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Evidence Summary MOTAC 7

A Quality Initiative of the Program in Evidence-Based Care (PEBC), Ontario Health (Cancer Care Ontario)

The Use of Molecular Tools for Identifying and Guiding Treatment of Cancers of Unknown Primary

S. Saibil, X. Yao, D. Sivajohanathan, M. Deodat, M Vickers, P. Wheatley-Price, J. Yoon, H. Feilotter

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For information about this document, please contact Dr. Samuel Saibil, the lead author, through the PEBC via:
Phone: 905-527-4322 ext. 42822 Fax: 905 526-6775 E-mail: ccopgi@mcmaster.ca

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Phone: 905-527-4322 ext. 42822 Fax: 905 526-6775 E-mail: ccopgi@mcmaster.ca

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Evidence Summary

THE PROGRAM IN EVIDENCE-BASED CARE

The Program in Evidence-Based Care (PEBC) is an initiative of the Ontario provincial cancer system, Ontario Health (Cancer Care Ontario) (OH (CCO)). The PEBC mandate is to improve the lives of Ontarians affected by cancer through the development, dissemination, and evaluation of evidence-based products designed to facilitate clinical, planning, and policy decisions about cancer control.

The PEBC is a provincial initiative of OH (CCO) supported by the Ontario Ministry of Health (OMH). All work produced by the PEBC is editorially independent from the OMH.

INTRODUCTION

Cancer of unknown primary (CUP) is defined as a histologically confirmed metastatic cancer where the primary tumour remains unidentified despite comprehensive diagnostic evaluations [1,2]. CUP accounts for approximately 3% to 5% of all cancer diagnoses globally, with an estimated incidence of seven to 12 cases per 100,000 people per year [2]. Despite its rarity, CUP represents a significant clinical challenge due to its heterogeneity and the poor prognosis often associated with the disease. Notably, CUP excludes certain malignancies such as sarcomas, melanomas, germ cell tumours, neuroendocrine tumours, and hematological cancers where the exact site of origin is undetermined [2]. The vast majority of patients with CUP, 80-85%, fall into the unfavourable risk group of tumours that are carcinomas with no clear tissue of origin from histological analysis and present with multiple sites of metastatic disease [3]. This stands in contrast to a subset of CUP patients, the favourable risk group, that present with limited disease amenable to curative intent, treatment with local therapies or a clinical presentation highly suggestive of tissue of origin, such as women with isolated axillary lymph nodes [4].

Patients diagnosed with unfavourable-risk CUPs frequently face limited treatment options, often relying on empiric chemotherapy regimens such as taxanes and platinum-based therapies [5]. However, these treatments have yielded only modest improvements in outcomes, with median overall survival (OS) ranging from six to 15 months [5]. The one-year survival rate for CUP patients has remained relatively stagnant at approximately 20%, underscoring the urgent need for more effective diagnostic and therapeutic strategies [2,6].

Molecular profiling has emerged as a promising approach to address the challenges associated with CUP [7]. For example, it was reported that Next-Generation Sequencing (NGS) can enhance personalized medicine and the treatment of autoimmune disorders and cancer by tailoring therapies to a patient's unique genetic profile, using whole genome and whole exome sequencing to guide treatment decisions [8]. By analyzing the genetic and molecular characteristics of the tumour, molecular tools can potentially identify the tissue of origin, possibly identify tumour agnostic actionable mutations, predict treatment response, and offer a more personalized treatment approach based upon the identification of targetable mutations. The integration of molecular diagnostics into standard care for CUP patients holds the potential for significant improvements in clinical outcomes, including prolonged progression-free survival (PFS) and enhanced quality of life [9].

The purpose of this evidence summary, developed by OH (CCO) in collaboration with the PEBC, is to systematically evaluate the existing evidence on the value of different types of biomarkers on the diagnosis and treatment of CUP. The categories of biomarkers include (1) gene expression using microarray, NGS or polymerase chain reaction (PCR)-based platforms, (2) simple DNA mutations, measured by targeted PCR or NGS approaches, (3) broad DNA mutations and fusions using NGS approaches, and (4) protein biomarkers measured by immunohistochemistry (IHC). The studies for patients with unknown primaries of neuroendocrine tumours, head and neck, and melanoma are excluded from this evidence summary because they represent a different pathological entity with established diagnostic and treatment algorithms. Based on the objective of this document, the Working Group derived the research question outlined below. This systematic review has been registered on the website of the International Prospective Register of Systematic Reviews (www.crd.york.ac.uk/prospero) as CRD42023493381.

OBJECTIVES

To provide a synthesis and summary of evidence surrounding the utility of molecular tools in patients with CUP.

RESEARCH QUESTIONS

This research question was developed to direct the search for available evidence on the use of molecular tools for diagnosing and guiding the treatment of CUP:

- Can clinical outcomes, such as OS and PFS, and/or diagnostic outcomes (such as sensitivity and specificity) be improved through molecular profiling in patients with a diagnosis of cancer of unknown primary?

TARGET POPULATION

Adult patients with a diagnosis of CUP.

INTENDED USERS

This evidence summary is intended for:

- Clinicians, laboratory physicians, and scientists involved in the care and testing of patients with cancers of unknown primary
- Policy makers, health care administrators, and the OMH

METHODS

This evidence summary was developed by a Working Group consisting of medical oncologists, a pathologist, a molecular geneticist and a health research methodologist at the request of the Molecular Oncology and Testing Advisory Committee (MOTAC).

The Working Group was responsible for reviewing the identified evidence and drafting the summary. Conflict of interest declarations for all authors are summarized in Appendix 1 and were managed in accordance with the *PEBC Conflict of Interest Policy*.

This evidence review was conducted in two planned stages, including a search for systematic reviews followed by a search for primary literature. These stages are described in subsequent sections.

Search for Systematic Reviews

A search was conducted for existing systematic reviews. This included original systematic reviews and systematic reviews published as a component of practice guidelines. The MEDLINE (January 2020 to May 2024) and EMBASE (January 2020 to May 2024) databases, as well as the

Cochrane Database of Systematic Reviews (January 2020 to July 20, 2024) were searched. The full search strategy is available in Appendix 2. Systematic reviews were included if they met the following criteria:

- The review addressed the research question with similar inclusion/exclusion criteria; and
- The review had a low risk of bias as assessed with the ROBIS tool or a moderate/high overall rating as assessed with the AMSTAR 2 tool; and
- The review had a literature search cut-off after 2020.

If more than one systematic review met the inclusion criteria, then one systematic review for each outcome per comparison was selected based on its age, quality, and the best match with our study selection criteria stated below.

For each outcome per comparison, if no systematic review was included, then a search for primary literature was conducted. For any included systematic review, an updated search for primary literature was performed if the literature search was older than six months. If any included systematic review was limited in scope, then an updated search of the systematic review and a new search for primary literature to address the limitation in scope were conducted.

Search for Primary Literature

Literature Search Strategy

The MEDLINE (from January 2013 to May 2024) and EMBASE (from January 2013 to May 2024) databases were searched for studies related to the use of molecular profiling tests in the clinical management of patients with CUP. The full search strategy is available in Appendix 2. Reference lists of included randomized controlled trials (RCTs) and comparative studies were scanned for additional citations. Moreover, the literature search of MEDLINE and EMBASE was updated until August 21, 2024 for RCTs only.

Study Selection Criteria and Process

Inclusion Criteria

1. Studies assessing patients with a diagnosis of cancer of unknown primary; and
2. Studies that reported on metrics representing a change in clinical management with the use of any of the following four categories of biomarkers: (1) gene expression using microarray, NGS or PCR-based platforms (2) simple DNA mutation measured by targeted PCR or NGS approaches, (3) broad DNA mutations and fusions using NGS approaches, and (4) protein biomarkers measured by IHC; and
3. Studies with the following study design: RCTs, comparative studies, and single-arm studies with a sample size of ≥50 patients of interest; and
4. Studies reporting any of the following outcomes: predicted cancer sites, theoretically actionable alterations, management changes after any of the above four biomarker tests, or survival outcomes; and
5. Studies that only reported the predicted cancer sites should report at least one diagnostic outcome, such as sensitivity, specificity, or detection rate; or be calculable based on the data provided.

Exclusion Criteria

1. Studies assessing patients with unknown primaries of neuroendocrine tumours, head and neck, melanoma; or
2. Conference abstracts of non-randomized studies; or

3. Abstracts of interim analyses; or
4. Papers or abstracts not available in English; or
5. Papers and abstracts published before 2013; or
6. The reference standard was not clarified for studies that only reported the predicted primary cancer sites (i.e., diagnostic information).

A review of the titles and abstracts was conducted by DS, XY, and MD, independently. For studies that warranted full-text review, two of the three reviewers reviewed each study independently following the inclusion and exclusion criteria, then discussed with the Working Group members to confirm the study inclusion.

Data Extraction and Assessment of the Certainty of the Evidence

All included primary studies underwent data extraction by one of the three reviewers (DS, XY, and MD), independently, with all extracted data and information audited subsequently by a different reviewer among the three of them, independently. MD conducted a data audit.

For treatment studies, the risk of bias for each outcome in the included RCTs was assessed using the Cochrane Collaboration Risk of Bias 2.0 tool [10]; for the included non-randomized comparative studies, the Risk of Bias in Non-randomised Studies of Interventions tool was utilized to evaluate the risk of bias for each outcome [11]. After that, the certainty of the evidence per outcome, taking into account the risk of bias, inconsistency, indirectness, imprecision, and publication bias was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach [12].

