert panel in expertise center unless necessary for regular pathology assessment.

5.1 Diagnostic time frame

In principle, the diagnostic process must be aimed to be finished within 4 weeks, but no later than 6 weeks, of first CT-scan or PET-CT (whichever came first), including consultation with the medical oncologist of the CUP expert panel of the expertise center concerning the interpretation of additional molecular testing (WGS or broad NGS) results when applicable.

5.2 Diagnostic workup – Blood collection and laboratory screening

Baseline laboratory measurements have been performed as part of regular diagnostics and used for the eligibility screen. Previously, serum assessment of potential tumor markers were performed upon indication. In this protocol, data on the following biomarkers will be standardly collected according to local laboratory practice: LDH, aFP, b-HCG, CEA, CA19.9, PSA (men), CA15.3 (women), CA125 (women).

Two samples of 10ml whole blood sample will be taken to be stored for potential WGS analysis and biomarker analyses, if patient consented see study procedures.

5.3 Diagnostic workup – radiological examination, endoscopy and other examinations

A diagnostic CT-scan of the thorax and abdomen or PET-CT of the body should be present given the patient population of this study. Whether additional radiological examination (respectively PET-CT or diagnostic CT-scan, or specific MRI) is of added value, should be decided by the treating physician. Endoscopies or other diagnostic procedures (i.e. gynecological examinations etc) should not routinely be performed and should only take place when symptom-, laboratory-, radiological or pathological examination shows reasonable probability of finding the primary tumor with this procedure.

Regardless of number of additional procedures, the timeframe of total diagnostic workup within 4-6 weeks should be attempted to be met.

5.4 Diagnostic workup - tissue collection and pathology assessment

An assessment will be made on the availability of a safely accessible metastatic lesion. Of the most safely accessible metastatic lesion a histological biopsy will be obtained according to local institutional guidelines. In case of a biopsy, multiple tumor biopsies are obtained from all patients as part of the routine standard of care diagnostic procedure. In case of patients with CUP in the differential diagnosis from start of the diagnostic process, one of the multiple histological biopsies obtained as routine standard of care diagnostic procedure will be fresh frozen and stored (see 5.6.3) in the participating centers with adequate storage and mailing logistics for fresh frozen tissue. When pathological assessment cannot be performed on the regular diagnostic biopsy samples, the stored fresh frozen sample will be used for standard diagnostics.

Pathological assessment will be according to standard local pathology practice. Immunohistochemical stains will be performed in a pre-specified tiered fashion summarized in Appendix I. Not being able to perform all necessary immunohistochemical analyses defined in Appendix I should result in timely referral to an affiliated CUP expertise center with a specific molecular tumor board, because of the diagnostic time-frame (including potential WGS) of 4-6 weeks.

In case of patients whom already have had a biopsy because of suspected specific primary tumor but on pathological examination no primary tumor was identified (patients suspected of CUP during the diagnostic process), no additional biopsy should be taken. Pathological assessment should be checked for completeness in accordance with this protocol and extended if necessary.

5.5 Results of diagnostic work-up

After full diagnostic work-up patients are classified in two groups, see figure 1:

A. Patients in whom after above-mentioned diagnostic work-up either of the following is identified:

    a. primary tumor;

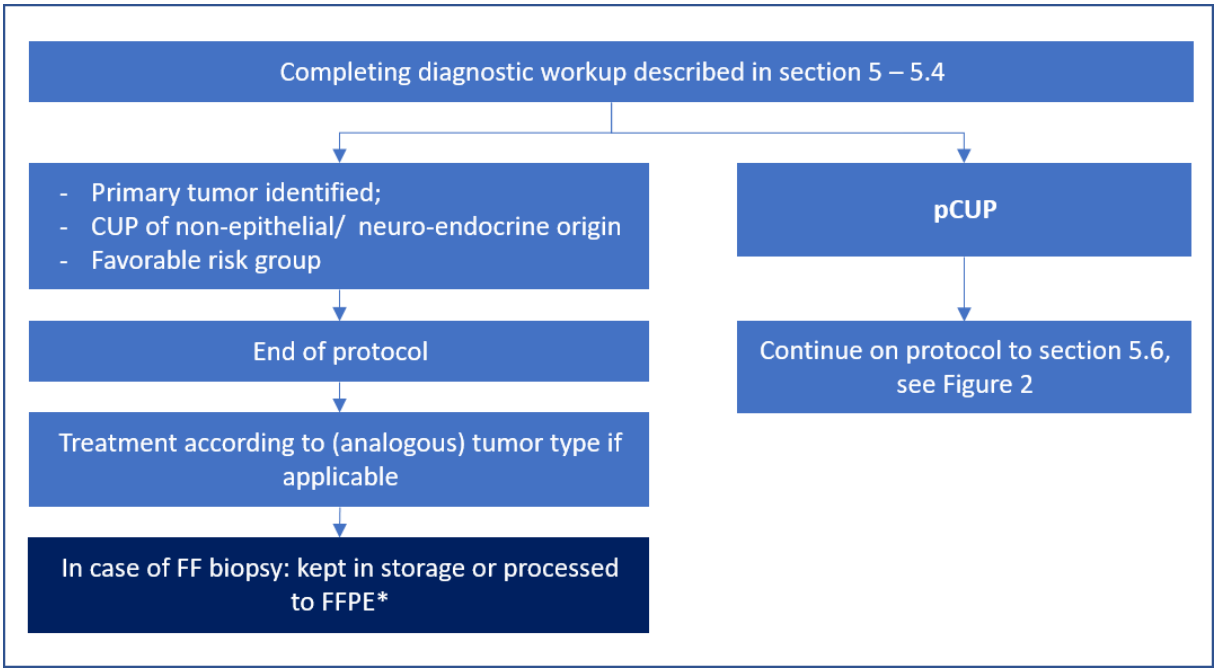
    b. tumor of non-epithelial, but clearly defined origin (lymphoma, sarcoma, melanoma);

