Site-specific therapy in cancers of unknown primary site: a systematic review and meta-analysis

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Background: Cancer of unknown primary site (CUP) is a term applied to characterize pathologically confirmed metastatic cancer with unknown primary tumor origin. It remains uncertain whether patients with CUP benefit from site-specific therapy guided by molecular profiling.

Patients and methods: A systematic search in PubMed, Web of Science, Embase, Cochrane Library, and ClinicalTrials.gov, and of conference abstracts from January 1976 to January 2021 was performed to identify studies investigating the efficacy of site-specific therapy on patients with CUP. The quality of included studies was evaluated using the Cochrane risk of bias tool and Newcastle-Ottawa scale. Eligible studies were weighted and pooled for meta-analysis. Hazard ratios (HRs) for overall survival (OS) and progression-free survival (PFS) were assessed to compare the efficacy of site-specific therapy with empiric therapy in patients with CUP. In addition, subgroup analyses were conducted.

Results: Five studies comprising 1114 patients were identified, of which 454 patients received site-specific therapy, and 660 patients received empiric therapy. Our meta-analysis revealed that site-specific therapy was not significantly associated with improved PFS [HR 0.93, 95% confidence interval (CI) 0.74-1.17, P = 0.534] and OS (HR 0.75, 95% CI 0.55-1.03, P = 0.069), compared with empiric therapy. However, during subgroup analysis significantly improved OS was associated with site-specific therapy in the high-accuracy predictive assay subgroup (HR 0.46, 95% CI 0.26-0.81, P = 0.008) compared with the low accuracy predictive assay subgroup (HR 0.93, 95% CI 0.75-1.15, P = 0.509). Furthermore, compared with patients with less responsive tumor types, more survival benefit from site-specific therapy was found in patients with more responsive tumors (HR 0.67, 95% CI 0.46-0.97, P = 0.037).

Conclusions: Our results suggest that site-specific therapy is not significantly associated with improved survival outcomes; however, it might benefit patients with CUP with responsive tumor types.

Key words: cancer of unknown primary site, site-specific therapy, empiric therapy, meta-analysis

INTRODUCTION

Cancer of unknown primary site (CUP) is a term used to characterize pathologically confirmed metastatic cancer for which clinicians are unable to identify a primary tumor, despite a standard and comprehensive diagnostic work-up.1,2 CUP accounts for 1%-5% of all malignancies,3-7 and it has been reported to be the fourth most common cause of cancer-related deaths worldwide.8 The prognosis for patients with CUP is dismal: survival of most patients is generally <1 year after diagnosis.9,10 At present, no consensus has been reached on the mechanisms underlying the pathogenesis of CUP. One predominant hypothesis is that CUP originates from a small, dormant, or later regressed primary lesion.11 CUP remains an under-researched entity with limited treatment options.2,12 Up to now, there is no specific regimen that can be recommended as a standard of care. Most patients with CUP have to be treated with empiric chemotherapy, such as taxane- or platinum-based regimens,13,14 which result in a low response rate and poor survival.15,16 Patients with CUP have been documented to have worse outcomes than patients with metastatic cancer originating from a known primary tumor.1,17
Accordingly, it might be beneficial to identify the potential primary tumor and select the treatment approach. Identifying the tissue of origin (TOO) of CUP is a crucial step toward more optimized and precise treatment schemes. Gene expression profiling (GEP) is a novel diagnostic approach that allows the prediction of the site of tumor origin based on gene expression patterns retained from the known primary tumor. Based on GEP analysis, we also developed several new bioinformatics methods for identifying putative primary tumors. In addition, multiple methods based on various omics, including genomics and epigenomics, have also been harnessed to predict the TOO of CUP.

The continued development of technology has enabled the emergence of site-specific treatment for CUP. Several studies have investigated the possible role of site-specific treatment as a treatment option in patients with CUP; however, there are inconsistencies within the published results. One meta-analysis from Rassy et al. found no significant improvement in overall survival (OS) with site-specific compared with empiric treatment for CUP. However, this review screened articles up to November 2019; details on the screening process were not provided, and analyses assessing publication bias, sensitivity analysis, and subgroup analysis were not performed. Moreover, another study that performed by Hasegawa et al. demonstrated the benefit of site-specific treatment for CUP by improving OS rates. However, this study was not included in the previous meta-analyses.

