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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

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Table of Contents

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

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 1% to 2% of all cancer diagnoses globally [3]. 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% to 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 [4]. This stands in contrast to a subset of CUP patients, the favourable risk group, that present with limited disease amenable to curative intent or treatment with local therapies or a clinical presentation highly suggestive of tissue of origin, such as women with isolated axillary lymph nodes [5].

Patients diagnosed with unfavourable-risk CUPs frequently face limited treatment options, often relying on empiric chemotherapy regimens such as taxanes and platinum-based therapies [6]. However, these treatments have yielded only modest improvements in outcomes, with median overall survival (OS) ranging from six to 15 months [6]. 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 [2,7].

Molecular profiling has emerged as a promising approach to address the challenges associated with CUP [8]. 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 [9]. 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 on the identification of targetable mutations. The integration of molecular diagnostics into standard care for CUP patients holds the potential for substantial improvements in clinical outcomes, including prolonged progression-free survival (PFS) and enhanced quality of life [10].

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

molecular tools for the diagnosis and treatment of CUP. The categories of molecular analysis 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 QUESTION

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 up to 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 molecular tools: (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 the following outcomes: predicted cancer sites or theoretically actionable alterations with management changes and 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, or 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 [11]; 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 [12]. 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 [13].

For studies that only reported diagnostic outcomes, the QUADAS-2 tool was used to assess the quality [14].

Synthesizing the Evidence

A meta-analysis was not conducted due to the heterogeneity of the trials. For studies that did not provide a hazard ratio (HR) with a 95% confidence interval (CI), the HR and the 95% CI were calculated, whenever possible, using data provided in the paper (i.e., measuring data in a Kaplan-Meier curve). When the 95% CI was incalculable, the p-value between the two comparative groups is presented, as reported.

RESULTS

Literature Search Results

The search for systematic reviews retrieved 197 articles, 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 [1,6,10,15-51]. Five publications were excluded as more detailed follow-up publications were available [6,36-39]. Ultimately, 35 studies were analyzed [1,10,15-35,40-51]. A PRISMA flow diagram [52] detailing the reasons for study exclusion is included in Appendix 3.

Among the 35 eligible studies, 34 [1,10,15-35,40-50] investigated the clinical utility of molecular testing and one study [51] focused on the diagnostic accuracy outcomes of molecular profiling for identifying primary tumour sites.

Of the 34 treatment-related studies, there were four RCTs [10,16,40], of which one is currently available in abstract form [17], and one comparative study [45]. In these five studies, patients in the experimental group (EG) underwent molecular profiling. Molecular profiling was used to potentially identify the tissue of origin or identify tumour agnostic actionable mutations to refine the treatment received from empiric chemotherapy. In patients where molecular profiling was unable to refine treatment, treatment similar to empiric chemotherapy (control group) was provided. The survival outcomes of all patients in the experimental group were compared with those in the control group.

All patients in each of the 29 single-arm studies received molecular testing (i.e., everyone was in the experimental group). Two [1,31] of the 29 single-arm studies reported

comparative survival outcomes between patients who received site-specific therapy versus those who, despite having had molecular profiling, received empirical therapy. 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 seven included studies. The biomarker categories assessed by each study are shown in Table 1.

The remaining 27 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 information may be useful, it does not provide any decision-making data and the studies are not further discussed in this document.

Table 1. Molecular tool categories for the seven 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 [16], Hayashi et al, 2019 [40], Fizazi et al, 2019 [17]	No studies met inclusion criteria	Kramer et al, 2024 [10] Fusco et al, 2022 [1]	No studies met inclusion criteria	Nishikawa et al, 2022 [31] Hasegawa et al, 2018 [45]

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 [10,16,40] and one non-randomized comparative study [45]. The fourth RCT was currently published in abstract form and could not be assessed [17]. 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 single-arm studies, a risk of bias assessment was not conducted as these studies have a high risk of bias due to having no control group in the study design, 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 [51] (Table A4-3, Appendix 4).

