



SEOM-GECOD clinical guideline for cancer of unknown primary (update 2025)

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Abstract

Cancer of unknown primary (CUP) is defined as a heterogeneous group of tumors that appear as metastases for which a standard diagnostic work-up fails to identify the tissue of origin. There is now high-level evidence showing that actionable genomic alterations should be routinely determined in patients with CUP to enable molecularly guided therapy as appropriate. In this guideline (updated in 2025), we summarize diagnostic processes and therapeutic options for CUP, as well as new developments in molecular medicine that will help to improve the poor outcomes associated with this unique disease entity.

Keywords Cancer · Diagnosis · Treatment · Unknown primary site

Introduction

Cancer of unknown primary (CUP) represents a heterogeneous group of histologically confirmed metastatic carcinomas or undifferentiated neoplasms for which a thorough diagnostic work-up (including clinical history, physical examination,

blood tests and biochemistry, and computed tomography [CT]) cannot identify the origin of the primary tumor [1, 2].

CUP currently accounts for approximately 2–5% of cancer diagnoses globally [2]. Probably because of improvements in diagnostic techniques for the identification of primary tumor sites, the incidence of CUP has been declining,

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but it remains a leading cause of cancer-related death worldwide [1, 2].

Given the poor prognosis of CUP, it is necessary to incorporate molecular testing into the diagnostic work-up to help predict the putative primary (tissue of origin) and identify actionable therapeutic targets, thereby facilitating site-specific and molecularly guided therapy (MGT) options [2, 3]. Given recent advances in molecular profiling and MGT, the optimal management of CUP is changing rapidly [4, 5]. This updated guideline incorporates data from recently published milestone trials demonstrating the potential of MGT in the evolving management of CUP [6, 7].

Methodology

This updated guideline has been developed with the consensus of ten oncologists highly experienced in the diagnosis and treatment of CUP, and members of the Spanish Research Group on Cancer of Unknown Origin (GECOD) and the Spanish Society of Medical Oncology (SEOM). The updates in this guideline (developed in December 2024) reflect the results of clinical trials reported after the publication of the previous version of the guideline (2022); the updated version of these guidelines has been approved by all the authors. The updated recommendations are provided in Table 1, which also include the changes made compared with the previous version of the guidelines.

Table 1 SEOM clinical practice guidelines for cancer of unknown primary (2025): summary of recommendations

Recommendation	Category, grade
<i>Molecular profiling for tumor-agnostic targeted therapy or immunotherapy</i>	
Tumor-agnostic targeted treatment is recommended, with NTRK inhibitors for <i>NTRK1,2,3</i> fusions, PD-1 checkpoint inhibitors for MSI-H, RET inhibitors for <i>RET</i> fusions, BRAF inhibitors + MEK inhibitors for <i>BRAF</i> mutations, pan-FGFR TKIs for <i>FGFR1/2/3</i> fusions/mutations, and PD-1/PD-L1 checkpoint inhibitors for TMB-H (> 10 mutations per megabase), as well as CUP-specific indications for ALK TKIs for ALK fusions and PD-1 inhibitors plus anti-CTLA4 inhibitors for TMB-H	I-III, B (by biomarker status)
<i>Recommendations for the use of molecular profiling</i>	
NGS may be carried out routinely in CUP	II, B
If there are no clear results with immunohistochemistry, NGS should be used to characterize genomic alterations that enable matched targeted treatments or immunotherapy, especially in fit patients with a 0–1 ECOG PS and without markedly elevated LDH	III, B
<i>Site-specific treatment according to histopathologic and/or clinical criteria</i>	
Certain subgroups of patients may benefit from a more specific treatment, similar to that for the primary tumors which their conditions resemble, such as renal-cell-like CUP, lung-like CUP, and colorectal-like CUP	III, B
In patients with favorable CUP, a genomic platform could help identify a genomic profile and/or an actionable genomic alteration, or biomarkers of response to immunotherapy to help inform site-specific therapy	III, B
<i>Surgical resection and radiotherapy</i>	
While the role of neoadjuvant or adjuvant chemotherapy in CUP is undefined, empirical adjuvant chemotherapy is reasonable, particularly in patients with poorly differentiated carcinoma or if indicated in a predicted tumor	IV, B
<i>Treatment of unfavorable CUP</i>	
The treatment of choice for unfavorable CUP is an empirical combination chemotherapy with a platinum agent plus another cytotoxic agent (taxane, gemcitabine, irinotecan)	II, A
Targeted therapies that have received approval for tumor-agnostic treatment of refractory and unresectable solid tumors can be considered, including NTRK inhibitors (larotrectinib, entrectinib) for NTRK fusion tumors and pembrolizumab for MSI-H and TMB-high solid tumors	I-III, B (by biomarker status)
<i>Other recommendations</i>	
Recommendation added to routinely use NGS in CUP	II, B
Recommendation for the use of tumor-agnostic therapy has been expanded to include RET inhibitors for <i>RET</i> fusions, BRAF inhibitors + MEK inhibitors for <i>BRAF</i> mutations, pan-FGFR TKIs for <i>FGFR1/2/3</i> fusions/mutations, and PD-1 checkpoint inhibitors for MSI-H tumors, as well as CUP-specific indications for ALK TKIs for ALK fusions and PD-1 inhibitors plus anti-CTLA4 inhibitors for TMB	I-III, B (by biomarker status)

ALK: Anaplastic lymphoma kinase; BRAF: B-Raf proto-oncogene; CTLA4: Cytotoxic T-lymphocyte-associated protein 4; CUP: Cancer of unknown primary; ECOG PS: Eastern Cooperative Oncology Group performance status; FGFR: Fibroblast growth factor receptor; LDH: Lactate dehydrogenase; MEK: Mitogen-activated extracellular signal-regulated kinase; MSI-H: High microsatellite instability; NGS: Next-generation sequencing; NTRK: Neurotrophic tropomyosin receptor kinase; PD-1: Programmed death-1; PD-L1: Programmed death-ligand 1; RET: Rearranged during transfection; TKIs: Tyrosine kinase inhibitors; TMB-H: High tumor mutational burden

Table 2 Strength of recommendation and quality of evidence score [8]

