Progress in refining the clinical management of cancer of unknown primary in the molecular era

Elie Rassy[1] ✉ *and Nicholas Pavlidis*[2] ✉

Abstract | Cancer of unknown primary (CUP) is an enigmatic disease entity encompassing heterogeneous malignancies without a detectable primary tumour, despite a thorough diagnostic workup. A minority of patients with CUP (15–20%) can be assigned a putative primary tissue of origin according to clinical and histopathological findings and typically have a more favourable prognosis with the use of corresponding tumour type-specific therapies. Thus, the majority of patients with CUP have disease that cannot be assigned to a culprit primary tumour, are treated with empirical chemotherapy and have a poor prognosis. In the molecular era, the use of (epi) genomic or transcriptomic CUP classifiers and DNA or RNA sequencing offers two, sometimes overlapping, therapeutic strategies: tumour type-specific therapy and biomarker-guided therapy. Published data reveal that the accuracy of site-of-origin predictions made using CUP classifiers ranges between 54% and 98% when compared with the assignment made according to the recommended clinicopathological criteria. These advances have led to promising results in non-randomized prospective studies evaluating the efficacy of tumour type-specific therapy; however, the favourable outcomes were not confirmed in randomized controlled studies comparing this approach with standard empirical chemotherapy. Currently, the evidence supporting the use of biomarker-guided therapies is limited to case reports and small case series. In this Review, we discuss the clinical management of CUP in the era of precision medicine. We focus on the advances in understanding the biology of CUP, the implications for the diagnosis and classification of CUP according to the tissue of origin and the shift away from empirical therapy towards tailored therapy.

Cancer of unknown primary (CUP) is a clinically well-recognized but biologically enigmatic disease entity that encompasses a heterogeneous group of metastatic cancers that lack an identifiable primary tumour, despite extensive diagnostic investigations[1]. Patients with CUP have a median age of 65 years, with only a marginal difference in incidence between men and women[2–4]. Since the 1960s, the incidence of CUP in adolescents and young adults has generally been stable, whereas wide variations have been observed in older adults (aged >50 years)[4,5]. In the early 1990s, CUP accounted for 3–5% of all malignancies, was the seventh or eighth most frequent cancer and ranked as the fourth most common cause of cancer-related deaths[3,4,6]. In the current era, however, several advances in radiological and molecular assessments have yielded a higher identification rate of primary tumour sites and have, therefore, decreased the proportion of patients with cancer who are diagnosed with CUP to 1–2%[7].

Despite these advances, the mechanisms underlying the carcinogenesis and progression of CUP remain enigmatic, which raises questions about the accuracy of the diagnostic workup performed at each encounter[8]. Indeed, CUP can be falsely or prematurely diagnosed in patients who undergo suboptimal investigations at the time of presentation and in those in whom a primary tumour becomes detectable during the disease course after the initial diagnosis (less than 10% of patients with CUP)[9–11]. Interestingly, the available literature lacks any systematic evidence regarding this issue and is mainly based on personal experiences. The accuracy of the diagnostic tools can also be compromised by extensive immunoediting of cancer cells, whereby tumour clones recognizable by the immune system are eradicated, thus editing out immunogenic features and resulting in the persistence of poorly immunogenic or immunosuppressive clones[12]. This editing process might destroy the features relevant to immunohistochemistry

[1]*Department of Medical Oncology, Institut Gustave Roussy, Villejuif, Paris, France.*

[2]*Medical School, University of Ioannina, Ioannina, Greece.*

✉*e-mail: elie.rassy@hotmail.com; npavlid@uoi.gr*

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Key points

- Cancer of unknown primary (CUP) is a clinically well-recognized, but biologically enigmatic, disease entity that encompasses a heterogeneous group of metastatic cancers without an identifiable primary tumour, despite extensive investigations.
- The current era is witnessing a decrease in the proportion of patients with cancer who are diagnosed with CUP to 1–2%, in comparison to 3–5% in the early 1990s.
- The recommended diagnostic workup includes a thorough physical examination, basic blood analyses, evaluation of tumour biomarkers (guided by the clinical scenario) and CT scans of the thorax, abdomen and pelvis.
- Multiple assays have been developed to predict the putative tissue of origin by alignment with prominent molecular profiles established for cancers with a known primary.
- Together with the overall clinical picture and results of pathology investigations, molecular profiling of CUP can have therapeutic implications by guiding treatment decisions according to the putative primary tumour type and the detection of potential driver mutations.
- The design of future prospective randomized trials should address the minority of patients that present with targetable mutations, especially driver mutations, based on the findings of molecular analyses and the predicted primary tumour type.

and gene-expression profiling (GEP) assays used in the identification of the tissue of origin of suspected CUP. Nonetheless, the clinical reality supports the existence of a distinct group of tumours with a natural history different from that of metastases originating from known primary tumours[13–15]. The unique natural history of CUP is characterized by early dissemination, an aggressive clinical course, an unpredictable metastatic pattern, intrinsic treatment resistance and a dismal prognosis[16,17]. CUP tumours have been hypothesized to possess not only a genetic signature specific for their primary tissue of origin, but also a second, independent genetic signature that is likely to reflect the different biology of the unknown primary tumours relative to that of known primary tumours[18].

The foundations for a role of molecular diagnostics in the management of CUP are formed on evidence from familial studies revealing robust associations between the location of tumour sites in patients with CUP and primary tumour sites in first-degree relatives[10,19,20]. Assays in which GEP is used to predict the primary tumour site, which are based on transcriptional signatures of the non-malignant tissue of origin that are retained in most cancers, might be useful in guiding treatment decisions[21]. Published data, mainly from case reports, case series and non-randomized prospective studies, have suggested that the use of tumour type-specific therapies leads to favourable outcomes in patients with CUP predicted to have originated from a particular type of primary tumour[22–25]; however, randomized controlled trials have failed to show improvements in outcomes with tumour type-specific therapy versus empirical chemotherapy[26,27].

Herein, we review the current standards in the diagnosis and classification of CUP, as well as the advances in understanding the biology of CUP. With the aim of facilitating progress in refining the clinical management of CUP in the molecular era, we also discuss the available data relating to the role of molecular profiling in the diagnosis and treatment of CUP.

Diagnosis of patients with CUP

The most appropriate approach to the diagnostic workup of a patient with suspected CUP depends on the influence of the ultimate diagnosis for treatment decisions[18]. Some experts consider CUP as a unique clinical entity with distinct features that obviate the need to identify the culprit primary tumour and favour the use of CUP-specific treatments[1]. By contrast, other experts consider CUP to be an artificial classification of malignant metastases from undetected and perhaps undetectable primary tumours and suggest intensive diagnostic evaluations to identify the culprit primary tumour and administer the corresponding disease-oriented therapy[18]. The clinical experience to date suggests that neither approach is superior over the other; however, oncologists are required to make a quick decision on how to treat the disease, instead of potentially spending much of the patient's limited remaining lifetime — typically ~1 year — performing diagnostic tests.