Given the limitations of previous reviews and the availability of additional data, we aimed to conduct an up-to-date systematic review and meta-analysis to better elucidate the role of site-specific treatment in patients with CUP.

**METHODS**

**Literature sources and search strategy**

A systematic literature search was performed in PubMed, Web of Science, Embase, Cochrane Library, and Clinical-Trials.gov from January 1976 to January 2021, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The conference abstracts of the American Society of Clinical Oncology (ASCO) and European Society of Medical Oncology (ESMO) meetings were also reviewed from January 2010 to January 2021. The search terms were as follows: [(cancer OR carcinoma* OR neoplas* OR malignan*) AND ('unknown primary' OR 'occult primary' OR 'primary metastatic')] AND [(Therapeutics) OR (Therapeutic) OR (Therapy) OR (Therapies) OR (Treatment) OR (Treatments)] AND [(overall survival) OR (os) OR (progression-free survival) OR (pfs) OR (survival progression free) OR (event free survival) OR (survival event free) OR (median survival time) OR (median survival times)]. Articles in the references were also searched if they were potentially relevant to this topic.

**Inclusion and exclusion criteria**

Published studies included in this meta-analysis had to meet the following inclusion criteria: (i) patients diagnosed with CUP (defined as metastatic tumors for which the standard diagnostic work-up failed to identify the site of origin at the time of diagnosis); (ii) studies comparing the difference of survival outcomes between site-specific therapy and empiric therapy in patients with CUP; and (iii) available survival data. Excluded studies included case reports, case series, reviews, meta-analyses, letters to the editor, and comments. Two independent researchers (YD and JJ) removed the duplicates, screened all titles and abstracts, and obtained full texts of eligible studies. Points of disagreement were reconciled by a discussion with a third researcher (JX).

**Data extraction and quality assessment**

Two independent researchers (YD and JJ) collected, extracted, and summarized the data from the eligible studies. The extracted information consisted of the following: (i) the name of the first author; (ii) the year of publication; (iii) country/region; (iv) study type; (v) patient sex; (vi) therapy type; (vii) assay for predicting the site of tumor origin; (viii) accuracy of predictive assay; (ix) predicted site of tumor origin; (x) OS and progression-free survival (PFS). The predictive level of 80% was adopted as the cut-off with high confidence for classifying molecular assays to the groups with high (≥80%) versus low (<80%) TOO prediction accuracy. More responsive tumor types were defined as those with a median survival of >12 months with standard treatment, such as colorectal cancer, breast cancer, ovarian cancer, kidney cancer, prostate cancer, bladder cancer, and non-small-cell lung cancer. Less responsive tumor types included cholangiocarcinoma, pancreatic cancer, gastroesophageal cancer, liver cancer, sarcoma, and cervical cancer. Hazard ratio (HR) with the corresponding 95% confidence interval (CI) for OS was directly extracted from the original articles. When the articles did not provide HR and 95% CI, we contacted the corresponding authors by e-mail to retrieve missing data. If no reply was received, we measured the Kaplan–Meier curves of these articles using Engauge Digitizer version 4.1 (free software downloaded from http://sourceforge.net) and extracted the HR and 95% CI into an Excel workbook (Microsoft, Redmond, WA) as Tierney et al. reported. The quality of the studies was assessed using the Cochrane risk of bias tool for randomized controlled clinical trials (RCTs) and Newcastle–Ottawa scale (NOS) for non-randomized controlled clinical trials (NRCTs). Two independent researchers (YC and WJ) evaluated the bias risk independently. Points of disagreement were reconciled by a discussion with a third researcher (YJ).