    c. CUP of the favorable risk group (see Table 1, p7)

    These patients go of protocol from this moment, the paragraphs below are not applicable for them; potential further work-up and/or treatment can be started accordingly.

B. Patients in whom after above-mentioned diagnostic work-up does not result in a clear classification in one of the three categories (a-c) above and a tumor of epithelial or undifferentiated lineage of unknown origin has been identified and hence can be labeled as having a **provisional CUP (pCUP)** . These patients will continue on protocol following the steps of the rest of the protocol (see section 5.6 and onwards; and Figure 2).

Figure 1. Results of diagnostic work-up (section 5 – 5.5)



Abbreviations: CUP: carcinoma of unknown primary; SOC, standard of care; FF: fresh frozen; FFPE: formalin-fixed paraffin embedded; pCUP: provisional CUP. * According to preference participating center.

5.6 Molecular testing related procedures

5.6.1 Indication WGS by CUP expert panel of an expertise center with a specific molecular tumor board (PATH – MTB)

If no primary tumor has been identified according to above-mentioned criteria (see section 5.5 and Figure 1) after regular diagnostic work up including radiological examination and pathological assessment (see section 5.4), the patients' diagnosis is changed to "provisional CUP" (pCUP). Subsequently, patients' case is formally presented for the indication of WGS to a specific CUP expert panel consisting of a dedicated medical oncologist and dedicated pathologist potentially as part of the MTB, within expertise centers with specific MTB's. These centers with specific MTB's have been predefined by the health insurance companies to be the MTB's registered as "PATH-MTB's" (https://www.netwerk-path.nl/index.php).

The presentation of a patients' case will be submitted digitally (by email or website, as determined by expert center) and contains concise clinical data (minimal patient data, see Appendix II), the full report of all radiological examinations and endoscopy if applicable and the full pathology report. The CUP expert panel will review the case and decide whether in accordance with the referral center the case should be considered a pCUP and hence WGS should be performed or the case should be interpreted differently in which case fitting advice will be given. This decision will be reported back to the treating physician in principle within 2 working days.

5.6.2 Interpretation CUP expert panel: pCUP

If the CUP expert panel rules the case to be a pCUP and hence the indication for WGS has been given, the following steps need to be organized:

a. Proceed with WGS (see below: 5.6.3)

b. Full referral to dedicated CUP medical oncologist of affiliated expertise center of which the CUP expert panel was consulted, including referral letter and imaging (full report on imaging already present) for a one-time (video)consultation summarizing all results (clinicopathological data and WGS interpretation). In case tissue collection for WGS needs to be performed in the CUP expert center, an extra consultation before biopsy will take place.

c. Send pathology slides and blocks for revision to dedicated CUP pathologist of affiliated expertise center of which the CUP expert panel was consulted

Both referring center as well as expertise center will take care to have all information complete before the consultation with the patient in the expertise center for the feedback on WGS (see below: 5.6.5). Given the average lead time of WGS of 10 working days, this should be feasible.

If no logistics for fresh frozen tissue are present in the referring center, patient should be referred on short notice and a consultation with the CUP medical oncologist should take place and additional biopsy should be arranged all within the set time-frame of consultation of WGS results within total diagnostic process of 4-6 weeks.

5.6.3 Tissue collection for WGS

In case of patients with CUP in the differential diagnosis from start of the diagnostic process, one of the multiple histological biopsies obtained as routine standard of care diagnostic procedure will be fresh frozen and stored to be potentially used for WGS (see Table 2 and Figure 2) in the participating centers with adequate logistics. In this case there will be no extra burden for the patient since it is performed in the standard of care diagnostic procedure. When pathological assessment cannot be performed on the regular diagnostic biopsy samples, the stored fresh frozen sample will have been used for standard diagnostics. In this case and in the case of patients suspected of CUP during the diagnostic process an extra biopsy is taken if WGS is deemed applicable by the expert panel (see Table 2 and Figure 2) as part of current standard of care.

In the participating centers with no storage capacity, regular tumor biopsies will be taken and pathologically assessed. If WGS is deemed applicable, an additional histological biopsy is taken to be send fresh-frozen for WGS; this extra biopsy can be taken in another participating center if facilities for temporarily storage of fresh frozen samples or the logistics for transportation for WGS is unavailable (see Table 2 and Figure 2).