For studies that only reported diagnostic outcomes, the QUADAS-2 tool was used to assess the quality [13].

Synthesizing the Evidence

When results from two or more studies were clinically and methodologically homogeneous, a meta-analysis was performed using RevMan software version 5.4.1. If a meta-analysis was not appropriate, the results of each study were presented individually in a descriptive manner. The preferred statistic for meta-analysis was the hazard ratio (HR) for the survival outcomes. A HR of less than 1.0 indicated that patients in the experimental group (EG) had a lower probability of experiencing harmful outcomes such as death events, while a HR greater than 1.0 suggested that patients in the control group (CG) had a lower probability of experiencing harmful outcomes such as death events. For studies that did not provide a HR with its 95% confidence interval (CI), we calculated the HR with its 95% CI by the data provided in the paper, such as measuring data in a Kaplan-Meier curve. For the studies in which we were unable to calculate the HR's 95% CI, we presented the p-value between two comparative groups as reported.

RESULTS

Literature Search Results

There were 197 hits after searching for systematic reviews, but none met the inclusion criteria. A search for primary literature yielded 1556 publications after de-duplication; 332 publications underwent full-text screening with 40 publications meeting the preplanned study selection criteria [1,5,9,14-50]. Five publications were excluded as more detailed follow-up publications were available [5,35-38]. Finally, 35 studies were analyzed [1,9,14-34,39-50]. A PRISMA flow diagram [51] detailing the reasons for study exclusion is included in Appendix 3.

Among the 35 eligible studies, 34 [1,9,14-34,39-49] investigated the clinical utility of molecular testing and one study [50] focused on the diagnostic accuracy outcomes of molecular testing for identifying primary tumour sites.

Of the 34 treatment-related studies, there were four RCTs [9,15,39], of which one is currently available in abstract form [16], and one comparative study [44]. In these five studies, patients in the EG underwent molecular testing. However, current molecular technology does not identify specific therapies for all patients. Only those patients who had actionable biomarkers could receive molecular-guided therapy and access specific treatment options (either linked chemotherapy, targeted therapy/immunotherapy, or both). The other patients without results in the EG received similar empirical treatments to those in the CG. The survival outcomes of all patients in the EG were compared with those in the CG.

All patients in each of the 29 single-arm studies received molecular testing, i.e., everyone was in the EG. Four [1,30,45,47] of the 29 single-arm studies reported comparative survival outcomes between patients who received molecular-guided therapy (EG1) versus those who, despite having had molecular testing, received empirical therapy (EG2). As these data provide relevant decision-making data, these studies were summarized along with the four RCTs and one comparative study for a total of nine included studies. The biomarker categories assessed by each study are shown in Table 1.

The remaining 25 single-arm studies provided non-comparative data on predicted primary cancer types after molecular testing without confirmation by clinical follow-up, data on theoretically actionable alterations and/or linked to specific treatment options, and OS outcomes in cohorts where all patients received the same treatment strategies. While this may be useful, it does not provide any decision-making data and are not further discussed in this document.

Table 1. Molecular tool categories for the nine included studies

Molecular tool category	(1) Gene expression using microarray, NGS or PCR based platforms	(2) Simple DNA mutations, measured by targeted PCR or NGS approaches	(3) Broad DNA mutations and fusions using NGS approaches	(4) Protein biomarkers measured by IHC	(5) Mixed categories (1) and (3)
Studies	Lui et al, 2024 [15], Hayashi et al, 2019 [39], Fizazi et al, 2019 [16]	No studies met inclusion criteria	Kramer et al, 2024 [9] Fusco et al, 2022 [1]	Junior et al, 2024 [47], Errani et al, 2017 [45]	Nishikawa et al, 2022 [30] Hasegawa et al, 2018 [44]

Abbreviations: IHC, immunohistochemistry; NGS, Next-Generation Sequencing; PCR, polymerase chain reaction

Certainty of the evidence assessment

The risk of bias assessment was conducted for three fully published RCTs [9,15,39] and one non-randomized comparative study [44]. The fourth RCT was currently published in abstract form and could not be assessed [16]. The risk of bias for each outcome for the three RCTs was scored as 'some concerns' primarily due to patients, clinicians, and outcome assessors being aware of the intervention received by study participants. For OS, this lack of blinding is less likely to introduce bias as the assessment of this outcome is objective; however, for PFS, it could increase the potential for bias. The assessment details of each domain per outcome and per study are provided in Table A4-1 in Appendix 4. The overall risk of bias in the non-randomized comparative study was 'moderate' as unknown confounders were unable to be controlled in this study design and the authors did not register or publish the study's protocol (Table A4-2 in Appendix 4).

The aggregate certainty of evidence for each comparison of interventions under the molecular tools' category ranged from 'low' to 'very low'. This was after considering the seven other domains (inconsistency, indirectness, imprecision, and publication bias to downgrade;

and large effect, dose-response, all plausible confounding and bias to upgrade), together from the GRADE approach, for the RCTs and one comparative study (Appendix 5). For the 29 single-arm studies, a risk of bias assessment was not conducted as these studies have a high risk of bias due to having no CG in the study design by nature, which mainly led to ‘very low’ certainty per comparison after considering other domains of the GRADE approach.

The quality of one diagnostic study was assessed to be ’moderate’ based on the QUADAS-2 tool as it is unknown whether the interpretation of the reference standard introduced bias and whether patients received the same reference standard [50] (Appendix 6).

Treatment Outcomes

The study characteristics of the nine studies evaluating clinical utility and outcomes are summarized in Table 2.

Table 2. Outcomes of the included interventional studies

Study; Country	Sample size; number of cycles of previous systemic therapy (range)	Median age, yrs (range)	Sex	Predicted primary cancer type after molecular profiling[a], n (%)	Theoretically actionable alterations	Management	Survival outcomes
A. Gene expression using microarray, NGS or PCR based platforms							
Randomized Controlled Trials							
Liu et al, 2024 [15]; China	EG: 91; 0	57 (51-64)	Female, 42% Male, 58%	83 (91%) of pts received prediction of primary cancer type. Gastroesophagus, 14 Lung, 12 Ovary, 11 Cervix, 11 Breast, 9 Head and neck, 7 Urinary, 6	NR	82 (90%) pts started site-specific treatment - 50% pts received specific chemotherapy and 50% received non-chemotherapy or treatment combined with chemotherapy. 32 (35%) pts completed treatment.	**Median OS** EG vs. CG 28.2 mths (95% CI, 23.3 to 46.5) vs. 19.0 mths (95% CI, 17.1 to 26.4) HR, 0.75; 95% CI, 0.53 to 1.08 **Median PFS, mths** EG vs. CG 9.6 mths (95% CI, 8.4 to 11.9) vs. 6.6 mths (95% CI, 5.5 to 7.9) HR, 0.68; 95% CI, 0.5 to 0.94
	CG: 91; 0	59 (51-64)	Female, 43%, Male, 57%	NA	NA	85 (93%) pts started empirical chemotherapy for a maximum of six cycles. (taxane + cisplatin/carboplatin; or gemcitabine + cisplatin/ carboplatin). 50 (55%) pts completed treatment.	
Hayashi et al, 2019 [39]; Japan	EG (site-specific treatment): 65; 0	67 (33-80)	Female, 42% Male, 58%	All pts received prediction of primary cancer type. Pancreas, 11 Gastric, 9 Lymphoma, 7 Urothelium, 3 Cervix, 5 Ovary, 5	NR	50 (77%) pts received site-specific therapy (48 pts received site-specific chemotherapy and 2 pts received targeted therapy).	**Median OS, mths[b]** EG vs. CG 9.8 mths (95% CI, 5.7 to 13.8) vs. 12.5 mths (95% CI, 8.9 to 16.1) HR, 1.028; 95% CI, 0.678 to 1.560 **Median PFS, mths** EG vs. CG 5.1 mths (95% CI, 1.9 to 8.3) vs. 4.8 mths (95% CI, 3.3 to 6.5) HR, 0.884; 95% CI, 0.590 to 1.326
	CG (empirical therapy): 65; 0	60 (31-78)	Female, 42% Male, 58%	All pts received prediction of primary cancer type. Pancreas, 15 Gastric, 14 Lymphoma, 4 Urothelium, 5 Cervix, 2 Breast, 2	NA	51 (78%) pts received paclitaxel and carboplatin.	