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

Two fully published RCTs [16,40] and one conference abstract [17] met the study selection criteria. The overall certainty of the evidence result was 'very low' (Appendix 5, Table A5-1).

All three trials used a tumour of origin approach. The RCTs by Hayashi et al and Fizazi et al used microarray analysis to determine the tumour of origin, a methodology that has been rendered obsolete by NGS methodologies. These studies, however, are discussed because they provide evidence of proof of concept that even utilizing older molecular tools can potentially affect the treatment selection and clinical outcomes of patients with CUP. Patients in all three trials did not receive any systemic therapy before study enrollment.

In the RCT by Hayashi et al [40], patients with metastatic CUP were randomized to receive empirical chemotherapy (i.e., paclitaxel and carboplatin; n=65) or site-specific therapy based on microarray profiling (n=65) to determine the most likely tissue of origin. All patients received a prediction of primary tumour site with the five most common being pancreas (16.9%), gastric (16.9%), lymphoma (26.2%), cervix (7.7%) and ovary (7.7%) in the site-specific therapy arm. One hundred and one patients were evaluated in the efficacy analysis (n=51 and 50, respectively); however, a sample size of 114 was needed to provide 80% power. In the efficacy analysis, receiving site-specific therapy did not improve median OS (9.8 mo [95% CI, 5.7 to 13.8] vs. 12.5 mo [95% CI, 8.9 to 16.1]; HR, 1.028; 95% CI, 0.678 to 1.560; p=0.896) or median PFS (5.1 mo [95% CI, 1.9 to 8.3] vs. 4.8 mo [95% CI, 3.3 to 6.5]; HR, 0.884; 95% CI, 0.590 to 1.326; p=0.550) when compared with patients in the control group.

The RCT by Fiyazi et al [17] was published as a conference abstract. Patients with metastatic CUP were randomized to receive cisplatin plus gemcitabine (n=120) or site-specific therapy based on the results of a gene expression test (n=123). The most predicted primary cancer types were pancreaticobiliary (19%), squamous cell carcinoma (11%), kidney (8%), and lung (8%). Ninety-one of 123 (74%) patients received site-specific treatment; the treatment of the remaining 32 patients was not specified. Receiving site-specific therapy did not improve median OS (10.7 mo vs. 10 mo [HR, 0.92; 95% CI, 0.69 to 1.23]) or median PFS (5.3 mo vs. 4.6 mo [HR, 0.95; 95% CI, 0.72 to 1.27; p=0.7]).

The study by Liu et al 2024 used the contemporary 90-gene expression assay to predict tumour of origin in patients. Patients with CUP were randomized to receive empirical chemotherapy (n=91) or site-specific therapy (n=91) [16]. The primary cancer type was predicted in 83 of 91 patients. 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%). Forty-one (45%) of the patients in the site-specific therapy group received targeted therapy or immunotherapy. In the control group, 85 of 91 patients received empirical chemotherapy for a maximum of six cycles (taxane plus platinum; or gemcitabine plus platinum). The median PFS for the site-specific therapy and control groups was 9.6 months (95% CI, 8.4 to 11.9) versus 6.6 months (95% CI, 5.5 to 7.9; HR, 0.68; 95% CI, 0.49 to 0.93; p=0.017), respectively. The median OS for patients in the in the site-specific therapy group was 28.2 months (95% CI, 23.3 to 46.5) versus 19.0 months (95% CI, 17.1 to 26.4; HR, 0.75; 95% CI, 0.53 to 1.08; p=0.098) for the control group; however, this study was not powered to detect changes in overall survival.