Category, grade	Definition
<i>Strength of recommendation</i>	
A	Strong evidence for efficacy with a substantial clinical benefit; strongly recommended
B	Strong or moderate evidence for efficacy but with a limited clinical benefit; generally recommended
C	Insufficient evidence for efficacy or benefit does not outweigh the risk or the disadvantages (adverse events, costs, etc.); optional
D	Moderate evidence against efficacy or for adverse outcome; generally not recommended
E	Strong evidence against efficacy or for adverse outcome; never recommended
<i>Quality of evidence</i>	
I	Evidence from at least one large randomized, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomized trials without heterogeneity
II	Evidence from small randomized trials or large randomized trials with a suspicion of bias (lower methodological quality) or meta-analyses of such trials or of trials with demonstrated heterogeneity
III	Evidence from prospective cohort studies
IV	Evidence from retrospective cohort studies or case–control studies
V	Evidence from studies without control group, case reports, or expert opinions

Levels of evidence and the grades of recommendation were assigned to a selection of statements using the Infectious Diseases Society of America-US Public Health Service Grading System for ranking recommendations in clinical guidelines (Table 2) [8]. The authors consider statements without grading to be standard clinical practice.

Summary of changes in the 2025 guideline (Table 1)

There is one new recommendation in the 2025 guideline: “Next-generation sequencing (NGS) may be carried out routinely in CUP” (I, B). Supportive evidence for this recommendation is provided by the findings of the CUPISCO trial [7].

In the 2022 guideline, there was a tumor-agnostic therapy recommendation for NTRK fusion gene inhibitors, and for pembrolizumab for tumors with a high tumor mutation burden (TMB-high; III, B). In addition to tumor type-agnostic indications for TRK inhibitors for *NTRK1,2,3* fusions and PD-1/PD-L1 checkpoint inhibitors for TMB-H, this recommendation has now been expanded to include RET inhibitors for *RET* fusions, BRAF inhibitors + MEK inhibitors for *BRAF* mutations, pan-FGFR TKIs for *FGFR1/2/3* fusions/mutations, and PD-1 checkpoint inhibitors for high microsatellite instability (MSI-H), as well as CUP-specific indications for ALK tyrosine kinase inhibitors (TKIs) for ALK fusions and PD-1 inhibitors plus anti-CTLA4 inhibitors for TMB-H, and the level of evidence has changed in many cases from III to I or II.

Other notable changes in the 2025 guideline include removal of neuroendocrine carcinomas of unknown

primary site and poorly differentiated carcinoma with mid-line nodal distribution in men as favorable CUP subtypes, and addition of carcinoma with renal-cell histological and immunohistochemical profile as a favorable CUP subtype. Favorable CUP subtypes recognized in the current guideline are now aligned with those recognized by the European Society of Medical Oncology (ESMO) [1].

Incidence and epidemiology

The incidence of CUP, which accounts for approximately 2–5% of all diagnosed cancer globally, seems to be declining [1, 2, 9]. In addition to the rate of success of locating primary tumors, the incidence of CUP is influenced by the absence of international consensus on the definition and registration of CUP [9]. Unfortunately, there is a lack of a diagnostic process records for these patients, making it difficult to ascertain reasons for the declining incidence of CUP, although it is probably a consequence of improvements in diagnostic techniques that result in increasingly successful localization of primary tumors [9].

CUP occurs most frequently after the age of 60 years, and is slightly more prevalent in men than women [1]. Among individuals older than 60 years of age, the incidence rate for CUP in the digestive tract has increased markedly [9].

Pathogenesis and biology

Little is understood about CUP pathogenesis and biology. Two predominant theories exist. The first theory refers to the *parallel progression* model, in which dissemination of

tumor cells occurs early—before the formation of a detectable primary tumor—and subsequent clonal evolution leads to a metastatic phenotype distinct from that of the occult primary lesion [10]. In this scenario, the tumor microenvironment at the metastatic site may selectively support metastatic growth, while hindering proliferation at the primary site [11]. The second proposed scenario is that no clinically relevant primary tumor ever develops, and CUP represents a biologically distinct single metastatic entity, as described by Rassy et al. [11].

In the absence of a unique biology/molecular signature to identify CUP as a single cancer entity, it is most likely that CUP is associated with an undetectable primary tumor [10]. In a parallel progression scenario, the process that generates CUP is driven by multiple interdependent alterations in cell behavior, including chromosomal alterations, self-sufficiency in growth signals, resistance to growth-inhibitory signals, reprogramming of energy metabolism, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, and evasion from immune destruction [11].

Prognosis

Given that CUP is characterized by early metastasis with an aggressive disease course, the prognosis of patients with CUP is generally poor. However, there is a subgroup of patients (approximately 20%) with a more favorable prognosis (termed favorable CUP), in whom a greater benefit can be expected when treated appropriately [1]. This group of patients with favorable CUP often have disease with radiological and/or histological features that align with cancers with a known primary site (Table 3) and should be treated with the equivalent site-specific therapy [1]. In line with the 2023 ESMO guideline, the previous favorable subtype ‘poorly differentiated carcinoma with midline nodal distribution in men’ has been removed, as many such cases historically represented extragonadal germ cell tumors and a minority may correspond to underdiagnosed NUT midline carcinoma, an aggressive but rare entity. In addition, neuroendocrine carcinomas of unknown primary site are no

longer included among favorable CUP subtypes, as these tumors should be classified and managed as neuroendocrine malignancies, irrespective of whether a primary is detectable. In agreement with the ESMO clinical practice guideline for CUP, neuroendocrine carcinomas of unknown primary, which were formerly considered a favorable CUP subtype, should now be sub-classified as neuroendocrine malignancies, irrespective of the presence of an obvious primary tumor (elusive primaries are common), and treated accordingly [1]. Also in agreement with the ESMO guideline [1], poorly differentiated carcinoma with midline nodal distribution in men is also no longer acknowledged as a favorable CUP subtype because historically many of these patients had extragonadal germ cell tumors and some may have an underdiagnosed, aggressive NUT midline carcinoma [1].

