CLINICAL PRACTICE

Cancer of Unknown Primary Site

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This Journal *feature begins with a case vignette highlighting a common clinical problem. Evidence supporting various strategies is then presented, followed by a review of formal guidelines, when they exist. The article ends with the author's clinical recommendations.*

A previously healthy 47-year-old woman presented with abdominal bloating and discomfort that had worsened over the previous 3 months. Her examination was notable for abdominal distention with bulging flanks and shifting dullness consistent with ascites. Further workup revealed a normocytic anemia (hemoglobin level, 10.4 g per deciliter; reference range, 12.0 to 14.0), elevated serum cancer antigen 125 level (168 U per milliliter; reference value, <38), and elevated carcinoembryonic antigen level (14.7 ng per milliliter; reference value, <3.8). A computed tomographic (CT) scan of her chest, abdomen, and pelvis with the use of contrast material showed liver, lymph node, and peritoneal tumors with ascites. She was referred to an oncologist because of suspicion of cancer, and an omental biopsy revealed poorly differentiated carcinoma with immunohistochemical assays positive for CK20, CDX2, and SATB2 (lower gastrointestinal tract immunostains) and negative for multiple other immunostains. A subsequent fluorodeoxyglucose (FDG) positron-emission tomographic (PET) scan showed FDG-avid liver, lymph node, and peritoneal metastases. Further testing with mammography, colonoscopy, and upper endoscopy failed to identify any primary site. Molecular profiling to find the tissue of origin and identify targetable genomic alterations was deemed indeterminate. How would you further evaluate and treat this patient?

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CME

THE CLINICAL PROBLEM

Cancer of unknown primary site is an ever-evolving disease entity and one of the most challenging diagnoses to manage in oncology.[1-3] The term "cancer of unknown primary site" encompasses a diverse group of histologically confirmed cancers that have metastasized by the time of presentation, with the primary site of origin eluding detection despite a standard diagnostic workup.[4-6] Patients with cancer of unknown primary site use more health care resources, including more investigations, emergency visits, and hospitalizations, than patients with known primary sites.[7,8]

Cancer of unknown primary site is an uncommon diagnosis that accounts for 2 to 4% of all cancers.[9] The American Cancer Society estimates that approximately 37,370 new cases of cancer of unknown primary site will be diagnosed in the United States in 2025.[10] Worldwide, incidence rates have been declining and range between 2 and 15 cases per 100,000 person-years.[9] Factors that have possibly contributed to this decreasing trend include enhanced diagnostic methods together with the expanded use of molecular profiling that can more accurately identify the primary site, improved quality of reporting, and better identification of key differential diagnostic elements, such as recurrence of antecedent cancers and cholangiocarcinoma.[11-14] As with all cancers, smoking, alcohol consumption, diabetes, and family

KEY POINTS

CANCER OF UNKNOWN PRIMARY SITE

- Cancer of unknown primary site is a heterogeneous group of histologically confirmed metastatic cancers, with the primary anatomical site of origin remaining unidentified after a standard diagnostic workup.
- Baseline evaluation involves a detailed history; physical, laboratory, and imaging assessments (ideally, a contrast-enhanced computed tomographic scan of the chest, abdomen, and pelvis); and a thorough pathological workup of adequate tumor tissue.
- Although immunophenotyping is the mainstay of diagnosis, recent advances have led to the integration of molecular profiling in predicting the tissue of origin and identifying targetable alterations in the management of cancer of unknown primary site.
- Both site-specific therapy (treatment of a putative primary site) and empirical chemotherapy (with a platinum-based cytotoxic regimen) are acceptable options for treatment.
- The overall prognosis for patients with cancer of unknown primary site remains poor. Participation in clinical trials should be encouraged.

history have been implicated as risk factors for cancer of unknown primary site.[15] Typically, patients present with incidental findings or clinical signs and symptoms of multiorgan metastases. Imaging and pathological examination reveal diverse histologic findings (adenocarcinoma in 59% of cases, poorly differentiated or undifferentiated carcinoma or neoplasm in 31%, and squamous carcinoma in 9%) with varied metastatic patterns (multiple sites in 33% of cases, liver in 25%, and lymph nodes in 7%, among others).[16,17]

Although cancer of unknown primary site has been classified into "favorable" and "unfavorable" subsets, these labels are evolving in the current era of molecular diagnostics and tailored therapies. Favorable cancer of unknown primary site refers to distinct clinicopathological manifestations in which the pattern of metastatic disease is typical of certain known cancers, such as breast cancer occurring with axillary lymphadenopathy (Table 1).[4-6] The favorable subset comprises 20% of cases of cancer of unknown primary site and is associated with a better prognosis than unfavorable disease.[18] The larger unfavorable subset of cancer of unknown primary site is associated with poor survival, although modest improvement has been noted in the past several years.[17-22] Survival outcomes in patients with cancer of unknown primary site appear to be worse than those in patients with metastatic cancers of known primary site, which suggests a more aggressive behavior of the former.[22] Male sex, poor performance status, histologic findings indicating adenocarcinoma, a high number of metastases, the presence of liver or peritoneal metastases, a high neutrophil–lymphocyte ratio, and molecular alterations (*KRAS* or *NRAS* mutation and *CDKN2A* deletion) have been implicated as adverse prognostic factors.[23-25]

Although the pathophysiologic mechanisms underlying cancer of unknown primary site remain unclear, a broad postulate has emerged, fostered by our understanding of the biologic underpinnings of metastases from known primary sites (Fig. 1).[26,27] One hypothesis is that cancer of unknown primary site is a metastatic syndrome with early and rapid dissemination from a primary tumor that either is very small or has regressed and is below the threshold of detection by contemporary diagnostic methods. This remarkably divergent behavior suggests a core programming distinct from cancers of known primary site that is driven by a prometastatic phenotype and ecosystem.[28,29] Evidence of heterogeneity and of biologic subtypes that are associated with distinct metastatic patterns, immunophenotypic and molecular profiles, and survival among persons with diverse cancers of known primary site furthers this hypothesis.[30-33]

STRATEGIES AND EVIDENCE

CLINICAL EVALUATION

The diagnosis of cancer of unknown primary site relies on a comprehensive workup aimed at evaluating for the presence of any primary lesion (Figs. 2 and 3).[4-6] However, the extent of diagnostic testing may be restricted by resource constraints, coexisting medical conditions, and the therapeutic window (the urgency of treatment dictated by the extent and pace of disease and the symptom burden). The clinical evaluation begins with obtaining a careful history, including

Table 1. Key Subsets within Cancer of Unknown Primary Site and the Therapeutic Implications.*