The diagnostic and staging guidelines for patients with an anticipatory CUP diagnosis recommend a thorough physical examination, basic blood analyses and CT scans of the thorax, abdomen and pelvis as the standard diagnostic workup[1,28–30] (BOX 1). The evaluation of serum tumour markers has no independent diagnostic, prognostic or predictive value in patients with CUP, although some markers might be helpful in certain clinicopathological subsets of patients, such as cancer antigen 15-3 (CA-15-3; also known as mucin-1) in women with isolated axillary nodal adenocarcinomas and CA-125 (mucin-16) in women with primary peritoneal adenocarcinomas[31]. Further investigations, such as endoscopies and advanced imaging assessments, can be considered according to each clinical scenario[8,32]. For example, ^{18}F-fluorodeoxyglucose (FDG)-PET–CT scans might be a promising tool for identification of the primary tumour in patients with CUP. A meta-analysis of this approach indicated an overall primary detection rate of 34% (locations of primary tumours detected by FDG-PET–CT), but a sensitivity of 84% (true-positive FDG-PET–CT detection of the primary tumour) and a specificity of 84% (true-negative FDG-PET–CT findings) in comparison to histopathological analysis of tissue obtained by biopsy or surgery, which was considered as the reference standard[33].

A pathology review of a good-quality tissue sample is often recommended in the workup of patients with an anticipatory CUP diagnosis. On histopathological analysis, CUP tumours are most commonly categorized as adenocarcinoma of well-to-moderate differentiation, accounting for 50% of cases, followed by poorly or undifferentiated adenocarcinomas, squamous-cell carcinomas (SCCs) and undifferentiated neoplasms in 30%, 15% and 5%, respectively[17]. An initial assessment of cytokeratin 7 (CK7) and CK20 using immunohistochemistry can be very useful in identifying the primary tumour origin of metastatic adenocarcinomas of unknown primary (TABLE 1). The evaluation of human papilloma virus (HPV) status by PCR or p16 expression by immunohistochemistry can be of prognostic value in patients with squamous cell CUP of the head and neck or the abdomen, pelvis and/or retroperitoneum, with patients with

HPV-positive tumours generally having better outcomes than those with HPV-negative tumours[34,35].

Classification of patients with CUP

CUP comprises a broad spectrum of disease subsets with different prognoses; some patients clearly have better outcomes than the 'average' patient with CUP. More specifically, patients with CUP can be categorized into two main subgroups according to clinicopathological criteria[13].

The first subgroup comprises a minority of patients (15–20%) who present with a constellation of clinical and histological findings that are highly suggestive of a specific tissue of origin. Traditionally, this subgroup includes women with isolated axillary lymph node metastases from adenocarcinoma or with papillary serous carcinoma restricted to the peritoneum, men with osteoblastic bone metastasis and an elevated level of serum prostate-specific antigen, and patients with SCC restricted to cervical lymph nodes or inguinal lymph nodes, neuroendocrine CUP, metastatic melanoma of unknown primary or CUP restricted to a single metastatic site[1,13]. Other emerging disease subsets can also be included in this subgroup, such as squamous-cell CUP of the abdomen, pelvis and/or retroperitoneum, renal-cell CUP, lung CUP and colorectal CUP[34,36,37]. Generally, patients diagnosed with these entities have chemosensitive tumours and a favourable prognosis when treated according to the suggested putative primary tumour type[1,13,34,36]. For example, in general, patients with CUP have poor overall survival (OS) outcomes with chemotherapy regimens used in the treatment of colorectal cancer, such as folinic acid, 5-fluorouracil and oxaliplatin (FOLFOX) and capecitabine and oxaliplatin (CAPOX) (median OS duration of 3–9.7 months), whereas the subset of patients with colorectal CUP are commonly sensitive to such regimens (median OS duration of 21–37 months)[36,37].

The second subgroup encompasses the remaining majority of patients (80–85%) who present with disseminated disease that does not fit with any of the favourable-prognosis subsets[1,13]. Liver CUP is the most common subset (30–40%) and has the most dismal prognosis (median OS duration of 1–2 months and 12-month OS of 5–12%)[13,16,17,38,39]. Other CUP-involved organs include lymph nodes (35%), bones (28%) and brain (15%)[13,16,17,38,39]. The most frequent histological subtype in this subgroup is adenocarcinoma (in 64% of patients), followed by undifferentiated carcinoma (20%), neuroendocrine (9%) and SCC (3%)[13,16,17,38,39]. Unfortunately, patients diagnosed with this disease entity have chemoresistant tumours and are generally suboptimally treated according to the individual oncologist's best guess of a primary tumour type[1]. Prospective clinical trials in this subgroup of patients with CUP have revealed median OS durations of 3–11 months, 1-year OS of 25–40% and 5-year OS of 3–15%[40–42]. Real-world data from population-based studies suggest more dismal outcomes, with median OS durations of only 1–3 months and 1-year OS of 19%[3,43,44].

A comprehensive review of CUP epidemiology has revealed that patients can also be categorized according to age groups, which are associated with differences in pathology, metastatic patterns and survival outcomes[45]. In particular, adults and young adults seem to constitute a distinct subgroup[46]; in comparison to the general population of patients with CUP, adolescent and young adult patients have a higher incidence of SCCs (29% versus 10%) and undifferentiated neoplasms, including neuroendocrine tumours (39% versus 5%), as well as a longer median OS duration[46].

The pathogenesis of CUP

The mechanisms underlying the development and progression of CUP have not been fully elucidated[47]. DNA alterations in non-malignant stem cells or non-stem cells can enable type 2 progression of neoplasia, which yields a clonal proliferation of stationary and motile cells leading to local tumour growth and metastatic dissemination[48,49] (FIG. 1). Metastasization in the

Box 1 | **Recommended investigations for patients with an anticipatory CUP diagnosis**[1,28–30]

- Thorough physical examination, including the head and neck, in addition to a rectal examination for all patients; additional examination of the testes in males, and the pelvis gynaecological organs and breasts in females
- Basic blood analyses, including complete blood counts, liver and kidney function tests, and measurement of serum electrolyte, calcium and lactose dehydrogenase levels in all patients
- Analysis of serum tumour markers: prostate-specific antigen in men with osteoblastic bone metastases (suggestive of prostate cancer); cancer antigen 125 (CA-125; also known as mucin-16) in women with primary peritoneal adenocarcinoma (suggestive of ovarian cancer); CA-15-3 (mucin-1) in women with axillary nodal adenocarcinoma (suggestive of breast cancer); α-fetoprotein and human chorionic gonadotropin in patients with midline undifferentiated carcinoma (suggestive of germ cell tumours)
- CT scans of the thorax, abdomen and pelvis in all patients
- Mammography and breast MRI in women with axillary nodal adenocarcinoma
- FDG-PET–CT scans in patients with head and neck squamous-cell carcinoma and those with a single cancer of unknown primary (CUP) site
- Endoscopies, dependent on the clinical scenario: laryngoscopy in patients presenting with cervical lymph node involvement; bronchoscopy in patients with hilar or mediastinal lymph node involvement and pulmonary symptoms; gastroscopy in patients with abdominal symptoms or a positive faecal occult blood test; colonoscopy in patients with abdominal symptoms or a positive faecal occult blood test, or a biopsy sample with an immunohistochemical staining showing $CK20^{+}$, $CK7^{-}$ and $CDX2^{+}$ phenotype

CDX2, caudal type homeobox 2; CK, cytokeratin; FDG, ^{18}F-fluorodeoxyglucose.