**Statistical analysis**

The meta-analysis was performed using R software (version 3.6.1; R Foundation, Vienna, Austria), and P < 0.05 was considered statistically significant. Considering the limited
number of eligible studies, two RCTs and three high-quality NRCTs (NOS score ≥6) were all enrolled for data synthesis. Meanwhile, due to the potential heterogeneity, we further performed separate meta-analyses for the RCTs and NRCTs in the subgroup analysis. For four included studies, the value of HR and corresponding 95% CI were directly recorded according to the original report. For the study reported by Hainsworth et al., the value of HR and corresponding 95% CI were extracted from the Kaplan—Meier curves using Engauge Digitizer version 4.1 and into an Excel workbook as Tierney et al. reported. For OS and PFS analysis, pooled HR and corresponding 95% CI were used to assess the survival benefit of site-specific therapy in comparison with empiric therapy in patients with CUP. Random-effects models were fitted with inverse variance weighting to combine data from different studies for meta-analysis. Sensitivity analysis was performed to verify the robustness of the results. For subgroup analysis, patients were stratified according to the variables, including study type, the predictive accuracy of assay, country/region, and duration of the enrollment. The Cochran Q statistic and the \( I^2 \) value were calculated to measure the interstudy heterogeneity, which was defined as low (\( I^2 < 25\% \)), moderate (\( I^2 = 25\% - 75\% \)), or high (\( I^2 > 75\% \)). Publication bias was visualized by funnel plot and estimated using Begg’s and Egger’s tests, where \( P < 0.10 \) was considered statistically significant.

RESULTS

Eligible studies and study characteristics

A total of 2835 potentially eligible studies were initially identified from the systematic literature search, as shown in Figure 1. After removing the duplicates from the different databases (n = 1074), irrelevant studies (n = 1738) were excluded by title and abstract screening. A total of 23 studies were assessed for eligibility. Fourteen were excluded due to unavailable survival data. Two uncompleted clinical

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of the search process in the meta-analysis.
Table 1. Study characteristics

| Characteristics          | Hainsworth et al. (2012)\(^{31}\) | Moran et al. (2016)\(^{26}\) | Hasegawa et al. (2018)\(^{30}\) | Hayashi et al. (2019)\(^{28}\) | Fizazi et al. (2019)\(^{15}\) |
|--------------------------|----------------------------------|-----------------------------|-------------------------------|-------------------------------|-------------------------------|
| Study design             | NRCT                             | NRCT                        | NRCT                          | RCT                           | RCT                           |
| Study type               | Prospective                      | Retrospective               | Prospective                   | Prospective                   | Prospective                   |
| Included time            | October 2008 to December 2011    | March 2011 to December 2015 | January 2010 to January 2016  | October 2008 to February 2015 | March 2012 to February 2018   |
| Country/region           | America                          | Japan                       | America/Europe                | Japan                         | Europe                        |
| Research center\(^a\)   | Multiple                         | Single                      | Multiple                      | Multiple                      | Multiple                      |
| Sex, n (%)               | NA                               | 56 (61)                     | NA                            | 59 (58)                       | NA                            |
|                          | Male                             | 36 (39)                     | NA                            | 42 (42)                       | NA                            |
|                          | Female                           | NA                          | NA                            | NA                            | NA                            |
| Therapy received\(^b\), n (%) | Empiric 396 (67)               | 61 (66)                     | 32 (36)                      | 51 (50)                       | 120 (49)                     |
|                          | Site-specific 194 (33)           | 31 (34)                     | 56 (64)                      | 50 (50)                       | 123 (51)                     |
| Assay\(^c\)              | RNA-92-gene assay               | DNA methylation             | Organ-specific immunohistochemical markers and gene analysis | Microarray gene expression | RNA-92-gene assay             |
| Accuracy in known tumors, % | 74%-77%                        | 87%-100%                    | Not provided in this study and reached >80% in previous reports.\(^d\) | 78.6%                         | 74%-77%                      |
| Top 3 predicted sites, % | 1 Biliary tract cancer (21%)    | Breast cancer (19%)         | Gastrointestinal cancer (36%) | Lymphoma (26%)                | Pancreaticobiliary cancer (19%) |
|                          | 2 Urothelium cancer (12%)       | Non-small-cell lung cancer (16%) | Gynecological cancer (21%)  | Gastric cancer (17%)           | Squamous cell carcinoma (11%) |
|                          | 3 Colorectal cancer (11%)/lung cancer (11%) | Hepatocellular cancer (13%) | Non-small-cell lung cancer (9%) | Pancreatic cancer (17%) | Kidney cancer (8%)/lung cancer (8%) |
| Predicted tumor types\(^e\), n (%) | Less responsive 79 (41)       | NA                          | NA                            | 71 (70)                       | NA                            |
|                          | More responsive 115 (59)        | NA                          | NA                            | 30 (30)                       | NA                            |
| Median OS (months)       | Empiric 9.1                      | 6                           | 10.7                          | 12.5                          | 10                            |
|                          | Site-specific 12.5              | 13.6                        | 20.3                          | 9.8                           | 10.7                          |
| Median PFS (months)      | Empiric NA                      | NA                          | 4.2                           | 4.8                           | 5.3                           |
|                          | Site-specific NA               | NA                          | 5.1                           | 5.1                           | 4.6                           |