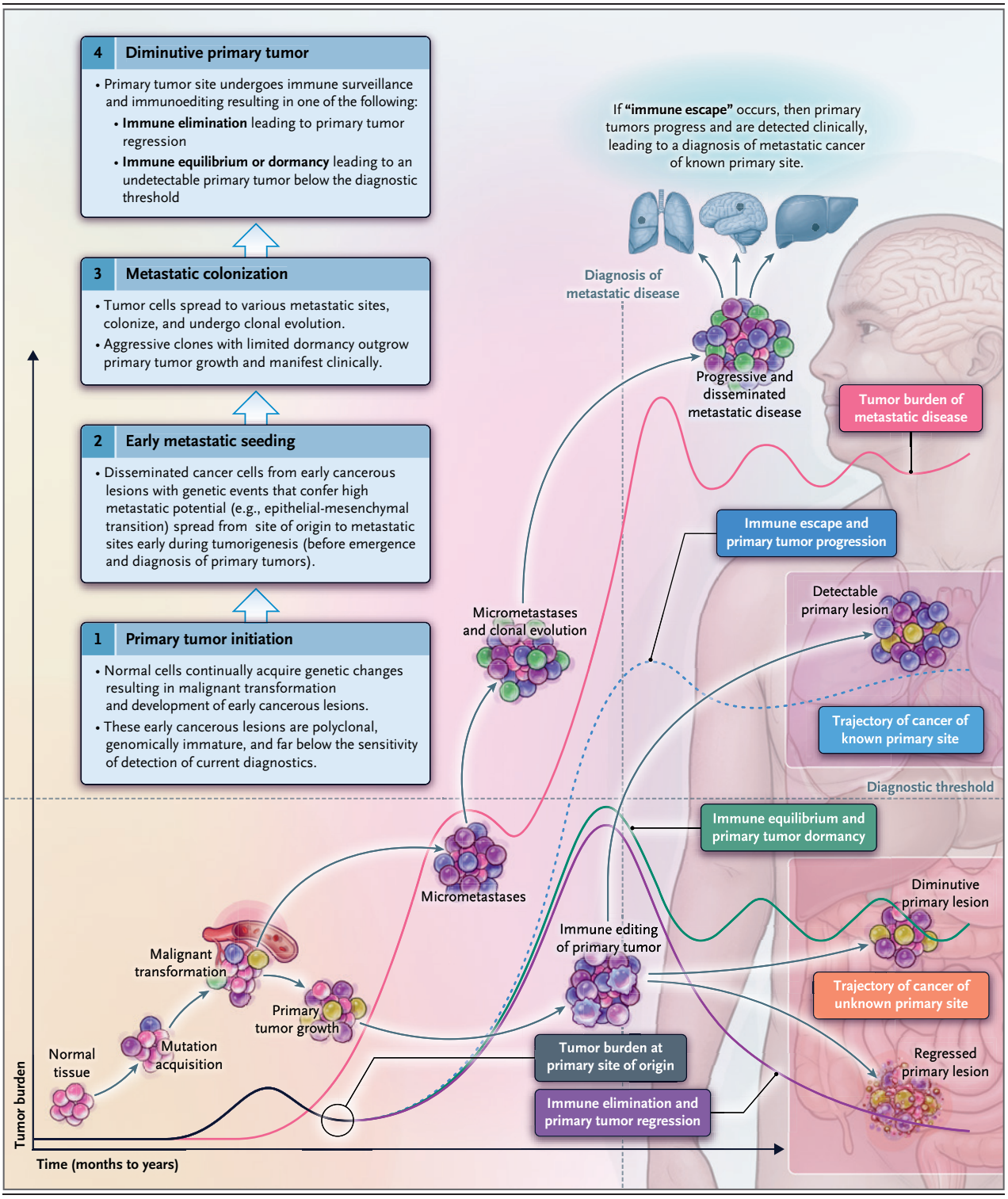
Manifestation	Analogous Known Primary Site	Therapeutic Strategy
Clinicopathological		
Blastic bone metastases with elevated prostate-specific antigen level (in men)	Prostate cancer	Combination androgen-deprivation therapy
Carcinoma (serous) with peritoneal carcinomatosis (in women)	Ovarian cancer	Chemotherapy (paclitaxel and carboplatin with or without bevacizumab)
Carcinoma with isolated axillary lymphadenopathy (in women)	Breast cancer	Chemotherapy, surgery, and radiation therapy (according to breast cancer guidelines)
Solitary or oligometastatic disease	Any tissue of origin	Chemotherapy with or without radiation therapy or chemoradiation with or without surgery
Squamous-cell carcinoma with cervical lymphadenopathy	Head and neck squamous-cell cancer	Surgery with or without radiation therapy or chemoradiation with or without chemotherapy (according to head and neck squamous-cell cancer guidelines)
Immunohistochemical		
CK20+ and CDX2+	Lower gastrointestinal or colorectal cancer	FOLFOX, XELOX, FOLFIRI, or FOLFOXIRI
Molecular		
BRAF V600E mutation	Any tissue of origin	Dabrafenib with trametinib
Fusions		
NTRK	Any tissue of origin	Entrectinib, larotrectinib, or repotrectinib
RET	Any tissue of origin	Selpercatinib
HER2 amplification or overexpression†	Any tissue of origin	Trastuzumab deruxtecan
Immunotherapy eligible		
Mismatch repair–deficient and microsatellite instability–high	Any tissue of origin	PD-1 and PD-L1 monoclonal antibodies
High tumor mutational burden‡	Any tissue of origin	Pembrolizumab

* FOLFIRI denotes folinic acid, fluorouracil, and irinotecan; FOLFOX fluorouracil, leucovorin, and oxaliplatin; FOLFOXIRI fluorouracil, leucovorin, oxaliplatin, and irinotecan; HER2 human epidermal growth factor receptor 2; PD-1 programmed cell death protein 1; PD-L1 programmed death ligand 1; and XELOX capecitabine and oxaliplatin.
† HER2 amplification or overexpression was defined as an immunohistochemical score of 3+.
‡ A high tumor mutational burden was defined as at least 10 mutations per megabase.

a review of family and personal history of previous cancers. A physical examination and baseline laboratory evaluation can help direct further workup. All patients should undergo imaging (ideally a CT scan with the use of contrast material) of the chest, abdomen, and pelvis. The use of adjunct diagnostic methods, such as PET scan, magnetic resonance imaging, mammography, testicular ultrasonography (in young men with midline lymphadenopathy), or invasive procedures (upper endoscopy, colonoscopy, bronchoscopy, laryngoscopy, or cystoscopy), should be guided by symptoms, imaging, or pathological findings indicative of a primary origin.[1] The diagnostic usefulness of any one test is limited, especially in the face of atypical biologic characteristics and patterns; therefore, the development of a management plan on the basis of the overall manifestation of the disease and the expertise of the multidisciplinary team is warranted (Figs. 2 and 3).

Immunophenotyping

Light microscopic examination of tumor tissue with immunophenotyping remains the mainstay in the diagnosis of cancer of unknown primary site. The accuracy of immunohistochemical testing for diagnosing tissue type is high, especially if adequate tumor tissue is assessed.[34,35] When possible, a core-needle biopsy is preferred to preserve features of tissue architecture that assist with diagnosis. During this process, close communication with a pathologist as a part of a multidisciplinary team can enable directed and judicious use of tissue for the immunohistochemical assay



since sufficient tumor tissue is also required for molecular profiling. Pathologists use an average of 8.8 immunostains (range, 0 to 20) in evaluating metastatic tumors, and nearly two thirds of patients with cancer of unknown primary site will have insufficient tissue left for molecular profiling during the course of their clinical care.[35,36] Despite this heavy reliance on immunohistochem-

Figure 1 (facing page). Development of Cancer of Unknown Primary Site.

