# Site-specific therapy guided by a 90-gene expression assay versus empirical chemotherapy in patients with cancer of unknown primary (Fudan CUP-001): a randomised controlled trial

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## Summary

**Background** Empirical chemotherapy remains the standard of care in patients with unfavourable cancer of unknown primary (CUP). Gene-expression profiling assays have been developed to identify the tissue of origin in patients with CUP; however, their clinical benefit has not yet been demonstrated. We aimed to evaluate the efficacy and safety of site-specific therapy directed by a 90-gene expression assay compared with empirical chemotherapy in patients with CUP.

**Methods** This randomised controlled trial was conducted at Fudan University Shanghai Cancer Center (Shanghai, China). We enrolled patients aged 18–75 years, with previously untreated CUP (histologically confirmed metastatic adenocarcinoma, squamous cell carcinoma, poorly differentiated carcinoma, or poorly differentiated neoplasms) and an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, who were not amenable to local radical treatment. Patients were randomly assigned (1:1) by the Pocock and Simon minimisation method to receive either site-specific therapy or empirical chemotherapy (taxane [175 mg/m² by intravenous infusion on day 1] plus platinum [cisplatin 75 mg/m² or carboplatin area under the curve 5 by intravenous infusion on day 1], or gemcitabine [1000 mg/m² by intravenous infusion on days 1 and 8] plus platinum [same as above]). The minimisation factors were ECOG performance status and the extent of the disease. Clinicians and patients were not masked to interventions. The tumour origin in the site-specific therapy group was predicted by the 90-gene expression assay and treatments were administered accordingly. The primary endpoint was progression-free survival in the intention-to-treat population. The trial has been completed and the analysis is final. This study is registered with ClinicalTrials.gov (NCT03278600).

**Findings** Between Sept 18, 2017, and March 18, 2021, 182 patients (105 [58%] male, 77 [42%] female) were randomly assigned to receive site-specific therapy (n=91) or empirical chemotherapy (n=91). The five most commonly predicted tissues of origin in the site-specific therapy group were gastro-oesophagus (14 [15%]), lung (12 [13%]), ovary (11 [12%]), cervix (11 [12%]), and breast (nine [10%]). At the data cutoff date (April 30, 2023), median follow-up was 33·3 months (IQR 30·4–51·0) for the site-specific therapy group and 30·9 months (27·6–35·5) for the empirical chemotherapy group. Median progression-free survival was significantly longer with site-specific therapy than with empirical chemotherapy (9·6 months [95% CI 8·4–11·9] *vs* 6·6 months [5·5–7·9]; unadjusted hazard ratio 0·68 [95% CI 0·49–0·93]; p=0·017). Among the 167 patients who started planned treatment, 46 (56%) of 82 patients in the site-specific therapy group and 52 (61%) of 85 patients in the empirical chemotherapy group had grade 3 or worse treatment-related adverse events; the most frequent of these in the site-specific therapy and empirical chemotherapy groups were decreased neutrophil count (36 [44%] *vs* 42 [49%]), decreased white blood cell count (17 [21%] *vs* 26 [31%]), and anaemia (ten [12%] *vs* nine [11%]). Treatment-related serious adverse events were reported in five (6%) patients in the site-specific therapy group and two (2%) in the empirical chemotherapy group. No treatment-related deaths were observed.

**Interpretation** This single-centre randomised trial showed that site-specific therapy guided by the 90-gene expression assay could improve progression-free survival compared with empirical chemotherapy among patients with previously untreated CUP. Site-specific prediction by the 90-gene expression assay might provide more disease information and expand the therapeutic armamentarium in these patients.

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For the Chinese translation of the abstract see **Online** for appendix 1

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## Research in context

### Evidence before this study

We conducted a literature review including searches of PubMed, international guidelines, and major congresses (eg, American Society of Clinical Oncology and European Society for Medical Oncology) for clinical study reports (with no date restrictions) evaluating diagnosis and treatment in patients with cancer of unknown primary (CUP). The search was done on Nov 15, 2023, and included the search terms "cancer of unknown primary" or "unknown primary cancer" and "diagnosis", "classification", or "treatment". The current first-line standard of care for unfavourable CUP is empirical cytotoxic treatment, but the majority of patients with CUP have dismal survival, with a median overall survival of approximately 6–12 months. Regarding the tumourigenesis of CUP, there are two main hypotheses. The first hypothesis considers CUP as a counterpart of cancer of known primary, with the primary lesion too small or too difficult to locate. The other hypothesis considers CUP as an independent entity of cancer, in which absence of the primary lesion is real and will persist during the whole course of disease, possibly due to its early and sustained regression or dormancy. Consequently, there are two different treatment approaches directed by predicted primary site or detected genetic alteration within the lesion. However, the clinical benefit of either of them has not been clearly defined. There have been some successes with targeted therapy and immunotherapy for CUP, but they were mostly in later-line treatment settings.

### Added value of this study

We did the randomised Fudan CUP-001 trial to compare site-specific therapy guided by a 90-gene expression assay versus empirical chemotherapy in patients with previously untreated CUP. Our study showed that site-specific therapy confers a progression-free survival benefit in patients with CUP compared with empirical chemotherapy. In this study, only 26% of patients in the site-specific therapy group received the same regimen as listed for the empirical chemotherapy group. Adverse events of grade 3 or worse were similar between the two groups. To our knowledge, our trial is the first randomised study showing that using a primary site classifier based on gene-expression profiling improves prognosis for patients with CUP. This finding has important theoretical and practical implications. Theoretically, it supports the hypothesis that CUP is composed of multiple cancers of known primary, rather than being an independent cancer entity. Clinically, the positive results will promote research investigating gene-expression profiling to predict the primary site of CUP, and exploring applications of standard biomarker testing and the corresponding treatment for the predicted primary sites in patients with CUP.

### Implications of all the available evidence

Another phase 3 trial (CUPISCO) also showed a positive result in this clinical setting, but using comprehensive genomic profiling. Both our study and the CUPISCO trial are positive studies, which will promote research interest in the tumourigenesis of CUP, whether it is a counterpart of cancer of known primary or an independent cancer entity. If it is the latter, all new drugs approved in cancers of known primary will have to be independently assessed in clinical trials in patients with CUP. The success of the present trial is in sharp contrast with two previous negative randomised trials in this setting, possibly due to more use of immunotherapy and targeted therapy, which are already part of the standard of care in other cancers, in the current paper. Therefore, it is a reasonable approach to explore using a primary site classifier, such as the 90-gene expression assay, as a starting point and then ordering standard biomarker testing or next-generation sequencing once the primary site has been proposed.