In case WGS results are not interpretable (due to for example, but not limited to: low tumor cell percentage or failed analyses) one extra biopsy procedure is allowed if considered to have a high success rate by repeating procedure.

In all above-mentioned cases in which an extra biopsy is needed, this will only be performed when a safely accessible metastatic lesion is present.

Table 2. Different routes to WGS in case of patients with suspected CUP at start or during diagnostic process: with and without FF tissue logistics

	CUP in differential diagnosis at start diagnostic process	At start likely primary tumor, during diagnostic work up no primary tumor found: suspected CUP
Center with storage capacity and logistics for sending FF tissue	Extra biopsy taken and stored at first pathological assessment	Extra biopsy taken and stored at first pathological assessment (by coincidence for example for study of said likely primary tumor)
	Extra biopsy allowed if FF tissue has been used for standard diagnostic work-up after indication by CUP team expertise center *	Extra biopsy specifically for WGS after indication by CUP team expertise center*
	Extra biopsy allowed if WGS not interpretable*	Extra biopsy allowed if WGS not interpretable*
Center with no storage capacity or logistics for sending FF tissue	Referral to center with facilities for WGS or WGS logistics	Referral to center with facilities for WGS or WGS logistics

CUP: carcinoma of unknown primary; FF: fresh frozen; WGS: whole genome sequencing; * perform only when a safely accessible metastatic lesion is present.

5.6.4 Blood collection

A 10ml whole blood sample will be obtained preferably in combination with routine care. The whole blood sample is needed to discriminate somatic mutations from the patient's germline DNA background variations for the WGS.

5.6.5 WGS

The tumor cell percentage will be estimated by the pathologist of the center where the biopsy has taken place on a frozen section of the fresh or fresh frozen biopsy. Alternatively, the frozen biopsy can also be send in to the expertise center for tissue selection for DNA and RNA isolation. A minimum of 20% tumor cell percentage is mandatory for WGS and will be used together with the whole blood sample for DNA sequencing. In a later stage the DNA WGS analysis may be complemented by RNA sequencing to investigate the additional value of combining DNA and RNA analysis.

In case WGS is performed by a third party and the center sending the fresh frozen biopsy is not the same center as the center of the expertise center of CUP team, the name of the expertise center is specified in the application of the WGS.

In case the DNA extraction and sequencing will be performed by a third, nonacademic, party, leftover biomaterial will be send to center of the CUP expert panel and will be stored in the local biobank (for procedure of investigation with this material see Chapter 11).

In principle, the pipeline developed by Hartwig Medical Foundation for analysis of (A)CUP tumors, "CUPPA analysis", will be run in order to potentially identify the primary tumor site (article in preparation). Result from this analysis will be reported back in addition to found DNA aberrations.

5.6.6 In case of failure of WGS – NGS panels

If WGS cannot be performed due to i) failure based on inadequate material even after potential repeat biopsy; ii) new biopsy not thought to be successful or safe; iii) or any other reason (for example: refusal of patient): DNA isolated for WGS or DNA isolated from FFPE tissue can be used for broad NGS panels and if necessary supplemented with other molecular assays (i.e. Archer, etc) as deemed of additional value by the pathologist of the CUP expert panel. Examples of broad gene panels to be used, are:

- TSO500 (TruSight Oncology 500 Assay | For pan-cancer biomarkers in DNA and RNA (illumina.com))
- OCA+ in combination with Archer fusion panel CTL (Oncomine Comprehensive Assay Plus | Thermo Fisher Scientific – NL Archer FusionPlex Comprehensive Thyroid & Lung (CTL) | Diagnóstica Longwood (dlongwood.com))

- Foundation One (FoundationOne CDx | Foundation Medicine).

5.6.7 Interpretation and feedback of results by affiliated expertise center

Results of the revision of the clinical data by the dedicated medical oncologist of the CUP expert panel, who will consult specific (nuclear) radiologists when considered of added value, result of the revision of pathology by the dedicated pathologist of the CUP expert panel and result of the WGS will be discussed in a multidisciplinary consultation of the expertise center. In this consultation both the medical oncologist and pathologist of the CUP expert panel having reviewed the case and the MTB will be present. During this consultation 5 items will be standardly recorded:

1) Revision of pathology, tumor type upon revision; if not one specific type was found, this item will be listed as not determined.

2) Revision of pathology, primary tumor upon revision; if not one specific primary tumor could be recorded, this item will be listed as not determined.

3) Consultation of CUP expert panel: was the DI-CUP protocol of any clinical value for this patient: yes or no.

4) Consultation of CUP expert panel: if applicable, was WGS of any additional value in this process: yes or no.