The hallmark of cancer of unknown primary site is the presence of detectable metastatic disease without an identifiable primary lesion. The biologic features of cancer of unknown primary site, as opposed to cancer of known primary site, favor an aggressive phenotype with a propensity for early metastases and metastatic tumor outgrowth. While the metastatic disease grows, the primary tumor undergoes extensive immunoediting that is orchestrated by diverse anti- and protumorigenic immune cells and cytokines. Unlike cancer of known primary site, in which the primary tumor will progress by evading the immune system, cancer of unknown primary site involves a process that results in either immune elimination with regression of the primary tumor or an immune equilibrium (a state of dormancy) that leads to a subclinical primary lesion below the limits of diagnostic sensitivity. The graph shows tumor-burden growth over time in both metastatic and primary disease. Biologic features of the disease leading to differences between the primary tumor and the metastatic disease with respect to growth trajectory and diagnostic sensitivity result in the entities known as cancer of known primary site and cancer of unknown primary site.

ical testing, its accuracy in identifying a primary organ is limited, especially in cases of poorly differentiated tumors.[35] Because no one immunostain is pathognomonic of the tissue of origin, a panel of tests performed in a stepwise fashion may be warranted.[37] The use of deep-learning methods for determining the tissue of origin may be the next frontier in the pathology of cancer of unknown primary site.[38]

Molecular Profiling for the Prediction of Tissue of Origin

Molecular profiling in cancer of unknown primary site has been used to predict a putative primary site (tissue-of-origin profiling) and to identify targetable genomic alterations (genomic profiling) amenable to targeted therapies.[1,2] Several tissue-of-origin assays have been developed on the premise that cancer of unknown primary site and metastases from known primary sites are alike. Therefore, a biomarker set can be generated on the basis of commonalities in genomic, transcriptomic, and epigenetic profiles with the use of a discovery cohort of known primary sites to predict the probability of a match between the signature of cancer of unknown primary site and that of cancer of known primary site.[1,2] Various strategies that use RNA, microRNA, DNA, and methylation coupled with machine learning have been developed, with accuracy in predicting the tissue of origin ranging from 65 to 99%.[39] Among these strategies, only gene-expression profiling has been evaluated in randomized trials, with mixed results (see below).[21,39-42]

PRINCIPLES OF TREATMENT

The goal of treatment in most patients with cancer of unknown primary site is largely palliative owing to the disseminated nature of the disease, except for a small subpopulation of patients who present with single-site or limited metastatic disease. In these patients, a multidisciplinary approach incorporating radiation therapy or surgery can be potentially curative (Fig. 4).

Site-Specific Therapy and the Potential Role of Molecular Profiling for Tissue of Origin

Patients with cancer of unknown primary site who present with a characteristic, recognizable, clinicopathological pattern that is analogous to specific known primary cancers can be treated according to guidelines pertaining to those specific tumors, a treatment approach that simply emulates a site-specific approach to therapy for cancer of unknown primary site (Table 1). However, most patients with cancer of unknown primary site do not have a typical presentation. In these cases, clinicopathological clues can be used to discern a putative primary site, despite the clear absence of a primary lesion. Tissue-of-origin profiling for discerning primary sites may also be helpful. For cancer of unknown primary site with a discernible primary site, preference should be given to first-line site-specific therapy, which mirrors the standard care for the corresponding metastatic cancer of known primary site. For instance, a patient with adenocarcinoma and mediastinal lymphadenopathy positive for thyroid transcription factor 1 and with liver metastases without a lung lesion who is a long-term smoker may be treated for putative primary lung cancer, although high-quality evidence to support this strategy is lacking.

Several retrospective studies and prospective trials have investigated the benefit of site-specific therapy over empirical chemotherapy with conflicting results (Table 2). Two multicenter trials, the Next Generation Sequencing for Patients with Cancer of Unknown Primary Site (CUP-NGS) trial (involving 130 patients) and the Groupe d'Etude

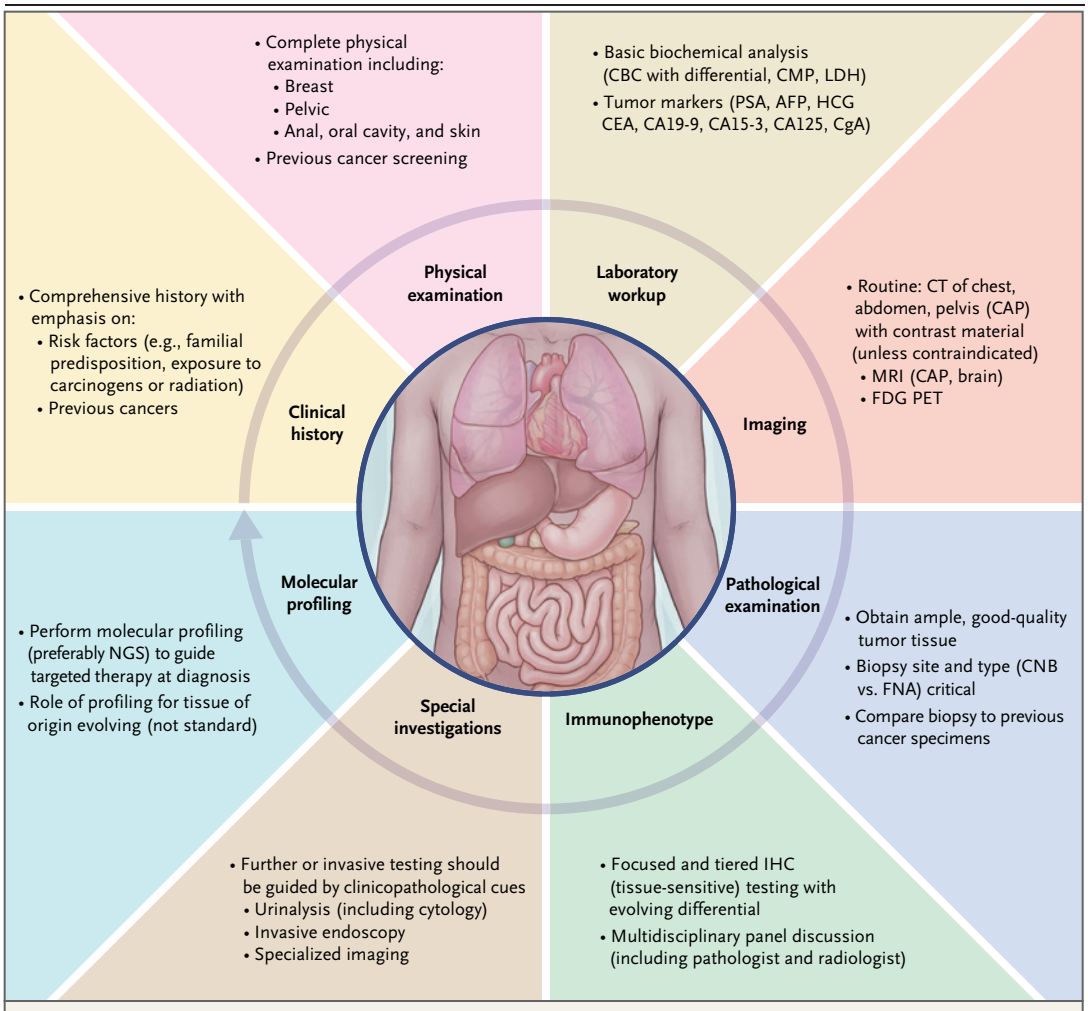


Figure 2. Diagnostic Workup for Patients with Cancer of Unknown Primary Site.

