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Tumour Review

Redefining cancer of unknown primary: Is precision medicine really shifting the paradigm?



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ABSTRACT

The concept of Cancer of Unknown Primary (CUP) has evolved with the advent of medical oncology. CUP can be difficult to diagnose and represents 2 to 5% of new cancers, therefore not exceptionally rare. Within CUPs can be identified a subset of favourable prognosis tumours, however the vast majority of CUP patients belongs to a poor prognosis group.

CUP features significant oncological challenges, such as unravelling biological and transversal issues, and most importantly, improving patient's outcomes. In that regard, CUP patients' outcomes regrettably showed minimal improvement for decades and CUP remains a cancer group of very poor prognosis.

The biology of CUP has two main hypotheses. One is that CUP is a subgroup of a given primary cancer, where the primary is present but cannot be seen due to its small size. The other, the "true" CUP hypothesis, states that CUP share features that make them a specific entity, whatever their tissue of origin. A true biological signature has not yet been described, but chromosomal instability is a hallmark of poor prognosis CUP group.

Precision oncology, despite achieving identifying the putative origin of the CUP, so far failed to globally improve outcomes of patients. Targeting molecular pathways based on molecular analysis in CUP management is under investigation. Immunotherapy has not shown ground-breaking results, to date. Accrual is also a crucial issue in CUP trials.

Herein we review CUP history, biological features and remaining questions in CUP biology, the two main approaches of molecular oncology in CUP management, in order to draw perspectives in the enormous challenge of improving CUP patient outcomes.

Introduction

An enigmatic entity, cancer of unknown primary (CUP), has evolved alongside the development of modern oncology. The first mention of cancer with "unknown primary" dates back to 1946 [1]. Since that time, a shift in the definition and diagnosis occurred, associated with the emergence of ever more sophisticated diagnostic tools which aim to trace biological and molecular similarities to the elusive, possibly

dormant or regressed, primary [2].

Epidemiology - prognosis

Epidemiology

CUP is defined by the absence of a clinically identified primary lesion (the primary is not "seen") at the time of diagnosis despite standardised

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diagnostic work-up [3–5].

Confirmed CUPs represent 2 to 5% of all cancers [5] and 15% of all new cancers are presenting as malignancy of unknown origin (MUO) [4].

CUPs incidence is varying across countries [5]. Differences may be explained by coding rules and methodology in cancer registries [6]. Cost issues can explain underreporting of CUP: in the US, costs are better covered for specific cancers in comparison with CUPs [7]. In contrast, CUP incidence has been related to be higher in patient with lower income, possibly due to insufficient diagnostic inquiry [7,8].

Incidence has increased since CUP concept was introduced, to reach a peak at the end of 1990's in most countries [5]. After the tipping point, the decreasing number of diagnosed CUPs is mainly explained by better identification of small primary lesion. Decreased incidence of some primary cancers (lung) could also account in the decrease in CUPs incidence.

Favourable And Unfavourable Subgroups Of CUPs

CUP can be divided in two subgroups with very different prognosis, treatment, and expected outcomes: the favourable (15–20%) and the unfavourable (80 – 85%) subgroups [3,9].

For patients presenting with CUPs belonging to the favourable subgroup, principles of treatment are mostly derived from the treatment of their corresponding known primaries. Nine specific situations are identified to belong to the favourable subgroups, with a corresponding tailored treatment recommendation based on the equivalent known primary tumour. This is well described in international guidelines [3,9,10], this situations are:

- poorly differentiated neuroendocrine carcinomas of unknown primary

- well-differentiated neuroendocrine tumour of unknown primary
- peritoneal adenocarcinomatosis of a serous papillary in females
- isolated axillary nodal metastases in females
- squamous cell carcinoma (SCC) involving non-supraclavicular cervical lymph nodes (head-and-neck)
- CUP with a colorectal profile (immunohistochemistry (IHC) or molecular)
- single metastatic deposit from unknown primary
- men with blastic bone metastases or IHC/serum PSA expression,
- isolated inguinal adenopathy (SCC)

Prognosis is better in these entities, with curative intent in some of them. As an example, the isolated axillary nodal metastases will be treated like a primary breast cancer with 3-year survival rate up to 97%, and women with peritoneal carcinomatosis of a serous papillary adenocarcinoma will have outcomes in the range of stage III and IV ovarian cancer patients [10].