## Introduction

Cancer of unknown primary (CUP) is defined as pathologically diagnosed metastatic cancer without identification of the primary tumour after a comprehensive diagnostic approach.[1] CUP accounts for 2–5% of all malignancies and has a high mortality rate.[2] The fact that the diagnostic work-up often changes with the availability of new diagnostic technology, and the high heterogeneity of CUP in terms of involved sites, pathology, treatment, and prognosis, have limited investigation into the disease and slowed progress in clinical treatment.[3–7] Approximately 80–85% of patients with CUP have unfavourable features with poor prognosis. Prospective studies intended to explore the benefit of molecularly tailored treatment are scarce and heterogeneous.[8] Until now, platinum-based empirical chemotherapy remains the standard upfront treatment for these patients, with a median overall survival of less than 1 year.[9–12]

In the past few decades, gene-expression profiling assays have been developed to identify the tissue of origin in patients with CUP.[13–15] A large phase 2 study by Hainsworth and colleagues published in 2013 showed that site-specific therapy had a median overall survival of 12·5 months (95% CI 9·1–15·4), which was better than the predefined historical cohort.[9] This study also showed improved survival for the subset of patients with molecularly diagnosed cancers that were responsive to therapy.[9] However, clinical benefit of site-specific therapy has not been shown in randomised trials. As immunotherapy and targeted therapy have demonstrated clinical benefit in many types of cancer, including CUP, in recent years,[16–18] it is increasingly likely that the latest site-specific therapies will be accessible to patients with CUP with predicted primaries and improve their outcomes.

The newly developed 90-gene expression assay (Canhelp-Origin Test [Canhelp Genomics, Hangzhou, China]) is a

real-time PCR-based assay that uses differential gene-expression patterns to assign tumours to one of 21 tumour types in its spectrum.[19] The 90-gene expression assay has previously been validated in a large-scale multicentre study involving 1417 samples, achieving an accuracy of 94·4% and a specificity exceeding 99%.[14] In addition, many studies have showcased the excellent performance of the 90-gene expression assay in difficult-to-diagnose tumours, including triple-negative breast cancer, brain metastases, liver metastases, and multiple primary tumours, with reported accuracy ranging from 92·0% to 97·4%.[20–23] Based on its performance in identifying the tissue origin of tumours, the 90-gene expression assay obtained the Conformité Européenne (CE) mark on March 25, 2022, and obtained approval from the National Medical Products Administration (NMPA) in China on July 21, 2022. Here, we report a single-centre randomised controlled trial (the Fudan CUP-001 study) to investigate the efficacy and safety of site-specific therapy directed by the 90-gene expression assay compared with empirical chemotherapy in patients with CUP.

## Methods

### Study design and participants

This randomised controlled trial was conducted at Fudan University Shanghai Cancer Center (FUSCC; Shanghai, China). The protocol (appendix 2 pp 12–39) and all amendments were approved by the institutional ethics review board. Amendments included changing the name of the assay from "the 96-gene expression assay" to "the 90-gene expression assay" on Sept 13, 2017, and excluding neuroendocrine tumours from the inclusion criteria on Feb 23, 2021.

All patients with CUP had undergone a standard evaluation, including medical history, physical examination, blood counts, chemistry profile, chest–abdomen–pelvis CT scans, PET/CT scans (optional), endoscopic examination of symptomatic areas, and pathological examination. The diagnostic procedure of CUP included the aforementioned standard diagnostic work-up and a group decision by a multidisciplinary team of Cancer of Multiple and Unknown Primaries in our institution. All patients with CUP had to meet the following main criteria: aged 18–75 years; histologically confirmed metastatic adenocarcinoma, squamous cell carcinoma, poorly differentiated carcinoma, or poorly differentiated neoplasms; no previous systemic therapy; an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; measurable or evaluable disease (according to the Response Evaluation Criteria in Solid Tumours [RECIST] version 1.1); expected survival time longer than 3 months; adequate organ function as defined by a neutrophil count of at least $1·5\times10^9$ cells per L, platelet count of at least $90\times10^9$ platelets per L, haemoglobin concentration of at least 90 g/L (with no blood transfusion within the past 14 days), serum bilirubin concentration of no more than 1·25 times the upper limit of normal (ULN), alanine aminotransferase and aspartate aminotransferase concentrations of no more than 2·5 times the ULN (≤5 times the ULN in patients with hepatic metastasis), and serum creatinine concentration of no more than 1·25 times the ULN; and sufficient formalin-fixed paraffin-embedded (FFPE) specimens to perform the 90-gene expression assay. Patients were excluded if their cancer was amenable to curative surgery or radiotherapy, including female patients with adeno-carcinoma limited to axillary lymph nodes, female patients with resectable peritoneal carcinomatosis, and patients with other single-site metastasis that could be surgically resected. The other main exclusion criteria were: a history of malignancy in the last 5 years (except for cured cervical carcinoma or skin basal cell carcinoma); active bleeding with clinical significance; symptomatic brain or meningeal metastasis; clinically significant organ failure; other severe diseases within 6 months before enrolment including coronary artery disease, cardiovascular disease, myocardial infarction, congestive heart failure, unstable angina pectoris, symptomatic cardiac effusion, or unstable arrhythmia; or any concurrent medical disorder that could endanger the patient's safety or affect the completion of the study based on the researchers' consideration. The full exclusion criteria are listed in appendix 2 (pp 19–20).

The trial was performed according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all the patients before enrolment. Patients could withdraw consent at any time after enrolment. This study is registered at ClinicalTrials.gov (NCT03278600).

See Online for appendix 2

### Randomisation and masking

After eligibility screening by the research coordinator, all patients were assigned a unique randomisation code. Randomisation was done using a random allocation system by a research nurse at FUSCC. The randomisation system allocated patients (1:1) to either empirical treatment or site-specific treatment. The randomisation system applied the Pocock and Simon minimisation method, using CentOS Linux release 7.9.2009 with Apache version 2.4.6, Jdk version 1.6, and MySQL version 5.5.32. The minimisation factors were ECOG performance status score (0–1 *vs* 2) and the extent of the disease (limited to lymph node metastasis *vs* extra-lymph node metastasis) and were equally weighted. A random element of 80% was incorporated in the minimisation algorithm. Individuals with a direct role in the conduct and analysis of the trial did not have access to the randomisation schedule. Clinicians and patients were not masked to interventions.