Table 1 | **A step-by-step approach to immunohistochemistry evaluation in the diagnosis of CUP**

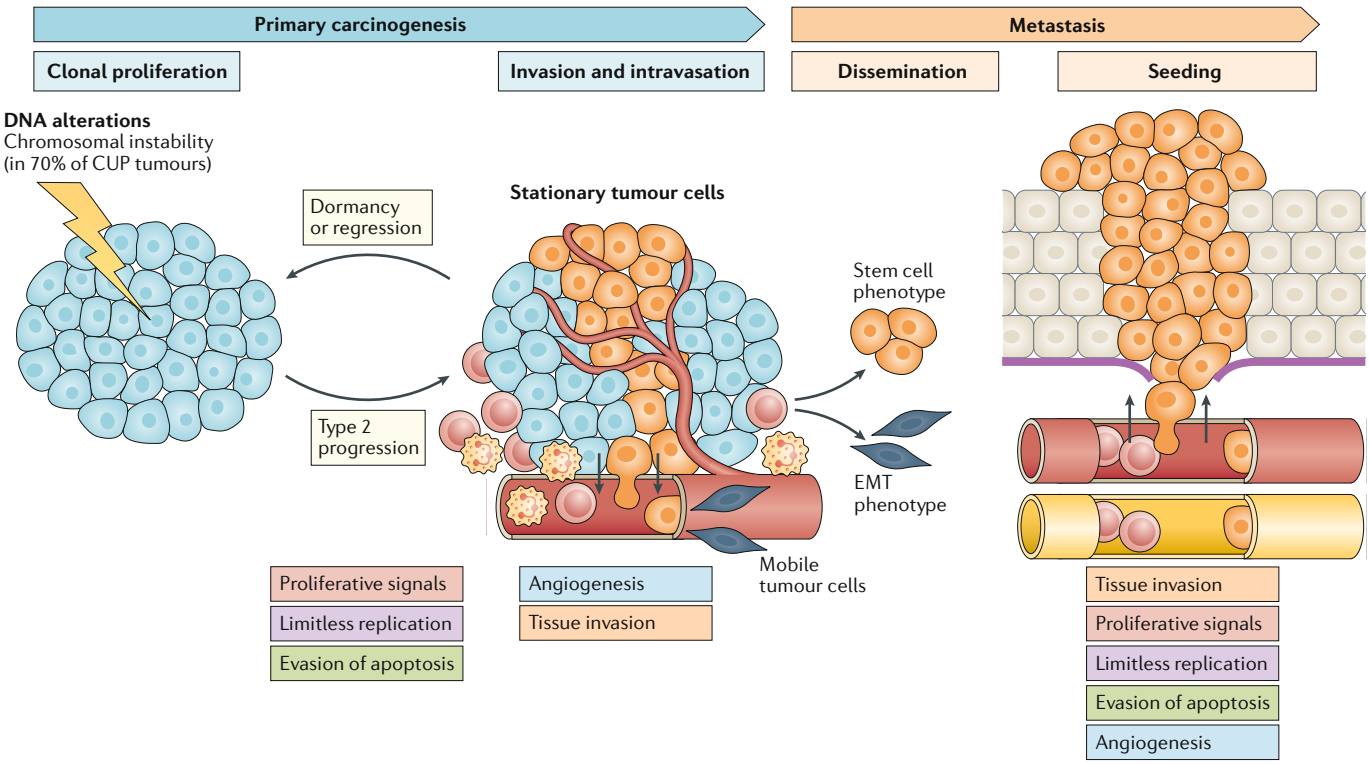
Immunohistochemical marker[a]	Potential cancer type
Step 1: detects broad types of cancer	
Pan-cytokeratin and/or EMA	Carcinoma
CLA and/or CD45RB and EMA$^-$	Lymphoma
Vimentin, desmin, S100, αSMA, myoD1, CD34, KIT and/or CD99	Sarcoma
S100, HMB45 and/or Melan-A	Melanoma
Step 2: detects broad types of carcinoma	
PAS, CK7 and/or CK20	Adenocarcinoma
CK5, CK6 and/or p63	SCC
Chromogranin, synaptophysin, PGP9.5 and/or CD56	Neuroendocrine carcinoma
PLAP, OCT4, AFP and/or hCG	Germ-cell carcinoma
Step 3: categorizes carcinomas into subgroups according to CK7 and CK20 expression	
CK7$^+$ and CK20$^+$	Ovarian mucinous or pancreatic adenocarcinoma, urothelial carcinoma, or cholangiocarcinoma
CK7$^+$ and CK20$^-$	Lung adenocarcinoma, cholangiocarcinoma, or breast, thyroid, endometrial, ovarian, cervical, salivary gland or pancreatic carcinoma
CK7$^-$ and CK20$^+$	Colorectal or Merkel cell carcinoma
CK7$^-$ and CK20$^-$	SCC or hepatocellular, renal cell, prostate, small-cell lung or head and neck carcinoma
Step 4: suggests potential origin of adenocarcinoma	
ER, GCDFP-15 and/or mammaglobulin (CK7$^+$ and CK20$^-$)	Breast carcinoma
CA-125, mesothelin, WT1 and/or ER (CK7$^+$ and CK20$^-$)	Ovarian cancer (serous papillary)
CA-125 and/or ER (CK7$^+$ and CK20$^-$)	Endometrial carcinoma
PSA and/or PAP (CK7$^-$ and CK20$^-$)	Prostate carcinoma
CDX2 and/or CEA (CK7$^-$ and CK20$^+$)	Colon carcinoma
CA-125 and/or mesothelin (CK7$^+$ and CK20$^\pm$)	Pancreatic adenocarcinoma
Hep Par-1, AFP, polyclonal CEA, CD10 and/or CD13 (CK7$^-$ and CK20$^-$)	Hepatocellular carcinoma
TTF1 (CK7$^+$ and CK20$^-$)	Non-small-cell lung cancer (lung adenocarcinoma)
CD10 (CK7$^-$ and CK20$^-$)	Renal cell carcinoma
TTF1 and/or thyroglobulin (CK7$^+$ and CK20$^-$)	Thyroid carcinoma

αSMA, α-smooth muscle actin; AFP, α-fetoprotein; CA-125, cancer antigen 125; CDX2, caudal type homeobox 2; CEA, carcinoembryonic antigen; CK, cytokeratin; CLA, cutaneous lymphocyte-associated antigen; CUP, cancer of unknown primary; EMA, epithelial membrane antigen; ER, oestrogen receptor; GCDFP-15, gross cystic disease fluid protein 15; hCG, human chorionic gonadotropin; Hep Par-1, hepatocyte-specific antigen; myoD1, myoblast determination protein 1; OCT4, octamer-binding transcription factor 4; PAP, prostatic acid phosphatase; PAS, periodic acid Schiff; PGP9.5, protein gene product 9.5; PLAP, placental alkaline phosphatase; PSA, prostate-specific antigen; SCC, squamous-cell carcinoma; TTF1, thyroid transcription factor 1; WT1, Wilms tumour protein.
[a]Potential cancer type designation is determined by positivity for marker, unless otherwise indicated.

context of CUP might occur according to two scenarios: 1) spread of the motile cells before uncontrolled local proliferation of non-motile neoplastic cells at the primary site of cell transformation[50–52]; and 2) dissemination of migratory cells from a primary tumour that formed initially but has subsequently been selectively eliminated or restrained by microenvironmental factors, whereas the outgrowth of the tumour cells at the metastatic site is favoured[53,54]. However, the available literature describing the mechanism of tumour cell emigration from the primary anatomical site in the setting of CUP is sparse. One hypothesis is that epithelial cells dedifferentiate and acquire mesenchymal features that facilitate motility, invasiveness and increased resistance to apoptosis[55]. The epithelial-to-mesenchymal transition phenotype, defined by the expression of N-cadherin and/or vimentin and SNAIL, together with a partial loss of E-cadherin expression, has been reported in 7.3–16% of patients with CUP, which might reflect the transient nature of the phenomenon[56,57]. This phenotype is associated with high histological grade, the presence of visceral metastasis and unfavourable survival in patients with CUP[39]. Another, potentially overlapping, hypothesis is that tumour cells with a stem cell phenotype, defined by the immunohistochemistry expression of CD133 and octamer-binding transcription factor 4, underlie rapid metastasization. The

expression of aldehyde dehydrogenase 1 in circulating tumour cells occurs in 50% of patients with CUP, which indicates that the acquisition of a stem cell phenotype in CUP might be rare, transient or dynamic[58,59].