Study; Country	Sample size; number of cycles of previous systemic therapy (range)	Median age, yrs (range)	Sex	Predicted primary cancer type after molecular profiling[a], n (%)	Theoretically actionable alterations	Management	Survival outcomes
Fizazi et al, 2019 [16] *Abstract*; France, Denmark, Netherlands, Spain	EG: 123; 0	NR	NR	Most predicted primary cancers: Pancreatico-biliary, 19% Squamous cell carcinoma, 11% Kidney, 8% Lung, 8%	NR	91 pts (74%) received site-specific treatment; treatment of remaining 32 pts was not specified.	**Median OS** EG vs. CG 10.7 mths vs. 10 mths HR, 0.92; 95% CI, 0.69 to 1.23 **Median PFS** EG vs. CG 5.3 mths vs. 4.6 mths HR, 0.95; 95% CI, 0.72 to 1.25
	CG: 120; 0	NR	NR		NR	All pts received cisplatin + gemcitabine	
B. Broad DNA mutations and fusions using NGS approaches							
Randomized Controlled Trial							
Krämer et al, 2024 [9]; 34 countries (mainly from Europe and Asia)	EG: 326; 3 cycles of platinum-based chemotherapy during the induction period	61 (53-70)	Female, 49% Male, 51%	NR	88 (27%) pts had genomic alterations or fit a genomic signature	88 (27%) pts received molecular-guided therapies (mainly targeted therapy or immune checkpoint inhibitors) and remaining 238 pts received atezolizumab + chemotherapy for at least three cycles until loss of clinical benefit, or unacceptable toxicity	**Median OS (interim analysis)** EG vs. CG 14.7 mths (95% CI, 13.3 to 17.3) vs. 11.0 mths (95% CI, 9.7 to 15.4) HR, 0.82; 95% CI, 0.62 to 1.09. **Median PFS, ITT analysis** EG vs. CG 6.1 mths (95% CI, 4.7 to 6.5) vs. 4.4 mths (95% CI, 4.1 to 5.6) HR, 0.72; 95% CI, 0.56 to 0.92.
	CG: 110; 3 cycles of platinum-based chemotherapy during the induction period	63 (55-69)	Female, 48% Male, 52%	NA	NA	110 pts received carboplatin–paclitaxel, cisplatin–gemcitabine, or carboplatin–gemcitabine until loss of clinical benefit, unacceptable toxicity, patient or investigator decision to discontinue, or death	

Study; Country	Sample size; number of cycles of previous systemic therapy (range)	Median age, yrs (range)	Sex	Predicted primary cancer type after molecular profiling[a], n (%)	Theoretically actionable alterations	Management	Survival outcomes
Retrospective single-arm study							
Fusco et al, 2022 [1]; USA	95; 1 line (0-8)	68 (18-92)	Female, 52% Male, 48%	14 (15%) of pts received a diagnosis with NGS. Intrahepatic cholangiocarcinomas, 5 (*confirmed 4 cholangiocarcinoma and 1 pancreaticobiliary*) Pancreas, 2 (*confirmed 1 pancreas and 1 pancreaticobiliary*) Basal cell carcinoma, 2 (*confirmed*) Lung adenocarcinomas, 2 (*confirmed 2 NSCLC*) Upper GI adenocarcinomas, 1 (*confirmed gastroesophageal adenocarcinoma*) MSI-High colon cancer, 1 (*confirmed colon*), Atypical rhabdoid/teratoid: 1 (*confirmed*)	OncoKB[d] Version 2: Level 1, 18 (19%) pts Level 3b, 30 (32%) pts Level 3c, 4 (4%) pts 68 clinically actionable alterations in 52 patients (55%) with therapeutic options including checkpoint immunotherapy (18 pts) and targeted therapy 34 pts).	17 (18%) pts received molecularly guided therapy while the remaining 78 pts (82%) received standard treatment options.	**Median OS** EG1 (n=17) vs. EG2 (n=78) 23.6 mths vs 14.7 mths HR, 0.568; 95% CI, 0.268 to 1.205[c]
C. Protein biomarkers measured by IHC							
Retrospective single-arm studies							
Junior et al, 2024 [47]; Brazil	109[d]; <50% of pts received chemotherapy (most with carboplatin+ paclitaxel)	Mean, ~60.4y	Female, 56% Male, 44%	NR	20 pts with PD-L1 expression.	Patients did not receive any immune checkpoint inhibitor treatment.	**Median OS** PD-L1 expression (n=20) vs. no PD-L1 expression (n=89) 18.7 mths vs. 3.0 mths HR: 0.42, 95% CI, 0.23 to 0.76[c]

Study; Country	Sample size; number of cycles of previous systemic therapy (range)	Median age, yrs (range)	Sex	Predicted primary cancer type after molecular profiling[a], n (%)	Theoretically actionable alterations	Management	Survival outcomes
Errani et al, 2017 [45]; Italy, Greece	67[e]; NR	63 (39-87)[f]	NR	35 (52%) of pts received a diagnosis of tumour origin after organ-specific IHC markers test on bone metastasis: Lung, 12 Breast, 7 Kidney, 6 Rectum, 4 Prostate, 4 Stomach, 2	NR	NR	**OS at 1 year** EG1 (n=35) vs. EG2 (n=32) 55.3% vs. 27.1%[c] HR: 0.34, 95% CI (0.15 to 0.75)[c]
D. Mixed Simple DNA mutation and Protein biomarkers measured by IHC							
Comparative study							
Hasegawa et al, 2018 [44]; Japan	EG: 90; 0	63 (29-82)	Female, 41% Male, 59%	56 (62%) of pts received prediction of primary cancer type. Gastrointestinal, 20 Gynaecological, 12 NSCLC, 6 Pancreas, 4 Neuroendocrine, 4 Urothelial, 3 Biliary tract, 2	NR	56 (62%) pts received site-specific chemotherapy; 34 (38%) pts received platinum empiric chemotherapy	**Median OS** EG (n=90) vs. CG (n=32) 15.7 mths vs. 10.7 mths; p=0.07. EG (n=56 with site-specific therapy) vs. CG (n=32) 20.3 mths vs. 10.7 mths HR, 0.57; 95% CI, 0.34 to 0.94; p=0.03. Multivariable analysis controlled ECOG performance status PFS, bone metastasis and number of metastatic sites,
	CG: 32; 0	63 (31-77)	Female, 41% Male, 59%	NA	NR	32 pts received platinum empiric chemotherapy	
Retrospective single-arm study							
Nishikawa et al, 2022 [30]	177 (33 favourable, 144 unfavourable pts[g]); ≥1 regimen of chemotherapy or chemoradiotherapy for CUP	Favourable, 69 (36-83)	Female, 67%, Male, 33%	SCLC, 10 Ovary, 10 Breast, 6, Head and neck cancer, 4	NR	NR	**Median OS** 24.2 mths (95% CI, 10.6-61.7)
		Unfavourable, 64.5 (35-84)	Female, 44%, Male, 56%	Non-SCLC, 13 Gastric, 12 Colon, 8 Pancreas, 7 Ovary, 4 SCLC, 2 Head and neck, 2	NR	60 (42%) pts in the unfavourable group received site-specific treatment while the remaining 84 (58%) received empiric treatment.	**Median OS:** EG1 (n=60) vs. EG2 (n=84) 10.0 vs. 10.1 mths HR, 1.01, 95% CI, 0.70 to 1.45; p=0.95

Abbreviations: CG, comparative group (patients in this group without biomarker testing group); CI, confidence interval; CUP, cancer of unknown primary; DNA, deoxyribonucleic acid; ECOG, Eastern Cooperative Oncology Group; EG, experimental group (patients in this group experienced NGS test); EG1, experimental group 1 (patients in this group received NGS guided therapy); EG2, experimental group 2 (patients in this group received empirical therapy (Although they received the biomarker test, they did not receive specific therapy guided by biomarker test)); FFPE, formalin-fixed, paraffin-embedded; GI, gastrointestinal; HR, hazard ratio; IHC, immunohistochemistry; ITT, intent to treat; Mb, Megabase; MSI, microsatellite Instability; mths, months; NA, not applicable; NGS, Next-Generation Sequencing; NR, not reported; NSCLC, non-small cell lung cancer; OncoKB, Oncology Knowledge Base; OS, overall survival; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; PFS, progression-free survival; pts, patients; RCT, randomized controlled trial; SCLC, small cell lung cancer; USA, United States of America; vs., versus; Yr, year.

[a] Only the 5 most common types are presented.

[b] Data from the efficacy analysis with 50 patients in each group

[c] Working Group calculated HR based on the data and figures provided in the original study

[d] OncoKB (Oncology Knowledge Base) system includes Level 1 genomic alterations (FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication), Level 2A (Standard care biomarker predictive of response to an FDA-approved drug in this indication), Level 2B (Standard care biomarker predictive of response to an FDA-approved drug in another indication but not standard care for this indication), Level 3A (Compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication, but neither biomarker nor drug is standard care), Level 3B (Compelling clinical evidence supports the biomarker as being predictive of response to a drug in another indication, but neither biomarker nor drug is standard care), Level 4 (Compelling biologic evidence supports the biomarker as being predictive of response to a drug, but neither biomarker nor drug is standard care), Level R1 (Standard care biomarker predictive of resistance to an FDA-approved drug in this indication), R2 (Compelling clinical evidence supports the biomarker as being predictive of resistance to a drug, but neither biomarker nor drug is standard care), and Level R3 (Compelling biologic evidence supports the biomarker as being predictive of resistance to a drug, but neither biomarker nor drug is standard care).