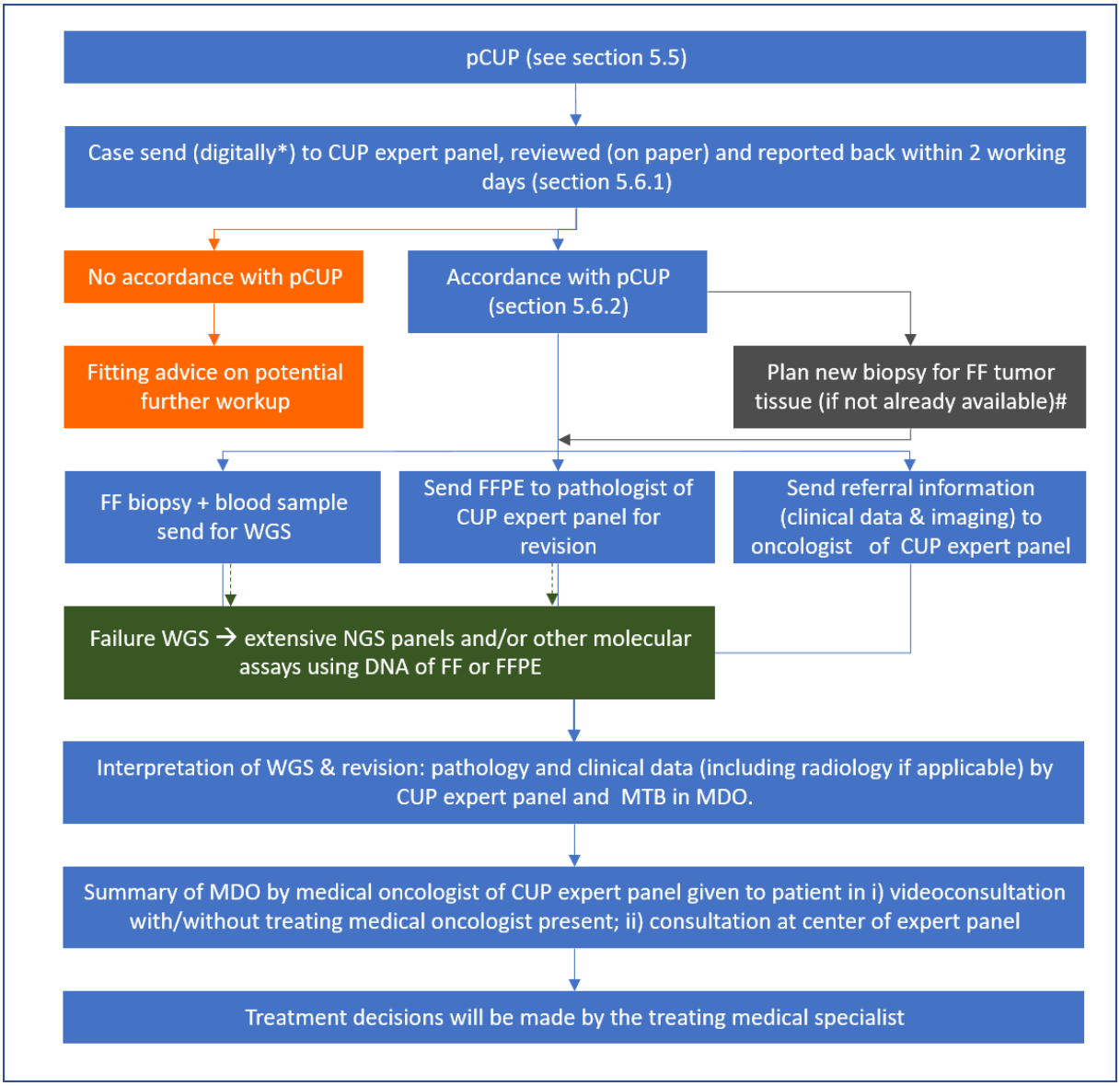
5) Consultation of CUP expert panel: if applicable, was patient diagnosed with confirmed CUP (cCUP): yes or no.

Outcomes of this discussion relevant to the patient will be summarized by the medical oncologist of the CUP expert panel in a single consultation, depending on wish of treating medical oncologist and coordination with the patient, in either: i) a video consultation with the patient in the presence of his/her treating medical oncologist; ii) a video consultation with only the patient; or iii) a consultation with the patient at the outpatient clinic of the medical oncologist of the CUP expert panel. The consultation of the medical oncologist of the CUP expert panel with the patient will take place within the diagnostic time frame of 4-6 weeks.

An advisory document with the summary of the multidisciplinary consultation and of the consultation with the patient will be provided by the medical oncologist of the CUP expert panel to the treating, referring medical oncologist.

In case WGS or broad NGS panel (when applicable) analysis has not identified a primary tumor, the patient will be diagnosed with a “confirmed CUP” (cCUP).

Figure 2. Schematic flowchart study procedures in case of pCUP



*Abbreviations: FF: fresh frozen; FFPE: formalin-fixed paraffin embedded; pCUP: provisional CUP; WGS: whole genome sequencing; MDO: multidisciplinary consultation (overleg). * Digitally: via dedicated email, website, etc: to be determined by each MTB individually; # In original participating center or referral to another participating center with facilities for sending out biopsies for WGS.*

6 METHOD AND DATA REGISTRY

6.1 Study Endpoint

6.1.1 Primary endpoint

Number of patients in whom a primary tumor was identified after following the protocol initially diagnosed with pCUP.