Unfortunately, most patients diagnosed with CUP (approximately 80%) have an unfavorable subtype, with a poor response to treatment (empiric chemotherapy) and a poor prognosis, with a median overall survival (OS) of approximately 6–12 months [6, 7]. The prognosis of unfavorable CUP is classified primarily by Eastern Cooperative Oncology Group (ECOG) performance status (PS) and serum lactate dehydrogenase (LDH) level [1]. Those with a good ECOG PS (0–1) and a normal LDH value have a better prognosis than those with ECOG PS > 1 and/or elevated LDH [1, 12].

In addition to unfavorable CUP subtype, poor ECOG PS, and elevated LDH, other independent risk factors predictive of a poor outcome include male sex, underlying comorbidity, a higher number of metastatically involved organs, the presence of liver metastases or visceral metastases, low serum albumin concentrations, high alkaline phosphatase concentrations, and lymphopenia or an elevated neutrophil versus lymphocyte ratio [1, 13].

No randomized trials have been conducted to demonstrate superiority of standard-of-care platinum-based doublet chemotherapy over best supportive care in patients with unfavorable CUP [1]. Whereas fit patients should receive standard-of-care chemotherapy and molecularly targeted therapy as appropriate (II, A), we generally suggest that unfit

Table 3 Favorable cancer of unknown primary subtypes

Adenocarcinoma with a colon cancer IHC (CK20 ⁺ , CK7 [−] , CDX2 ⁺) or molecular profile (colon-like CUP)
Squamous cell carcinoma with head and neck lymph node involvement (head and neck-like CUP)
Papillary adenocarcinoma of the peritoneal cavity in women (ovary-like CUP)
Isolated axillary lymph node metastases in women (breast-like CUP)
Blastic bone metastases and elevated PSA in men (prostate-like CUP)
Carcinoma with renal-cell histological and IHC profile (renal cell carcinoma-like CUP)
Single or potentially resectable oligometastatic disease, including squamous cell carcinoma with inguinal nodes

CDX2: Caudal-type homeobox 2; CK: Cytokeratin; CUP: Cancer of unknown primary; IHC: Immunohistochemistry; PSA: Prostate-specific antigen

Table 4 Diagnostic work-up in cancer of unknown primary

Assessment	Patient subset
Complete clinical history and physical examination, including head and neck and rectal examination; CBC, LDH, and serum markers; and CT of thorax, abdomen, and pelvis	All patients
Serum tumor markers	
αFP, βHCG	Midline presentation
PSA	Men with adenocarcinoma and bone metastasis
CA-125	Women with peritoneal adenocarcinoma
Mammography	All women
Breast MRI	Women with axillary adenocarcinoma
PET/CT	Selected cases: Cervical squamous cell carcinoma If radical treatment is possible Sign/symptom/IHC oriented Neuroendocrine tumor
Endoscopy	
Octreoscan/DOTATOC and chromogranin A	Suspected head and neck tumors, brain metastases, and suspected pelvic neoplasms
MRI	
Chromogranin A	Suspected neuroendocrine malignancy

αFP: α-fetoprotein; βHCG: β-human chorionic gonadotropin; CA-125: Cancer antigen 125; CBC: Complete blood count; CT: Computed tomography; CUP: Cancer of unknown primary site; IHC: Immunohistochemistry; LDH: Lactate dehydrogenase; MRI: Magnetic resonance imaging; PET: Positron emission tomography; PSA: Prostate-specific antigen

patients (ECOG PS > 2) with unfavorable CUP receive best supportive care (III, B).

Diagnostic work-up

The diagnostic process in patients with CUP seeks to identify subgroups that can benefit from a specific therapeutic procedure, avoiding prolonged, expensive diagnostic processes that provide little therapeutic benefit for the patient [14]. The diagnosis work-up is summarized in Table 4.

Anamnesis and physical examination

The standard clinical work-up includes a complete medical history, with attention to smoking history (smokers are at increased risk of developing CUP), other diseases, and a patient or family history of neoplasms. A thorough physical examination must also be carried out. This should include head and neck and rectal examination, examination of the testes in males, and pelvic/gynecologic and breast examination in women [15].

Laboratory tests

Required laboratory tests include complete blood count, liver and kidney function tests, electrolytes (including calcium), and LDH, given that this is an important prognostic factor [1, 14].

Serum tumor markers are often elevated in a non-tumor type-specific manner in patients with CUP, so their routine measurement offers no diagnostic or prognostic assistance. However, serum tumor marker tests are indicated in certain clinical situations: determination of serum prostate-specific antigen (PSA) in male patients with bone metastasis (to exclude occult metastatic prostate cancer); germ cell tumor markers (α-fetoprotein [AFP] and β-human chorionic gonadotropin [β-HCG]) in males with midline disease; cancer antigen 125 (CA-125) in women with peritoneal involvement; and chromogranin A in patients with a possible neuroendocrine malignancy [1]. Importantly, serum AFP is not recommended as a diagnostic test to suggest hepatocellular carcinoma in CUP with liver-dominant disease, as this indication is not supported by the 2023 ESMO guidelines due to its limited sensitivity and specificity.

Imaging tests

CT of the thorax, abdomen, and pelvis is routinely performed as part of the initial diagnostic work-up [1]. In addition to attempting to detect the primary, this can locate lesions that can be biopsied [16]. Mammography should be performed in cases of adenocarcinoma in women. Magnetic resonance imaging (MRI) is recommended in cases of suspected brain metastasis, head and neck tumors, and pelvic neoplasms.

The value of positron emission tomography (PET)–CT in the CUP diagnostic work-up remains to be fully evaluated in large-scale prospective studies. Given that it is more effective in detecting additional metastases rather than primary

tumors, PET–CT should generally only be performed to rule out the possibility of more widespread metastases before radical locoregional therapy for localized CUP, such as single site/oligometastatic lesions and cervical lymph node metastases indicative of head and neck-like CUP [17, 18].

Indications for further procedures

Further procedures include: (i) laryngoscopy: useful in cases with cervical lymph node involvement; (ii) bronchoscopy: in cases with hilar or mediastinal lymph node involvement, and pulmonary symptoms; (iii) gastroscopy: if the patient has abdominal symptoms or positive fecal occult blood test; (iv) colonoscopy: if the patient has abdominal symptoms or positive fecal occult blood test, or biopsy with colorectal immunohistochemistry (IHC) (cytokeratin [CK]20⁺/CK7⁻/caudal-type homeobox 2 [CDX2⁺]); (v) testicular ultrasound: if a retroperitoneal or mediastinal mass is present; (vi) gynecologic ultrasound: if pelvic or peritoneal metastases are present or CK7⁺ on the biopsy tissue; and (vii) breast MRI: if adenocarcinoma with negative mammography and metastasis to axillary lymph nodes are present.