CUP, cancer of unknown primary site; NA, not available; NRCT, nonrandomized controlled clinical trial; OS, overall survival; PFS, progression-free survival; RCT, randomized controlled clinical trial.
\(^a\) Multiple, patients from two or more research centers; single, patients from one research center.
\(^b\) Site-specific therapy, standard treatments for predicted sites of origin; empiric therapy, treatments without consideration of primary sites, which was based on oncologists’ experience in treating other people with similar characteristics (usually with a taxane plus platinum, or gemcitabine plus a platinum regimen).
\(^c\) The method for predicting the tissue of the origin in CUP.
\(^d\) The IHC panel could correctly classify primary sites with an accuracy of >80% in previous reports.
\(^e\) More responsive, the cancer types with more response to treatment (colorectal, breast, ovary, kidney, prostate, bladder, non-small-cell lung cancer, germ cell, poorly differentiated neuroendocrine, lymphoma, and small-cell lung cancers); less responsive, the cancer types with less response to treatment (biliary tract, pancreatic, gastroesophageal, liver, sarcoma, cervical, carcinoid, endometrial, mesothelioma, melanoma, skin, thyroid, head and neck, and adrenal cancers).
trials, one duplicate clinical cohort and one single-arm study, were also excluded. Finally, five studies, including 1114 patients, were considered in the meta-analysis.26-28,30,35 Table 1 shows a summary of included studies. All patients were diagnosed with CUP based on clinical and radiologic evaluation and immunohistochemistry analysis according to institutional standards.36,37 As shown in Table 1, 40.8% (454/1114) patients in the site-specific group received first-line chemotherapy based on the predicted tumor origin (details for site-specific treatment regimens are given in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2022.100407), whereas 59.2% (660/1114) patients in the empiric group received chemotherapy based on the oncologists’ experience (usually a taxane plus platinum, or gemcitabine plus a platinum regimen). The top three predicted primary sites in these five studies are shown in Figure 2 and exhibited a markedly inconsistent distribution. The quality assessment of included studies indicated that the data were of satisfactory quality for further analysis (RCTs shown in Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2022.100407; NRCTs shown in Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2022.100407).

The comparison of survival outcomes between patients receiving site-specific therapy and empiric therapy

Five studies, including 1114 patients, reported OS as a prognostic variable. Pooled estimates revealed that site-specific therapy was not significantly associated with improved OS (HR 0.75, 95% CI 0.55-1.03, P = 0.069; \( I^2 = 57\%), \( P = 0.05 \); Figure 3A), compared with empiric therapy. We found that PFS was reported in two studies (Fizazi et al.35 and Hayashi et al.28), and the pooled effect