### Procedures

For site-specific therapy, an archived FFPE tumour specimen was analysed in an approved clinical laboratory using the Canhelp-Origin 90-gene expression assay. The results of the test are usually available within 1 week.

Additional biomarker testing was done if it was included in the standard diagnostic procedure of the predicted tumour. For example, for patients with a predicted origin of breast, oestrogen receptor, progesterone receptor, and HER2 status were further tested. Standard treatment for predicted tumour types was determined by the investigators referring to the relevant practice guidelines (appendix 2 p 1). If the assay did not determine the tissue of origin, patients received empirical chemotherapy. For the empirical chemotherapy group, patients were given taxane (175 mg/m² by intravenous infusion on day 1) plus platinum (cisplatin 75 mg/m² or carboplatin area under the curve 5 by intravenous infusion on day 1), or gemcitabine (1000 mg/m² by intravenous infusion on days 1 and 8) plus platinum (same as above), per physician's choice, administered every 3 weeks until disease progression, death, unacceptable adverse events, withdrawal of informed consent, or a maximum of six cycles. The protocol permitted dose modifications and cycle interruptions. As multiple therapy regimens were involved, researchers were responsible for dose adjustments based on adverse events during the last therapy course. Two dose level reductions were permitted.

Sex and ethnicity were defined via electronic medical records. Laboratory examination, including complete blood count, liver and kidney function, and electrolytes, were assessed at baseline and during treatment (within 1 week before each treatment course). Serum tumour markers (per investigator's decision) were examined every 6 weeks. Adverse events were assessed at each follow-up visit and graded per National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. During the study, patients were evaluated by CT or MRI scans for treatment response every 6 weeks. Tumour response was assessed by investigators and was not centrally reviewed.

Patients had the option to withdraw from trial treatment or follow-up at any stage. Under the following circumstances, a patient might be removed from the study: pregnancy or preparing for pregnancy; poor compliance, not completing the treatment planned, or switching to other chemotherapy schemes; serious adverse events occurring during the trial, and inability to continue the trial by dose reduction; delayed treatment for more than 2 weeks for any reason; and disease progression. These patients would continue to be followed up.

Needle biopsy or surgically resected tissue samples were submitted for gene-expression analysis with the 90-gene expression assay. Two senior pathologists (QW and XZ) reviewed haematoxylin and eosin slides to pinpoint regions with the highest tumour cell content. For each specimen, five to 15 sections (5 µm thick) of FFPE blocks were collected. Tumour cells were then enriched by microdissection, and total RNA was isolated using a commercially available FFPE RNA Isolation Kit (Canhelp Genomics, Hangzhou, China). The 90-gene expression assay was performed on a real-time PCR platform as previously described.[19] For each case, the isolated total RNA was reverse-transcribed into complementary DNA. The expression pattern of 90 tumour-specific genes was analysed using a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Subsequently, the 90-gene algorithm was used to assess the similarity between the gene-expression pattern of each specimen and the gene-expression profile of each tumour type in the reference database.[19] This comparison generated a similarity score for each tumour type. The tumour type with the highest similarity score was then conveyed to the treating physicians to aid in clinical decision making.

### Outcomes

The primary endpoint was progression-free survival, defined as the interval from the time of randomisation to disease progression or death from any cause, whichever occurred first. Secondary endpoints were overall survival, objective response rate, safety, and biomarker investigation. Overall survival was calculated from the time of randomisation to death from any cause. Objective response rate was calculated as the number of patients with complete response or partial response divided by all patients with a measurable lesion at baseline according to RECIST 1.1. Patients who did not have measurable disease were defined as not assessable. Adverse events were assessed in the safety population (all patients who started planned treatment). Biomarker analyses are ongoing and will be reported in a future publication.

### Statistical analysis

This trial aimed to evaluate whether site-specific therapy guided by the 90-gene expression assay improved progression-free survival as compared with empirical chemotherapy. Assuming a progression-free survival of 4·5 months for the empirical chemotherapy group and 7·2 months for the site-specific therapy group, approximately 156 patients would need to undergo randomisation in a 1:1 ratio for 147 events to be observed for the primary analysis of progression-free survival. We estimated that with this calculated sample size, the trial would have 80% power using a log-rank test with a two-sided significance level of 0·05. The expected enrolment time was 3 years with a follow-up time of 12 months. We further assumed that 10% of the patients would be lost to follow-up or would prematurely discontinue the trial. This yielded a final sample size of 174 patients (87 patients per group). The study was not powered to detect improvements in the secondary outcome of overall survival. Primary analyses were done in the intention-to-treat (ITT) population, including all randomly assigned patients. Prespecified analyses were also performed in the per-protocol population, which included patients who completed at least one cycle of protocol-defined treatment. In the

main ITT analysis, patients who had used other antitumour therapies before disease progression were censored at the date of the last imaging assessment before the use of other antitumour therapies. Patients with no post-baseline imaging assessment were censored on the date of the first dose of study medication. In view of this strict censoring mechanism in the ITT population, the restrictive per-protocol population, and the resulting risk of bias in the survival analysis due to imbalance of the treatment groups, a post-hoc sensitivity analysis based on the treatment policy estimand was done according to the group to which patients were randomly assigned, regardless of off-trial treatment, early dropout, and primary site revelation. In this analysis, progression-free survival was calculated from the time of randomisation to disease progression or recurrence or death regardless of violations, discontinuation of study drug, or change of therapy, equivalent to the ITT principle. An additional post-hoc sensitivity analysis was done focusing on the patients who were censored in the first 12 months whereby participants without a clear date of progression were treated as progressing at the date of death based on the ITT principle. Adverse events were assessed in the safety population (all patients who started planned treatment).