Histology and immunochemistry

Pathological assessment of good quality tumor tissue samples is crucial for CUP diagnosis [1]. Core biopsy is preferred to fine-needle biopsy or cytology. Further procedures such as surgical biopsies may be considered when the initial sample is inadequate to confirm the diagnosis. Histological evaluation must rule out some special tumors with a specific therapeutic approach that are not classified as CUP, even if the site of origin is unknown (hematological malignancies, germ cell tumors, melanomas, sarcomas and neuroendocrine tumors) [1, 19]. Thereafter, CUP can be categorized into morphological subgroups [1] as follows: well to moderately differentiated adenocarcinoma (~50%); poorly differentiated adenocarcinoma or undifferentiated carcinoma (~30%); squamous cell carcinoma (~15%); and undifferentiated neoplasms (~5%).

IHC plays an essential role in the evaluation of metastatic tumor tissue specimens. It has a relatively low cost compared with other techniques, but it also has limitations. Scarce biopsied CUP tumor tissue has historically been used for extensive IHC analyses to determine the most likely tissue of origin, but it is now important to reserve tumor tissue for additional molecular studies [7]. IHC should be conducted to determine the most likely cell lineage, to exclude highly chemosensitive and potentially curable tumors, and/or to rule out hormone-sensitive malignancies amenable to specific therapy. For undifferentiated neoplasms, initial IHC screening typically includes a broad-spectrum keratin to identify tumors of epithelial origin [1]. Staining for chromogranin

A and synaptophysin is needed to profile neuroendocrine differentiation.

Once carcinoma has been confirmed and other treatable malignancies excluded (i.e., lymphoma and sarcoma), additional IHC can be conducted to help determine the likely tissue of origin [5]. Among keratin family members, CK7 and CK20 can be used to help predict primary sites for carcinomas [1]. Although these expression patterns may provide an indication of the primary site of origin and help to direct further work-up, cases that do not fit these profiles are encountered frequently. The CK7-positive and CK20-negative immunophenotype is the most common in CUP, but it is not particularly useful for suggesting a specific anatomical site of origin [20].

The most common CK7 and CK20 profiles and other positive markers are shown in Fig. 1 and Table 5. We propose a step-by-step algorithm to arrive at a CUP diagnosis according to CK7 and CK20 expression and other IHC markers that help to determine the most likely site of origin (Table 5).

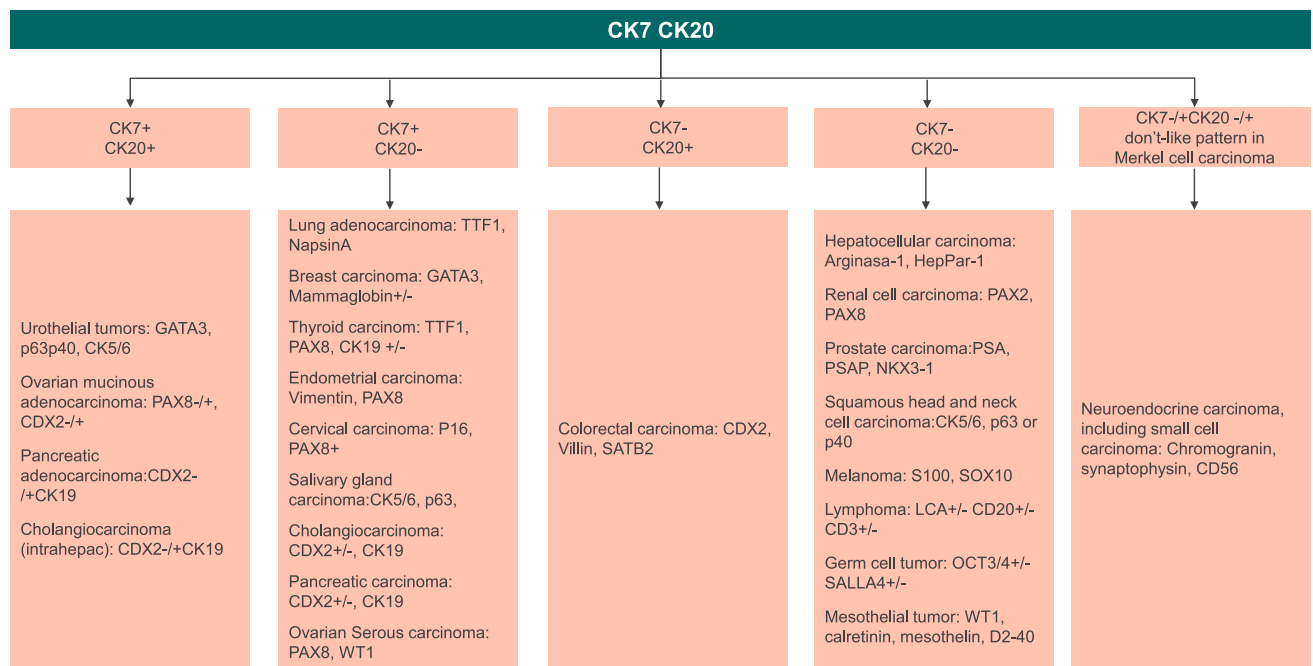
Molecular profiling strategies to guide treatment

Two types of molecular profiling strategies are possible in patients diagnosed with CUP: gene expression profiling aimed at identifying the likely primary tumor site (tissue of origin prediction) to help select site-specific therapies, and use of next-generation sequencing (NGS) platforms that characterize the tumor mutational profile to facilitate tumor-agnostic MGT [3, 5].

Molecular profiling aimed at identifying the primary tumor for site-specific therapy

Molecular profiling platforms that use DNA methylation-based or RNA expression-based techniques to predict the tissue of origin in patients with CUP generate similarity scores for the molecular profiles of CUP tumor specimens compared with the genetic or epigenetic characteristics of known primary tumor types [23–27]. Once the molecular profile of the tumor has been obtained, it is compared with the profiles of tumors of known origin contained in a reference database. The tumor type with the highest similarity score indicates the most likely CUP tissue of origin [6].