Cancer of unknown primary site is a diagnosis of exclusion, with workup designed to rule out any known primary site. Investigations should follow a tiered format with emphasis on tailoring the workup with each subsequent test. The critical decision is centered on striking the appropriate balance between the intensity of and time required for testing and the indication for and ability to start treatment guided by the patient's clinical status, disease trajectory, and goals of care. Immunophenotyping takes center stage in the diagnosis, but no one stain is sensitive or specific enough; therefore, a modular approach is necessary. With respect to imaging, magnetic resonance imaging (MRI), positron emission tomography (PET), or both can be performed in patients with contraindication to CT with the use of intravenous contrast material. The multidisciplinary team includes a medical oncologist, pathologist (with or without a molecular pathologist), radiologist, and in certain cases a surgical oncologist, radiation oncologist, and interventional radiologist. Special investigations include urinalysis, invasive endoscopy (involving colonoscopy and esophagogastroduodenoscopy [if clinical history or symptoms or immunohistochemical findings suggest a gastrointestinal primary cancer], cystoscopy, or panendoscopy with biopsies and tonsillectomy [for cases in which head and neck carcinoma is suspected]), and specialized imaging (such as breast imaging [mammography or breast MRI] and ^{18}F-fluorodeoxyglucose [FDG] PET), among others. Molecular profiling to determine the tissue of origin is useful as an adjunct to standard workup and preferably in a research context. The value of molecular profiling over the diagnostic methods currently used is yet to be established. AFP denotes α-fetoprotein, CA cancer antigen, CA19-9 carbohydrate antigen 19-9, CBC complete blood count, CEA carcinoembryonic antigen, CgA chromogranin A, CMP complete metabolic profile, CNB core needle biopsy, FNA fine-needle aspiration, HCG β-human chorionic gonadotropin, LDH lactate dehydrogenase, IHC immunohistochemical, NGS next-generation sequencing, and PSA prostate-specific antigen.

Français des Carcinomes de site Primitif Inconnu-04 (GEFCAPI-04) trial (involving 243 patients), randomly assigned patients with newly diagnosed cancer of unknown primary site to site-specific therapy (on the basis of gene-expression profiling to predict the primary site) or to empirical chemotherapy (carboplatin plus paclitaxel in the CUP-NGS trial and gemcitabine plus cisplatin in the GEFCAPI-04 trial).[21,41] Neither trial showed superiority of site-specific therapy over empirical chemotherapy.[21,41] Overall survival at 1 year was 44.0% in the site-specific therapy group and 54.9% in the empirical therapy group (P=0.26) in the CUP-NGS trial and was 41.3% and 40.7%, respectively (P=0.71), in the GEFCAPI-04 trial.[21,41] The corresponding median progression-free survival was 5.1 months and 4.8 months (P=0.55) in the CUP-NGS trial and 4.6 months and 5.3 months (P=0.71) in the GEFCAPI-04 trial.[21,41]

Contrary to these trials, the single-center, randomized Fudan CUP-001 trial (involving 182 patients) showed superiority of site-specific therapy guided by gene-expression profiling over platinum-based empirical chemotherapy (plus a taxane or gemcitabine).[40] The median progression-free survival was 9.6 months with site-specific therapy and 6.6 months with empirical chemotherapy (P=0.017).[40] Heterogeneity of the population and improved systemic therapies, specifically the incorporation of targeted therapies and immunotherapy (in nearly 45% of patients) in the experimental group, may have affected this advantage with respect to progression-free survival.

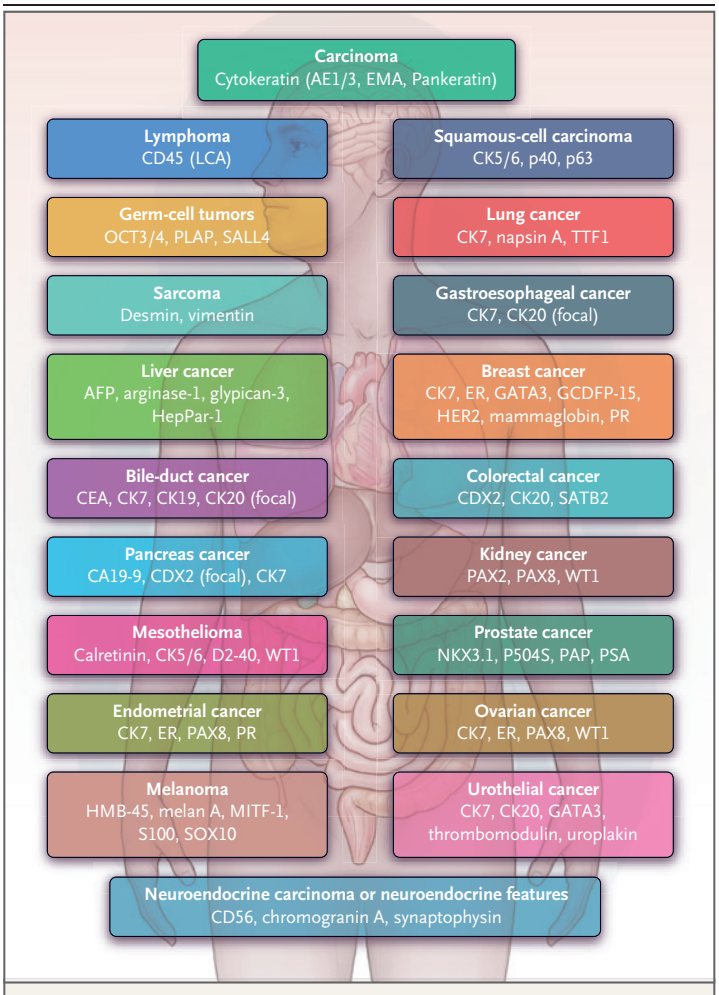


Figure 3. Immunophenotyping in Cancer of Unknown Primary Site.
Shown are key positive immunostains that are indicative of specific tumor types during phenotyping. Detailed descriptions of the immunostains are provided in Table S3.