Table 2: Outcomes of studies evaluating gene expression using microarray, NGS or PCR-based platforms

Study; country;	Sample size; number of cycles of previous systemic therapy	Median age, yrs (range)	Sex	Predicted primary cancer type after molecular profiling[a], n (%)	Intervention	Survival outcomes
Randomized controlled trials						
Liu et al, 2024 [16]; China FUDAN	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	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 mo (95% CI, 23.3 to 46.5) vs. 19.0 mo (95% CI, 17.1 to 26.4) HR, 0.75; 95% CI, 0.53 to 1.08 **Median PFS** EG vs. CG 9.6 mo (95% CI, 8.4 to 11.9) vs. 6.6 mo (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	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 [40]; Japan	EG: 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	50 (77%) pts received site-specific therapy (48 pts received site-specific chemotherapy and 2 pts received targeted therapy).	**Median OS[b]** EG vs. CG 9.8 mo (95% CI, 5.7 to 13.8) vs. 12.5 mo (95% CI, 8.9 to 16.1) HR, 1.028; 95% CI, 0.678 to 1.560 **Median PFS** EG vs. CG 5.1 mo (95% CI, 1.9 to 8.3) vs. 4.8 mo (95% CI, 3.3 to 6.5) HR, 0.884; 95% CI, 0.590 to 1.326
	CG: 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	51 (78%) pts received paclitaxel and carboplatin.	
Fizazi et al, 2019 [17] *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%	91 pts (74%) received site-specific treatment; treatment of remaining 32 pts was not specified.	**Median OS** EG vs. CG 10.7 mo vs. 10 mo HR, 0.92; 95% CI, 0.69 to 1.23 **Median PFS** EG vs. CG 5.3 mo vs. 4.6 mo HR, 0.95; 95% CI, 0.72 to 1.25
	CG: 120; 0	NR	NR	NA	All pts received cisplatin + gemcitabine	

Abbreviations: CG, comparative group (patients who didn't receive any molecular profiling); CI, confidence interval; CUP, cancer of unknown primary; EG, experimental group (patients who received molecular profiling); HR, hazard ratio; mo, months; NA, not applicable; NGS, next-generation sequencing; NR, not reported; OS, overall survival; PFS, progression-free survival; pts, patients; vs, versus

[a] The 5 most common types are presented

[b] Data from the efficacy analysis with 50 and 51 patients in each group, respectively

2. Broad DNA mutations and fusions using NGS approaches

One RCT [10] and one retrospective study [1] met the study selection criteria for this category. The overall certainty of the evidence result is 'low' (Appendix 5, Table A5-2).The CUPISCO trial by Kramer et al [10] used complete genome profiling, an NGS approach, to compare the efficacy of molecularly guided therapy with standard platinum-based chemotherapy in patients with unfavourable, non-squamous CUP. This study used a tumour agnostic approach by examining targetable genomic alterations. Six-hundred thirty-six patients were enrolled in this study and 436 patients reached randomization after induction chemotherapy without progression. Three hundred twenty-six patients were randomized to platinum-based chemotherapy plus molecularly guided therapy and 110 patients were randomized to platinum-based chemotherapy alone. Among the 326 patients, 88 patients received therapies targeting genomic alterations or fitting a genomic signature, and the remaining 238 patients received atezolizumab plus chemotherapy. The median PFS in the intention-to-treat population was longer in patients who received molecularly guided therapy (6.1 mo [95% CI, 4.7 to 6.5]) versus those in the control group (4.4 mo [95% CI, 4.1 to 5.6]; HR of 0.72; 95% CI, 0.56 to 0.92; p=0.0079). The mean difference of median PFS was 1.7 months (95% CI, 1.64 to 1.76). 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 receiving molecularly guided therapy compared with those in the control group, respectively, with an HR of 0.82 (95% CI, 0.62 to 1.09). The mean difference of median OS was 3.7 months (95% CI, 3.51 to 3.89).

The retrospective, single-arm study by Fusco et al [1] included 95 patients with CUP, who received NGS testing. While NGS identified options for molecularly guided treatment in 55% of the patients, 17 (18%) patients received molecularly guided therapy with 14 receiving a diagnosis with a predicted cancer type. The difference in median OS between patients who received molecularly guided therapy (23.6 mo) and those who did not (14.7 mo; HR, 0.57; 95% CI, 0.268-1.205; p=0.13) was 8.9 months (HR, 0.57; 95% CI, 0.27 to 1.20). Further, NGS assisted with a diagnosis in 14 (15%) patients with the assistance of cinical features, imaging and pathology. 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%).