Until recently, evidence in support of site-specific therapy based on molecular studies to predict the tissue of origin in CUP was limited to short series and retrospective phase II studies [26, 28, 29]. Two prospective randomized studies failed to demonstrate a significant benefit of tissue-of-origin testing and site-specific therapy compared with standard empiric chemotherapy [30, 31]. More recently, the Fudan CUP-001 trial, conducted at a single center in China in 182 patients with CUP not amenable to curative surgery or radiotherapy and an ECOG PS of 0–2, reported



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Fig. 1 CK7/CK20 profile and additional markers Adapted from Tomuleasa C, et al. [21]

significantly longer median progression-free survival (PFS) in patients randomized to site-specific therapy guided by a 90-gene expression assay compared with patients randomized to empiric chemotherapy (9.6 vs 6.6 months; hazard ratio [HR] 0.68; $p=0.017$) [6]. Median OS was also prolonged with site-specific treatment compared with empiric chemotherapy, but not to a statistically significant extent (28.2 vs 19.0 months; HR 0.75; $p=0.098$). Whereas patients treated with site-specific therapy in earlier randomized trials primarily received chemotherapy, 45% of patients in the CUP-001 trial site-specific treatment group received targeted agents or immunotherapy [6]. This reflects rapid advances in MGT and immunotherapy for various tumor types in recent years and may have contributed to the positive CUP-100 trial results [3, 6, 32]. It is, therefore, still not known whether site-specific treatment improves outcomes for patients with unfavorable CUP without an actionable molecular profile [33].

Molecular profiling for tumor-agnostic targeted therapy or immunotherapy

NGS techniques enable the identification of targetable mutations and genomic signatures that can help guide tumor-agnostic MGT. NGS can identify genomic alterations in 87% of patients, and 30–40% of these patients have actionable or potentially actionable molecular targets [34–45]. These

include *KRAS G12C*, *PIK3CA*, *FGFR2/3*, *BAP1*, *ERBB2*, *BRAF*, *IDH1/2*, *PTEN*, *GNAS*, *EGFR*, *MET*, *CDK4/6*, *NTRK1*, *BRCA1/2*, *PALB2*, *ROS1*, *RET*, *ALK*, *JAK2*, *ATM*, *CHEK2*, *NRAS*, *HRAS*, *SMO*, *PTCH1*, and *AKT1* [7, 33]. The main objection to an NGS approach without identifying the most likely tissue of origin stems from potential for the response to a drug targeting a given mutation to be influenced by the tumor type in which the mutation is found (i.e., the tumor histology and location may affect the response to MGT) [46–50].

Comprehensive genomic profiling (CGP) uses NGS to analyze a large number of genes and detect genomic alterations such as DNA insertions/deletions, copy number variations, and gene rearrangements, as well as genomic signatures involving microsatellite instability (MSI) and tumor mutational burden (TMB). These are all of paramount importance for clinical decision-making regarding targeted and/or immunotherapeutic treatment options [7, 35, 36, 41].

NTRK1,2,3 fusions, *RET* fusions. *FGFR1/2/3* fusions/mutations, *BRAF V600E* mutations, MSI-H and TMB-high are all ESMO scale for clinical actionability of molecular targets (ESCAT) level IC tumor-agnostic targets [50]. TMB-H is also ESCAT level IB specifically in the CUP setting, and *ALK* fusions are ESCAT level IIB [50]. There is currently consensus on a tumor-agnostic indication for the use of NTRK inhibitors for *NTRK1,2,3* fusions, PD-1 checkpoint inhibitors for MSI-H, RET inhibitors for *RET* fusions, BRAF inhibitors + MEK inhibitors for *BRAF* mutations,

Table 5 Step-by-step algorithm to arrive at a cancer of unknown primary diagnosis using immunohistochemistry

Diagnosis	
<i>Step one (most likely cell lineage)</i>	
CK ⁺ , S100 [−] , CD45 [−] , vimentin [±]	Carcinoma
CLA ⁺ , CD45 ⁺ , vimentin ⁺ , CK [−] , S100 [−]	Lymphoma
S100 ⁺ , vimentin ⁺ , CK [−] , CD45 [−]	Melanoma
S100 [−] , vimentin [±] , CK [−] , αSMA, CD45 [−]	Sarcoma
<i>Step two (categorizes carcinomas and into broad subgroups)</i>	
CK7 and/or CK20, PAS	Adenocarcinoma
PLAP, OCT4, αFP, βHCG	Germ cell tumor
Chromogranin, synaptophysin, PGP9.5, CD56	Neuroendocrine carcinoma
CK5 or CK6, p63	SCC
<i>Step three (categorizes carcinomas into subgroups according CK7/CK20 expression)</i>	
CK7 ⁺ and CK20 ⁺	Ovarian mucinous or pancreatic adenocarcinoma, urothelial carcinoma, cholangiocarcinoma
CK7 [−] and CK20 ⁺	Colorectal or Merkel cell carcinoma
CK7 ⁺ and CK20 [−]	Lung adenocarcinoma; cholangiocarcinoma; breast, thyroid, endometrial, ovarian, cervical, salivary gland or pancreatic carcinoma
CK7 [−] and CK20 [−]	SCC; hepatocellular, renal cell, prostate, small-cell lung cancer, head and neck carcinoma
<i>Step four (indicates potential origin)</i>	
TTF1, napsin A (CK7 ⁺ /CK20 [−])	NSCLC (adenocarcinoma)
GCDFP-15, mammaglobin, GATA3, ER (CK7 ⁺ /CK20 [−])	Breast carcinoma
PSA, PAP, NKX3.1 (CK7 [−] /CK20 [−])	Prostate carcinoma
CDX2, CEA, SATB2 (CK7 [−] /CK20 ⁺)	Colon carcinoma
ER, CA-125 (CK7 ⁺ /CK20 [−])	Endometrial carcinoma
ER, CA-125, mesothelin, WT1 (CK7 ⁺ /CK20 [−])	Serous ovarian cancer
CA-125, S100 (CK7 ⁺ /CK20 ⁺)	Pancreatic adenocarcinoma
CD10, marker for RCC (CK7 [−] /CK20 [−])	RCC
TTF 1, thyroglobulin, PAX8 (CK7 ⁺ /CK20 [−])	Thyroid carcinoma
Hep Par 1, αFP, polyclonal CEA, CD10, CD13, Arg 1 (CK7 [−] /CK20 [−])	Hepatocellular carcinoma