Empirical Chemotherapy

A meta-analysis of five studies (three observational studies and two randomized trials; the Fudan CUP-001 trial was excluded from the analysis) investigating the role of site-specific therapy and empirical chemotherapy in cancer of unknown primary site showed that site-specific therapy was not significantly associated with an improvement in either progression-free survival or overall survival, indicating that empirical chemotherapy remains the standard against which newer strategies should be compared.[42] The most common empirical chemotherapy used in practice is a combination of a platinum agent (carboplatin or cisplatin) with either paclitaxel or gemcitabine. Other regimens comprising gemcitabine plus docetaxel and a fluorouracil-based therapy are also used. A meta-analysis of 32 studies (including 7 randomized trials) evaluating 42 regimens did not show clear superiority of any one regimen.[50]

Molecularly Guided Targeted Therapy

Biomarker-driven therapies, specifically those with proven efficacy regardless of the specific tissue or organ where the cancer originated (sometimes termed tissue-agnostic therapy), are endorsed by guidelines for cancer of unknown primary site (Table 1).[4,6] Genomic aberrations are common in cancer of unknown primary site, and response

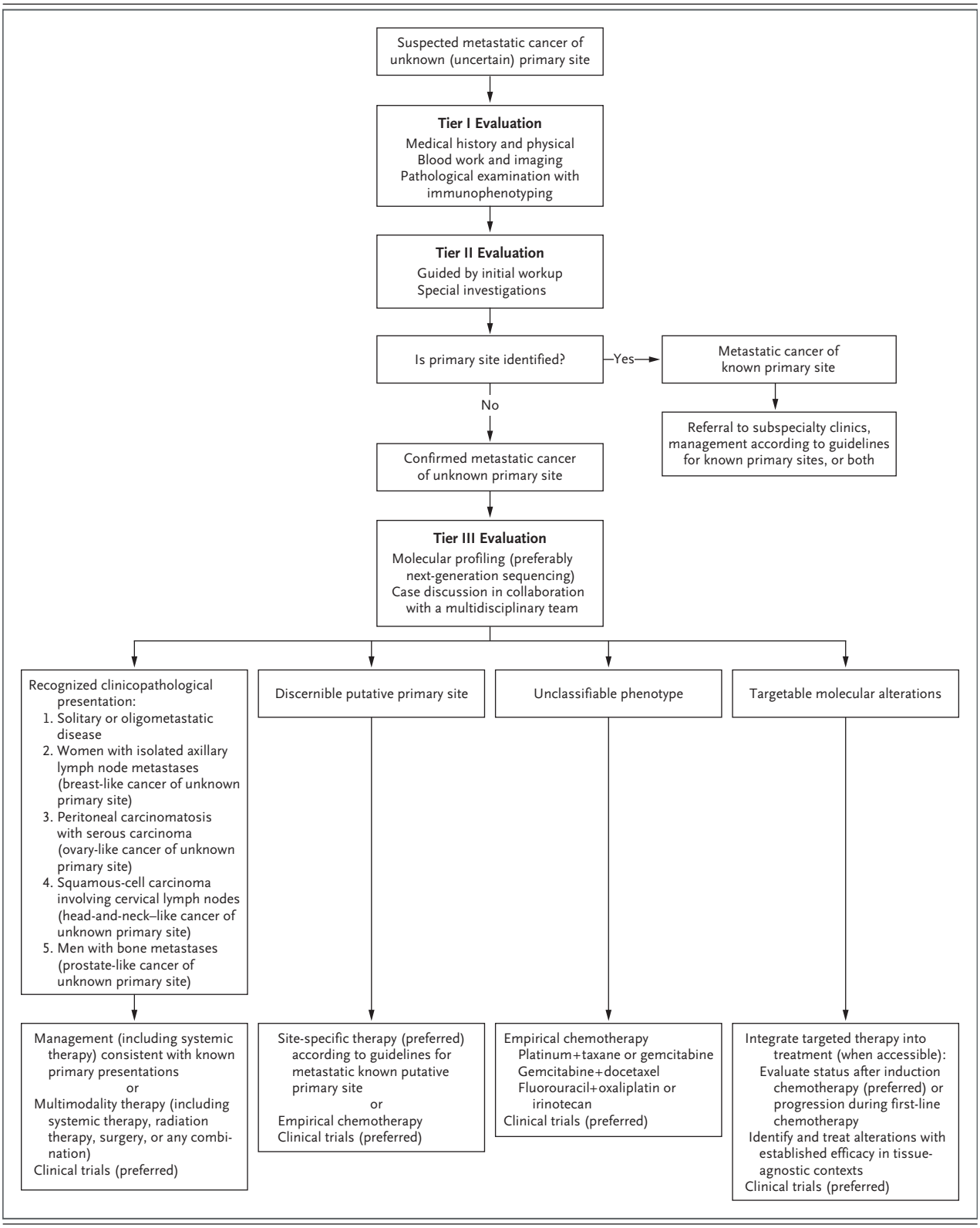


Figure 4 (facing page). Treatment Algorithm for Management of Cancer of Unknown Primary Site.

Confirmation and classification of cancer of unknown primary site for therapeutic purposes involve a tiered workup, which leads to the categorization of the disease into one of four discrete subgroups. These subgroups differ in their treatments and prognosis. Systemic cytotoxic chemotherapy with palliative intent is the mainstay of therapy for most patients with cancer of unknown primary site. In select cases, incorporation of targeted or biomarker-guided therapy, immunotherapy, or multimodality therapy (or a combination of these) with curative intent is appropriate. Despite these options, the prognosis is poor, and early referral and participation in clinical trials should be encouraged whenever possible. Multimodality therapy involving systemic therapy, radiation therapy, surgery, or a combination of these therapies is often used in patients presenting with solitary or oligometastatic disease. Empirical chemotherapy regimens include but are not restricted to platinum-based doublet therapies (cisplatin or carboplatin) combined with either a taxane (paclitaxel or docetaxel) or gemcitabine. Gemcitabine plus docetaxel and fluorouracil–based regimens are other options used empirically in the treatment of cancer of unknown primary site. Some overlap can occur within these subgroups of cancer of unknown primary site; therefore, molecular targets can manifest within any subgroup, and all attempts should be made to incorporate targeted therapies into the treatment plan. Biomarker-guided therapy is established for advanced solid tumors that harbor a *BRAF* V600E mutation, human epidermal growth factor receptor 2 overexpression (immunohistochemical score of 3+), *NTRK* fusion, *RET* fusion, high tumor mutational burden (≥10 mutations per megabase), and those that are mismatch repair–deficient and microsatellite instability–high.

to targeted therapies has been documented in case series and nonrandomized studies.[36,51-53] The CUPISCO trial (involving 436 patients) evaluated the clinical benefit of incorporating molecular profiling to identify targetable genomic alterations; the trial used tumor tissue, blood-based assay, or both to inform systemic therapy in cancer of unknown primary site.[54] In this trial, patients with cancer of unknown primary site who had disease control after three cycles of standard first-line platinum-based chemotherapy and were randomly assigned to receive therapy guided by molecular profiling had progression-free survival that was 6 weeks longer than those who were assigned to continued chemotherapy (median, 6.1 vs. 4.4 months; P=0.008), even though less than one third of the patients assigned to molecular profiling–guided therapy had an actionable target.[54]