Progression-free survival and overall survival curves were estimated with the Kaplan–Meier method. The primary analysis was done with unstratified log-rank tests in the ITT population. To summarise the difference between the empirical chemotherapy group and site-specific treatment group, we also estimated the hazard ratio (HR) and corresponding 95% CIs for progression-free survival and overall survival on the basis of a Cox proportional hazards model adjusted for extent of disease (limited to lymph node metastasis *vs* extra-lymph node metastasis). We did not adjust analyses by ECOG performance status because nearly all patients had an ECOG performance status of 0–1. The Cox proportional hazards model was also used to generate HRs and 95% CIs for multivariable analysis, and group comparisons were assessed using the Wald test. Unstratified Cox proportional hazard models were also fitted and presented in forest plots to assess the effect size across subgroups defined by baseline patient characteristics (patient sex, age, ECOG performance status, pathology, and disease involvement). Multiplicative interaction was estimated using the likelihood ratio test. To explore the effect of identified prognostic factors (patient age, pathology, sex, disease involvement, and treatment group) on progression-free and overall survival in the ITT population, a post-hoc analysis using unstratified Cox proportional hazard models was done. Proportionality of hazards was tested by analysis of scaled Schoenfeld residuals. The exact 95% CIs of objective response rates were calculated with the Clopper–Pearson method. Patients who did not have measurable disease were defined as not assessable. p values less than 0·05 were considered significant. All analyses were conducted in R version 3.3.3.

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Between Sept 18, 2017, and March 18, 2021, 182 patients with CUP were recruited at FUSCC and randomly assigned to the site-specific therapy group (n=91) or the empirical chemotherapy group (n=91). Baseline characteristics were generally similar between the two groups (table 1). Of the 182 patients, 105 (58%) were male and 77 (42%) female, and most patients (174 [96%]) had an ECOG performance status of 0–1. All patients were Asian. Half of the patients (91 [50%]) had lymph node-only disease. All the patients assigned to treatment were included in efficacy analyses and 167 (92%) patients who started protocol-defined treatment were included in the safety population. Nine (10%) of 91 patients in the site-specific therapy group and six (7%) of 91 in the empirical chemotherapy group did not initiate their assigned study therapy, and were therefore not included in the safety population (figure 1). The median time between randomisation and the start of treatment was 15 days (IQR 10–20) in the site-specific

| | Site-specific therapy (n=91) | Empirical chemotherapy (n=91) |
|---|---|---|
| **Age, years** | | |
| Median (IQR) | 57 (51–64) | 59 (51–64) |
| ≤60 | 55 (60%) | 51 (56%) |
| >60 | 36 (40%) | 40 (44%) |
| **Sex** | | |
| Male | 53 (58%) | 52 (57%) |
| Female | 38 (42%) | 39 (43%) |
| **Ethnicity** | | |
| Asian | 91 (100%) | 91 (100%) |
| **ECOG performance status** | | |
| 0 or 1 | 86 (95%) | 88 (97%) |
| 2 | 5 (5%) | 3 (3%) |
| **Histology** | | |
| Adenocarcinoma | 46 (51%) | 38 (42%) |
| Poorly differentiated carcinoma | 24 (26%) | 33 (36%) |
| Squamous cell carcinoma | 20 (22%) | 20 (22%) |
| Poorly differentiated neoplasm | 1 (1%) | 0 |
| **Metastatic sites** | | |
| Lymph node only | 46 (51%) | 45 (49%) |
| Extranodal involvement | 45 (49%) | 46 (51%) |

ECOG=Eastern Cooperative Oncology Group.