![Figure 2. Schematic diagram showing the heterogeneity of predicted site of origin in cancer of unknown primary site across five included studies (i.e., 26,28,30,31,35). Top three predicted sites of origin in five studies were labeled in the box using different colors.](https://doi.org/10.1016/j.esmoop.2022.100407)
was not statistically significant (HR 0.93; 95% CI 0.74-1.17, 
P = 0.534; \( I^2 = 0\% \), \( P = 0.76 \), Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2022.100407). In addition, bias analysis revealed that publication bias was not negligible (Begg's test: \( P = 0.05 \), Egger's test: \( P = 0.08 \), Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2022.100407); accordingly, the trim and fill method was used to adjust for publication bias. The pooled HR value was still not significant (HR 0.836, 95% CI 0.582-1.201, \( P = 0.33 \), Supplementary Figure S4, available at https://doi.org/10.1016/j.esmoop.2022.100407), consistent with results obtained before the trim and fill analysis. Sensitivity analysis showed that the pooled HRs were not affected after excluding one study at a time (Supplementary Figure S5, available at https://doi.org/10.1016/j.esmoop.2022.100407), indicating the robustness of the results. These findings suggested that patients with CUP did not receive additional benefits from the site-specific therapy than empiric therapy.

Subgroup analysis

Subgroup analyses were performed to explore any potential source of heterogeneity and identify the subpopulation of patients with CUP who might benefit from site-specific therapy. First, we conducted a subgroup analysis among different study types; although the NRCTs showed benefit from site-specific therapy (HR 0.57, 95% CI 0.35-0.93, \( P = 0.024 \); \( I^2 = 53\% \), \( P = 0.12 \), Figure 3B), the two recently published RCTs did not support this result (HR 0.95, 95% CI 0.75-1.21, \( P = 0.671 \); \( I^2 = 0\% \), \( P = 0.66 \), Figure 3B). Furthermore, site-specific therapy significantly improved the OS of patients with CUP in the high accuracy predictive assay subgroup (HR 0.31, 95% CI 0.14-0.69, \( P = 0.004 \); \( I^2 = 56\% \), \( P = 0.07 \), Figure 3C) compared with the low accuracy predictive assay subgroup (HR 0.93, 95% CI 0.75-1.15, \( P = 0.509 \); \( I^2 = 0\% \), \( P = 0.78 \), Figure 3C). Moreover, subgroup analysis of studies from American/European and Asian populations indicated no significant difference in
OS between site-specific therapy and empiric therapy for patients with CUP (Figure 3D). Besides, we did not observe the survival benefit of site-specific therapy in the subgroups with either long or short duration time of the enrollment (Figure 3E). Furthermore, patients were divided into two groups according to the predicted tumor types into less and more responsive tumor types. It was observed that, after receiving site-specific therapy, an improved OS was found in patients with more responsive tumor types than in those with less responsive tumor types (HR 0.67, 95% CI 0.46-0.97,

![Table A](image1)

| Study            | logHR | seTE | Hazard Ratio Random, 95%CI | HR   | 95% CI | Weight |
|------------------|-------|------|----------------------------|------|--------|--------|
| Haisworth 2012   | -0.46 | 0.3109 |                          | 0.63 | (0.34-1.15) | 37.4% |
| Hayashi 2019     | -0.38 | 0.2402 |                          | 0.69 | (0.43-1.10) | 62.6% |
| **Random effects model** | | | | **0.67** | **(0.46-0.97)** | **100.0%** |

Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $P = 0.83$

![Diagram B](image2)

**Figure 4.** Meta-analysis of the overall survival by comparing the more responsive group with the less responsive group receiving the site-specific therapy.

HR, hazard ratio; CI, confidence interval; CUP, cancer of unknown primary site.


**DISCUSSION**

CUP is a clinically well-recognized but enigmatic disease entity, which remains under-researched. Patients with CUP face dismal prognoses, and limited treatment options are available despite huge therapeutic advancements in other metastatic cancers. This study is the most up-to-date meta-analysis assessing the efficacy of site-specific treatment in patients with CUP to the best of our knowledge. Importantly, we found that site-specific therapy was not significantly associated with improved survival outcomes, compared with empiric chemotherapy. However, during subgroup analysis, we found that selecting an assay with reliable performance might improve survival outcomes and most importantly, site-specific therapy could improve outcomes in patients predicted with responsive tumor types compared with less responsive ones. These findings provide new insights on whether patients with CUP benefit from site-specific treatment and may help physicians and patients during treatment decision making.