Table 3. Outcomes of studies evaluating broad DNA mutations and fusions using NGS approaches

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	Intervention	Survival outcomes
Randomized controlled trial							
Krämer et al, 2024 [10]; 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	**Median OS (interim analysis)** EG vs. CG 14.7 mo (95% CI, 13.3 to 17.3) vs. 11.0 mo (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 mo (95% CI, 4.7 to 6.5) vs. 4.4 mo (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	
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 assistance from 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*)	OncoKB[b] 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 mo vs 14.7 mo HR, 0.568; 95% CI, 0.268 to 1.205

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	Intervention	Survival outcomes
				Upper GI adenocarcinomas, 1 (*confirmed gastroesophageal adenocarcinoma*) MSI-High colon cancer, 1 (*confirmed colon*) Atypical rhabdoid/teratoid: 1 (*confirmed*)			

Abbreviations: CG, comparative group (patients who didn't receive any molecular profiling); CI, confidence interval; EG, experimental group (patients who received molecular profiling); EG1, experimental group 1 (patients in this group received molecularly guided therapy); EG2, experimental group 2 (patients in this group received empirical therapy although they underwent molculer profiling); GI, gastrointestinal; HR, hazard ratio; ITT, intent to treat; MSI, microsatellite Instability; mo, months; NA, not applicable; NGS, next-generation sequencing; NR, not reported; NSCLC, non-small cell lung cancer; OncoKB, Oncology Knowledge Base; OS, overall survival; PFS, progression-free survival; pts, patients; RUSA, United States of America; vs, versus.

[a] The 5 most common types are presented

[b] 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).

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

Two studies were found which used mixed methods. The certainty of the evidence result is 'very low' (Appendix 5, Table A5-3).

One comparative study and one single-arm study were analyzed. Hasegawa et al [45] conducted a retrospective, comparative study of 122 patients with unfavourable CUP at two different time points. This study used an unvalidated IHC panel and gene analysis to predict the tumour of origin in 90 patients after July 2012 and compared them with 32 patients who received platinum-based empiric chemotherapy before June 2012. In this study, 56 of 90 patients had predicted primary sites and received site-specific chemotherapy and the remaining 34 patients received platinum empiric chemotherapy similar to the control group. 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 median OS was 15.7 months versus 10.7 months (p=0.07) between those receiving site-specific therapy and empiric chemotherapy, respectively. When comparing those who received site-specific chemotherapy (n=56) with the control group (n=32), the median OS was 20.3 months versus 10.7 months, respectively, with a multivariable analysis controlling for Eastern Cooperative Oncology Group performance status, PFS, bone metastasis, and number of metastatic sites. (HR, 0.57; 95% CI, 0.34 to 0.94; p=0.03)

The retrospective, single-arm study by Nishikawa et al [31] reported on 177 patients with CUP, 33 patients in the favourable subset and 144 patients in the unfavourable subset. All patients received ≥1 regimen of chemotherapy or chemoradiotherapy for CUP as first-line therapy. Patients in the unfavourable group received empiric or site-specific treatment. Site-specific treatment consisted of chemotherapy or chemoradiotherapy based on treatment guidelines for each site. Tumour of origin was estimated using IHC, gene mutation, and molecular analysis results with only 29 patients (16.4%) receiving gene mutation and molecular analysis. 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; p=0.45) with a mean difference of -0.1 months (Figure 5).

Table 4. Outcomes of studies evaluating mixed simple DNA mutations and protein biomarkers using IHC

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 (%)	Intervention	Survival outcomes
Retrospective, comparative study						
Hasegawa et al, 2018 [45]; 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	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 mo vs. 10.7 mo; p=0.07. EG (n=56 with site-specific therapy) vs. CG (n=32) 20.3 mo vs. 10.7 mo 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	32 pts received platinum empiric chemotherapy	
Retrospective, single-arm study						
Nishikawa et al, 2022 [31]	177 (33 favourable, 144 unfavourable pts[b]); ≥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	**Median OS** 24.2 mo (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	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 mo vs. 10.1 mo HR, 1.01, 95% CI, 0.70 to 1.45; p=0.95

Abbreviations: CG, comparative group (patients who didn't receive any molecular profiling); CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; EG, experimental group (patients who received molecular profiling);; EG1, experimental group 1 (patients in this group received site-specific therapy); EG2, experimental group 2 (patients in this group received empirical therapy although they underwent molecular profiling); HR, hazard ratio; mo, months; NA, not applicable; NR, not reported; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; pts, patients; SCLC, small cell lung cancer; vs, versus

[a] The 5 most common types are presented.