The carcinogenic process of CUP seems to be driven by chromosomal instability, which is characterized by a high rate of gains or losses of whole or large portions of chromosomes[60], thus leading to a form of genomic instability that is associated with a mutator phenotype[61]. In addition to chromosomal alterations, multiple aberrations in interdependent cellular signalling pathways can contribute to the pathogenesis of CUP, including self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, evasion of immune destruction and reprogramming of energy metabolism (TABLE 2). The predominant signalling pathway alterations have direct



	Reprogramming of energy metabolism and evasion of the immune system
Proliferative signals	RAS expression in 92%, EGFR in 75%, PDGFR in 50% and *MET* mutation in 30%
Limitless replication	p53 expression in 48–70% and phospho-AKT detected in 73.2%
Evasion of apoptosis	Bcl-2 expression in 65%
Angiogenesis	VEGF-A expression in 26–83% and tumoural hypoxic phenotype in 25%
Tissue invasion	MMP2 expression in 69%, MMP9 in 49% and TIMP2 in 79%
Evasion of the immune system	PD-1 expression in 63% , PD-L1 in 21% High tumour mutational load in 11.8% and microsatellite instability in 1.8%

Fig. 1 | **The development and dissemination of CUP.** The figure provides a schematic representation of the successive steps in the development and dissemination of cancer of unknown primary (CUP), with an overview of key pathobiological hallmarks of various stages of this process. A summary of the molecular features or phenotypes associated with these hallmarks, as well as their prevalence in CUP, is also shown (see TABLE 2 for a more complete list and associated references). During the carcinogenesis of CUP, the proliferation of cancer cells at the primary site generates a population of stationary tumour cells that are hypothesized to undergo a regression and/or dormancy process. In parallel, a subpopulation of invasive tumour cells invade the adjacent tissue, enter the lymphatic or circulatory systems, extravasate at distant sites, colonize this foreign microenvironment and proliferate to form a clinically detectable metastasis with the capacity to seed additional metastases in other tissues. In comparison to cancers of known primary, three hypotheses are considered in the carcinogenesis of CUP: 1) CUP does not undergo type 1 progression (from a premalignant to a malignant lesion) and rather undergoes type 2 progression (malignant lesion at the onset of the disease without prior formation of a nascent primary tumour); 2) CUP does not follow a linear progression model, whereby stepwise progression of accumulating genetic and epigenetic alterations occurs over the course of cancer development, and instead follows the parallel progression model, whereby metastases can arise early in the development of a malignancy; and 3) CUP might occur owing to the migration of deregulated, premalignant or cancerous stem cells away from their natural tissues and to form tumours in other locations. EMT, epithelial-to-mesenchymal transition; MMP, matrix metalloproteinase; TIMP, metalloproteinase inhibitor.

Table 2 | **Summary of proteins and genes reported to contribute to the hallmarks of CUP**

Hallmark	Gene mutation or protein expression in CUP	Prevalence of gene mutation or protein expression in CUP tumours (%)	Clinical implication	Refs
Self-sufficiency in growth signals	EGFR expression	74–75 (overexpression in 4–61)	Not associated with any clinical or pathological parameters; no prognostic implications	91,95–97
	PDGFRα and PDGFRβ expression	50 and 25, respectively	No prognostic implications	98,99
	KIT expression	11–81 (overexpression in 4–13)	No prognostic implications	98,99
	MYC expression	96 (overexpression in 23)	NR	100
	TRKA expression	5.9	NR	101
	MAPK phosphorylation (in ≥40% of cells)	54	Associated with a response to chemotherapy (median OS 9 months for phospho-MAPK ≥40% group vs 17 months for phospho-MAPK <40% group; $P=0.016$)	102
	RAS p21 expression	92 (overexpression in 23)	NR	100
	KRAS mutations	10.2–37.5	NR	63,103,104
	RAF mutations	3–4.5	NR	103,104
	PIK3CA mutations	6.7–37.5	NR	63,103,104
	MET mutations	1.6–30	Associated with fewer metastatic sites ($P<0.001$) and low-grade squamous tumours ($P<0.001$)	102,104,105
Insensitivity to antigrowth signals	$3p21^{CIP1}$ expression	60.6	Correlates with different CUP subgroups (high $p21^{CIP1}$ expression was reported in 76% of predominantly nodal versus 63% of predominantly visceral versus 44% of the peritoneal or pleural carcinomatosis subgroups; $P=0.025$) and is associated with favourable survival (RR 0.34, 95% CI 0.16–0.73)	106,107
Evasion of apoptosis	AKT phosphorylation	73.2	Associated with unfavourable survival (RR 2.39, 95% CI 1.23–4.66)	106,107
	Bcl-2 expression	65 (overexpression in 40)	No prognostic implications	108
Limitless replicative potential	p53 expression	48–70 (overexpression in 53)	No prognostic implications	62,91,108,109
Sustained angiogenesis	VEGF-A expression	26–83	No prognostic implications	109–112
	Tumoural hypoxic phenotype (expression of HIF-1α, GLUT1 and COX2)	25	Unfavourable prognosis (GLUT1, HIF-1α or COX2 expression was associated with a poor prognosis; $P=0.048$, $P=0.029$ and $P=0.042$, respectively)	99
	Thrombospondin-1 expression	80 (overexpression in 20)	Not associated with any clinical or pathological parameters	111
	Notch 1 expression	2	Unfavourable survival in patients with visceral CUP (median OS 3 months in those with high expression vs 7 months in those with low expression; $P=0.05$)	102
	Notch 2 expression	56	No prognostic implications	102
	Notch 3 expression	73	Unfavourable survival in patients with midline nodal CUP (median OS 12 months in those with high expression vs 31 months in those with low expression; $P=0.05$)	102
Tissue invasion and metastasis	MMP2 expression	69 (overexpression in 49)	Not associated with any clinical or pathological parameters	112
	MMP9 expression	49 (overexpression in 36)	No prognostic implications	112
	TIMP1 expression	79	Associated with a shorter survival (median OS 7.5 months in those with high expression vs 12 months in those with low expression; $P=0.016$)	112
Evasion of immune destruction	PD-1 expression	63	NR	91
	PD-L1 expression	21	NR	91
	High tumour mutational load (≥17 mutations/Mb)	11.8	NR	91
	Microsatellite instability	1.8	NR	91

Table 2 (cont.) | **Summary of proteins and genes reported to contribute to the hallmarks of CUP**