The present meta-analysis was conducted to provide comprehensive updated evidence on the outcomes of site-specific therapy versus empiric chemotherapy for CUP. Engauge Digitizer was used to correct data from previously published meta-analyses and new data were included. First, the study by Hainsworth et al., which was also included in a previous meta-analysis, assessed the efficacy of tumor site-specific therapies in patients with unknown primary predicted using a 92-gene GEP assay (CancerTYPE ID; bioTheranostics, San Diego, CA). According to the CancerTYPE ID prediction, a comparison of the survival benefit between 194 patients treated with tumor site-specific therapy and a historical series of 396 patients with CUP treated with empirical chemotherapy was performed. Although Kaplan—Meier survival curves (assay-directed versus empiric treatment) were plotted to assess patient prognosis, Hainsworth et al. did not report the HR and CI in their article. Notably, we found that the HR and CI value used in the prior meta-analysis (HR 0.63, 95% CI 0.60-0.65) was much different from the actual Kaplan—Meier curves (Figure 2D in ). Thus, the Engauge Digitizer software was applied to obtain data from the Kaplan—Meier survival curves according to a method previously described, and the value of HR and CI (HR 0.82, 95% CI 0.51-1.32) was different from that reported by Hainsworth et al. Moreover, we included another retrospective study by Hasegawa et al. that assessed the effect of site-specific treatment on CUP. In this study, the authors reported the outcomes of 56 patients who received therapy tailored according to the primary tumor type predicted through the molecular pathological algorithms. A statistically significant OS benefit was found in patients who received therapy tailored to the primary tumor type compared with those who received empiric chemotherapy regimens (median OS duration 20.3 months versus 10.7 months; HR 0.57, 95% CI 0.34-0.94, 95% CI = 0.028).

We found that site-specific therapy was not significantly associated with improved survival outcomes, compared with empiric chemotherapy, showing a similar trend with a prior meta-analysis. We further explored the complexity and heterogeneity of the role of tailored therapies by subgroup analysis. Interestingly, significant heterogeneity was found within the top three primary tumor types predicted by the molecular pathological algorithm among the included five studies (Figure 2). The presence of significant heterogeneity may partially account for the inconsistency in study findings from the literature. Accordingly, more studies with larger sample sizes and meta-analyses are needed to substantiate the role of tailored therapies in patients with CUP. The ORIGIN-PanCA OR trial, an ongoing phase III, randomized, and open-label trial in which patients with treatment-naïve unfavorable CUP are enrolled (NCT03278600), could provide additional insights into this issue. This study has been designed to include 176 participants with CUP and evaluate the value of site-specific therapy guided by 90-gene expression assay profiling.

Moreover, in recent years, several molecular and pathological profiling methods have been established, including messenger RNA analysis, microRNA analysis, DNA mutation analysis, copy number variation analysis, liquid biopsies, and artificial intelligence-based pathology; the prediction accuracy of these assays reportedly range from 73% to 94%. In studies by Hainsworth et al. and Fizazi et al., a 92-gene reverse transcriptase-polymerase chain reaction was used for cancer classification (CancerTYPE ID; bioTheranostics, San Diego, CA), and the reported prediction accuracy was only 74%-77%. In addition, a randomized prospective phase II trial by Hayashi et al. failed to demonstrate the efficacy of site-specific therapy guided by molecular profiling, which could be accounted by the fact that primary site prediction was based on a poorly validated molecular test with an average prediction accuracy of 78.6% (10-fold cross-validation). The prediction accuracy of molecular profiling was <80% in these three studies that failed to demonstrate the efficacy of site-specific therapy. Meanwhile, a DNA methylation-based assay (prediction accuracy 87%-100%) was developed by Moran et al. to guide site-specific therapy, and better patient outcomes were reported. In the present study, during subgroup analysis, no significant improvement in OS was found with site-specific treatment compared with empiric treatment, which could be accounted for by the low TOO prediction accuracy assay (<80%). However, in the group using high TOO prediction accuracy, patients that received tumor site-specific therapy showed improved OS compared with those who received empiric therapy (HR 0.31, 95% CI 0.14-0.69, 95% CI = 0.004).