Prognosis in the unfavourable subgroup of CUPs

Table 1 describes outcomes from randomized trials in CUPs. Early trials reported dramatically short median survival (few weeks). In more recent trials within the unfavourable subgroup, median overall survival is around 1 year, up to 13.6 months [11]. Such discrepancy may reflect changes in inclusion criteria, and a strong selection bias favouring enrolment of patients with better prognosis in recent trials.

Prognosis of CUP patients is generally worse than those with known primary cancers [12]. Among the unfavourable subgroup of newly diagnosed CUP patients, the most widely used prognostic model is based on the LDH level and the performance status (PS), separating a good prognosis group (PS = 0–1, normal LDH or absence of liver metastasis if LDH unknown) with a predicted 11.7 months median survival, and a

Table 1
Randomized clinical trials studying specifically CUP.

Author (year) (number of patients)	Histology	Regimens	Median overall survival (months)	Response rate	p
Woods et al. (1980) ⁷⁵ (n = 47)	Adenocarcinoma, undifferentiated	5-Fluorouracil, cyclophosphamide, methotrexate	1.6	4.5%	NS
Shildt et al. (1983) ⁷⁶ (n = 36)	Adenocarcinoma	Doxorubicin, mitomycin-C	4.1	36%	NS
		5-Fluorouracil, Doxorubicin, 5-fluorouracil, cyclophosphamide	3.1	0%	
Milliken et al. (1987) ⁷⁸ (n = 101)	Adenocarcinoma, undifferentiated	Doxorubicin, mitomycin-C	4.1	42%	NS
Eagan et al. (1987) ⁷⁷ (n = 55)	Carcinoma (50/55 adenocarcinomas)	Cisplatin, bleomycin, vinblastin	5.7	32%	NS
		Doxorubicin, mitomycin-C	5.5	14%	
Falkson et al. (1998) ⁷⁹ (n = 84)	Adenocarcinoma, undifferentiated	Cisplatin, doxorubicin, mitomycin-C	4.6	27%	0.05
		Cisplatin, epirubicin, mitomycin-C	9.4	50%	
Dowell et al. (2001) ⁸⁰ (n = 34)	Adenocarcinoma, undifferentiated	Mitomycin-C	5.4	17%	NS
		Paclitaxel, 5-fluorouracil, leucovorin	8.2	19%	
Assersohn et al. (2003) ⁸¹ (n = 88)	Carcinoma	Carboplatin, etoposide	6.4	19%	NS
		5-Fluorouracil	6.6	11.6%	
Culine et al. (2003) ⁸³ (n = 80)	Carcinoma	5-Fluorouracil, mitomycin-C	4.7	20%	NS
		Cisplatin, gemcitabine	8	55%	
Palmeri et al. (2006) ¹¹ (n = 66)	Carcinoma	Cisplatin, irinotecan	6	38%	NS
		Cisplatin, gemcitabine, vinorelbine	13.6	48.5%	
Huebner et al. (2009) ⁸⁴ (n = 92)	Adenocarcinoma, undifferentiated	Cisplatin, paclitaxel, gemcitabine	9.6	42.3%	NS
		Carboplatin, paclitaxel	11	23.8%	
Hainsworth et al. (2010) ⁸⁵ (n = 198)	Carcinoma	Gemcitabine, vinorelbine	6.9	20%	NS
		Paclitaxel, carboplatin, etoposide then gefitinib	7.4	18%	
Gross-Goupil et al. (2012) ⁸⁶ (n = 52)	Carcinoma	Gemcitabine, irinotecan then gefitinib	8.5	18%	NS
		Cisplatin	8	16%	
Hainsworth et al. (2015) ⁷⁴ (n = 89)	Carcinoma	Cisplatin, gemcitabine	11	19%	NS OS P less than 0.02 ORR
		Carboplatin, paclitaxel, belinostat	12.4	45%	
Hayashi et al. (2019) ⁸⁸ (n = 130)	Carcinoma	Carboplatin, paclitaxel	9.1	21%	NS
		Site-specific therapy	10.7	41.2%	
Fizazi et al. (2019) ⁸⁹ (n = 243)	Carcinoma	Cisplatin, gemcitabine	10	34.7%	NS
		Site-specific therapy	10.7	NA	

p = p-value for statistical significance for overall survival; NS = not statistically significant, OS = overall survival, ORR = overall response rate, NA = not available

poor prognosis group (PS > 1 or elevated LDH) with 3.9 months median survival [13]. This model was based on retrospective data, with a third of patients included being part of prospective trials, with probably overestimation of survival in comparison with real world outcomes.