αFP: α-fetoprotein; Arg 1: arginase 1; αSMA: α-smooth muscle actin; βHCG: β-human chorionic gonadotropin; CA-125: Cancer antigen 125; CDX2: Caudal-type homeobox 2; CEA: Carcinoembryonic antigen; CK: Cytokeratin; CLA: Cutaneous lymphocyte-associated antigen; ER: Estrogen receptor; GCDFP-15: Gross cystic disease fluid protein 15; HCG: Human chorionic gonadotropin; Hep Par 1: Hepatocyte paraffin 1; NKX3.1: NK3 homeobox 1; NSCLC: Non-small cell lung cancer; OCT4: Octamer-binding transcription factor 4; PAP: Prostatic acid phosphatase; PAS: Periodic acid Schiff; PAX8: Paired box gene 8; PGP9.5: Protein gene product 9.5; PLAP: Placental alkaline phosphatase; PSA: Prostate-specific antigen; RCC: Renal cell carcinoma; SATB2: Special AT-rich sequence-binding protein 2; SCC: Squamous cell carcinoma; TTF1: Thyroid transcription factor 1; WT1: Wilms tumor 1; NSCLC: Non-small-cell lung cancer

Adapted from Rassy E et al. [22]

pan-FGFR TKIs for *FGFR1/2/3* fusions/mutations, and PD-1/PD-L1 checkpoint inhibitors for TMB-H (> 10 mutations per megabase), as well as CUP-specific indications for ALK TKIs for *ALK* fusions and PD-1 inhibitors plus anti-CTLA4 inhibitors for TMB-H (I, B).

The CUPISCO trial was a large international phase II study that compared CGP-informed, tumor-agnostic MGT with standard platinum-based chemotherapy in > 600 newly diagnosed patients with unfavorable, non-squamous CUP and an ECOG PS of 0–1 [7]. Patients with disease control after three cycles of standard first-line platinum-based chemotherapy (thus, preventing treatment delay while awaiting CGP results) were randomized to MGT or continuation

of chemotherapy. In the MGT arm, 27% of patients had an actionable genomic alteration or signature and were stratified to receive one of eleven different MGT options, comprising targeted therapy or monotherapy with the immune checkpoint inhibitor atezolizumab; the remaining 73% received continued chemotherapy plus atezolizumab. A significant PFS benefit was reported for the overall MGT group compared with the chemotherapy group (6.1 months vs 4.4 months; HR 0.72, $p=0.0079$). Early OS results favored the MGT group (14.7 months vs 11.0 months for the chemotherapy group). When the analysis was restricted to patients with an actionable genomic alteration or signature, median PFS was 8.1 months in those treated with MGT

versus 4.7 months in those treated with chemotherapy (HR 0.65). In patients without an actionable molecular profile, median PFS was 5.5 months with chemotherapy plus atezolizumab versus 4.4 months with chemotherapy alone (HR 0.76). CUPISCO is the largest interventional, randomized trial in CUP performed to date, and the first study to show a survival benefit of MGT over platinum-based chemotherapy [7]. The results of this study highlight the importance of incorporating CGP into routine clinical practice for patients with unfavorable CUP [33, 50].

Recommendations for the use of molecular profiling

To be able to take advantage of potential treatment options involving targeted therapies or immune checkpoint inhibitors, there is consensus among experts that molecular profiling should be a routine part of the CUP diagnostic process [1, 34, 38, 39, 41, 42]. Indeed, NGS may be carried out routinely in CUP (II, B). This recommendation is based on the significantly improved PFS obtained with CGP-informed tumor-agnostic MGT versus empiric chemotherapy in patients with unfavorable CUP participating in the CUPISCO trial [7]. A meta-analysis of five prospective studies, including the CUPISCO and FUDAN CUP-001 trials, evaluating MGT versus empiric chemotherapy therapeutic approaches has since revealed significant OS and PFS benefits in favor of MGT versus empiric chemotherapy in patients with CUP [51].

The use of gene expression profiling to help identify CUP tissue of origin is not yet supported by high-level evidence [1]. Despite the promising results with such a

strategy in the FUDAN CUP-001 trial, positive results from a large prospective, international, randomized trial will be required before gene expression profiling to predict tissue of origin for site-specific therapy is recommended as standard practice in patients with CUP [2, 32]. Patients who are candidates for NGS should show an absence of clear clinical criteria for a known primary tumor, as well as an absence of clinical-IHC correlation. Initially, an anatomopathological morphological evaluation and IHC should be performed as precisely as possible, ensuring that after IHC analysis, there is sufficient tissue for molecular analysis. If there are no clear results with IHC, NGS should be used to characterize genomic alterations that enable matched targeted treatments or immunotherapy, especially in fit patients with a 0–1 ECOG PS and not very high LDH (III, B) (Table 1 and Fig. 2) [13].

Site-specific treatment according to histopathologic and/or clinical criteria

There is a subgroup of patients, between 10–15% of all CUP, who have a favorable CUP subtype based on clinical and histopathological similarities to cancers with a known primary (Table 3). Emerging non-randomized data favor the identification of certain subgroups of patients in whom a more specific treatment, similar to that for primary tumors which their conditions resemble, may be beneficial, such as renal-cell-like CUP, lung-like CUP, and colorectal-like CUP (III, B).