AREAS OF UNCERTAINTY

The rarity of cancer of unknown primary site and the paucity of research amplify the uncertainty in the treatment of patients with this morbid orphan disease. Although the debate on effective therapy for cancer of unknown primary site is shifting toward site-specific and molecularly guided therapies, high-level evidence regarding improvement in outcomes with these therapies over treatment guided by standard clinicopathological assessment is lacking.[39]

We know very little about the biologic differences between cancer of unknown primary site with a putative primary site and corresponding metastatic cancers of known primary site. For instance, should *BRAF* V600E–mutated cancer of unknown primary site be treated with BRAF–MEK inhibition (as for melanoma) or with BRAF–epidermal growth factor receptor (EGFR) inhibition (as for colorectal cancer), and how do we incorporate the tissue-of-origin context in this case?[55] Also crucial is understanding the molecular subsets that cancer of unknown primary site represents within the framework of known primary cancers and the response to standard therapies. For example, do cancers of unknown primary site with a colon profile behave as cancers on the right or left side of the colon or as consensus molecular subtype 4 tumors when treated with anti-EGFR–based chemotherapy?[31] Immunotherapy has also been investigated in cancer of unknown primary site, with responses in approximately 20% of patients (Table S2). However, in the absence of any validated biomarkers, a broad approach is not recommended in clinical guidelines, and immunotherapy is appropriate only for tumors that have high microsatellite instability or DNA mismatch repair deficiency (dMMR) or that have a high mutational burden or as part of site-specific therapy. More research on the efficacy and safety of immunotherapy in cancer of unknown primary site is needed.[4-6,56]

GUIDELINES

The National Comprehensive Cancer Network (NCCN), the National Institute for Health and Care Excellence (NICE), and the European Society for Medical Oncology (ESMO) publish and regularly update guidelines focused on the evaluation

Table 2. Summary of Studies Evaluating the Role of Site-Specific Therapy as Compared with Empirical Therapy in Cancer of Unknown Primary Site.*

Study†	Study Type and Primary End Point	Tissue-of-Origin Profiling	Sample Size (Site-Specific vs. Empirical Therapy)	Empirical Therapy Regimen	Sites of Common Predicted Tumors	Outcomes‡
Fudan CUP-001[40]	RCT, PFS	Canhelp-Origin, a 90-gene expression assay (archived FFPE)	182 (91 vs. 91)	Platinum and paclitaxel or gemcitabine	Stomach or esophagus, lung, ovary, cervix, breast	Median PFS, 9.6 vs. 6.6 mo (HR, 0.68; 95% CI, 0.49–0.93; P=0.017) Median OS, 28.2 vs. 19.0 mo (HR, 0.74; 95% CI, 0.52–1.06; P=0.09) Objective response, 49% vs. 46% (P=0.76)§
New South Wales[43]	Retrospective	Clinicopathological (tissue-based)	57 (26 vs. 31)	Platinum and gemcitabine or carboplatin and paclitaxel	Head and neck, colon or rectum, pancreas, lung, stomach or esophagus	Median PFS, 9.8 vs. 7.3 mo (HR, 0.70; 95% CI, 0.40–1.30; P=0.29) Median OS, 25.9 vs. 13.2 mo (P=0.30)
Japan-8 CUP[44]	Retrospective	Clinicopathological (tissue-based)	144 (60 vs. 84)	Various	Lung, stomach or esophagus, pancreas, colon or rectum, ovary	Median OS, 10.0 vs. 10.1 mo (HR, 1.01; 95% CI, 0.70–1.45; P=0.95)
CUP-NGS[21]	RCT, OS	Microarray-based gene expression analysis (fresh frozen)	101 (50 vs. 51)¶	Carboplatin and paclitaxel	Pancreas, stomach or esophagus, lymphatic system, bladder, cervix	Median PFS, 5.1 vs. 4.8 mo (HR, 0.88; 95% CI, 0.59–1.33; P=0.55) Median OS, 9.8 vs. 12.5 mo (HR, 1.03; 95% CI, 0.68–1.56; P=0.89) Overall response, 34.7% vs. 41.2% (P=0.50)§
GEFCAPI-04[41]	RCT, PFS	92-Gene RT-PCR (tissue-based)	243 (123 vs. 120)	Gemcitabine and cisplatin	Pancreas, gallbladder, or bile duct; squamous-cell carcinoma; kidney, lung, intestines	Median PFS, 4.6 vs. 5.3 mo (HR, 0.95; 95% CI, 0.72–1.25; P=0.71) Median OS, 10.7 vs. 9.9 mo (HR, 0.92; 95% CI, 0.69–1.23; P=0.72)
Aichi CUP[45]	Retrospective	Clinicopathological (tissue-based)	122 (90 vs. 32)	Platinum-based therapy	Colon or rectum, gynecologic system, lung, pancreas, neuroendocrine system	Median PFS, 5.1 vs. 4.2 mo (P=0.02) Median OS, 15.7 vs. 10.7 mo (P=0.07)
EPICUP[46]	Retrospective	Microarray DNA methylation signatures (tissue-based)	92 (31 vs. 61)	Various	Lung, head and neck, breast, colon or rectum, liver	Median OS, 13.6 vs. 6.0 mo (P=0.008)
Sarah Cannon[47]	Prospective, OS	92-Gene RT-PCR (tissue-based)	223 (194 vs. 29)	Various	Biliary tract, bladder, colon or rectum, lung, pancreas	Median OS, 12.5 vs. 9.1 mo (historical control)
Lower GI CUP[48]	Retrospective	Immunohistochemical test (tissue-based)	68 (53 vs. 15)	Gemcitabine- or taxane-based therapy	Lower gastrointestinal tract	OS (HR, 0.52; 95% CI, 0.22–1.22; P=0.13)
CancerTYPE ID-GI[49]	Retrospective	92-Gene RT-PCR (tissue-based)	42 (24 vs. 18)	Various	Colon or rectum	Median PFS, 8.5 vs. 6 mo (P=0.11)

* No primary end points were specified for the retrospective studies listed. CI denotes confidence interval; FFPE formalin-fixed, paraffin embedded; NS not specified; RCT randomized, control trial; and RT-PCR reverse-transcriptase polymerase chain reaction.
† Study titles and references are provided in Table S1 in the Supplementary Appendix (available with the full text of this article at NEJM.org).
‡ Values are site-specific therapy as compared with empirical therapy. Hazard ratios (HRs) for analyses of overall survival (OS) indicate the risk of death, and hazard ratios for the analyses of progression-free survival (PFS) indicate the risk of disease progression or death.
§ Response was according to Response Evaluation Criteria in Solid Tumors, version 1.1.
¶ The numbers shown are the number of patients who received treatment.

and treatment of patients with cancer of unknown primary site.[4-6] The recommendations in the present article are largely concordant with these key professional guidelines. Barring selected favorable subsets of cancer of unknown primary site, the guidelines mentioned here do not explicitly support the use of site-specific therapy over empirical chemotherapy. Adenocarcinoma with a colorectal immunophenotype (CK7-negative, CK20-positive, and CDX2-positive) or molecular profile (colon-like cancer of unknown primary site) is now classified by ESMO in the favorable subset of cancer of unknown primary site, and treatment analogous to that for metastatic colorectal cancer is generally recommended. Similarly, NCCN recommends fluorouracil-based regimens as one of the preferred treatment options for occult primary adenocarcinoma, primarily for disease with a presumed gastrointestinal primary site. Because of limited data supporting improved outcomes with the use of molecular profiling to guide site-specific therapy over conventional diagnostic approaches, clinical practice guidelines recommend against the routine use of molecular profiling to identify the tissue of origin in cancer of unknown primary site.[4,6] Given this limited availability of high-level clinical evidence, all guidelines encourage the participation of patients with cancer of unknown primary site in clinical trials.