6.1.2 Secondary endpoint

- A diagnostic process within 6 weeks in 75% of the patients with suspected CUP; and to compare the average time of diagnostic process to historical control, available from NKR database (2016-2019)
- Number of patients with pCUP or cCUP who could potentially be treated with (targeted) therapy based on the outcomes of broad molecular testing including WGS
- Number of patients with pCUP or cCUP who were treated with (targeted) therapy based on the outcomes of WGS
- Number of filled in questionnaires sufficient for performing PROM research
- Database of all (coded) data obtained using this protocol
- Database of liquid biopsies for potential biomarker analyses

6.2 Data registry – Registration of patients

A Patient Information Folder (PIF) and Informed Consent will be used to inform patients and ask their consent for analyzing and collecting data on their clinicopathological results, genomic results and separately on i) collecting an additional blood sample; ii) filling out QoL questionnaires; and iii) storing their data (clinical and genomics) in a database for future research purposes, which is made accessible for research purposes using an access-controlled procedure (see Chapter 11).

Informed consent will be asked as soon as CUP is suspected (at least after (PET)CT-scan, see above Chapter 5) but can also be asked later on in the diagnostic process. After the patient has signed informed consent:

- The treating medical oncologist or person delegated by the investigator registers the patient by completing the Registration Form (see Appendix III) and emailing this to the Netherlands Comprehensive Cancer Organisation (IKNL) trial office.
- IKNL will allocate an unique patient number to this particular patient, which will serve as the unique DI-CUP study number. This number will be the non-convertible link between the clinicopathological database (collected by IKNL) including informed consent details and the databases of molecular assays including WGS.

- This unique DI-CUP study number of the patient will be communicated back by the IKNL to the registering physician or person dedicated by the investigator, who will record this in the local electronic health record system.

- If consent was given the extra Cellsave blood sample will be send to NKI-biobank with a label with this unique DI-CUP study number.

- In case WGS will be performed:

    - Patient is part of a center **with** storage capacity and logistics for sending FF tissue: the unique DI-CUP study number of the patient will be added to the pathology application for WGS as well as a note that the patient participates in this study. If WGS is performed by a third party, the pathologist/KMBP will 1) add the unique DI-CUP study number as well as a note that the patient participates in this study and 2) include the name of the center of the CUP expert panel where WGS indication was set.

    - Patient is part of a center **without** storage capacity and logistics for sending FF tissue: this unique CI-DUP study number and a statement that patient participates in the DI-CUP study will be added to the referral letter to the center with facilities for WGS or WGS logistics. Preferably, this unique DI-CUP study number will also be communicated between sending and receiving pathology departments (in case revision of FFPE tissue will take place).

- All raw and analyzed genomic data will be stored under this unique DI-CUP study number by the party performing WGS. This data will be fully shared in a structured manner between the party responsible for WGS and this study team.

- In case of failure of WGS and additional molecular assays were performed (i.e. broad NGS panels), this data will be collected in a separate database by the performing party under this unique DI-CUP study number. All raw and analyzed molecular data will be fully shared in a structured manner between the party responsible for these molecular assays and this study team.

6.3 Data collection

6.3.1 Clinicopathological and genomic data

Clinical data will be collected through the Netherlands Cancer Registry (NKR) by IKNL and pathological data will be added by linkage with the national pathology digital archive, PALGA (“Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief"). Results of WGS or additional molecular assays will be collected by the party performing the analyses in a separate database using the unique DI-CUP study number . Two separate databases will be generated by the unique study number: i) clinicopathological data; ii) genomic data. Hence, databases can be linked in a pseudonymized fashion ensuring patient confidentiality and DNA sequencing data. All raw and analyzed genomic data will be fully shared in a structured manner between the party responsible for WGS and this study team. This is done in full compliance with the WGBO, WMO and GDPR and the local implementation thereof at the center sending the sample for WGS.

Decoding the unique study number will only be performed to find patients who have opted for the possibility to be informed in case advanced insights on the interpretation of genomic data could have consequences for the patient or her/his family.

No identifiable data will be transmitted in any case other than in communication with the treating physicians and only in the patients interest thereby protecting patients' privacy at all times. In case of biological samples of patients, these will be stored with this unique DI-CUP study number. All data will be gathered in a secure, not to individual patients convertible, database.

6.3.2 Blood collection

The data on additional biomarkers (aFP, b-HCG, CEA, CA19.9, PSA (men), CA15.3 (women), CA125 (women)) will be collected as part of routine clinical care. In case patient consented, additionally 3x10ml CellSave blood samples will be collected in combination with routine care. The CellSave blood samples will be send to NKI-biobank for storage for potential future biomarker analysis.