[b] 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 of a serous papillary histological type in females, osteoblastic bone metastases of adenocarcinoma with positive IHC staining of prostate-specific antigen or elevated serum PSA in males, liver or peritoneal metastases of adenocarcinoma with a colorectal cancer immunoprofile (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

Diagnostic Outcomes (Predicted Cancer Sites)

One study provided diagnostic outcome data of molecular profiling tools. Greco et al [51] compared molecular tumour profiling diagnoses with the latent primary sites found, the gold standard. 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 for molecular tumour profiling using a 92-gene RT-PCR, 144 received a predicted diagnosis of the tissue of origin. However, only 24 patients had anatomic primary sites identified. Among these 24 patients, the sensitivity was 25% for IHC and 75% for PCR (Table 5).

Table 5. 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 [51]; USA	171	59 (24-85)	Female, 53% Male, 47%	Molecular tumour profiling assay	144 of 149 (96%) pts with adequate tumour specimens had a predicted diagnosis.	Biopsy and imaging examination	24	75% (18/24)	14% (24/171)
				Single 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 6.

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. The studies included in this review used molecular tools to potentially identify the tissue of origin or identify tumour agnostic actionable mutations to refine the treatment received from empiric chemotherapy, which is known to offer little clinical benefit in terms of PFS and OS. 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. In the use of molecular profiling tools that target gene expression, two RCTs using microarray analysis were included, even though microarray analysis is no longer used. The rationale for this is that both studies demonstrated the ability to assign a putative tissue of origin for the majority of patients enrolled. Accordingly, it was deemed that these studies were of sufficient quality to provide insight into the potential for clinical benefit by leveraging a molecular tool that allows for a site-specific treatment approach for CUP patients, albeit an older molecular tool. While both studies showed no improvement in survival outcomes, it is important to note that even for those assigned to site-specific therapy, they primarily received chemotherapy rather than targeted therapy or immunotherapy. Further, for many predicted cancer types, the site-specific therapy was similar to the empirical chemotherapy regimen which would contribute to similar survival results. The efficacy analysis for the trial by Hayashi et al was not powered for analysis. The RCT by Liu et al used a contemporary method for gene analysis and reported longer median PFS in patients who received site-specific therapy. It is important to note that 45% of the patients in the site-specific therapy group received targeted therapy or immunotherapy. This trial demonstrates the positive impact on survival of CUP patients with access to targeted therapy and immunotherapy. This study was conducted in a single centre and was limited to Asian patients, which may limit its generalizability; future research in mixed populations is recommended.

The CUPISCO trial used complete genome profiling, using both tissue and liquid biopsies, for a tumour agnostic approach by determining the targetable genomic alterations in patients with unfavourable, non-squamous CUP after induction chemotherapy. In the experimental group, 27% of patients received therapies targeting genomic alterations or fitting a genomic signature, and the remaining 73% of patients received atezolizumab plus chemotherapy, whereas all patients in the control group received platinum-based chemotherapy. This study demonstrated an increase in median PFS (mean difference, 1.7 mo; 95% CI, 1.64 to 1.76). While the data must mature, the significance of the CUPISCO trial is that in a large cohort of poor risk CUP patients, it demonstrates that approximately one third of CUP patients have a targetable genomic alteration. Moreover, by leveraging modern molecular NGS approaches to identify these tissue agnostic alterations and to tailor treatment according to mutation, superior clinical outcomes for patients were seen.

The remaining studies in this review were retrospective and included comparative results. However, the outcome from these studies is subject to a high risk of bias and had small patient numbers. Further, as mentioned above, it is important to note that many patients who received site-specific therapy also received regimens similar to the empirical chemotherapy regimen.