***Table 1:*** **Baseline characteristics in the intention-to-treat population**

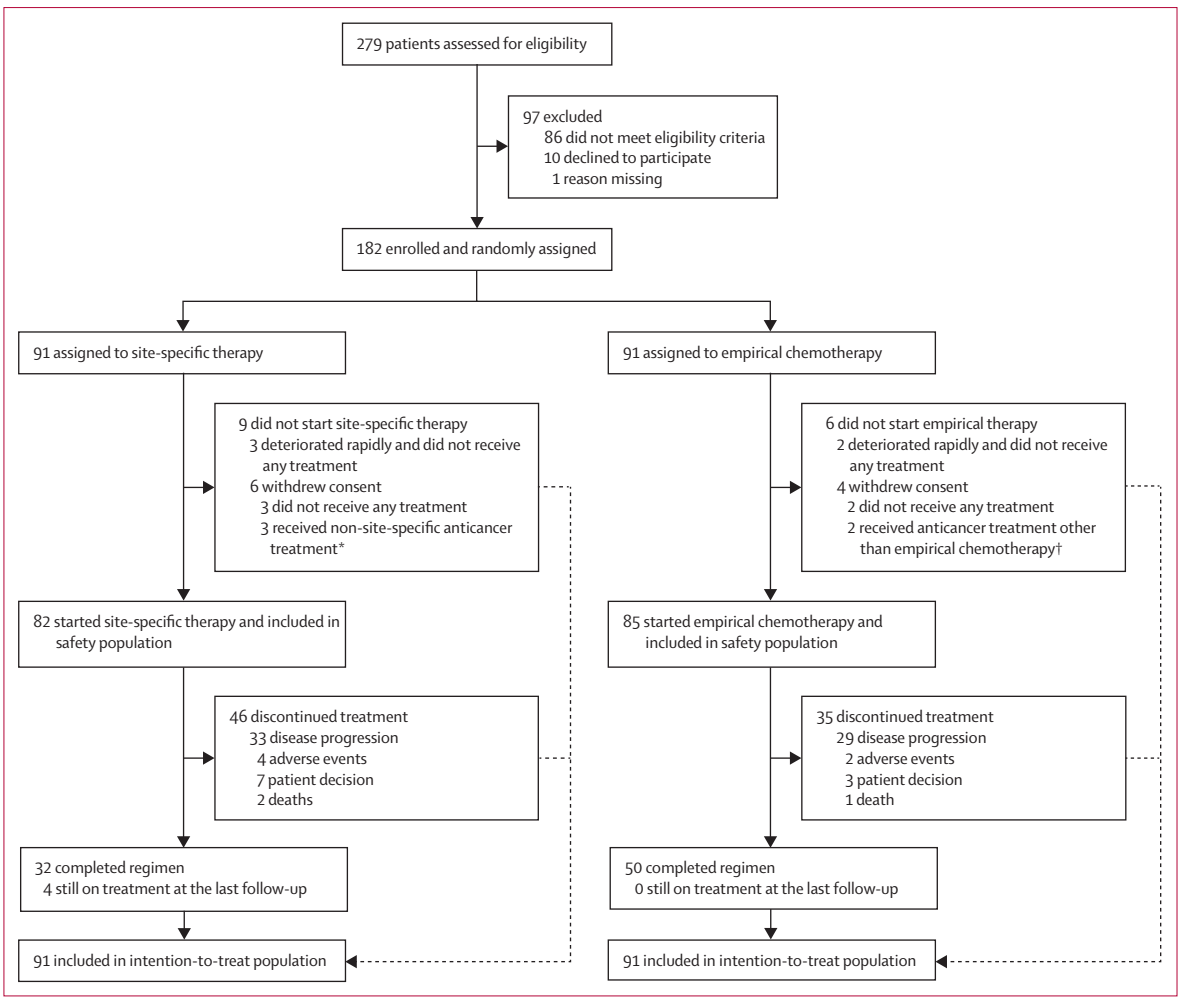


***Figure 1:*** **Trial profile**
*Two received chemotherapy and one received immunochemotherapy. †One received chemotherapy (FOLFIRI regimen) plus cetuximab, the other received immunochemotherapy.

therapy group and 6 days (3–7) in the empirical chemotherapy group.

The 90-gene expression assay was applied to predict the tissue of origin for 83 of the 91 patients in the site-specific therapy group. The 90-gene expression assay could not be successfully performed or interpreted in eight (9%) patients due to insufficient RNA quality or quantity of the biopsy specimen (n=4) and consent withdrawal (n=4). There were 14 (15%) patients with a predicted origin of gastro-oesophagus, for whom the site-specific therapy included chemotherapy (n=10), chemotherapy plus PD-1 inhibitor (n=2), and no treatment (n=2). Among the 12 (13%) patients with a predicted origin of lung, the site-specific therapy included chemotherapy (n=3), chemotherapy plus bevacizumab (n=3), chemotherapy plus endostatin (n=1), chemotherapy plus PD-1 inhibitor (n=3), and gefitinib (n=2, both harbouring *EGFR* 19 deletion). Among the 11 (12%) patients with a predicted origin of ovary, the site-specific therapy included chemotherapy (n=1), chemotherapy plus bevacizumab (n=7), chemotherapy plus bevacizumab with olaparib maintenance (n=2), and no treatment (n=1). Among the 11 (12%) patients with a predicted origin of cervix, the site-specific therapy included chemotherapy (n=1), chemotherapy plus bevacizumab (n=8), chemotherapy plus PD-1 inhibitor (n=1), and no treatment (n=1). Among the nine (10%) patients with a predicted origin of breast, the site-specific therapy included chemotherapy (n=7), chemotherapy plus trastuzumab (n=1), and chemotherapy with olaparib maintenance (n=1; this patient had *BRCA1* mutation). Among the seven (8%) patients with a predicted head and neck cancer, the site-specific therapy included chemotherapy (n=2), chemotherapy plus PD-1 inhibitor (n=3), and chemotherapy plus cetuximab (n=2). Details of all the treatment regimens for the site-specific therapy group are available in appendix 2 (p 2). In this group, 24 (26%) of 91 patients received the same regimen as

listed for the empirical chemotherapy group. 41 (45%) of the 91 patients received targeted agents or immunotherapy; targeted agents administered included antiangiogenic agents (n=23), immune checkpoint inhibitors (n=9), HER2 or EGFR monoclonal antibody therapy (n=3), EGFR or ALK tyrosine kinase inhibitors (n=3), and PARP inhibitors (n=3). Of the 91 patients in the empirical chemotherapy group, 57 (63%) received taxane plus platinum, 28 (31%) received gemcitabine plus platinum, four did not receive any treatment, and two did not receive protocol-defined treatment (one received chemotherapy plus immunotherapy and one received chemotherapy plus cetuximab).

At the data cutoff date (April 30, 2023), we recorded a total of 150 events of disease progression (82% of the 182 patients in the ITT population). In each group, 75 (82%) patients had disease progression events (the disease progression events were deaths in 12 patients, including seven in the site-specific therapy group and five in the empirical chemotherapy group), with a median follow-up time of 33·3 months (IQR 30·4–51·0) in the site-specific therapy group and 30·9 months (27·6–35·5) in the empirical chemotherapy group. The median progression-free survival was significantly longer with site-specific therapy than with empirical chemotherapy (9·6 months [95% CI 8·4–11·9] *vs* 6·6 months [5·5–7·9]; unadjusted HR 0·68 [95% CI 0·49–0·93]; adjusted HR 0·68 [0·50–0·94]; log-rank p=0·017; figure 2A) in the ITT population. At the time of the data cutoff, there were 55 deaths (60%) in the site-specific therapy group compared with 65 deaths (71%) in the empirical chemotherapy group, with median follow-up times of 43·6 months (IQR 34·4–52·9) and 42·9 months (33·9–51·9), respectively. Of these deaths, 117 (64%) were related to CUP (53 in the site-specific therapy group and 64 in the empirical chemotherapy group), one was due to vascular thrombosis or embolism (in the site-specific therapy group), and two resulted from other causes (one in each group). Median overall survival was 28·2 months (95% CI 23·3–46·5) in the site-specific therapy group and 19·0 months (17·1–26·4) in the empirical chemotherapy group (unadjusted HR 0·74 [95% CI 0·52–1·06]; adjusted HR 0·75 [0·53–1·08]; log-rank p=0·098) in the ITT population (figure 2B). The results of the prespecified analysis of progression-free survival in the per-protocol population (n=158) are in the appendix 2 (p 11).

In our post-hoc sensitivity analysis of progression-free survival following the ITT principle, the HR for progression-free survival changed only slightly (unadjusted HR 0·69 [95% CI 0·51–0·95]; adjusted HR 0·71 [0·52–0·97]; log-rank p=0·024; appendix 2 p 8). The additional post-hoc sensitivity analysis focusing on the patients who were censored in the first 12 months also yielded similar results (appendix 2 p 9). The proportional hazards assumption was met in the ITT progression-free survival and overall survival analyses, as well as in the sensitivity analysis of progression-free survival (appendix 2 p 3). A post-hoc analysis in the ITT population explored the effect of identified prognostic factors on progression-free and overall survival (appendix 2 pp 4–5). The second-line treatment of patients in the site-specific therapy and empirical chemotherapy groups is reported in appendix 2 (p 7).