6.3.3 Questionnaires

In case patients consented, standardized questionnaires will be collected to gather information about health-related quality of life. Validated and nationally and internationally accepted questionnaires will be used to ensure that comparison with other or future studies is possible. For measurement of Patient Reported Outcome Measures (PROMs) digital patient tracking system PROFILES (Patient Reported Outcomes Following Initial Long term treatment and Survivor Ship) will be used. Collaboration with the PROFILES group has several advantages including the opportunity to compare the PROMs of patients with CUP to those of large population based samples of cancer patients.

Patients will be asked to fill out questionnaires at 3 time-points in the protocol: 1) after signing ICF but before histological biopsy;2) after receiving the results of the regular pathological assessment; and 3) if applicable a after consultation about the WGS results. Those patients diagnosed as having a confirmed CUP will be invited to fill out a questionnaire 3-monthly for the following year. The questionnaires will be sent to the patients digitally. The process of completing the questionnaires will be coded and patients will be reminded to fill in the questionnaires by email or by letter.

6.4 Data reporting

The report of WGS results will be sent to both the center submitting the tumor material and to the corresponding center of expertise of the CUP team. This report will be processed according to local guidelines and made accessible in the electronic health record system of that center, but will be preferably added to PALGA by the center of the CUP expert panel.

In the patient information form unforeseen potential research purposes outside this protocol according to separate approval procedure outlined in chapter 11, will be explained.

6.5 Storage of samples

If material has been send out to a third, nonacademic, party all leftover biomaterial (DNA, RNA, tissue and/or blood) leftover biomaterial will be send to center that has send the material or to the corresponding CUP expert panel of that center if in accordance with local agreement. Centers can store leftover biomaterial in the local biobank if so preferred by that center (for procedure of investigation with this material see Chapter 11).

6.6 Storage of questionnaires

Questionnaires will be send digitally and will be stored automatically at the protected database of PROFIEL-systeem (www.profileregistry.nl). All questionnaires are coded so that they cannot be linked to the patient. This data can be shared with other parties for future research purposes.

6.7 Follow up

Follow up will be recorded by the NKR according to their regular procedures wherein treatment regimen, date of progression, potential switch of treatment and death will be noted. General procedure of response evaluation after 8-12 weeks depending on chosen regimen should be followed according to standard local practice.

7 STATISTICAL CONSIDERATIONS

This protocol is developed to prospectively collect as complete as possible clinicopathological information on patients with CUP and to monitor the outcome of the introduction of a new diagnostic entity (WGS and broad NGS panels). WGS is already approved by the Dutch Healthcare Authority under specific circumstances. Therefore, no sample size calculation is needed.

If during this protocol decisions regarding reimbursement of certain diagnostic procedures for patients with CUP need to be made, specific sample size calculation can potentially be added with data collected in the earlier phase of the protocol.

8 SAFETY REPORTING

Tumor material from a metastatic lesion will be obtained from patients as a standard routine of care diagnostic procedure. Whenever possible multiple biopsies will be taken within the same procedure of which one will be stored fresh frozen to ensure the least burden for the patient. Since WGS has been approved under specific circumstances by the Dutch Healthcare Authority for reimbursement, an extra biopsy for this diagnostic test should be considered part of the standard of care.

The risk of the blood draws for biomarker analysis is negligible.

9 ETHICAL CONSIDERATIONS

9.1 Regulation statement

The data registry part of this protocol is to be conducted according to the international standards of Good Clinical Practice, in full conformance with the "Declaration of Helsinki" (latest amendment), the Dutch laws and regulations and with the W.M.O. ("Wet Medisch-wetenschappelijk Onderzoek met mensen") in particular. Institutional research policies and procedures will also be followed. Review of the protocol and consent form by the accredited IRB will be obtained and documentation of IRB approval and the approved consent form will be provided to each site participating in the data registry part of this protocol. Each participating site will obtain local approval from their respective Board of Directors prior to enrollment of patients.

9.2 Recruitment and consent

All patients will be informed in the out-patient clinic of participating centers. Before patients agree to participation in the data registry part of this protocol, they will be provided with written information (Patient Information Sheet). It is the responsibility of the investigator, or a person designated by the investigator, to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Once the essential information has been provided to the patient and the investigator is satisfied with the candidate's understanding of the implication of participating in this study, the subject will be asked to give consent by signing and dating the informed consent form in the presence of the (sub)investigator. The informed consent must be obtained before initiation of any study (registry) specific procedures. Patients have the right to withdraw from the study at any time, without giving an explanation and without prejudice to their subsequent care. The Informed Consent Form as well as the Patient Information Sheet must be prospectively approved by the Independent Ethics Committee/Institutional Review Board (IEC/IRB) and must be in compliance with Good Clinical Practice, local regulatory requirements and legal requirements. The investigator will retain the original of each subject's signed Informed Consent Form.

Separate consent will be obtained for optional procedures which are: i) collecting an additional blood sample; ii) filling out QoL questionnaires; iii) future use of stored tumor tissue for future research at the local pathology departments; iv) inclusion of genomic results in a database for future research purposes, which is made accessible for research purposes using an access-controlled procedure. At the Informed Consent Form the patient can mark her/his choice whether to consent to these for options or not. Finally, patients will be asked whether they want to be informed on coincidental findings discovered during the analysis of the whole genome gene expression data that could have implications for the patient or her relatives. At the informed consent form the patient can mark her/his choice whether to consent to these separate issues (see appendix X).