With respect to identifying the site of the CUP, one study met our study selection criteria in reporting diagnostic outcomes [51]. Although the primary cancer site was predicted in 59 patients through protein biomarkers using IHC and in 144 patients by gene through molecular tumour profiling using a 92-gene RT-PCR, 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 [53], and accordingly, there is a need to assess whether the use of molecular profiling tools can be directly linked to changes in therapies and patient outcomes.

In future research, more high-quality RCTs are needed to focus on comparing 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, or writing of the report, as was the case in the study by Kramer et al [10]. 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, the inclusion of subgroup analyses for cancer types, levels of tumour mutational burden, and molecularly guided chemotherapy vs. targeted therapy/immunotherapy 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 for future research.

This systematic review has some limitations. This review focused on the use of molecular profiling tools and their effects on survival benefits, and data on adverse effects of the molecularly guided treatment options were not collected. Two considerations explain this exclusion: (1) Studies consist of heterogenous predicted primary cancer types for CUP patients leading to varying adverse effects from targeted 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) [54,55]. We included RCTs, and where RCTs were not available, studies presenting comparative results. While this allowed us to include the highest level of evidence, relevant single-arm studies were not discussed. 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 highlights the complexities of the existing literature in patients with CUP. While the use of molecular profiling tools shows promise in identifying the tissue of origin or tumour agnostic actionable mutations, its published effect on survival outcomes by guiding treatment has been limited due to study design; however, improved survival has been shown in patients who have received immunotherapy or targeted therapy. The results from future RCTs or high-quality comparative studies addressing known confounders will confirm and clarify the role of molecular profiling tools in patients with CUP.

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"

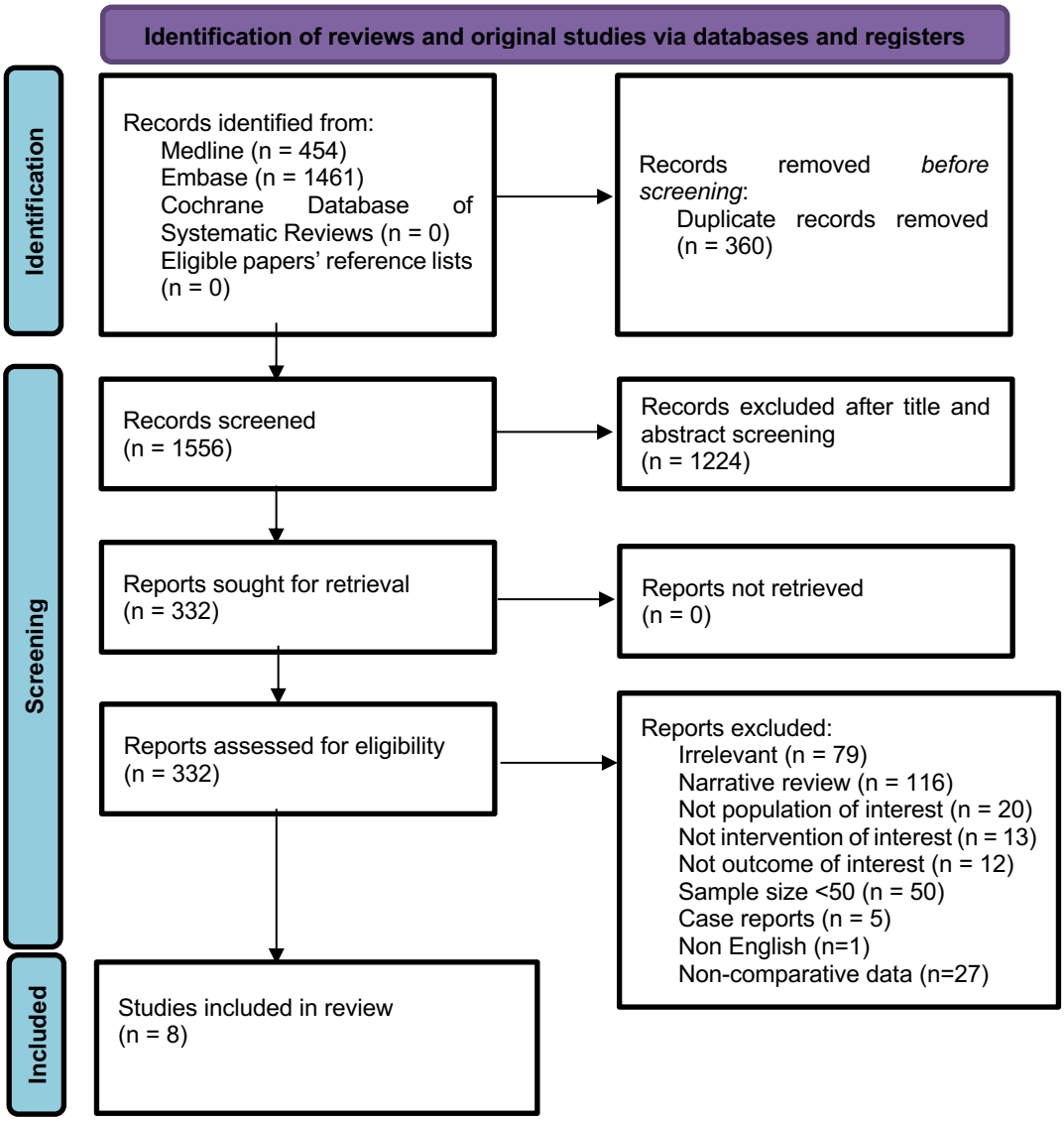
Database: Embase <1996 to May 2024>
Search Strategy:
---