Indeed, data from the Surveillance, Epidemiology, and End Results (SEER) registry analysis up to 2008 shows dismal survival after CUP diagnosis with a median survival of 3 months, making CUP the fourth more lethal cancer in the world [8].

Over time, there were almost no improvement in prognosis [14], with survival improving only in the subset of patients with squamous cell carcinoma [8]. Within this study, there is no distinction between the favourable and unfavourable subgroup of CUPs: it is likely that most of the patients were belonging to the unfavourable subgroup of CUPs. The longitudinal analysis of the SEER registry (1973 to 2008) showed a better survival (after multivariate analysis) for white race, female, age under 65, married people, squamous histology, diagnosis done in the most recent decade, and treatment with radiotherapy [8].

Diagnosis And The Tissue Of Origin Concept

Diagnosis is established after pathological examination of a good quality tissue sample (biopsy or, more rarely, cytology). Diagnostic workup aims to exclude non-carcinomatous tumours (lymphomas, melanomas, germ cell tumours, sarcomas), which account for less than 5% of findings, but point towards the elusive primary and require very specific treatments. Consequently, CUPs are almost exclusively carcinomas, explaining why the term CUP is also used for “carcinoma” of unknown primary origin.

The availability of advanced imaging and endoscopy technologies, serum marker testing, the development of immunohistochemical panel testing and the increasing access to gene profiling and other molecular analyses, including epigenetics [15,16] allow suspecting a tissue of origin in a growing subset of CUPs, even in the absence of anatomically identified primary [17,18]. The European Society of Medical Oncology (ESMO) guidelines suggest a basic immunohistochemical (IHC) work-up for CUPs, divided in a minimum set of primary markers, to initially direct towards subsets of potential primaries, followed by a set of additional markers towards the final identification of the putative primary tumour, endorsing a previously described two-step procedure [3,19]. Two CUP groups can be distinguished: tissue of origin-defined CUP and unclassifiable CUP [17], the latter corresponding to the confirmed CUP for which neither an identifiable primary site nor a suggested tissue of origin exists.

With the use of more advanced assays (such as microarrays and next

generation sequencing), a putative tissue of origin can be identified in approximately 80%-85% of cases [15,16].

An integrative illustration of these definitions and of the way the diagnostic work-up of CUP is currently shaped is presented in Fig. 1.

Tissue Of Origin Classifier Assays

Driven by the hypothesis that the identification of a primary tumour could lead to more specific treatment and therefore would improve patient’s outcomes, several molecular assays were developed in order to identify the tissue of origin in CUPs with more accuracy.

Based on gene expression profiling, miRNA expression or DNA methylation analysis, these tests compare the molecular features of the CUP to the molecular profile of tumours of known origin, therefore inferring a putative tissue of origin. Because no gold standard test defining the tissue of origin exists in CUP, the assessment and validation of the performance of these classifiers are challenging [2,20].

Several retrospective studies have attempted to assess the prediction accuracy of these tests performed on biopsy specimens from patients with CUP. Using correlation with clinicopathological features, the IHC profile or the identification of a latent primary as prediction comparator, these molecular based tissue of origin classifiers yield prediction accuracy from 60% to 92% [15,21–30]. This is corroborated by a prospective study demonstrating an 84% agreement of molecular profile with clinicopathological diagnosis [31].

One approach, illustrated by the work of Moran et al., is based on the epigenetic profiling via analysis of DNA methylation, developing an assay called EPICUP® DNA methylation profiling. This was developed from the analysis of 2790 tumour samples and validated in 7691 known tumour samples, with 87% prediction of a primary [27].

MicroRNA profiling is another technique, that has been studied in a prospective study [31]. The analysis of formalin-fixed paraffin-embedded (FFPE) metastatic tissues from 104 patients using a subset of 48 microRNAs led to a 71% accuracy in predicting the tissue of origin. Ferracin et al. used a similar approach with a 47-miRNA signature, reaching a 100% accuracy for primary tumours and 78% for metastasis from known primary tumours, with an accuracy for CUP tested in only 16 patients [24].