[e] Number of evaluable patients in study

[e] Number of patients with unknown tumour of origin, a subset of the total study population

[f] All patients at time of diagnosis of bone metastasis (n=125)

[g] According to the European Society for Medical Oncology (ESMO) guideline (2015), patients with CUP were classified as having favorable-risk CUP if they had one of the following: isolated axillary nodal metastases of adenocarcinoma in women, peritoneal adenocarcinomatosis (Peri ADC) of a serous papillary histological type in females, osteoblastic bone metastases of adenocarcinoma with positive IHC staining of prostate-specific antigen (PSA) or elevated serum PSA in males, liver or peritoneal metastases of adenocarcinoma with a colorectal cancer immunoprofle (CK7/CK20− /+and CDX2), well or poorly differentiated neuroendocrine tumor of unknown primary, squamous cell carcinoma in cervical lymph nodes, and a single metastatic lesion of unknown primary. All patients who did not fall into one of the favorable-risk subgroups were considered to have unfavorable-risk CUP

1. Gene expression using microarray, NGS or PCR-based platforms

Two fully published RCTs [15,39] and one conference abstract [16] met the study selection criteria. Patients did not receive any systemic therapy before study enrollment. In the study by Liu et al 2024 [15], the primary cancer type was predicted in 83 of 91 patients in the EG. The five most predicted primary cancer types were gastroesophagus (14 patients; 17%), lung (12 patients; 14%), ovary (11 patients; 13%), cervix (11 patients; 13%), and breast (9 patients; 11%). Among the 82 patients who received site-specific treatment, 50% received specific non-chemotherapy (such as targeted therapy or immune checkpoint inhibitors), with or without specific chemotherapy, and the other 50% received specific chemotherapy alone. In the CG, 85 of 91 patients received empirical chemotherapy for a maximum of six cycles (taxane plus cisplatin/carboplatin; or gemcitabine plus cisplatin/carboplatin). When patients in the EG were compared with those in the CG, the median OS for patients in the EG was 28.2 months (95% CI, 23.3 to 46.5) versus 19.0 months (95% CI, 17.1 to 26.4) for the CG; HR, 0.75; 95% CI, 0.53 to 1.08. The median PFS for the experimental and control groups were 9.6 months (95% CI, 8.4 to 11.9) versus 6.6 months (95% CI, 5.5 to 7.9), respectively; HR, 0.68; 95% CI, 0.5 to 0.9.

In the RCT by Hayashi et al [39], receiving site-specific therapy (n=50) did not lead to a better median OS (9.8 months [95% CI, 5.7 to 13.8] vs. 12.5 months [95% CI, 8.9 to 16.1]; HR, 1.028; 95% CI, 0.678 to 1.560) or median PFS (5.1 months [95% CI, 1.9 to 8.3] vs. 4.8 months [95% CI, 3.3 to 6.5]; HR, 0.884; 95% CI, 0.590 to 1.326) when compared with patients in the CG (n=51).

The RCT by Fiyazi et al [16] was published as a conference abstract. There were 123 patients in the EG and 120 patients in the CG. The most predicted primary cancer types were pancreaticobiliary (19%), squamous cell carcinoma (11%), kidney (8%), and lung (8%). In the EG, 91/123 (74%) patients received site-specific treatment. The treatment of the remaining 32 patients in the EG was not specified. In the CG, all 120 patients received cisplatin plus gemcitabine. Receiving site-specific therapy did not improve median OS (10.7 months vs. 10 months [HR, 0.92; 95% CI, 0.69 to 1.23]) or median PFS (5.3 months vs. 4.6 months [HR, 0.95; 95% CI, 0.72 to 1.27]).

The meta-analyses of these three RCTs showed that the pooled mean difference between the EG and CG was 2.39 months (95% CI, -2.73 to 7.52) for median OS and 1.34 months (95% CI, -0.37 to 3.05) for median PFS (Figures 1.1.1 and 1.2.1, respectively). The HR for median OS was 0.85; 95% CI, 0.67 to 1.07, and for median PFS was 0.89; 95% CI, 0.72 to 1.10. (Figures 1.1.2 and 1.2.2, respectively).

The certainty of the evidence result is 'very low' (Appendix 5, Table A5-1). Gene expression molecular testing may have little effect on median OS and median PFS.

Figure 1.1.1 A meta-analysis for median overall survival from studies using gene expression testing—the mean difference

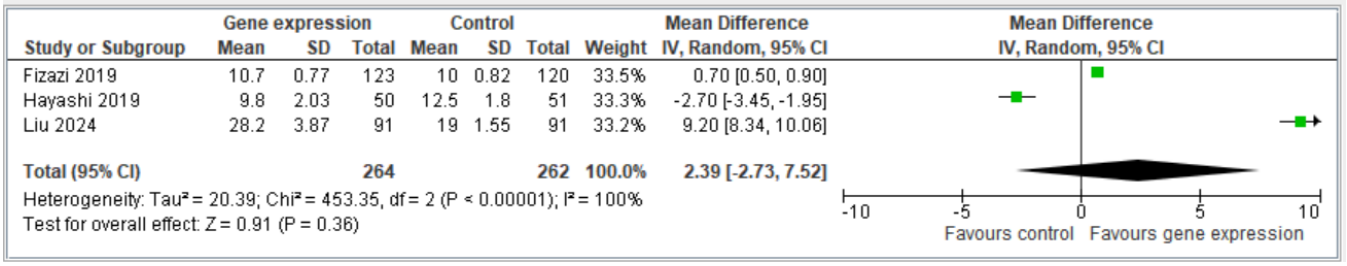


Figure 1.1.2 A meta-analysis for median overall survival from studies using gene expression testing—the pooled HR value

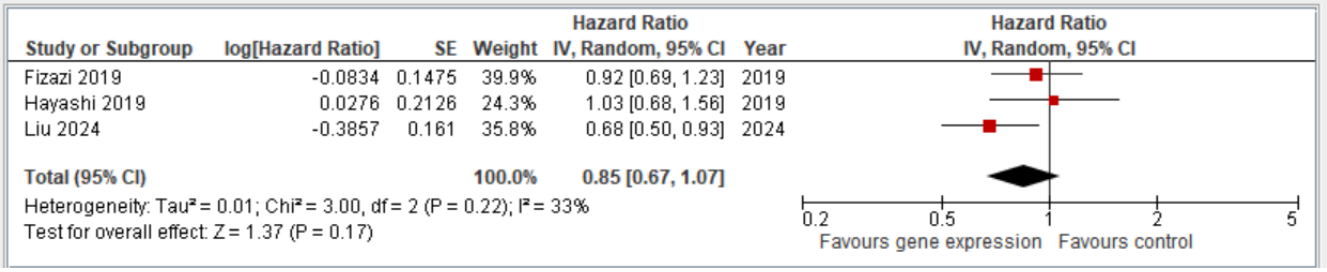


Figure 1.2.1 A meta-analysis for median progression-free survival from studies using gene expression testing—the mean difference

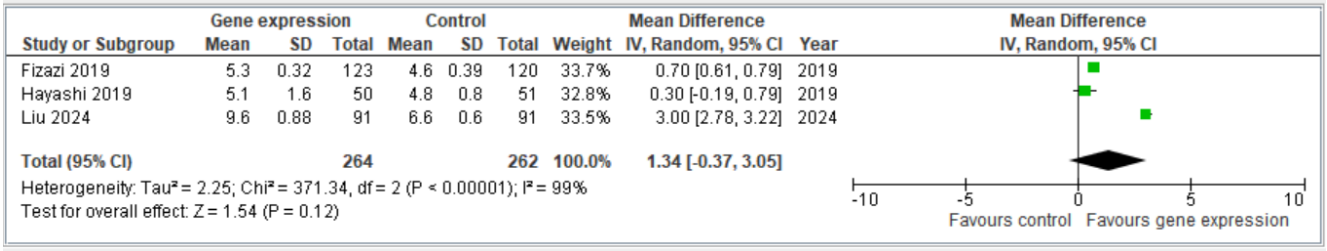
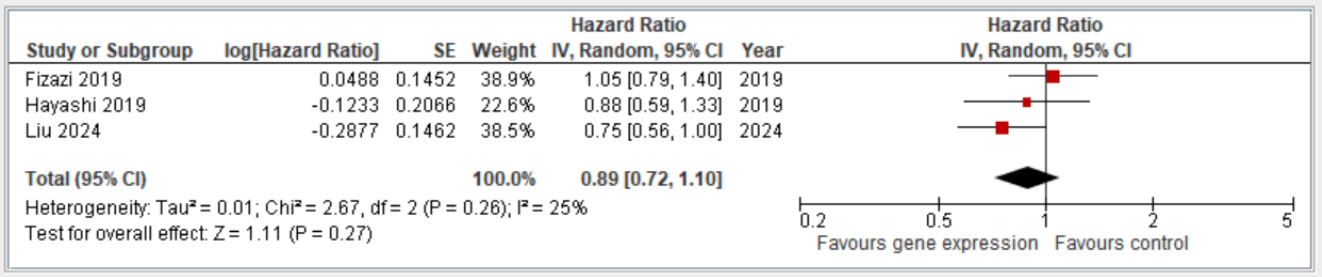


Figure 1.2.2 A meta-analysis for median progression-free survival from studies using gene expression testing—the pooled HR value



2. Broad DNA mutations and fusions using NGS approaches

One RCT [9] and one retrospective study [1] met the study selection criteria for this category. The CUPISCO Kramer et al [9] recruited 436 patients who received three cycles of platinum-based chemotherapy; 326 patients were randomized into the EG and 110 patients to the CG, where they continued receiving platinum-based chemotherapy until loss of clinical benefit, unacceptable toxicity, patient or investigator decision to discontinue, or death. Among the 326 patients in the EG, 88 had genomic alterations or fit a genomic signature that linked to targeted therapy or immune checkpoint inhibitors, and the remaining 238 patients received atezolizumab plus chemotherapy for at least three cycles until loss of clinical benefit or unacceptable toxicity. Currently, an interim analysis of median OS is available with a final analysis planned at study closure. The interim median OS was 14.7 months (95% CI, 13.3 to 17.3) versus 11.0 months (95% CI, 9.7 to 15.4) for patients in the EG and CG, respectively, with an HR of 0.82 (95% CI, 0.62 to 1.09). The mean difference of median OS was 3.7 (95% CI, 3.51 to 3.89) months (Figure 2.1). The median PFS was 6.1 months (95% CI, 4.7 to 6.5) versus 4.4 months (95% CI, 4.1 to 5.6) with a HR of 0.72; 95% CI, 0.56 to 0.92. The mean difference of median PFS was 1.7 (95% CI, 1.64 to 1.76) months (Figure 2.2).