9.3 In case of no consent

If patients do not want to participate in this study, no consent has been given, the same diagnostic workup and structure for WGS if applicable, can be followed. Referral information should clearly state that patient is not enrolled and hence no patient-number is available.

9.4 Benefits and risk assessment

Regarding the risk assessment and safety see above (part 8). Regarding risk assessment and the potential findings of actionable hereditary (germline) aberrations: patients will be explained of the risk of such findings in the patient information sheet and will be given the choice to be informed of said findings or not. This can lead to information about hereditary predisposition to cancer, which may also have consequences for patient's family members. In such case patients will be referred to the clinical geneticist for further counseling. Non-actionable germline variations will be automatically subtracted from the somatic mutations in the bioinformatics pipeline and will not be seen by the investigators and will not be reported.

Results from WGS can potentially lead to the identification of the primary tumor and thereby provide (regular) treatment options. Furthermore, WGS could potentially identify actionable DNA variants and thereby provide anticancer therapy options that target these specific alterations outside or in the context of a clinical trial. Lastly, the identification of a hereditary germline mutation and thereby initiating active surveillance could be seen as beneficial for the health of the patient's family by preventing related disease to occur.

10 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Monitoring and quality assurance

We qualify the risk of this study as 'negligible' (small chance of moderate damage). According to this negligible risk a 'minimally intensive monitoring' is advised (according to guideline NFU (Dutch Federation of University Medical Centers) and adapted guideline by the UMC Utrecht), which will be performed by a clinical research associate (CRA; monitor).

10.2 Handling and storage of data and documents

All raw and analyzed genomic data will be fully shared in a structured manner between the party responsible for WGS and this study team. This is done in full compliance with the WGBO, WMO and GDPR and the local implementation thereof at the center sending the sample for WGS. All data will be coded in a pseudo-anonymized manner by using a unique patient identification code. External researchers will not be able to trace back to the individual patient. The patient will be informed of this via the patient information form.

10.3 Amendments

This protocol aims to collect data on patients with CUP by offering a predefined diagnostic structure. However, since this predefined diagnostic structure has been devised to be able to implementable in every Dutch hospital and not based on previous studies, this protocol should not be considered fixed but to be dynamic. Hence, parts of the protocol might be subject to change if during the study results show that one of the procedures is for example not of added value or performed at suboptimal place in work-up. In this case or for other reasons an amendment could be made.

A 'substantial amendment' is defined as an amendment to the terms of the IRB application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the study;
- the scientific value of the study;
- the conduct or management of the study; or
- the quality or safety of any intervention used in the study.

All substantial amendments will be notified to the IRB and to the competent authority. Non-substantial amendments will not be notified to the accredited IRB and the competent authority, but will be recorded and filed by the principal investigator.

10.4 Annual progress report

If requested, the investigator will submit a summary of the progress of the trial to the accredited IRB once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included, other problems, and amendments.

10.5 Publication policy

The publication guidelines of the Dutch CCMO (Central Committee for Research in Humans, www.ccmo.nl) will be followed exactly. In adherence with these guidelines, results will be disclosed, and submitted to peer-reviewed scientific journals. Publications and oral presentations of any results from the study shall be in accordance with accepted scientific practice, academic standards and customs. The principal investigators and the steering committee need to give written consent before the whole or any part of the results of the study carried out under this protocol will be published or passed on to any third party.

The principle investigators decide after advice from the steering committee when study results will be published, who will be authors, and in which order. Authorship will include at least the principal investigators, the steering committee and any other person that made a significant contribution (as decided by the steering committee). General publication policy:

- Results on objectives: When results on one of the objectives of this protocol will be offered to a peer reviewed journal for publication. Collaborating centers who have enrolled ≥5% of patients included in the publication will be invited to propose one author; >10% will be invited to propose two authors. Collaborating centers who have enrolled < 5% of patients included in the publication will be mentioned in the acknowledgements. If the publication policy of the journal does not allow above-mentioned list of authors, the list of authors will be finished by mentioning “on behalf of the DI-CUP study team”. In case above-mentioned rule does not fit the publication requirements authorship will be decided on by the principal investigators and steering committee.

- Other study results: during or after the trial, new research questions (that were not contained within the study’s objectives) may arise. All participating parties and site members could apply for such a research project. A separate procedure for approval will be followed (see part 11). Once approved, the applicant will prepare the manuscripts together with a statistician, a molecular biologist and at least two other members of the protocol (principal investigators and/or steering committee) and those investigators (one per center) that contributed substantially to the project. First author will be the person who writes the paper. The other investigators will be co-authors. All other participating centres/physicians will be acknowledged.

11 USE OF MATERIAL OF PROTOCOL FOR SPECIFIC RESEARCH QUESTIONS

All data is accessible for research purposes using an access-controlled procedure in which scientific, legal (fit within informed consent context) and ethical aspects of data requests are assessed by an independent scientific and data access board.