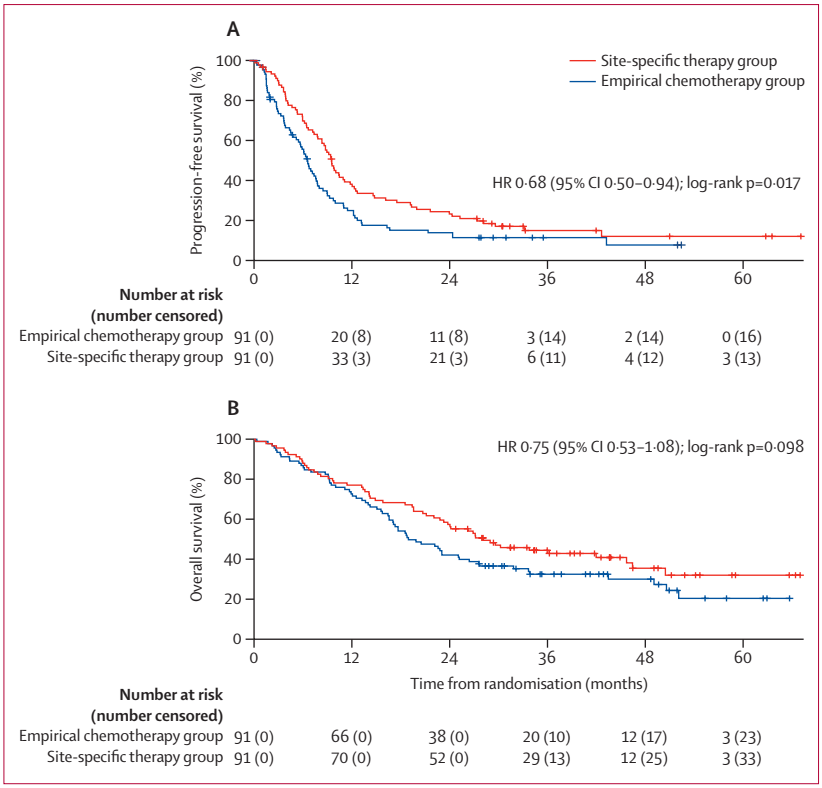


***Figure 2:*** **Kaplan–Meier plots for progression-free survival (A) and overall survival (B) according to site-specific or empirical therapy in the intention-to-treat population**
Adjusted HRs are shown. HR=hazard ratio.

Of 158 patients with measurable disease, 38 (49% [95% CI 37–60]) of the 78 patients who received site-specific therapy had an objective response, compared with 37 (46% [35–58]) of the 80 patients who received empirical chemotherapy (p=0·756; appendix 2 p 6). The subgroup analyses of progression-free survival (figure 3) and overall survival (appendix 2 p 10) in the ITT population are shown in forest plots.

Of the 167 patients in the safety population, 155 (92%) patients had adverse events of any grade (75 in the site-specific therapy group and 80 in the empirical chemotherapy group). 46 (56%) of 82 patients in the site-specific therapy group and 52 (61%) of 85 patients in the empirical chemotherapy group had grade 3 or 4 treatment-related adverse events. The most frequently reported grade 3 or worse treatment-related adverse events in the site-specific therapy and empirical chemotherapy groups were decreased neutrophil count (36 [44%] *vs* 42 [49%]), decreased white blood cell count (17 [21%] *vs* 26 [31%]), and anaemia (ten [12%] *vs* nine

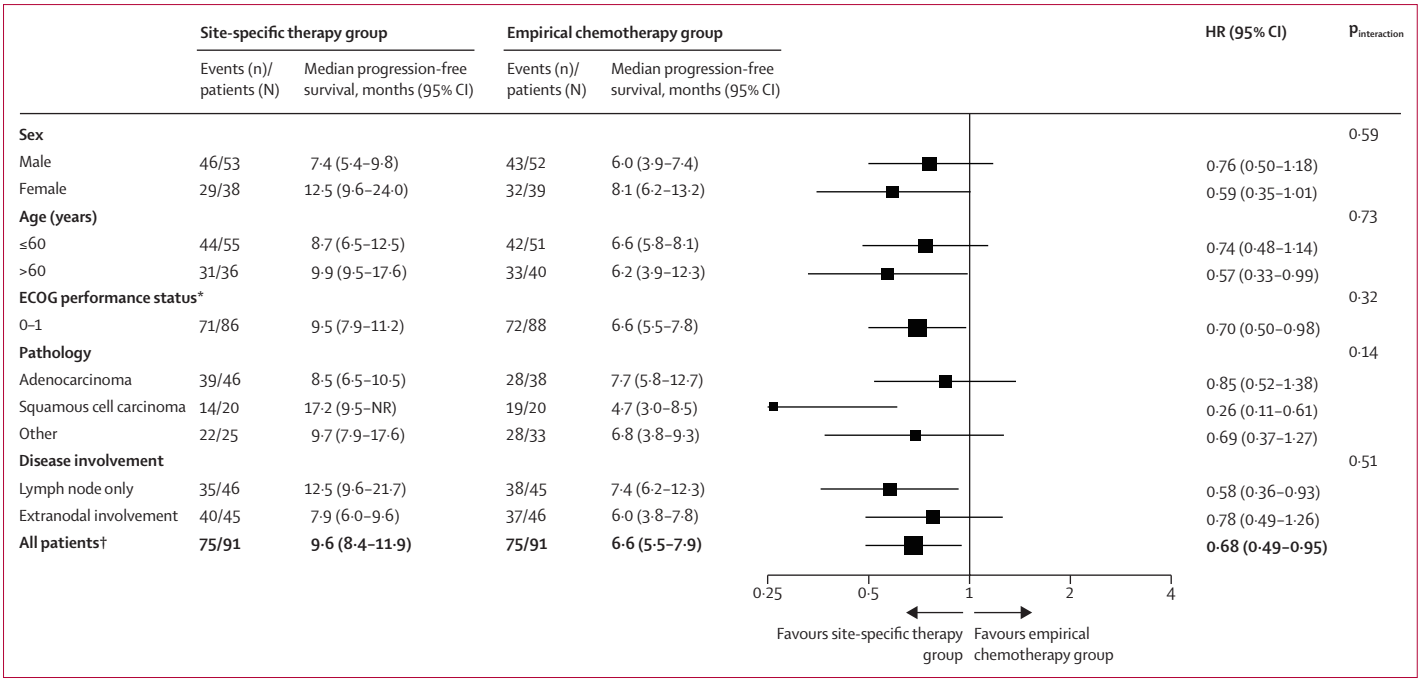


***Figure 3:*** **Forest plot of progression-free survival in patient subgroups**
ECOG=Eastern Cooperative Oncology Group. HR=hazard ratio. NR=not reached. *ECOG 2 is not included due to the limited patient number (n=8). †Results are presented after adjustment for the patient characteristics listed in the forest plot.