Another important factor that influences the comparison of therapeutic effect is the significant delay (the TOO prediction usually takes 2-3 weeks) before standard site-specific therapy for advanced cancer was administered. Accordingly, this emphasizes the necessity and importance of selecting an assay with quick, efficient, and reliable performance in the
future. In addition, we noticed the difficulty of recruiting patients with CUP due to its low prevalence and the relatively strict inclusion and exclusion criteria. For instance, the latest phase II and phase III randomized clinical trials took ≥6 years to enroll patients.28,35 The treatment in some site-specific arms, such as single-agent gemcitabine for pancreatic cancer or biliary tract cancers,28,31 is no longer considered the first-line treatment regimen.48,49 Therefore, refined cooperation at national and international level is essential to conduct more efficient clinical trials and improve our knowledge of this rare disease.

In a nonrandomized prospective study, Hainsworth et al.31 documented the significantly improved outcomes of patients with responsive tumor types (defined as those with a median survival of >12 months with standard treatment, such as colorectal, breast, ovary, kidney, prostate, bladder, and non-small-cell lung cancers) who received tumor type-specific therapy, compared with patients with less responsive tumor types (such as biliary tract, pancreas, gastroesophageal, liver, sarcoma, and cervix). However, in another randomized phase II trial,28 no statistically significant difference in the median OS duration was demonstrated in patients with responsive and less responsive tumor types (HR 0.69, 95% CI 0.43-1.10). Given the inconsistency of these results, we conducted a meta-analysis and found that site-specific therapy might improve outcomes in patients predicted with responsive tumor types based on molecular profiling diagnosis, compared with patients with less responsive tumor types (Figure 4). This finding suggested that site-specific therapy should be considered in patients with CUP predicted with more responsive tumor types. Accordingly, future prospective randomized trials should be carried out to substantiate our findings.

In recent years, with the development of next-generation sequencing technology, many effective molecularly targeted therapies have been developed for treating numerous types of tumors, such as therapies targeting neurotrophin receptor kinase (NTRK)50 or B-Raf proto-oncogene (BRAF).51 Therefore, in addition to site-specific therapy, next-generation sequencing profiling-based molecularly targeted therapy is another strategy that should be incorporated during the design of CUP trials in future. In addition, the advances made in the immunotherapy field have transformed the treatment landscape for many anatomically defined cancers.52-54 High microsatellite instability, programmed death-ligand 1 (PD-L1) expression, and high tumor mutational burden have been identified as pan-cancer biomarkers that can be used to assess the efficacy of immunotherapy.14,55 Interestingly, Haratani et al.56 demonstrated PD-L1 expression and tumor-infiltrating lymphocyte density in CUP and revealed potential benefits from immunotherapy. An ongoing phase II multicenter randomized trial (named CUPISCO trial, NCT03498521) is enrolling treatment-naïve unfavorable patients with CUP assigned to the targeted or immunotherapy treatment arms by genomic profiling. This trial will provide significant insights into whether targeted or immunotherapy treatment regimens could improve outcomes using comprehensive genomic profiling in previously untreated patients with CUP.

**Limitation**

Several limitations of the present study should be considered during the interpretation of our findings. Although this is the most up-to-date meta-analysis, it is still limited by the number of studies and patients. The nonrandomized nature of some included studies and heterogeneity in reporting could lead to missing significant confounding factors. Moreover, the results of one included RCT is currently available only in abstract form. Finally, the potential association between treatment benefit and toxicity was not evaluated.

**Conclusion**

This study provides comprehensive, up-to-date evidence on this important controversial subject. Site-specific therapy guided by molecular profiling may improve OS only when high-accuracy assays assign CUP to responsive tumor types. Our findings might provide the basis for the next generation of CUP trials.

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**DISCLOSURE**

The authors have declared no conflicts of interest.

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