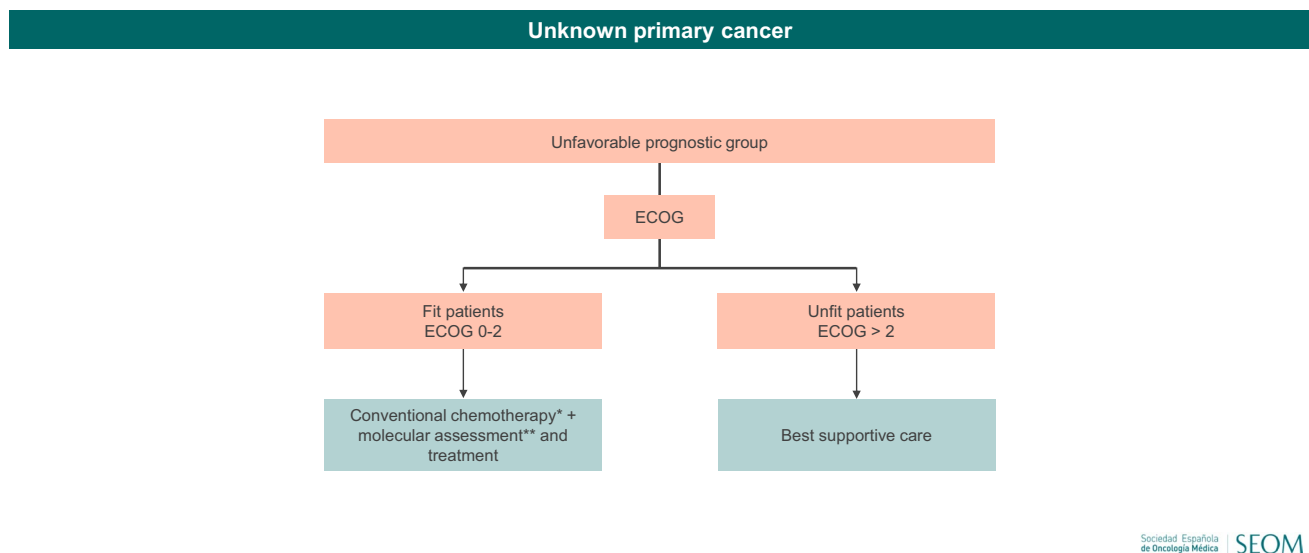


Fig. 2 Management of unfavorable CUP according to ECOG performance status. Fit patients (ECOG 0–2) are candidates for chemotherapy and molecularly guided treatment, whereas unfit patients

(ECOG > 2) should receive best supportive care *Platinum-based chemotherapy doublet. **See Fig. 1

It is important to point out that IHC results are only indicative of the location of a primary tumor and by themselves should be considered insufficient to guide site-specific treatment, hence the concept of “suggestive primary cancer profile”. It is, therefore, a reasonable approach to complement IHC tissue-of-origin information with NGS to detect mutations that can help to identify the putative primary or other actionable genomic alterations. In patients with favorable CUP, a genomic platform could help identify a genomic profile and/or an actionable genomic alteration, or biomarkers of response to immunotherapy to help inform site-specific therapy (III, B) [6, 36, 52].

Surgical resection and radiotherapy

When CUP is identified as localized disease, after complete staging (including PET) [53], local treatment can result in long disease-free intervals and improve prognosis. The most common sites include liver, bone, lung, skin, adrenal gland, and lymph nodes.

Resection of solitary lesions is preferred. If resection is not feasible, definitive local radiation therapy should be considered. Radiation therapy should also be considered for patients with risk factors for residual disease (e.g., multiple involved nodes or extracapsular spread) to maximize the chance of local control.

The role of neoadjuvant or adjuvant chemotherapy in CUP is undefined. Empirical adjuvant chemotherapy is reasonable in this setting, particularly in patients with poorly differentiated carcinoma or if indicated in a predicted tumor (IV, B).

Treatment of unfavorable CUP

The majority of patients with CUP (80–85%) are not included in the favorable CUP subgroups and their prognosis and treatment options are poor. The treatment of choice for patients with unfavorable CUP is empirical combination chemotherapy with a platinum agent plus another cytotoxic

agent (taxane, gemcitabine, irinotecan) [54], which is associated with low response rates of 15–20% and OS of about 9 months (Table 6) (II, A) [55–63].

Due to the poor prognosis of unfavorable CUP, the discovery of potential therapeutic molecular targets is essential to guide treatment away from conventional platinum-based chemotherapy and improve survival outcomes.

Before the CUPISCO trial [7], actionable genomic alterations matched to targeted therapies (Table 7) had mainly been studied retrospectively in patients with CUP [35, 64–73]. Biomarkers of response to immunotherapy such as TMB-high, MSI-high, and PD-L1 expression have also been studied and determined to be important predictors of treatment response to immune checkpoint inhibitors in patients with unfavorable CUP [33].

Targeted therapies that have received approval for tumor-agnostic treatment of refractory and unresectable solid tumors include NTRK inhibitors, such as larotrectinib and entrectinib in NTRK fusion tumors with remarkable response [70], and pembrolizumab for MSI-high and TMB-high solid tumors (III, B) [73]. Other tumor-agnostic treatments now include BRAF inhibitors plus MEK inhibitors for *BRAF V600E* mutations, RET inhibitors for *RET* fusions, and pan-FGFR tyrosine kinase inhibitors for *FGFR1/2/3* fusions or mutations [52]. ALK TKIs and PD-1 inhibitors can be used in unfavorable CUP with *ALK* fusions or TMB-H, respectively [52].

The CUPISCO trial was not designed to statistically analyze individual cohorts, but compared with chemotherapy, the greatest PFS and OS benefits were observed in unfavorable CUP patients with TMB-high or MSI-high treated with atezolizumab, those with *BRAF* mutations treated with vemurafenib plus cobimetinib, and those with *FGFR* alterations treated with pemigatinib [7]. Further investigation is needed before any recommendations can be made in this regard. Likewise, although the CUPISCO trial findings indicate that the PFS benefit of tumor-agnostic MGT was mainly driven by matched targeted therapies (median PFS of 8.1 in patients with an actionable molecular target vs 4.7 months with chemotherapy in such patients) [74], the results suggest

Table 6 Prospective trials with chemotherapy regimens in patients with unfavorable cancer of unknown primary site

Ref	CT schedule	ORR (%)	OS (months)
Culine 2023 [56]	Cisplatin + gemcitabine vs cisplatin + irinotecan	55 vs 38	8 vs 6
Greco 2020 [57]	Cisplatin + docetaxel vs carboplatin + docetaxel	26 vs 22	8 vs 8
Huebner 2009 [58]	Carboplatin + paclitaxel vs gemcitabine + vinorelbine	23.8 vs 20	11 vs 7
Dowel 2001 [59]	Carboplatin + etoposide vs paclitaxel + 5-FU + leucovorin	19 vs 19	8.3 vs 6.4
Briasoulis 2008 [60]	Irinotecan + oxaliplatin	13	2.7
Schuetz 2009 [61], Møller 2010 [62], Hainsworth 2010 [63]	Capecitabine + oxaliplatin	11.7–19	3.9–9.7