Hallmark	Gene mutation or protein expression in CUP	Prevalence of gene mutation or protein expression in CUP tumours (%)	Clinical implication	Refs
Reprogramming of energy metabolism	Chromosome 11 region containing *DHCR7*, *NADSYN1* and *KRTAP5–7*	NR	Lipid metabolic disturbance increases the risk of CUP	113
Chromosomal alterations	Chromosomal instability (enrichment for transcripts of proteins that function in DNA damage and homologous recombination repair networks, such as *BRCA1*, *ATM* and *CHEK2*)	70	Poor prognosis and resistance to chemotherapy (2-year OS 18% in patients with diploid CUP vs 9% in those with aneuploid CUP)	114

CI, confidence interval; COX2, cyclooxygenase-2; CUP, cancer of unknown primary; GLUT1, glucose transporter type 1; HIF-1α, hypoxia-inducible factor 1α; MMP, matrix metalloproteinase; NR, not reported; OS, overall survival; RR, relative risk; TIMP1, metalloproteinase inhibitor 1.

implications in the characteristics of each tumour. For example, SCCs have a higher prevalence of variants of genes encoding proteins with roles in cell cycle regulation (such as *TP53*, *CDKN2A* and *MLH1*) than other forms of CUP, whereas adenocarcinomas have the largest number of cellular signalling pathway variants (in genes such as *MET*, *EGFR*, *HRAS*, *KRAS* and *BRAF*)[62].

Molecular profiling in CUP management

Diagnosis. The diagnosis of cancer in routine clinical practice requires a comprehensive synthesis of the clinical and pathological findings. Morphological and immunohistochemistry assessments, performed according to the recommendations discussed previously, form the basis of a CUP diagnosis (TABLE 1). This approach is the cheapest and fastest means of identifying a primary tumour or predicting the putative primary tumour type; however, the diagnosis of patients with CUP is not straightforward because tumours often lack the typical features characteristic of differentiation towards a particular cell lineage, which complicates assignment of the primary tumour type. The failure to identify the culprit primary tumour often raises questions over the histopathological diagnosis assigned to CUP tumours and can eventually lead to the integration of molecular CUP classifiers in the diagnostic workup (FIG. 2).

Several assays have been developed to predict the putative tissue of origin of a CUP lesion by alignment with prominent molecular profiles established for cancers with a known primary (TABLE 3). Many of these assays involve the comparison of the gene-expression profile of a CUP lesion, as determined by quantitative reverse transcription PCR (qRT-PCR), microarray analysis or microRNA profiles, to prototypical gene-expression profiles of metastases originating from particular types of primary tumours and/or the primary tumours themselves. Accordingly, a similarity score can be calculated, and a primary tumour type can be predicted. The accuracy of the CUP classifiers ranges between 54% and 98% in comparison with primary tumour type assignments made according to the recommended clinicopathological criteria (TABLE 3). These molecular diagnostics are commonly performed after the failure of the conventional pathology assessments in an attempt to identify the primary tumour type; thus, the tests should be applicable to formalin-fixed paraffin-embedded tissues.

Next-generation sequencing can also be used in CUP diagnostics, with massively parallel sequencing enabling predictions based on typical sets of recurrent mutations that are enriched in particular types of primary tumour; perhaps more importantly, this technique can reveal targetable mutations in individual tumours, potentially enabling a rational, biomarker-based approach to the treatment of CUP[63,64]. Interestingly, artificial intelligence has been applied to the assessment of clinical DNA sequencing data to predict the site of origin, with promising results[65]. In a cohort of 141 patients with CUP, the likely tissue of origin was predicted for 95 patients (67.4%) by applying a machine learning algorithm to data obtained through prospective targeted sequencing of up to 468 cancer-associated genes[65]. Moreover, prospective use of the algorithm prompted a change in diagnosis for two patients with CUP initially attributed to breast cancer, with corresponding changes to treatment resulting in clinical responses in both patients[65].

Thus, GEP and next-generation sequencing provide reliable tools to classify ambiguous tumours. However, the results obtained using classifiers based on these technologies should be interpreted in conjunction with the clinical presentation, imaging investigations and pathology review to establish a clinically meaningful diagnosis[66].

Treatment according to the putative primary tumour type. Patients with poor-prognosis subsets of CUP are commonly treated with various empirical chemotherapy regimens, which are mostly the historical standard-of-care regimens used in the treatment of the primary tumour types that are commonly predicted in patients with CUP, mainly non-small-cell lung cancer (NSCLC) and biliary tract cancers[67]. In prospective randomized controlled studies, patients with CUP treated with empirical chemotherapy regimens, mainly platinum-based doublets, were shown to have median OS durations of 2.7–10.7 months (TABLE 4). The dismal survival of patients with CUP relative to patients with metastatic cancer originating from a known primary tumour suggests that identifying the culprit primary tumour type and treating the disease accordingly would be beneficial[68]. Moreover, the efficacy of tumour type-specific treatments in patients with favourable-prognosis subsets of CUP provides compelling evidence supporting

investigations to identify the primary tumour type in patients with unfavourable subsets of CUP[69]. Indeed, advances in molecular classifications have led to the reassignment of some patients with unfavourable-prognosis subsets of CUP into the favourable-prognosis subset, thereby underlining the potential role of tumour type-specific therapies[36]. For example, CUP with features characteristic of renal-cell carcinoma (CUP-RCC) is usually resistant to the empirical chemotherapy regimens but is often highly responsive to tyrosine kinase inhibitors (such as sunitinib and pazopanib) typically used in patients with metastatic RCC[70–73]. In a case series of 24 patients with CUP-RCC, identified by histology and/or a molecular signature, 16 patients received front-line RCC-specific therapies, which resulted in an objective response rate of 19%, a median progression-free survival (PFS) of 8 months and a median OS duration of 14 months[70]. A further eight patients received first-line empirical chemotherapy, yielding an objective response rate of 12.5% and a median OS duration of 13 months (three of these patients received second-line RCC-specific therapy)[70]. The median OS duration of all 20 patients who received RCC-specific treatment at some time during the disease course was 16 months[70].