The retrospective single-arm study by Fusco et al [1] included 95 patients with CUP, who received molecular testing. Seventeen (18%) patients received molecularly guided therapy,

with 14 patients receiving a diagnosis with a predicted cancer type. The most predicted primary cancer types were intrahepatic cholangiocarcinomas (5 patients; 36%), pancreas (2 patients; 14%), basal cell carcinoma (2 patients; 14%), lung adenocarcinomas, (2 patients; 14%), and upper gastrointestinal adenocarcinomas (1 patient; 7%). The difference in median OS between patients who received molecularly guided therapy (23.6 months) and those who did not (14.7 months) was 8.9 months (HR, 0.57; 95% CI, 0.27 to 1.20) (Figure 3).

The certainty of the evidence result is 'low' (Appendix 5, Table A5-2). Identifying broad DNA mutations and fusions through NGS approach molecular testing may result in a slight increase in median OS and median PFS.

Figure 2.1 Median overall survival using broad DNA mutations and fusions—the mean difference

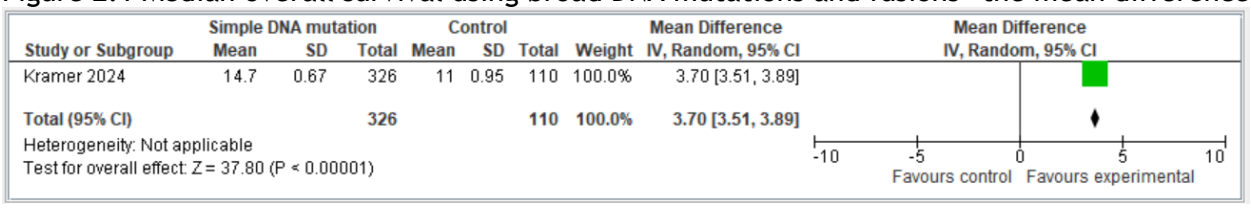


Figure 2.2 Median progression-free survival using broad DNA mutations and fusions —the mean difference

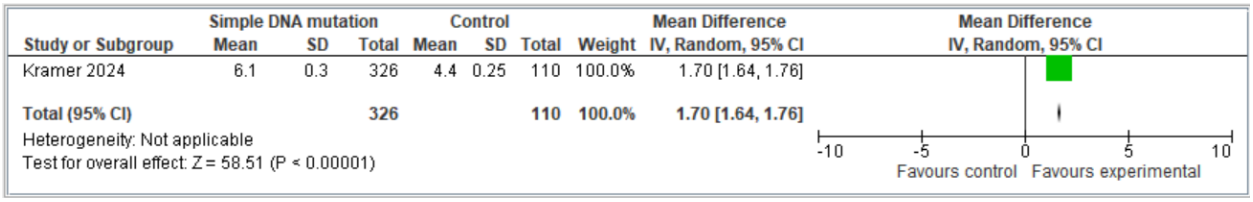
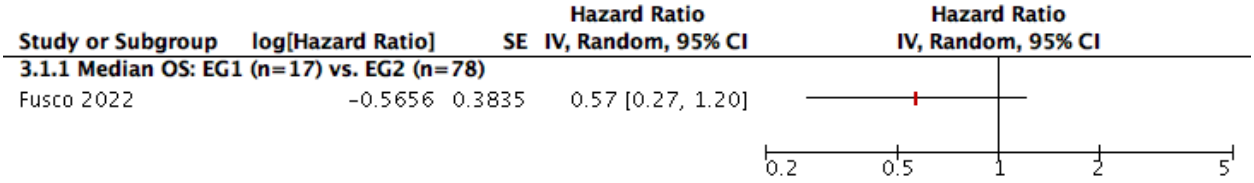


Figure 3. Retrospective study using broad DNA mutations and fusions using NGS approaches



3. Protein biomarkers measured by IHC

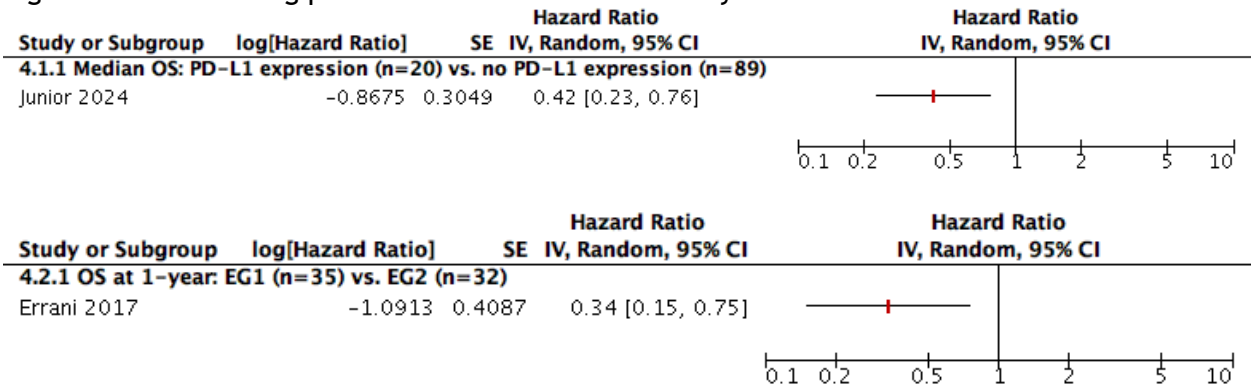
No RCTs or comparative studies met the study selection criteria. Two retrospective single-arm studies were analyzed here. The first, by Junior et al [47], included 109 evaluable CUP patients who were analyzed for programmed death-ligand 1 (PD-L1) expression by IHC. Of these, 20 patients were positive for PD-L1 expression, while the remaining 89 patients were negative for PD-L1 expression. Neither group was treated with immune checkpoint inhibitors. The median OS in those who were positive for PD-L1 expression was 18.7 months, while those who were negative had a median OS of 3.0 months (HR, 0.42; 95% CI, 0.23 to 0.76) (Figure 4).

The second single-arm study by Errani et al [45] reported that 35 of the 67 CUP patients (52%) who had protein biomarkers measured by IHC had a diagnostic prediction of the tumour origin. The most predicted primary cancer sites were lung (12 patients; 34%), breast (7 patients; 20%), kidney (6 patients; 17%), rectum (4 patients; 11%), and prostate (4 patients; 11%). Thus, 35 patients were treated based on the putative primary site and 32 patients were treated by empiric chemotherapy. The one-year OS rates were 55.3% versus 27.1% (HR, 0.34; 95% CI, 0.15

to 0.75) for the patients in the IHC-guided therapy group compared with patients in the empirical therapy group (Figure 4).

The overall certainty of the evidence is 'very low'. Patients whose primary site of tumour was identified using protein biomarkers by IHC and received molecularly guided therapy may have a better OS outcome when compared with those whose primary site of tumour was not identified using IHC.

Figure 4. Studies using protein biomarkers measured by IHC



4. Mixed gene expression and broad DNA mutations and fusions using NGS approaches

One comparative study and one single-arm study were analyzed. In the comparative study by Hasegawa et al [44], 56 of 90 patients in the EG who had gene expression and/or broad DNA mutations were predicted to have certain cancers and received site-specific chemotherapy. The most predicted primary cancer sites were gastrointestinal (20 patients; 36%), gynecological (12 patients; 21%), non-small cell lung cancer (6 patients; 11%), pancreas (4 patients; 7%), and neuroendocrine (4 patients; 7%). The remaining 34 patients in the EG received platinum empiric chemotherapy. All 32 patients in the CG did not have molecular testing and received platinum empiric chemotherapy directly. The median OS was 15.7 months versus 10.7 months (p=0.07) between the EG and CG, respectively. When comparing those who received site-specific chemotherapy (n=56) with the CG (n=32), the median OS was 20.3 months versus 10.7 months, respectively (HR, 0.57; 95% CI, 0.34 to 0.94) (Figure 5). Multivariable analysis controlled for Eastern Cooperative Oncology Group performance status, PFS, bone metastasis, and number of metastatic sites.