CONCLUSIONS AND RECOMMENDATIONS

Regarding the patient in the vignette with cancer of unknown primary site, I would manage her care according to the most likely putative cancer of known primary site given the phenotype and genotype. She had an immunophenotype suggestive of a gastrointestinal primary cancer (colon-like cancer of unknown primary site) on the basis of assays positive for CK20, CDX2, and SATB2, which are immunostains used for samples from the lower gastrointestinal tract. I would start by obtaining a repeat biopsy and sending tissue for genomic profiling to identify targetable alterations. Alternatively, genomic profiling with blood-based assays can also be used to detect potential actionable genetic alterations (e.g., dMMR, *BRAF* V600E, human epidermal growth factor receptor 2 amplification).

I would then recommend palliative systemic chemotherapy that corresponded to the best treatments known for the presumed primary cancer. In this case, I would use fluorouracil (plus leucovorin), oxaliplatin, and irinotecan (FOLFOXIRI), drawing from evidence supporting the role of triplet cytotoxic chemotherapy in metastatic colorectal cancer. The use of doublet fluorouracil-based therapy with oxaliplatin (FOLFOX) or irinotecan (FOLFIRI) is also defensible, but a 47-year-old patient should be able to receive the more aggressive and more effective FOLFOXIRI therapy.[57] The results of molecular profiling can enable the integration of molecularly guided therapy in a treatment continuum that is based on response to therapy, the level of evidence that supports targeting the alteration, and the availability of molecularly guided therapies, preferably within the context of clinical trials. I would also seek early referral to a center with a multidisciplinary program focused on cancer of unknown primary site and enroll the patient in a clinical trial, if she were willing.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

AUTHOR INFORMATION

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REFERENCES

1. Varadhachary GR, Raber MN. Cancer of unknown primary site. N Engl J Med 2014;371:757-65.
2. Rassy E, Pavlidis N. Progress in refining the clinical management of cancer of unknown primary in the molecular era. Nat Rev Clin Oncol 2020;17:541-54.
3. Pauli C, Bochtler T, Mileshkin L, et al. A challenging task: identifying patients with cancer of unknown primary (CUP) according to ESMO guidelines: the CUPISCO trial experience. Oncologist 2021;26(5):e769-e779.
4. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: occult primary. April 29, 2024 (https://www.nccn.org/guidelines/guidelines-detail?category=1&id=1451).
5. National Institute for Health and Care Excellence. Metastatic malignant disease of unknown primary origin in adults: diagnosis and management. April 26, 2023 (https://www.nice.org.uk/guidance/cg104).
6. Krämer A, Bochtler T, Pauli C, et al. Cancer of unknown primary: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann Oncol 2023;34:228-46.
7. Gordon LG, Wood C, Tothill RW, et al. Healthcare costs before and after diag-