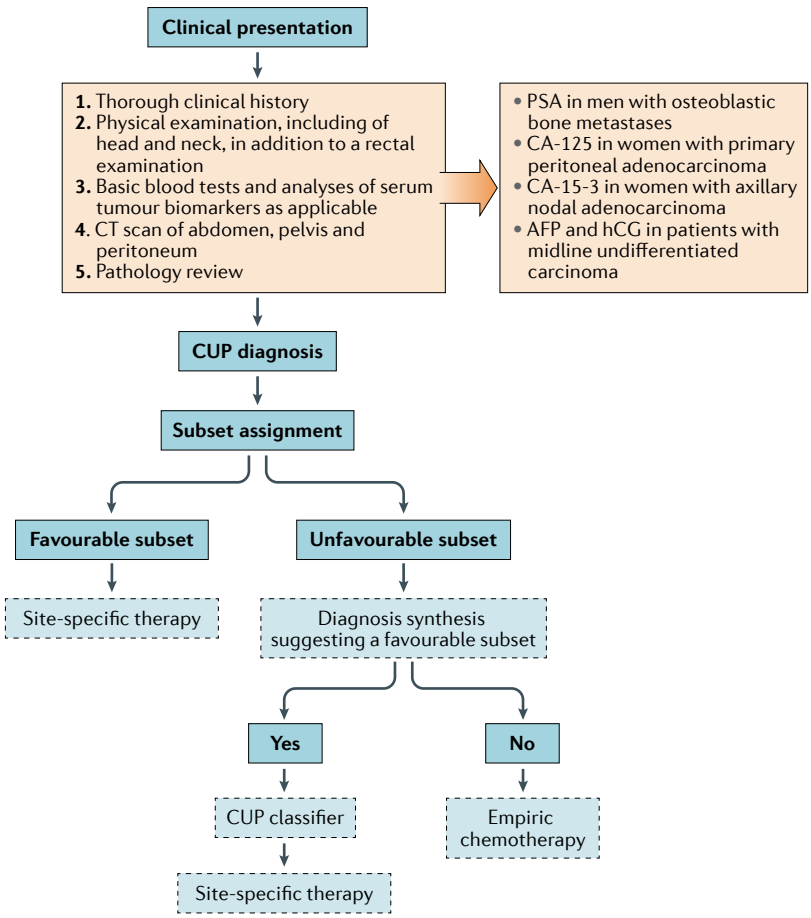


Fig. 2 | **Proposed algorithm for the diagnosis and management of CUP.** The clinical evaluation starts with a thorough patient history and physical examination, followed by analyses including basic blood tests, assays of select tumour biomarkers and CT scans of the thorax, abdomen and pelvis. The complete workup also requires a core needle/excisional biopsy and expert review to confirm the diagnosis (see TABLE 1 for a detailed pathology algorithm). In the absence of detection of a primary tumour site, the cancer of unknown primary (CUP) diagnosis is retained. Patients with CUP that can be classified into the favourable disease subset (15–20%) are treated according to the equivalent primary tumours. For example, patients with poorly differentiated neuroendocrine CUP are treated with platinum plus etoposide; those with well-differentiated neuroendocrine CUP are treated with somatostatin analogues, streptozocin plus 5-fluorouracil, sunitinib or everolimus; women with peritoneal adenocarcinomatosis of a serous papillary histological type are treated with optimal surgical debulking and platinum–taxane-based chemotherapy; women with isolated axillary nodal metastases are treated with axillary nodal dissection, mastectomy or breast irradiation and chemohormonotherapy; patients with squamous cell carcinoma involving cervical lymph nodes are treated with neck dissection and/or irradiation of bilateral neck and head–neck axis (or for those with advanced-stage disease, induction chemotherapy with platinum-based combination or chemoradiation); patients with a single metastatic deposit from unknown primary are treated with local treatment with or without systemic therapy; and men with osteoblastic bone metastases and prostate-specific antigen (PSA) expression are treated with androgen-deprivation therapy with or without chemotherapy, novel anti-hormonal therapy and/or radiotherapy. Patients who are classified with an unfavourable subset of CUP (80–85%) are treated with empirical chemotherapy regimens (such as paclitaxel plus carboplatin or gemcitabine plus cisplatin). In patients presenting with clinical features suggestive of a particular primary tumour type, according to the histological characteristics and the pattern of metastatic spread, a CUP classifier can be applied to facilitate the choice of a tumour type-specific therapy. AFP, α-fetoprotein; CA, cancer antigen; hCG, human chorionic gonadotropin.

The efficacy of tumour type-specific therapies in patients with CUP has been assessed in two non-randomized prospective studies (TABLE 5). The first study involved 247 patients with a primary tumour type predicted using a 92-gene qRT-PCR GEP classifier (CancerTYPE ID), including biliary tract carcinoma (in 21% of patients), urothelial carcinoma (12%), colorectal carcinoma (11%) and NSCLC (11%)[22]. Among the patients who received tumour type-specific therapy, the median OS duration was better for those with more responsive tumour types (colorectal, breast, ovarian, renal, prostate, bladder, germ cell, poorly differentiated neuroendocrine or small-cell lung cancer, NSCLC or lymphoma) than for those with less responsive types (biliary tract, pancreatic, gastro-oesophageal, liver, cervical, carcinoid, endometrial, skin, thyroid, head and neck or adrenal cancer, mesothelioma or sarcoma) (13.4 months versus 7.6 months; $P = 0.04$)[22]. Moreover, patients classified with high probability predictions (≥80%) had a better median OS duration than patients with lower probability predictions (12.5 months versus 10.8 months; $P = 0.03$)[22]. These findings illustrate the improved outcome of patients with responsive tumour types who receive tumour type-specific therapy. In a retrospective comparison with data from a historical series of 396 patients treated with empirical chemotherapy, 194 patients treated with tumour type-specific therapy according to the CancerTYPE ID prediction had a statistically significant OS benefit (median OS duration 12.5 months versus 9.1 months[22]; HR 0.63, 95% CI 0.60–0.65 (REF.[21])). In the second study, investigators reported the outcomes of 31 patients who received therapy tailored according to the primary tumour type predicted using the EPICUP DNA-methylation microarray classifier, including breast carcinoma in six patients (19%), NSCLC in five (16%), hepatocellular carcinoma in four (13%) and ovarian carcinoma in three (10%), among others[23]. This group of 31 patients had a statistically significant OS benefit in comparison with 61 patients treated with empirical chemotherapy regimens (median OS duration 13.6 months versus 6.0 months; HR 0.31, 95% CI 0.13–0.70)[23].

The efficacy of tumour type-specific therapy in patients with CUP has been further addressed in two prospective randomized controlled trials (TABLE 5). In a phase II study[26], 101 patients were randomly assigned (1:1) to receive tailored therapy or empirical paclitaxel and carboplatin chemotherapy. All patients underwent tumour GEP by microarray analysis, which revealed pancreatic cancer (in 21% of patients), gastric cancer (21%), lymphoma (20%), urothelial carcinoma (6.2%), cervical cancer (5.4%) and ovarian cancer (4.6%) as the most common putative primary tumour types[26]. The median OS duration was similar in both treatment groups (9.8 months with tumour type-specific therapy versus 12.5 months with empirical therapy; HR 1.03, 95% CI 0.68–1.56; $P=0.90$), as was the median PFS duration (5.1 months versus 4.8 months; HR 0.88, 95% CI 0.59–1.33; $P=0.55$)[26]. Moreover, no statistically significant difference in the median OS duration of patients with 'responsive' tumour types (colorectal, breast, ovarian, renal, prostate, bladder or germ cell cancer, NSCLC or lymphoma) and less-responsive types (biliary tract, pancreatic, gastro-oesophageal, liver, cervical, endometrial, thyroid or head and neck cancer or mesothelioma) was demonstrated. In the phase III GEFCAPI 04 trial[27], 243 patients were randomly assigned (1:1) to receive tumour type-specific therapy or empirical cisplatin plus gemcitabine chemotherapy. The most commonly reported putative primary tumour types, mostly assigned according to GEP with CancerTYPE ID, included pancreaticobiliary tumours (in 19% of patients), SCCs (11%), lung cancers (8%) and kidney cancers (8%). Neither PFS (median 4.6 months with tailored therapy versus 5.3 months with empirical therapy; HR 0.95, 95% CI 0.72–1.25) nor OS (median 10.7 months versus 10.0 months; HR 0.92, 95% CI 0.69–1.23) differed significantly between the two treatment arms[27]. However, patients might not have received the optimal tumour type-specific therapy. For example, patients with RCC, NSCLC or melanoma were not treated with immune-checkpoint inhibitor-based combinations, which are the current standard of care[74,75]. Similarly, patients with pancreatic or biliary tract cancers, which usually confer a dismal prognosis regardless of the treatment administered, did not receive treatment regimens with demonstrated survival benefits[76]. Nevertheless, certain selected subgroups of patients diagnosed with, for example, melanoma, NSCLC or colorectal cancer on the basis of molecular CUP classifiers might benefit from tumour type-specific therapy[27].