5-FU: 5-fluorouracil; CT: Chemotherapy; ORR: Objective response rate; OS: Overall survival

Table 7 Targeted therapies in relation to genomic alterations

Identified actionable genome alterations	Associated targeted therapies
<i>ALK</i>	Crizotinib, ceritinib, alectinib, brigatinib, lorlatinib
<i>RET</i>	Selpercatinib, pralsetinib
<i>ROS1</i>	Crizotinib, ceritinib, lorlatinib, repotrectinib
<i>NTRK</i>	Entrectinib, larotrectinib
<i>PI3K</i>	Temsirolimus, everolimus, alpelisib
<i>BRCA1/2</i>	Olaparib, niraparib, rucaparib, talazoparib
<i>EGFR</i>	Gefitinib, erlotinib, afatinib, dacomitinib, osimertinib
<i>FGFR1/2/3</i>	Pazopanib, ponatinib, erdafitinib, pemigatinib
<i>MET</i>	Crizotinib, tepotinib, capmatinib
<i>BRAF V600, BRAF K601E</i>	Vemurafenib, encorafenib, dabrafenib, regorafenib
<i>KRAS G12C</i>	Sotorasib, adagrasib
<i>ERBB2</i>	Trastuzumab, lapatinib, pertuzumab, afatinib, neratinib, trastuzumab-deruxtecan, trastuzumab emtansine, tucatinib
<i>SMO, PTCH1</i>	Vismodegib
<i>AKT1, PIK3CA, PTEN</i>	Ipatasertib, capivasertib
<i>IDH1</i>	Ivosidenib
TMB-high	Immune checkpoint inhibitors
MSI-high	Immune checkpoint inhibitors

BRAF: B-Raf proto-oncogene; EGFR: Epidermal growth factor receptor; FGFR: Fibroblast growth factor receptor; IDH1: Isocitrate dehydrogenase 1; MSI: Microsatellite instability; NTRK: Neurotrophic tropomyosin receptor kinase; PI3K: Phosphoinositide 3-kinase; PTCH1: Patched 1; PTEN: Phosphatase and tensin homolog; RET: Rearranged during transfection; SMO: Smoothed; TMB: Tumor mutational burden

that chemotherapy plus cancer immunotherapy combinations should be further investigated in patients without an actionable target (median PFS of 5.5 months with chemotherapy + atezolizumab vs 5.5 months for those treated with chemotherapy alone) [7].

Conclusion

CUP represents 2–5% of all tumor diagnoses and is characterized by an aggressive clinical course, an unpredictable metastatic pattern, early dissemination, intrinsic resistance to treatment, and poor prognosis. It is a biologically complex entity, with intrinsic genetic alterations that condition a behavior different from that of putative primaries [2, 11].

Given the aggressiveness of most CUPs, it is important to make a diagnosis as early as possible, performing the diagnostic process in a standardized manner, both clinically and pathologically, reserving part of the tumor sample, if possible, to determine actionable mutations, especially in patients with ECOG PS 0–2.

The accurate diagnosis and optimal treatment of CUP remain major challenges. Improvements in diagnostic techniques, including the latest generation of IHC markers and molecular profiling platforms, have, however, helped to refine the prediction of the possible primary in many cases. New molecular profiling platforms based on CGP have the

potential to set a new standard for precision oncology in CUP by enabling the detection of actionable genomic alterations for which personalized targeted treatments are available. The Fudan CUP-001 and CUPISCO trials have, for the first time, demonstrated survival benefits when molecular profiling is used to inform site-specific treatment and tumor-agnostic MGT in patients with CUP [6, 7]. Together, these treatment strategies will provide a more tailored approach to the management of patients diagnosed with CUP in the future [2, 3]. Routine NGS will increase treatment options and be especially beneficial for patients with unfavorable CUP who are found to have actionable genomic alterations and/or a putative primary site, and whose treatment options would otherwise be restricted to empiric platinum-based chemotherapy.

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Declarations

Conflict of interest Ferrán Losa has acted in an advisory or consultancy role for Amgen and Roche; has participated in a speaker bureau for Amgen, Merck, Roche, Sanofi, and Servier; and has received travel/accommodation fees or expenses from Amgen, Merck, Roche, and Sanofi. Olatz Etxaniz has acted in an advisory or consultancy role for Ipsen, Merck, Pfizer, and Roche; has participated in a speaker bureau for BMS, Ipsen, and Pfizer; and has received expenses from BMS, Clovis, Janssen, and Pfizer. Alejandra Giménez has acted in an advisory or consultancy role for Ipsen; has participated in a speaker bureau for Ipsen, Merck, and Sanofi; and has received expenses from Amgen, Merck, and Roche. Lara Iglesias has acted in an advisory or consultancy role for Bayer, BMS, Lilly, MSD, Roche, and Sanofi; and has participated in a speaker bureau for Bayer, BMS, Eisai, Kyowa-kirin, Merck, MSD, Roche, and Sanofi. Federico Longo has acted in an advisory or consultancy role for Ferre and Roche. Esteban Nogales has acted in an advisory or consultancy role for Daiichi Sankyo and Roche; and has received speaking fees from AstraZeneca, Kyowa Kirin, Pfizer, and Roche. Gemma Soler has acted in an advisory or consultancy role for Amgen, Kiowa, and Roche; has participated in a speaker bureau for Amgen, Merck, Roche, Sanofi, and Servier; and has received travel/accommodation fees or expenses for Amgen, Merck, Roche, and Sanofi. Isaura Fernández has acted in an advisory or consultancy role for AstraZeneca and Roche; and has participated in a speaker bureau for AstraZeneca, Clovis, Glaxo, MSD, Pfizer, and Roche. Paula Gomila and Antonio Sánchez have nothing to disclose.

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Informed consent The manuscript does not contain clinical studies or patient data.

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