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

RCT search strategy:
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 for RCTs using the Cochrane Collaboration Risk of Bias 2.0 tool

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 et al, 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 et al, 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 et al, 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 using the ROBINS-I tool

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 et al, 2018	OS	Moderate to serious	Low	Low	Low	Low	Low	Moderate	Moderate	Moderate

Abbreviations: OS, overall survival

Table A4-3. 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 et al, 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 ≥2 items are "H", the overall quality of the study is "Low"; if one item is "H" and ≤2 items are "U", or ≥3 items are "U", the overall quality of the study is "Moderate"

Appendix 5. GRADE summary of finding tables

Table A5-1: Studies evaluating gene expression using microarray, NGS or PCR-based platforms

Certainty assessment							№ of patients		Effect	Certainty	Importance
№ of studies	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Experimental**	Control	**Mean difference; HR (95% CI)**		
Median overall survival (months)											
3	RCTs	not serious	serious[a]	not serious	extremely serious[b]	not serious	279	276	Not calculated	⨁◯◯◯ very low	Critical
Median progression-free survival (months)											
3	RCTs	not serious[c]	serious[a]	not serious	extremely serious[b]	not serious	279	276	Not calculated	⨁◯◯◯ 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 one level.

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

[c] Since this outcome is subjective, we can downgrade one level for the risk of bias due to blinding. However, realistically, it is unlikely to lead to risk of bias. Further, whether or not we downgrade this domain, the overall certainty is still "very low". Thus, we did not downgrade for this domain.

Table A5-2: Study evaluating broad DNA mutations and fusions using NGS approaches

Certainty assessment							№ of patients		Effect	Certainty	Importance
Study	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Experimental**	Control	**Mean difference; HR (95% CI)**		
Median overall survival											
Kramer et al, 2024	RCT	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 et al, 2024	RCT	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 two threshold lines (i.e., HR=0.75 and HR=1). Thus, the imprecision domain was downgraded 2 levels.

b. Since this outcome is subjective, and "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 downgraded one level for the risk of bias domain.

c. The 95% CI of HR for median progression-free survival crossed one threshold line (i.e., HR=0.75). Thus, the imprecision domain wasdowngraded one 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	Experimental	Control			
Median overall survival											
Hasegawa et al, 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, unknown confounders cannot be controlled. Thus, we downgraded one level.

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

Appendix 6. 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