The material collected as part of this protocol will not be used beyond the scope of this protocol. In case of a new research purpose, an appropriate application has to be submitted to at least two members of the steering committee or principal investigators. Since this project is a collaboration with IKNL, all data requests for research purposes will be judged by the steering committee of the DI-CUP study and by the NCR Board of Control (Commissie van Toezicht). The coordinating investigator of the DI-CUP study is responsible for gathering the reviews of both committee and board and will report the result to the applicant. In case of a positive decision, an agreement is made with the applicant including detailed information on which data will be exchanged as restrictions on handling these data.

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13 APPENDICES

I. Pathology assessment

The tables below depicts the basic immunohistochemical approach for analysis of a histological specimen suspected for CUP. The approach consists of a two step procedure. In the first step the broad cancer type is investigated and depending on the results of this first step a further analysis is performed.

If more than one biopsy is available, it is advised to embed the biopsies in separate blocks. Since it is foreseeable that limited material will be available in many patients, it is advised to consider tissue saving procedures, such as cutting extra unstained tissue slides. The recommended IHC stainings should be interpreted as a minimal dataset for submitting patients to the CUP expert panel. Each CUP expert center is allowed to add specific stains to this basic approach.

IHC first step

Marker	Carcinoma	Melanoma	Lymphoma	Sarcoma
CK pan	+	-	-	-
CK 8/18	+	+	-	+/-
S100	-	+		-
HMB45	-	+	-	-
Melan-A	-	+	-	-
CD20	-	-	+/-	-
CD3	-	-	+/-	-
CD138	+	-	+/-	-
Vimentin	-/+	+	+	+
SMA	-	-	-	-
Desmin	-	+	-	-

IHC second step group typing carcinoma: CK 7 and CK20

CK7 - / CK20 -	CK7 + / CK20 -	CK7 - / CK20 +	CK7 + / CK20 +
Prostate: PSA, PSAP, NKX3.1	Lung: TTF-1	Colon: CDX2	Lung muc: TTF-1, CDX2
RCC clearcell: PAX8, CD10	Breast: GATA3, ER, PR	Skin merkelcel ca.: CD56, synaptohysine, chromogranin	UCC: p40, GATA3
PCC: p40, p63	UCC: p40, GATA3	Bladder: CDX2 +/-	Bladder adenoca
	Subset UCC, RCC (papillary)		
Adrenal ca: MelanA, Calretinin, Inhibin A	Serous Ovarian ca: PAX8, WT1		.

CK7 - / CK20 -	CK7 + / CK20 -	CK7 - / CK20 +	CK7 + / CK20 +
HCC: HepPar1	Endometrium: PAX8, ER, PR, vimentin		Muc ovarian ca
stomach	Oesophagus, stomach, pancreas: CDX2	Stomach , appendix, small bowel, colorectal	Stomach, pancreas
	Cholangioca: CA19-9		Cholangioca: CA19-9
Non-seminoma germcell tumor: CD117, CD30, OCT4, PLAP	Germcell ca: Oct 4		
Mesothelioma: calretinin, WT1	Mesohelioma: calretinin, WT1, D2-40		
SCLC: TTF-1, CD56, synaptopysine, chromogranine	NE tumor: CD56, synaptopysine, chromogranine		
	Thyroid pap. ca.: TTF-1, PAX8, Thyroglobin Thyroid med. ca.: TTF-1, calcitonine, CEA		
	Thymus		
	Salivary duct ca.: GATA3, AR		

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II. Digital referral CUP expert center

Email send to referral CUP expert center consultation

Type of data	Containing following information
Clinical data	Sex
	Age
	Performance status
	Summarized medical history: - relevant cardiac or pulmonary condition - relevant auto-immune conditions for which oral immunosuppressive drugs are used other than prednisolone ≤10mg - previous malignancy(ies): (including year of diagnosis)
Radiological/ endoscopic data	Full report of either CT-thorax/abdomen and/or whole body PET-CT.
	In case additional scans performed (MRI, other), full report
	In case of endoscopy: date performed and conclusion
Pathology assessment	Full pathological report, including immunohistochemistry of all obtained biopsies.

III. Registration form

Registratieformulier DI protocol CUP

[Uitgebreide titel studie]

Naam aanmelder:……

Ziekenhuis:……

Bereikbaar op telefoonnummer:……..

Geboortemaand en jaar patient: __ __ / __ __ __ __

Datum Informed consent: __ __ / __ __ / __ __ __ __

Doorhalen wat niet van toepassing is:

Geslacht	Man	Vrouw
Leeftijd ouder dan 18 jaar	JA	NEE (niet eligible)
Verdenking CUP	JA	NEE (niet eligible)
Getekend informed consent	JA	NEE (niet eligible)

Handtekening:…….. Datum: __ __ / __ __ / __ __ __ __

► Vul dit formulier volledig in en mail dit naar IKNL trialbureau: trialbureau@iknl.nl

► U ontvangt per mail een bevestiging van de aanmelding.

► Bewaar dit originele registratieformulier samen met het originele informed consent formulier in de Investigator Site File.