A meta-analysis of data from these four prospective studies showed a trend towards a statistically significant OS benefit with tumour type-specific therapy versus empirical chemotherapy (HR 0.73, 95% CI 0.52–1.02)[21]; a pooled analysis of data from the two randomized controlled trials indicated similar PFS (HR 0.93, 95% CI 0.74–1.17) and OS (HR 0.95, 95% CI 0.75–1.21) with each of the two approaches to therapy for patients with CUP[21]. However, these comparisons between tumour type-specific therapy and empirical chemotherapy regimens for CUP are limited by heterogeneity in the characteristics of the patients enrolled, the CUP classifiers used, the predicted primary tumour types and the types of systemic therapies administered[22,23,26,27]. Notably, the population of patients with CUP included in clinical trials has changed substantially in the past decade. The initial trials in this context included patients who had not undergone optimal diagnostic investigation and, therefore, included many patients with lung, breast, ovarian or upper gastrointestinal tract cancers. More recently, oncologists have tended to accept the suggested primary tumour types as a definite diagnosis and treat the patients accordingly, instead of enrolling them in clinical trials, given the favourable outcomes achieved for several tumours in the era of targeted and immunotherapies. For example, in GEFCAPI 04, in which patients were recruited between 2012 and 2018, lung and intestinal (mostly colorectal) cancers were each reported in <10% of patients, and breast and ovarian cancers in <5%[27], which is below

Table 3 | **Methods used to identify the tissue of origin of CUP and their associated predictive accuracy**

Method	*n*	Type of tissue	Analyte	Predictive accuracy (%)	Refs
GEP (microarray)	222	FF/FFPE	RNA	64–94	115–121
GEP (whole-genome expression by qRT-PCR)	58	FFPE	RNA	78	122
qRT-PCR of 10 genes	104	FFPE	RNA	61	123
CancerTYPE ID (92-gene qRT-PCR)	2,277	FFPE	RNA	54–98	22,124,125
MTP (bioTheranostics)	171	FFPE	RNA	84	126
Signature of 47 miRNAs	16	FFPE	miRNA	75	127
Signature of 48 miRNAs	87	FFPE	miRNA	84	128
Array of 64 miRNAs	192	FFPE	miRNA	86	129
Methylation array (comprising 1,505 CpG sites)	42	FF	DNA	78	130
DNA-methylation microarray (EPICUP)	216	FFPE	DNA	87	23
386-gene PeterMac Comprehensive Cancer Panel (CCP) and CUPguide (a gene-expression microarray assay)	124	NA	DNA and RNA	86.6	131

Predictive accuracy was generally determined by comparison with designations made using gold-standard clinicopathological criteria. CUP, cancer of unknown primary; FF, fresh frozen; FFPE: formalin-fixed paraffin-embedded; GEP, gene-expression profiling; miRNA, microRNA; MTP, molecular tumour profiling; *n*, number of patients; NA, not applicable; qRT-PCR, quantitative reverse transcription PCR.

Table 4 | **Prospective trials of palliative chemotherapy regimens in patients with unfavourable CUP subsets**

Chemotherapy	ORR (%)	Median OS (months)	Refs
Cisplatin + gemcitabine vs cisplatin + irinotecan	55 vs 38	8 vs 6	132
Cisplatin + docetaxel vs carboplatin + docetaxel	26 vs 22	8 vs 8	133
Carboplatin + paclitaxel vs gemcitabine + vinorelbine	23.8 vs 20	11 vs 7	42
Carboplatin + etoposide vs paclitaxel + 5-fluorouracil + leucovorin	19 vs 19	8.3 vs 6.4	134
Irinotecan + oxaliplatin	13	2.7	135
Capecitabine + oxaliplatin	11.7–19	3.9–9.7	136–138

CUP, cancer of unknown primary; ORR, objective response rate; OS, overall survival.

the proportions of patients with these cancers included in the non-randomized trials (lung cancer in 11–21%, intestinal cancer in 9–11%, breast cancer in 5–9% and ovarian cancer in 4–10%)[22,23]. Moreover, the inclusion of large percentages of patients with pancreaticobiliary cancers and SCCs has been a consistent feature of many molecular studies of CUP and is in part attributable to the lack of diagnostic immunohistochemistry markers for these tumour types. Consequently, the cohorts of randomized studies have been enriched with relatively resistant and unresponsive cancer types, including biliary tract cancers and metastatic SCCs, which will always have similar survival outcomes in the absence of standard therapies for these cancers that are superior to empirical chemotherapy — this situation will only change if better therapies become available for patients with these tumour types. Such improvements in therapeutic paradigms have occurred for many other cancer types, such as non-small-cell lung CUP, CUP-RCC and colorectal CUP, and these diseases should be the focus of randomized trials designed to test tumour type-specific therapies. In the GEFCAPI 04 trial[27], the administered tumour type-specific therapies were predetermined in 2011, and substantial improvements in the treatment of many types of metastatic cancer have been achieved over the past decade. Similarly, in their randomized phase II trial, Hayashi et al.[26] required 7 years to accrue 101 patients and did not update the tumour type-specific therapies according to therapeutic advances; this study also had the major caveat of using a proprietary GEP assay that required fresh biopsy tissue. Notably, 20% of the patients enrolled had lymphoma, which is not common in studies of CUP and might imply methodological issues with either the standard pathology assessments and/or GEP assay used[26].

Treatment according to a biomarker-based approach. Beyond identifying the tissue of origin and/or putative primary tumour type, the molecular advances in the field of CUP diagnosis have opened up the potential for biomarker-directed therapy, which can be effective even in patients with treatment-refractory tumours[77,78]. Indeed, extensive work by The Cancer Genome Atlas Network has revealed that the tissue of origin of a particular cancer is much less relevant to prognosis and response to therapy than the causative mutations, thus underlining the likely importance of molecularly targeted therapies used in combination with the optimal predictive biomarkers[79,80]. Moreover, CUP is most often predicted to emanate from tumour types, such as biliary tract, lung, colorectal and breast cancers, that commonly harbour actionable or potentially actionable oncogenic drivers[15]. Biomarker-guided targeted therapy with BRAF or IDH inhibitors holds promise in patients with biliary tract cancers, which are considered chemoresistant[81]. Molecularly targeted agents have already transformed the treatment landscape of other tumour types, including EGFR, ALK, ROS1 and BRAF inhibitors for NSCLC, KRAS and BRAF inhibitors for colorectal cancer and HER2 inhibitors for breast cancer[82,83].