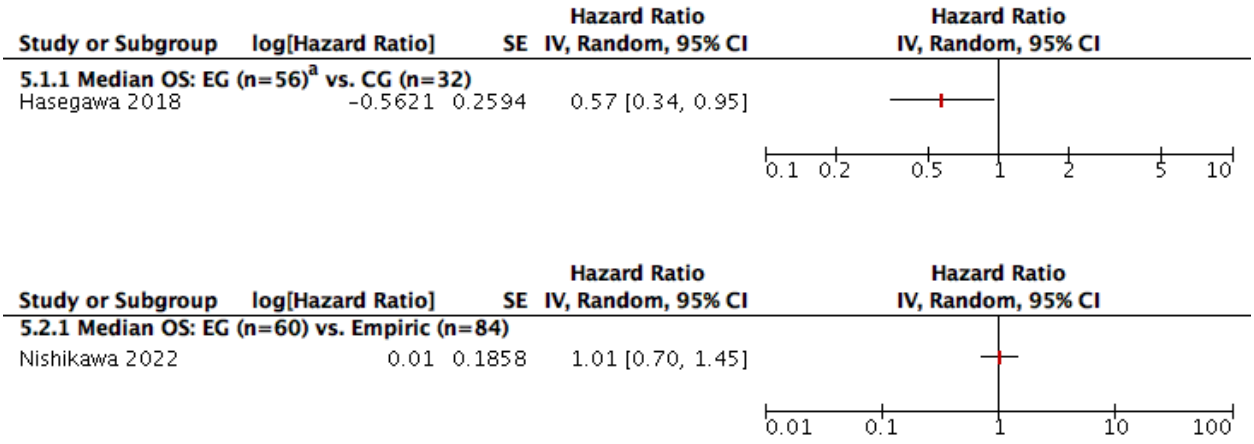
The certainty of the evidence result is 'very low' (Appendix 6.C). Mixed gene expression and broad DNA mutations assessed using NGS approaches may have a small effect on median OS; however, it is important to note the data come from a comparative study contributing to very low confidence in the overall results.

The retrospective single-arm study by Nishikawa et al [30] reported on 177 patients, 33 patients with favourable CUP and 144 patients with unfavourable CUP, who received ≥1 regimen of chemotherapy or chemoradiotherapy for CUP as first-line therapy. Patients in the unfavourable group received empiric or site-specific treatment. Of the 33 patients in the favourable group, 30 patients (91%) had a cancer site prediction, including small cell lung cancer (10 patients, 33%), ovarian (10 patients, 33%), breast (6 patients, 20%), and head and neck (4 patients, 13%). Of the 144 patients in the unfavourable group, 60 patients (42%) had a cancer site prediction, with the most predicted being non-small cell lung cancer (13 patients,

22%), gastric (12 patients, 20%), colon (8 patients, 13%), pancreatic (7 patients, 12%), and ovarian (4 patients, 7%). In the unfavourable group, the median OS in patients who received site-specific therapy (n=60) compared with those who received empiric therapy (n=84) was 10.0 months versus 10.1 months (HR, 1.01, 95% CI, 0.70 to 1.45) with a mean difference of -0.1 months (Figure 5).

The certainty of the evidence result is 'very low' (Appendix 5, Table A5-3). Mixed gene expression and broad DNA mutations assessed using NGS approaches may have a small effect on median OS; however, it is important to note the data come from comparative and retrospective studies contributing to very low confidence in the overall results.

Figure 5. Studies using mixed gene expression and broad DNA mutations and fusions using NGS approaches



[a] n=56 patients with site-specific chemotherapy

Diagnostic Outcomes (Predicted Cancer Sites)

The study by Greco et al [50] provided diagnostic outcome data of molecular profiling tools. Of the 171 patients who had protein biomarker expression measured by IHC, a single diagnosis of the tissue of origin was made in 59 patients; and among 149 patients who had adequate tumour specimens to run gene expression using PCR-based platforms, 144 received a predicted diagnosis of the tissue of origin. However, only 24 patients had the final clear diagnosis of primary cancer type. Among these 24 patients, the sensitivity was 25% for IHC and 75% for PCR (Table 3).

Table 3. Outcomes of diagnostic study

Study; Country	Number of patients (n_1)	Median age, yrs (range)	Sex	Molecular profiling tool	Number of patients with predicted cancer site after molecular profiling (n_2)	Reference standard	Number of patients with final diagnosis after reference standard (n_3)	Sensitivity in patients who had final diagnosis of tumour sites	Prevalence of final diagnosis (n_3/n_1)
Greco et al, 2013 [50]; USA	171	59 (24-85)	Female, 53% Male, 47%	Gene expression using microarray, NGS or PCR based platforms	144 of 149 (96%) pts with adequate tumour specimens had a predicted diagnosis.	Clinical follow-up including biopsy and imaging examination	24	75% (18/24)	14% (24/171)
				IHC	59 of 171 (35%) pts were predicted to have one cancer site.			25% (6/24)	

Abbreviations: IHC, immunohistochemistry; NGS, Next-Generation Sequencing; PCR, polymerase chain reaction; pts, patients; yrs, years

Ongoing, Unpublished, or Incomplete Studies

The Clinical Trials Registration database (http://www.clinicaltrials.gov/) was searched on August 16, 2024 using the terms "Cancers of Unknown Primary" OR "CUP" for trials meeting the inclusion criteria for this systematic review. Three trials were found and are summarized in Appendix 8.

DISCUSSION

To our knowledge, this is the first systematic review that investigates the roles of molecular profiling tools in the diagnosis and management of patients with CUP. Although the certainty of the evidence is 'low' or 'very low' for each comparison or per study on the management topics and 'moderate' for the diagnostic study, several messages from the evidence are consistent. First, with respect to identifying the site of the CUP, only one study met our study selection criteria to report diagnostic outcomes [50]. Although the primary cancer site was predicted in 59 patients through protein biomarkers measured by IHC and in 144 patients by gene expression using microarray or PCR-based platforms, only 24 patients had a confirmed diagnosis of the primary cancer. However, it should be noted that this study was published 10 years ago. Thus, more high-quality diagnostic studies are necessary to investigate this area. It is important to recognize that diagnostic outcomes serve as proxies for patient outcomes [52], and accordingly, to assess whether the use of molecular profiling tools can be directly linked to changes in therapies and patient outcomes.

In the use of molecular profiling tools that target gene expression, a meta-analysis of the three RCTs demonstrates that the molecular testing group has little effect on increasing median OS (mean difference, 2.39 months; 95% CI, -2.73 to 7.52) and median PFS (mean difference, 1.34 months; 95% CI, -0.37 to 3.05) [15,16,39]. In molecular tools targeting broad DNA mutations and fusions using NGS approaches, one RCT showed that molecular testing may result in a slight increase in median OS (mean difference, 3.7 months; 95% CI, 3.51 to 3.89) and in median PFS (mean difference, 1.7 months; 95% CI, 1.64 to 1.76) [9]; a retrospective study also shared similar findings in OS [1]. For molecular profiling tools targeting mixed gene expression and broad DNA mutations and fusions, a comparative study reported that molecular testing may increase the median OS (mean difference, 5 months; 95 CI%, not calculable) [44]. Secondly, when targeting protein biomarkers by IHC, three single-arm studies indicated that after molecular testing, those who received molecularly guided therapy may have an improved OS outcome compared to those who did not receive molecularly guided therapies [45,47]. However, another single-arm study found that when targeting mixed gene expression and/or broad DNA mutations, patients with unfavourable CUP cancers who received molecularly guided therapy may have similar OS outcomes when compared to those who did not receive molecularly guided therapy, but the evidence is weak [30].

In future research, more high-quality RCTs are needed to focus on comparing patients' survival outcomes between patients with and without molecularly guided therapies. To avoid bias, the funders of the RCT should not have a role in study design, safety monitoring, data collection, data analysis, data interpretation, and writing of the report, as was the case in the study by Kramer et al [9]. It should also be noted that survival outcomes can vary based on the predicted primary cancer types. For example, study populations with an increased predicted primary cancer type of breast cancer would have better survival outcomes due to established treatment protocols and generally improved prognosis. As a result, an RCT study design is ideal as single-arm study designs present a high risk of bias. Additionally, including subgroup analyses for different cancer types, responsive types, levels of tumour mutational burden, molecularly guided chemotherapy vs. target therapy/immunotherapy, etc. will be beneficial for improved decision-making for patient care. Finally, not all patients who undergo molecular profiling will

receive molecular guided therapy. Improving molecular profiling tests to indicate more linked treatments is another critical area.

This systematic review has some limitations. As this review focused on the use of molecular profiling tools and their effects on survival benefits, adverse effects data of the molecularly guided treatment options were not collected. The rationale for this exclusion was that (1) Studies consist of heterogenous predicted primary cancer types for CUP patients leading to varying adverse effects from the target therapies, making it challenging to compare them among studies. (2) In general, biomarker status linked to the targeted therapy or immunotherapy has fewer adverse effects than empiric therapy (platinum-based) [53,54]. The literature search was limited to English-language publications which may have led to the exclusion of relevant articles published in other languages. Therefore, readers should consider these limitations when applying the results to their clinical practice and research.

CONCLUSIONS

This systematic review indicates that the use of molecular profiling tools show promise in identifying the cancer of origin and may improve survival outcomes by guiding treatment. The results from future RCTs or high-quality comparative studies addressing known confounders will confirm and clarify the roles of molecular profiling tools in guiding the treatment of CUP to improve patient outcomes.

INTERNAL REVIEW

The evidence summary was reviewed by Chika Arinze. The Working Group was responsible for ensuring any necessary changes were made.

Acceptance by the Molecular Oncology and Testing Advisory Committee

MOTAC has reviewed the document throughout the document development stages as well as the final systematic review, and formally accepted the document.