| | Site-specific therapy (n=82) | | | Empirical chemotherapy (n=85) | | |
|---|---|---|---|---|---|---|
| | Grade 1–2 | Grade 3 | Grade 4 | Grade 1–2 | Grade 3 | Grade 4 |
| Nausea | 26 (32%) | 0 | 0 | 29 (34%) | 2 (2%) | 0 |
| Vomiting | 11 (13%) | 1 (1%) | 0 | 22 (26%) | 2 (2%) | 0 |
| Decreased appetite | 26 (32%) | 0 | 0 | 15 (18%) | 0 | 0 |
| Constipation | 14 (17%) | 0 | 0 | 11 (13%) | 0 | 0 |
| White blood cell count decreased | 35 (43%) | 12 (15%) | 5 (6%) | 26 (31%) | 23 (27%) | 3 (4%) |
| Neutrophil count decreased | 15 (18%) | 21 (26%) | 15 (18%) | 14 (16%) | 19 (22%) | 23 (27%) |
| Anaemia | 34 (41%) | 9 (11%) | 1 (1%) | 44 (52%) | 9 (11%) | 0 |
| Thrombocytopenia | 27 (33%) | 3 (4%) | 2 (2%) | 17 (20%) | 4 (5%) | 1 (1%) |
| ALT increased | 19 (23%) | 0 | 0 | 11 (13%) | 1 (1%) | 0 |
| AST increased | 17 (21%) | 0 | 0 | 7 (8%) | 1 (1%) | 0 |
| Creatinine increased | 12 (15%) | 0 | 0 | 15 (18%) | 0 | 0 |
| Weight loss | 20 (24%) | 0 | 0 | 16 (19%) | 0 | 0 |
| Fatigue | 28 (34%) | 1 (1%) | 0 | 20 (24%) | 1 (1%) | 0 |
| Sensory neuropathy | 13 (16%) | 1 (1%) | 0 | 9 (11%) | 4 (5%) | 0 |
| Alopecia | 45 (55%) | 0 | 0 | 58 (68%) | 0 | 0 |

ALT=alanine aminotransferase. AST=aspartate aminotransferase.

***Table 2:*** **Treatment-related adverse events that occurred at any grade in at least 5% of patients in the safety population**

[11%]). The treatment-related adverse events of patients in each group in the safety population are listed in table 2. Adverse events leading to dose reduction occurred in 17 (21%) of 82 patients in the site-specific therapy group compared with 17 (20%) of 85 patients in the empirical chemotherapy group. Adverse events leading to treatment discontinuation occurred in four (5%) of 82 patients in the site-specific therapy group, including grade 2 vomiting (n=1), persistent grade 3 thrombocytopenia and anaemia (n=1), grade 4 anaemia (n=1), and grade 2 dyspnoea (n=1). Two (2%) of 85 patients in the empirical chemotherapy group discontinued treatment due to treatment-related grade 3 sensory neuropathy. Treatment-related serious adverse events were reported in five (6%) of 82 patients in the site-specific therapy group, including grade 4 thrombocytopenia (n=1, 1%), grade 3 febrile neutropenia (n=1, 1%), grade 2 dyspnoea (n=1, 1%), grade 4 anaemia (n=1, 1%), and grade 3 anaemia (n=1, 1%). Treatment-related serious adverse events were reported in two (2%) of 85 patients in empirical chemotherapy group (grade 3 vomiting in both cases). No treatment-related deaths were observed.

## Discussion

As far as we are aware, the present randomised controlled trial is the first to show that site-specific therapy directed by a gene-expression assay could improve the clinical outcome of patients with CUP, compared with standard platinum-based chemotherapy. Progression-free survival was significantly longer in patients receiving site-specific therapy than patients receiving empirical chemotherapy (9·6 months *vs* 6·6 months), which met the primary endpoint of our study. Additionally, the incidence of grade 3 or worse adverse events associated with site-specific therapy was similar to that with empirical chemotherapy.

Over the past few decades, molecular tumour profiling has been developed to predict primary sites in patients with CUP. The clinical utility of several tissue-of-origin assays has been investigated. Hayashi and colleagues did a randomised trial to evaluate the efficacy of site-specific therapy based on the results of tissue of origin testing versus empirical carboplatin–paclitaxel in patients with CUP. No benefits were observed in progression-free survival or overall survival for site-specific therapy. This finding might be due to the study being underpowered for efficacy analysis and the unclear ability of microarray-based expression profiling to identify the primary site, which had not been robustly validated. Moreover, the uncommon patient mix (eg, lymphoma accounting for 10·9% of predicted cancers among patients in the efficacy analysis) might also have resulted in bias. Additionally, the uncommercialised microarray assay used in this study required a fresh frozen tumour specimen obtained through repeat biopsy, resulting in a 3-week treatment delay. Another randomised trial, GEFCAPI-04, also did not show survival benefits of site-specific therapy guided by the results of two commercial assays, CancerTYPE ID (Hologic, Marlborough, MA, USA) and the Tissue of Origin test (Pathwork Diagnostics, Sunnyvale, CA, USA).[11] It is worth noting that patients enrolled in the aforementioned trials, even for those assigned to site-specific therapy, primarily received chemotherapy rather than targeted therapy or immunotherapy. In fact, in the study conducted by Hayashi and colleagues, site-specific therapy was cytotoxic drugs in almost all cases, except for two patients with kidney cancer receiving sunitinib. Using a different approach, Moon and colleagues reported that tailoring treatment of patients with CUP based on the predicted tumour types by Oncology next generation sequencing-based primary cancer-type classifier (OncoNPC) resulted in a 2·2-times increase in the number of patients receiving genomically guided therapies, and patients who received first palliative intent treatments concordant with their OncoNPC-predicted cancers had significantly better outcomes than those who received discordant treatments, although it was not a randomised study.[24]

With the development of molecular biomarkers and advances in immune checkpoint inhibitors and targeted agents, treatment paradigms have changed substantially in recent years. Patients in the present study had the chance to receive molecularly targeted agents, antiangiogenic agents, or immune checkpoint inhibitors. In this study, only 26% of the patients in the site-specific therapy group received the same regimen as listed for the empirical chemotherapy group. Indeed, 45% of patients in this group received targeted agents or immunotherapy. In terms of safety, the proportion of patients with grade 3 or worse adverse events linked to site-specific therapy was similar to that of standard empirical chemotherapy (56% *vs* 61%).