Indeed, patients with CUP have been reported to harbour clinically relevant genomic alterations[84]. The precision oncology knowledge base, OncoKB, might be helpful to oncologists in interpreting genomic alterations and making optimal treatment decisions[85]. This online resource, which is curated and maintained by a group at the Memorial Sloan Kettering Cancer Center, classifies predictive genetic biomarkers into those linked with oncology drug approvals or recommended for use with standard-of-care therapies by expert panels, as well as those with investigational or hypothetical therapeutic implications based on promising clinical and/or biological evidence[85]. In 2018, the ESMO Translational Research and Precision Medicine Working Group published the ESMO Scale of Clinical Actionability for molecular Targets (ESCAT)[86]. Using this framework, genomic alterations are ranked as targets for precision medicine on a six-point scale according to the level of available clinical evidence of implications for patient management[86]. The prevalence of clinically relevant mutations in patients with CUP varies according to the definition of clinical actionability[84]; clinically relevant druggable mutations have been reported in 85–96% of patients, of which 13–64% can be targeted using FDA-approved therapies[63,87–89]. When defined on the basis of confirmed therapeutic responses associated with an FDA approval or some other form of clinical evidence, clinically relevant mutations have been reported in 30% of patients with CUP (most commonly *HER2* amplification or $BRAF^{V600E}$ mutation); this figure rises to 55% when preclinical evidence for a response to a specific drug is included in the definition of actionability[90]. Biomarkers relevant to immune-checkpoint inhibitors were found in 28% of the patients with CUP, notably a high total mutational load in 11.8%, a microsatellite instability high status in 1.8% and tumour PD-L1 expression in 22%[91]. A small percentage of patients with CUP harboured predictors of hyperprogression, including

Table 5 | **Summary of the studies comparing empirical and tailored therapy for patients with CUP**

Study	Recruitment period	Study design	*n*		Median OS duration (months)		HR (95% CI)
			Empirical treatment	Tailored treatment	Empirical treatment	Tailored treatment	
Hainsworth et al.[22]	NR	Not randomized	396[a]	194	9.1	12.5	0.63 (0.60–0.65)
Moran et al.[23]	2011–2015	Not randomized	61[a]	31	6	13.6	0.31 (0.13–0.70)
Hayashi et al.[26]	2008–2015	Randomized	51	50	12.5	9.8	1.028 (0.68–1.56)
Fizazi et al.[27]	2012–2018	Randomized	120	123	10.0	10.7	0.92 (0.69–1.23)

The empirical treatment was usually a taxane plus a platinum-based agent or gemcitabine plus a platinum-based agent; the tailored treatment was guided by the putative primary tumour as suggested by the results of tumour molecular profiling. CUP, cancer of unknown primary; *n*, number of patients; NR, not reported; OS, overall survival. [a]The empirical treatment arm comprised a historical control group.

MDM2 amplification in 2% and loss-of-function *JAK2* mutation in 1%[91].

We have previously reported that patients with CUP can benefit from targeted therapies, although the available evidence is limited to case reports and small series[25]; examples include erlotinib and gefitinib in patients with activating *EGFR* mutations, crizotinib in patients with *MET* amplifications or *ALK* rearrangements and dabrafenib in patients with $BRAF^{V600E}$ mutation[25]. Other case reports have described the successful management of patients using targeted therapies, such as sunitinib, lenvatinib, axitinib and gefitinib, based on the clinical prediction of a potential primary tumour according to the histological features and the pattern of metastatic spread[25]. Two phase II trials evaluating the combination of the anti-VEGF-A antibody bevacizumab with the EGFR inhibitor erlotinib, with or without paclitaxel plus carboplatin chemotherapy, revealed disappointing outcomes in patients with CUP who had features of the poor-prognosis subset or had previously received chemotherapy (median OS durations of 7.4 months without paclitaxel plus carboplatin and 12.6 months with paclitaxel plus carboplatin); however, these studies were performed more than a decade ago, and the study designs did not include evaluation for the presence of the targetable mutations[92,93]. Better designed trials taking into consideration the importance of molecular alterations with respect to the biology of the predicted primary tumour are needed to better understand the role of targeted therapies in CUP, and such trials are eagerly awaited.

The CUPISCO trial (NCT03498521) will provide useful insights into this issue, owing to the categorization of patients according to biomarker-defined subsets for which advances in molecularly targeted therapy have transformed the treatment landscape. The CUPISCO study is a phase II, randomized, open-label, active-controlled, multi-centre trial in which patients with treatment-naive unfavourable CUP subsets are being enrolled. Patients are categorized after three cycles of platinum-based chemotherapy according to RECIST v1.1 into a group of patients with progressive disease or group of patients achieving stable disease or a partial or complete response. Patients in the first group will receive molecularly guided therapy according to the advice of a molecular tumour board. Patients in the second group are being randomized (3:1) to receive either molecularly guided therapy or platinum-based chemotherapy for an additional three cycles. The molecularly guided therapies include: 1) targeted therapies, such as alectinib for patients with *ALK* or *RET* rearrangements, vismodegib for those with inactivating *PTCH1* and activating *SMO* mutations, ipatasertib for those with *AKT*, *PIK3CA* or *PTEN* alterations, olaparib for those with BRCA1, BRCA2 or another homologous recombination deficiency based on loss of heterozygosity, erlotinib plus bevacizumab for those with actionable *EGFR* alterations, vemurafenib plus cobimetinib for those with $BRAF^{V600}$ alterations, and trastuzumab and pertuzumab plus chemotherapy for patients with actionable *HER2* (*ERBB2*) and/or *ERBB3* alterations; 2) immune-checkpoint inhibitors, such as atezolizumab for patients with a tumour mutational burden ≥16 mutations/Mb or microsatellite instability high tumours or atezolizumab plus chemotherapy for those with a tumour mutational burden <16 mutations/Mb; and 3) alternative commercially available targeted therapies with a strong biological rationale in patients with a strong suspicion of a relevant primary tumour type provided by data from comprehensive genomic profiling and with a negative predictor of response to immune-checkpoint inhibitors.

Conclusions

In the era of molecular diagnostic workup, further tools beyond histology and immunohistochemistry with which to characterize cancers have become available; however, the current molecular advances have not yet resulted in the expected improvements in the management of CUP. CUP remains a valid diagnosis in the absence of a putative primary tumour, even when molecular studies enable prediction of the probable primary tumour type. The addition of a CUP classifier to the standard diagnostic workup might avoid failures in recognizing atypical presentations among patients with favourable-prognosis disease subsets who often benefit from tumour type-specific therapies. Currently, an overall trend favours the refinement of the clinical management of patients with CUP according to the advances afforded by assessment of the molecular biology of the tumours, although this approach has not yet been proven to improve outcomes. The available literature shows discordant results between randomized and non-randomized trials evaluating tumour type-specific therapies, which might be explained by the difference

in the clinicopathological characteristics of the study cohorts. Looking into the details of the GEFCAPI 04 trial[27], subgroup analyses suggest that selected patients with CUP (namely, those diagnosed with tumour types that are sensitive to novel targeted agents) might benefit from this approach. Certainly, physicians should consider the information from molecular analyses together with the overall clinical picture and results of pathology investigations before deciding whether to perform additional investigations in patients with CUP.

We believe that the ongoing advances in molecular biology methodologies will enable a better understanding of the carcinogenesis of CUP and the potentially targetable pathway alterations. Large-cohort prospective studies designed to validate the feasibility and utility of molecular profiling assessments should be considered, bearing in mind that patients with aggressive forms of CUP tend to quickly succumb to the disease and are typically under-represented in clinical trials. GEFCAPI 04 has partially addressed this issue: 28% of the eligible patients had an Eastern Cooperative Oncology Group performance status of 2. However, despite the fact that study treatment was initiated within 1 month after enrolment in most patients (range 6–33 days), 26% of patients did not receive the planned tumour type-specific therapy, in particular owing to dramatic clinical deterioration (in 10%) and an urgent need for treatment (in 11%)[27]. In addition, the design of future prospective randomized trials should also be reconsidered to best exploit the benefits associated with the molecular advances. Moreover, future studies should address the minority of patients that present with novel potentially targetable mutations, especially driver mutations, as determined based on genetic comparisons with their putative tissue of origin[94].

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