nosis of cancer of unknown primary versus ovarian cancer in Australia. Pharmacoecon Open 2023;7:111-20.
8. Schaffer AL, Pearson S-A, Dobbins TA, Er CC, Ward RL, Vajdic CM. Patterns of care and survival after a cancer of unknown primary (CUP) diagnosis: a population-based nested cohort study in Australian Government Department of Veterans' Affairs clients. Cancer Epidemiol 2015;39:578-84.
9. Rassy E, Pavlidis N. The currently declining incidence of cancer of unknown primary. Cancer Epidemiol 2019;61:139-41.
10. American Cancer Society. Key statistics for cancers of unknown primary. January 16, 2025 (https://www.cancer.org/cancer/types/cancer-unknown-primary/about/key-statistics.html).
11. Hemminki K, Liu H, Heminki A, Sundquist J. Power and limits of modern cancer diagnostics: cancer of unknown primary. Ann Oncol 2012;23:760-4.
12. Vajdic CM, Er CC, Schaffer A, et al. An audit of cancer of unknown primary notifications: a cautionary tale for population health research using cancer registry data. Cancer Epidemiol 2014;38:460-4.
13. Javle M, Lee S, Azad NS, et al. Temporal changes in cholangiocarcinoma incidence and mortality in the United States from 2001 to 2017. Oncologist 2022;27:874-83.
14. Greco FA, Lennington WJ, Spigel DR, Hainsworth JD. Molecular profiling diagnosis in unknown primary cancer: accuracy and ability to complement standard pathology. J Natl Cancer Inst 2013;105:782-90.
15. Hermans KEPE, Kazemzadeh F, Loef C, et al. Risk factors for cancer of unknown primary: a literature review. BMC Cancer 2023;23:314.
16. Zolotykh MA, Mingazova LA, Filina YV, et al. Cancer of unknown primary and the «seed and soil» hypothesis. Crit Rev Oncol Hematol 2024;196:104297.
17. Brewster DH, Lang J, Bhatti LA, Thomson CS, Oien KA. Descriptive epidemiology of cancer of unknown primary site in Scotland, 1961-2010. Cancer Epidemiol 2014;38:227-34.
18. Wong B, Vickers MM, Wheatley-Price P. The diminishing importance of primary site identification in cancer of unknown primary: a Canadian single-center experience. Front Oncol 2021;11:634563.
19. Riihimäki M, Hemminki A, Sundquist K, Hemminki K. Time trends in survival from cancer of unknown primary: small steps forward. Eur J Cancer 2013;49:2403-10.
20. Briasoulis E, Kalofonos H, Bafaloukos D, et al. Carboplatin plus paclitaxel in unknown primary carcinoma: a phase II Hellenic Cooperative Oncology Group Study. J Clin Oncol 2000;18:3101-7.
21. Hayashi H, Kurata T, Takiguchi Y, et al. Randomized phase II trial comparing site-specific treatment based on gene expression profiling with carboplatin and paclitaxel for patients with cancer of unknown primary site. J Clin Oncol 2019;37:570-9.
22. Kim CS, Hannouf MB, Sarma S, et al. Survival outcome differences based on treatments used and knowledge of the primary tumour site for patients with cancer of unknown and known primary in Ontario. Curr Oncol 2018;25:307-16.
23. Raghav K, Hwang H, Jácome AA, et al. Development and validation of a novel nomogram for individualized prediction of survival in cancer of unknown primary. Clin Cancer Res 2021;27:3414-21.
24. Bochtler T, Reiling A, Endris V, et al. Integrated clinicomolecular characterization identifies RAS activation and CDKN2A deletion as independent adverse prognostic factors in cancer of unknown primary. Int J Cancer 2020;146:3053-64.
25. Qi P, Sun Y, Liu X, et al. Clinicopathological, molecular and prognostic characteristics of cancer of unknown primary in China: an analysis of 1420 cases. Cancer Med 2023;12:1177-88.
26. Hu Z, Li Z, Ma Z, Curtis C. Multi-cancer analysis of clonality and the timing of systemic spread in paired primary tumors and metastases. Nat Genet 2020;52:701-8.
27. Klein CA. Parallel progression of primary tumours and metastases. Nat Rev Cancer 2009;9:302-12.
28. Ganesh K, Massagué J. Targeting metastatic cancer. Nat Med 2021;27:34-44.
29. Vikeså J, Møller AK, Kaczkowski B, et al. Cancers of unknown primary origin (CUP) are characterized by chromosomal instability (CIN) compared to metastasis of know origin. BMC Cancer 2015;15:151.
30. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol 2010;28:3271-7.
31. Lenz HJ, Ou F-S, Venook AP, et al. Impact of consensus molecular subtype on survival in patients with metastatic colorectal cancer: results from CALGB/SWOG 80405 (Alliance). J Clin Oncol 2019;37:1876-85.
32. Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 2014;25:152-65.
33. Bijlsma MF, Sadanandam A, Tan P, Vermeulen L. Molecular subtypes in cancers of the gastrointestinal tract. Nat Rev Gastroenterol Hepatol 2017;14:333-42.
34. Lin F, Liu H. Immunohistochemistry in undifferentiated neoplasm/tumor of uncertain origin. Arch Pathol Lab Med 2014;138:1583-610.
35. Handorf CR, Kulkarni A, Grenert JP, et al. A multicenter study directly comparing the diagnostic accuracy of gene expression profiling and immunohistochemistry for primary site identification in metastatic tumors. Am J Surg Pathol 2013;37:1067-75.
36. Huey RW, Shah AT, Reddi HV, et al. Feasibility and value of genomic profiling in cancer of unknown primary: real-world evidence from prospective profiling study. J Natl Cancer Inst 2023;115:994-7.
37. Kawaguchi KR, Lu F-I, Kaplan R, et al. In search of the ideal immunopanel to distinguish metastatic mammary carcinoma from primary lung carcinoma: a tissue microarray study of 207 cases. Appl Immunohistochem Mol Morphol 2014;22:266-74.
38. Lu MY, Chen TY, Williamson DFK, et al. AI-based pathology predicts origins for cancers of unknown primary. Nature 2021;594:106-10.
39. Ma W, Wu H, Chen Y, et al. New techniques to identify the tissue of origin for cancer of unknown primary in the era of precision medicine: progress and challenges. Brief Bioinform 2024;25(2):bbae028.
40. Liu X, Zhang X, Jiang S, et al. Site-specific therapy guided by a 90-gene expression assay versus empirical chemotherapy in patients with cancer of unknown primary (Fudan CUP-001): a randomised controlled trial. Lancet Oncol 2024;25:1092-102.
41. Fizazi K, Maillard A, Penel N, et al. A phase III trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression analysis in patients with carcinomas of an unknown primary (CUP) site (GEFCAPI 04). Ann Oncol 2019;30:Suppl 5:v851 (https://www.annalsofoncology.org/article/S0923-7534(19)60360-1/fulltext).
42. Ding Y, Jiang J, Xu J, et al. Site-specific therapy in cancers of unknown primary site: a systematic review and meta-analysis. ESMO Open 2022;7:100407.
43. Boys EL, Gao B, Grimison P, et al. Retrospective analysis of clinical characteristics and outcomes of patients with carcinoma of unknown primary from three tertiary centers in Australia. Cancer Med 2024;13(6):e7052.
44. Nishikawa K, Hironaka S, Inagaki T, et al. A multicentre retrospective study comparing site-specific treatment with empiric treatment for unfavourable subset of cancer of unknown primary site. Jpn J Clin Oncol 2022;52:1416-22.
45. Hasegawa H, Ando M, Yatabe Y, et al. Site-specific chemotherapy based on predicted primary site by pathological profile for carcinoma of unknown primary site. Clin Oncol (R Coll Radiol) 2018;30:667-73.
46. Moran S, Martínez-Cardús A, Sayols S, et al. Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis. Lancet Oncol 2016;17:1386-95.
47. Hainsworth JD, Rubin MS, Spigel DR, et al. Molecular gene expression profiling to predict the tissue of origin and direct

site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon Research Institute. J Clin Oncol 2013;31:217-23.
48. Varadhachary GR, Karanth S, Qiao W, et al. Carcinoma of unknown primary with gastrointestinal profile: immunohistochemistry and survival data for this favorable subset. Int J Clin Oncol 2014;19:479-84.
49. Hainsworth JD, Schnabel CA, Erlander MG, Haines DW III, Greco FA. A retrospective study of treatment outcomes in patients with carcinoma of unknown primary site and a colorectal cancer molecular profile. Clin Colorectal Cancer 2012;11:112-8.
50. Lee J, Hahn S, Kim D-W, et al. Evaluation of survival benefits by platinums and taxanes for an unfavourable subset of carcinoma of unknown primary: a systematic review and meta-analysis. Br J Cancer 2013;108:39-48.
51. Ross JS, Wang K, Gay L, et al. Comprehensive genomic profiling of carcinoma of unknown primary site: new routes to targeted therapies. JAMA Oncol 2015;1:40-9.
52. Kato S, Krishnamurthy N, Banks KC, et al. Utility of genomic analysis in circulating tumor DNA from patients with carcinoma of unknown primary. Cancer Res 2017;77:4238-46 .
53. Hayashi H, Takiguchi Y, Minami H, et al. Site-specific and targeted therapy based on molecular profiling by next-generation sequencing for cancer of unknown primary site: a nonrandomized phase 2 clinical trial. JAMA Oncol 2020;6:1931-8.
54. Krämer A, Bochtler T, Pauli C, et al. Molecularly guided therapy versus chemotherapy after disease control in unfavourable cancer of unknown primary (CUPISCO): an open-label, randomised, phase 2 study. Lancet 2024;404:527-39.
55. Gouda MA, Subbiah V. Expanding the benefit: dabrafenib/trametinib as tissue-agnostic therapy for *BRAF* V600E-positive adult and pediatric solid tumors. Am Soc Clin Oncol Educ Book 2023;43:e404770.
56. Raghav KP, Stephen B, Karp DD, et al. Efficacy of pembrolizumab in patients with advanced cancer of unknown primary (CUP): a phase 2 non-randomized clinical trial. J Immunother Cancer 2022;10(5):e004822.
57. Loupakis F, Cremolini C, Masi G, et al. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. N Engl J Med 2014;371:1609-18.