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Appendix 1. Affiliations and Conflict of Interest Declarations

Table A1-1. Members of the Cancer of Unknown Primary Guideline Development Group

Name	Affiliation	Declarations of interest
Samuel Saibil Medical oncologist	Princess Margaret Cancer Centre Toronto, ON	Has received $500 or more in a single year to act in a consulting capacity (i.e., Advisory Boards) for Medison, BMS and Novartis
Harriet Feilotter Molecular geneticist	Kingston Health Sciences Centre Kingston, ON	None declared
Michael Vickers Medical oncologist	The Ottawa Hospital Ottawa, ON	Has received $500 or more in a single year in a consulting capacity from Merck
Paul Wheatley-Price Medical oncologist	The Ottawa Hospital Ottawa, ON	Has received $500 or more in a single year in a consulting capacity (i.e., Advisory Boards or speaker honoraria) from Merck, Astra Zeneca, BMS, Roche, Novartis, Pfizer, Bayer, Janssen, Jazz Pharmaceuticals, Guardant, Sanofi, Abbvie, Amgen, Lilly, EMD Serono, and Takeda; has been a principal investigator of a multicentre chart review of patients with CUP, and concordance with guidelines (unfunded study)
Ju-Yoon Yoon Pathologist	Unity Health Toronto Toronto, ON	Has received $500 or more in a single year to act in a consulting capacity (i.e., Advisory Boards) for Roche. Honoraria were received from Amgen. Grants were received from AstraZeneca, Amgen, Bayer, Merck, and Pfizer.
Duvaraga Sivajohanathan Health Research Methodologist	Program in Evidence-Based Care McMaster University Hamilton, Ontario	None declared
Xiaomei Yao Health Research Methodologist	Program in Evidence-Based Care McMaster University Hamilton, Ontario	None declared
Marisa Deodat Master's Student	Department of Public Health Sciences Queen's University Kingston, ON	None declared

Appendix 2. Literature Search Strategy

Database: OVID Medline Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid
MEDLINE(R) 1946 to Present
Search Strategy:
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1 (meta-analy: or metaanaly: or meta analy: or systematic review: or systematic overview:).mp. or ((exp "review"/ or exp "review literature as topic"/ or review.pt. or (review: or overview:).tw.) and (systematic: or selection criteria or data extraction or quality assessment or methodologic: quality or (study adj selection) or Cochrane or Medline or Embase or PubMed or Med-line or Pub-med or hand search: or hand-search: or manual search: or reference list: or bibliograph: or pooled analys: or statistical pooling or mathematical pooling or statistical summar: or mathematical summar: or quantitative synthes?s).tw.)
2 exp practice guideline/ or exp guideline/ or guideline.pt. or consensus development conference/ or practice guideline$.tw. or (guideline: or recommend: or consensus or standards).ti,kw.
3 1 or 2
4 (comment or news or newspaper article or historical article or editorial or note or letter or short survey).pt.
5 (exp animals/ or exp animal experiment/) not (humans/ or exp human/)
6 4 or 5
7 3 not 6
8 Neoplasms, Unknown Primary.mp.
9 (cancer of unknown primary or carcinoma of unknown primary).mp.
10 8 or 9
11 exp High-Throughput Nucleotide Sequencing/ or High-Throughput Nucleotide Sequencing.mp.
12 next generation sequencing.mp.
13 exp Immunohistochemistry/ or Immunohistochemistry.mp.
14 exp Gene Expression Profiling/ or Gene Expression Profiling.mp.
15 comprehensive genomic profiling.mp.
16 exp Biomarkers, Tumor/ or Biomarkers, Tumor.mp.
17 molecular profiling.mp.
18 exp In Situ Hybridization, Fluorescence/ or fluorescence in situ hybridization.mp. or FISH.mp.
19 exp Polymerase Chain Reaction/ or polymerase chain reaction.mp. or PCR.mp.
20 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21 10 and 20
22 7 and 21
23 limit 22 to yr="2018 -Current"
24 21 not 6
25 limit 24 to yr="2013 -Current"

Database: Embase <1996 to May 2024>
Search Strategy:
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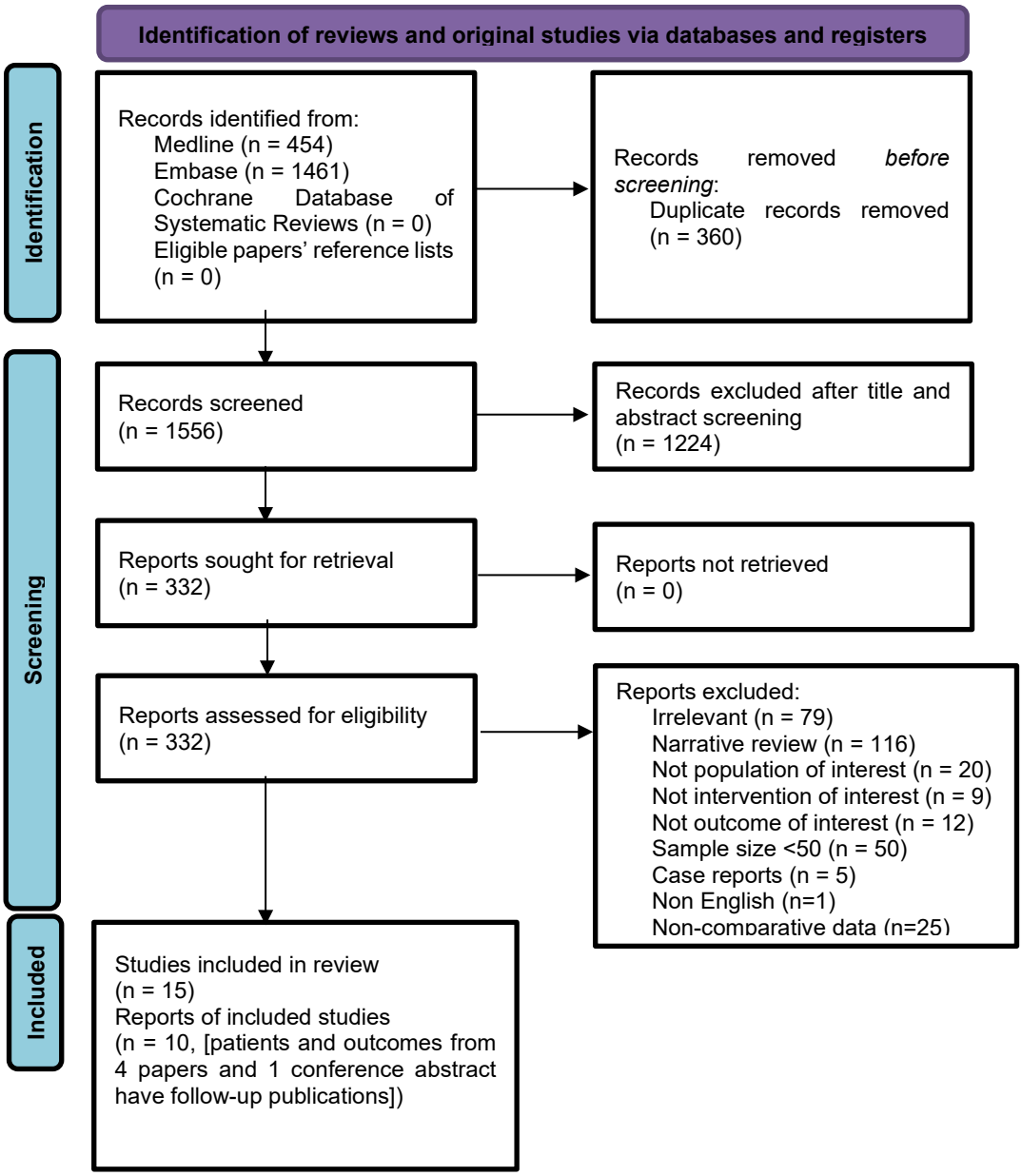
1 (meta-analy: or metaanaly: or meta analy: or systematic review: or systematic overview:).mp. or ((exp "review"/ or exp "review literature as topic"/ or review.pt. or (review: or overview:).tw.) and (systematic: or selection criteria or data extraction or quality assessment or methodologic: quality or (study adj selection) or Cochrane or Medline or Embase or PubMed or Med-line or Pub-med or hand search: or hand-search: or manual search: or reference list: or bibliograph: or pooled analys: or statistical pooling or mathematical pooling or statistical summar: or mathematical summar: or quantitative synthes?s).tw.)
2 exp practice guideline/ or exp guideline/ or guideline.pt. or consensus development conference/ or practice guideline$.tw. or (guideline: or recommend: or consensus or standards).ti,kw.
3 1 or 2
4 (comment or news or newspaper article or historical article or editorial or note or letter or short survey).pt.
5 (exp animals/ or exp animal experiment/) not (humans/ or exp human/)
6 4 or 5
7 3 not 6
8 cancer of unknown primary.mp. or exp "cancer of unknown primary site"/
9 carcinoma of unknown primary.mp.
10 8 or 9
11 exp high throughput sequencing/
12 next generation sequencing.mp.
13 immunohistochemistry.mp. or exp immunohistochemistry/
14 gene expression profiling.mp. or exp gene expression profiling/
15 comprehensive genomic profiling.mp.
16 biomarker$1.mp. or exp biological marker/
17 molecular profiling.mp. or exp molecular fingerprinting/
18 exp polymerase chain reaction/ or polymerase chain reaction.mp. or PCR.mp.
19 exp fluorescence in situ hybridization/ or fluorescence in situ hybridization.mp. or FISH.mp.
20 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21 10 and 20
22 7 and 21
23 limit 22 to yr="2018 -Current"
24 21 not 6
25 limit 24 to yr="2013 -Current"

Note: On Aug 21, We updated the literature search of Medline and Embase from Jan to Aug 2024 using the above search terms plus the following RCT search strategies and got 12 results. After reviewing titles and abstracts, two RCTs met our study selection criteria.