The 90-gene expression assay used in our study employs real-time PCR technology, providing a cost-effective solution with relatively low expenses. Multiple studies have shown that incorporating molecular classifier assays into clinical practice is a cost-efficient strategy for streamlining diagnostic methods and enhancing patient care.[25,26] Therefore, molecular classifier assays, such as the 90-gene expression assay, hold promise for integration into the standard work-up for diagnosing CUP, alongside clinical features, serum tumour marker profiling, and standard pathology, to enable the most precise identification of the tissue of origin in CUP.[27,28]

The 90-gene expression assay is an NMPA-approved commercial assay, with several studies demonstrating a robust performance of 90–97·4% accuracy in pan-cancer and several difficult-to-diagnosis tumour types.[14,19,20,22,23] Notably, among different histological types, concordance rates between the 90-gene expression assay predictions and the reference diagnosis reached 91·0% (151 of 166) for squamous cell carcinoma and 94·5% (656 of 694) for poorly differentiated or undifferentiated tumours.[14] For those pathological subtypes, routine diagnostic approaches including morphological and immunohistochemical assessments will provide limited information in identifying a tumour origin. The 90-gene expression assay is deemed to be more objective and less likely to be affected by the observer. However, there are some limitations of the assay, such as the possibility of acquiring insufficient good-quality RNA for the assay possibly resulting from substantial tumour necrosis; pre-analytical failure; and post-analytical failure. Another intrinsic weakness is that other cancers besides the 21 tumour types predicted with this assay, such as rare occult primary cancers, can also present as CUP.

The five most common predicted primary sites in this study were gastro-oesophagus, lung, ovary, cervix, and breast, accounting for about 60% of all patients with CUP in the site-specific therapy group. This cancer type distribution was similar to our previous epidemiological study, which identified breast, gastro-oesophageal, ovary, lung, and colorectal cancer as the most common tumour types.[6] Of note, the cancer types identified through molecular diagnosis in our study differed from the cancer types diagnosed in Europe and the USA by other molecular classifier assays. The biliary tract, urothelium, and colorectum were the most commonly predicted tissues of origin in the large phase 2 study by Hainsworth and colleagues.[9] Another difference was predominant lymph node involvement, a more favourable site of metastasis, which was seen in about 50% of patients in each group of our study, higher than the proportion (9–17·3%) reported in other studies.[29,30] However, in a study by Hayashi and colleagues, the stomach was the predicted site in 22·8% of the total population, and 41 (41%) of 101 patients had lymph node-only disease;[12] these findings are consistent with our study. It is worth

mentioning that the 90-gene expression assay has also obtained CE certification, opening the possibility for further validating its clinical significance in non-Asian populations.

The CUPISCO trial[31] compared targeted therapy or cancer immunotherapy guided by comprehensive genomic profiling versus platinum-based chemotherapy in newly diagnosed patients with unfavourable CUP. The results of the study indicated that the targeted therapy or immunotherapy based on the advice of the molecular tumour board conferred a progression-free survival benefit (6·1 months [95% CI 4·7–6·5] *vs* 4·4 months [4·1–5·6], HR 0·72 [95% CI 0·56–0·92]).[31] By contrast with our study, CUPISCO included only patients with unfavourable non-squamous CUP. Additionally, the schedule of CUPISCO included an induction period allowing for the results of genomic profiling to become available and preventing treatment delay. Moreover, patients who were initially resistant to platinum-based chemotherapy (ie, had progressive disease on induction chemotherapy before being switched to molecularly guided therapy) were not included in the survival analysis. The positive results of both studies in patients with CUP will arouse research interest in the tumourigenesis of CUP, whether it is a counterpart of cancer of known primary or an independent cancer entity. Based on our data, future studies could explore the use of genomic profiling (eg, with the 90-gene expression assay) as the primary tumour classifier, followed by corresponding standard biomarker testing or next-generation sequencing (or both) for the predicted primary as a new diagnostic work-up for CUP.

This study has several limitations. First, it is a single-centre study involving only Asian participants, which might limit the generalisability of the results. Second, because of the heterogeneity of this disease, patient consent withdrawal and protocol violation could introduce bias into the interpretation of results. Additionally, due to drug availability, only a minority of patients received immunotherapy in the experimental group of the study. It is also worth mentioning that the statistical power is inadequate for overall survival analysis, which warrants further validation. Finally, biomarkers are under investigation and not reported here.

In summary, this randomised controlled trial demonstrated that site-specific therapy guided by the 90-gene expression assay resulted in longer progression-free survival compared with empirical chemotherapy in previously untreated patients with CUP who were not amenable to local radical treatment. Our findings suggest that identifying the tissue of origin as a guide for site-specific therapy has the potential to be the new standard of care in patients with CUP in the era of precision medicine.

#### Contributors

XLi, SJ, ZL, and XH drafted the manuscript. XLi, XH, and ZL conceptualised and designed the study. MM and JL provided statistical input into the implementation and statistical analysis plan, under the supervision of XH. QX and YS contributed to the 90-gene expression assay. XLi, XZha, SJ, QW, YaW, LZ, SH, HW, YH, YC, XLu, YuW, XZho, WL, CC, XY, KC, JC, ZL, and XH contributed to participant enrolment and data acquisition. ZL and XH co-conceived, implemented, and supervised the Fudan CUP-001 study. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. ZL and XH verified the data. All authors read, commented on, and approved the final manuscript.

#### Declaration of interests

QX and YS are employees of Canhelp Genomics, Hangzhou, China. All other authors declare no competing interests.

#### Data sharing

Trial data shall not be disclosed. We encourage investigators interested in data sharing and collaboration to contact the corresponding author.

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