RCT search strategies:
exp phase 3 clinical trial/ or exp "phase 3 clinical trial (topic)"/ or exp clinical trial, phase iii/ or exp clinical trials, phase iii as topic/ or exp phase 4 clinical trial/ or exp "phase 4 clinical trial (topic)"/ or exp clinical trial, phase iv/ or exp clinical trials, phase iv as topic/ or exp randomized controlled trial/ or exp "randomized controlled trial (topic)"/ or exp randomized

controlled trials as topic/ or exp controlled clinical trial/ or "controlled clinical trial (topic)"/ or controlled clinical trials as topic/ or exp randomization/ or exp random allocation/ or exp double-blind method/ or exp single-blind method/ or exp double blind procedure/ or exp single blind procedure/ or exp triple blind procedure/ or exp placebos/ or exp placebo/ or ((exp phase 2 clinical trial/ or exp "phase 2 clinical trial (topic)"/ or exp clinical trial, phase ii/ or exp clinical trials, phase ii as topic/ or exp clinical trial/ or exp prospective study/) and random$.tw.) or (((phase II or phase 2 or clinic$) adj3 trial$) and random$).tw. or ((singl$ or double$ or treble$ or tripl$) adj3 (blind$ or mask$ or dummy)).tw. or (placebo?).tw. or (allocat: adj2 random:).tw. or (rct or phase III or phase IV or phase 3 or phase 4 or randomi$: or randomly).tw. or (random$ adj3 trial$).mp. or "clinicaltrials.gov".mp.

Appendix 3. PRISMA Flow Diagram



Appendix 4. Risk of bias assessment

Table A4-1. Risk of bias assessment table for RCTs

Study		Domain 1: Randomization Process	Domain 2: Deviation from Intervention	Domain 3: Missing Outcome Data	Domain 4: Measurement of Outcome	Domain 5: Reported Results	Overall Risk of Bias	
							Per outcome	Per study if needed
Hayashi 2019	Median OS	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
	Median PFS	Low	Some concerns	Low	Some concerns	Low	Some concerns	
Kramer 2024	Median OS	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
	Median PFS	Low	Some concerns	Low	Some concerns	Low	Some concerns	
Liu 2024	Median OS	Low	Some concerns	Low	Low	Low	Some concerns	Some concerns
	Median PFS	Low	Some concerns	Low	Some concerns	Low	Some concerns	

Abbreviations: OS, Overall survival; PFS, Progression-free survival; RCT, Randomized controlled trial

Table A4-2. Risk of bias assessment for comparative study

Study	Outcome	Domain 1: Bias due to confounding	Domain 2: Bias in selection of participants into the study	Domain 3: Bias in classification of interventions	Domain 4: Bias due to Deviation from Intended Intervention	Domain 5: Bias due to Missing Data	Domain 6: Bias in Measurement of Outcome	Domain 7: Bias in selection of the Reported Results	Overall Risk of Bias	
									Per outcome	Per study if needed
Hasegawa 2018	OS	Moderate to serious	Low	Low	Low	Low	Low	Moderate	Moderate	Moderate

Appendix 5. GRADE summary of finding tables

Table A5-1: Studies using gene expression

Certainty assessment							№ of patients		Effect	Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Gene expression**	Empirical	**Mean difference;** HR **(95% CI)**		
Median overall survival (months)											
3	randomised trials	not serious	serious[a]	not serious	extremely serious[b]	not serious	279 participants	276 participants	2.39 (-2.73 to 7.52) months; 0.85 (0.67 to 1.07)	⊕◯◯◯ very low	Critical
Median progression-free survival (months)											
3	randomised trials	not serious[d]	serious[a]	not serious	extremely serious[b]	not serious	279 participants	276 participants	1.34 (-0.37 to 3.05) months; 0.89 (0.72 to 1.10)	⊕◯◯◯ very low	Critical

Abbreviations: CI, Confidence interval; HR, Hazard ratio; RCT, Randomized controlled trial

Explanations

[a.] The point estimate of the mean difference from these three RCTs fell in different directions in Figure 1.1.1 (two RCTs fell on the right side of the mean difference of "0" and one RCT fell on the left side). Thus, the inconsistency domain was downgraded by 1 level of certainty.

[b.] The 95% CI of HR for median overall survival crossed 2 threshold lines (i.e., HR=0.75 and HR=1). Thus, the imprecision domain was downgraded by 2 levels.

[c] Since this outcome is not an objective judgment outcome, due to the blinding issue, we can downgrade 1 level for the risk of bias domain. However, in real life, we think it is unlike to lead to the risk of bias, and also, no matter whether we downgrade this domain or not, the overall certainty is "very low". Thus, we did not downgrade for this domain.

Table A5-2: Study using broad DNA fusions

Certainty assessment							№ of patients		Effect	Certainty	Importance
Study	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Simple DNA mutation	Empirical	Mean difference; HR (95% CI)		
Median overall survival											
Kramer 2024	randomised trials	not serious	not serious	not serious	very serious[a]	none	326	110	3.70 (3.51 to 3.89) months; 0.82; 95% CI, 0.62 to 1.09	⊕⊕◯◯ Low	Critical
Median progression-free survival											
Kramer 2024	randomised trials	Serious[b]	not serious	not serious	serious[c]	none	326	110	1.70 (1.64 to 1.76) months; 0.72; 95% CI, 0.56 to 0.92	⊕⊕◯◯ Low	Critical

Abbreviations: CI, Confidence interval; HR, Hazard ratio

Explanations

a. The 95% CI of HR for median overall survival crossed 2 threshold lines (i.e., HR=0.75 and HR=1). Thus, the imprecision domain can downgrade 2 levels.

b. Since this outcome is not an objective outcome, and since "The funder of the study had a role in study design, provision of study drugs, protocol development, regulatory and ethics approvals, safety monitoring, data collection, data analysis, data interpretation, and writing of the report, in collaboration with the study authors.", we downgrade 1 level for the risk of bias domain.

c. The 95% CI of HR for median progression-free survival crossed 1 threshold line (i.e., HR=0.75). Thus, the imprecision domain can downgrade 1 level.

Table A5-3: IHC-guided treatment compared with non-IHC treatment for patients with CUP

Certainty assessment							№ of patients		Effect	Certainty	Importance
Study	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	IHC-guided treatment	Conventional treatment			
Median overall survival											
Hasegawa 2018	Non-randomized comparative study	serious[a]	not serious	not serious	very serious[b]	none	90	32	20.3 mths vs. 10.7 mths; HR, 0.57; 95% CI, 0.34 to 0.94	⨁◯◯◯ Low	Critical

Abbreviations: CI, confidence interval; HR, Hazard ratio; IHC, Immunohistochemistry; mths, months; OS, Overall survival

Explanations

[a.] Due to the flaw of the study design, the unknown confounders can not be controlled. Thus, we downgraded 1 level.

[b.] The 95% CI of HR for median OS crossed 2 threshold lines (i.e., HR=0.5 and HR=0.75). Thus, the imprecision domain can downgrade 2 levels.

Appendix 6. Assessing risk of bias and overall quality for the diagnostic study

Table A6-1. Risk of bias assessment using QUADAS-2

Study	Risk of Bias				Applicability Concerns			Overall
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard	
Greco 2013	L	L	U	U	L	L	U	Moderate

Abbreviations: H = high risk, L = low risk, U = unclear.
The QUADAS-2 tool was used and we **assumed** that if any two or more items are "H", the overall quality of the study is considered as "Low"; if one item is "H" and less than or equal to two items are "U", or any three or more items are "U", the overall quality of the study is considered as "Moderate"; for the rest of studies, the overall quality of the study is considered as "High".

Appendix 7. Ongoing, unpublished or incomplete trials

Website: https://clinicaltrials.gov/

Search terms: Cancers of Unknown Primary OR CUP

Search dates: Aug 16, 2024 (361 hits)

PaCIFiC-CUP: Pan-Cancer Integrated Fingerprinting Classifier for Identifying the Origin of Cancer of Unknown Primary: A Multi-Center Bidirectional Cohort Study	
Protocol ID:	NCT06140992
Type of trial:	Observational
Primary endpoint:	Overall survival
Accrual:	160
Sponsorship:	Sun Yat-sen University
Status:	Recruiting
Date last updated:	November 21, 2023
Estimated study completion date:	December 2025
The Value of Molecular Biological Analysis of Blood Samples in Standardized Care Procedures in Suspected Cancer (SCAN) and Cancer of Unknown Primary (CUP)	
Protocol ID:	NCT04025970
Type of trial:	Observational
Primary endpoint:	Possibility of cellular and genomic sampling as part of the standardised care process
Accrual:	200
Sponsorship:	Christer Ericsson
Status:	Unknown
Date last updated:	October 4, 2019
Estimated study completion date:	December 2021 (contacted the author and received a reply on Aug 19th, 2024 that they don't have any results to publish, the trial is still ongoing)
Enabling Genomic Testing in Cancer of Unknown Primary (EGGCUP)	
Protocol ID:	NCT06695494
Type of trial:	Observational
Primary endpoint:	The utility of cfDNA molecular profiling in patients diagnosed with CUP
Accrual:	100
Sponsorship:	The Christie NHS Foundation Trust
Status:	Recruiting
Date last updated:	November 19, 2024
Estimated study completion date:	December 2027

Abbreviations: CUP, cancer of unknown primary