Another approach is based on the analysis of gene expression profiling. Horlings et al. obtain whole gene expression data using microarray technology from FFPE classifying correctly 81% of metastasis to their known primaries. A primary origin was assigned in almost 94% of ACUP (adenocarcinoma of unknown primary origin) [22]. Even if the microarray technology is not the most used today, this study

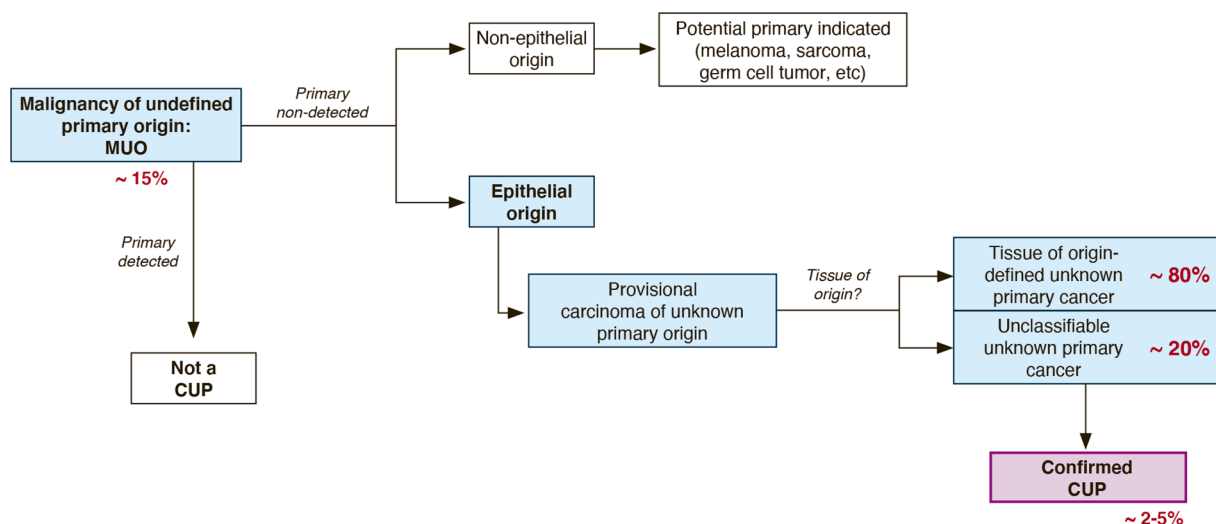


Fig. 1.

confirmed that gene expression analysis can yield high predictive accuracy in determining tissue of origin in CUPs.

Some limitations in the analysis of gene expression in CUP could be overcome by performing more sophisticated analysis, allowing more comparison with available datasets for all tumour cases. These types of approaches were implemented in a recent study, with machine learning analysing 77,044 DNA sequencing and whole transcriptome data. Even with such sophisticated tools, this yields in 71.7% of prediction in CUPs. This approach also have the originality to provide, at the same time, results about potential targetable genomic alteration [30].

Biological landscape

Biological hypothesis

Tumorigenesis of CUPs, and how to explain the absence of a detectable primary, remain matters of debate, with two main hypotheses [32,33]. The first hypothesis considers each CUP as a subtype of its corresponding known primary cancer with a primary lesion too small or too difficult to detect. This disease would, therefore, share clinical features with the corresponding primary and could benefit from the same treatment. An example is high-grade serous peritoneal carcinoma that could arise from early lesions in the Fallopian tube. These early lesions are undetectable if extensive sampling of the Fallopian tubes is not undertaken.

The second hypothesis suggests that the absence of the primary lesion is real, and will persist during the course of the disease, most probably due to its early and maintained regression or dormancy. CUPs, in relation to these early biological events, would be a specific entity, and share similarities whatever their tissue of origin, making them a complete distinct entity from known primary tumours: this is the “true” or “genuine” CUP hypothesis [32,33]. The parallel progression model of tumorigenesis could support this hypothesis, as independent clonal evolution under differential selection pressure could explain the regression of a primary lesion (that could be immune-mediated [34]) along with growing metastatic lesions [35,36].

Favouring the first hypothesis, a number of CUP patients (ranging from 4% to 25% in the reported literature) will subsequently, during the course of the disease, present a primary lesion [2]. Furthermore, autopsy studies led to identification of a primary lesion in 73% of CUPs cases, with variation across studies [20]. Nevertheless, a substantial group of CUP patients remains without an identified primary lesion.

The ability of molecular classifiers to infer a tissue of origin in the vast majority of cases argues in favour of biological proximity between CUPs and known primary cancers [37]. Moreover, a study that compared microRNA profiles of CUPs metastases with those from primary tissue-matched metastases of known primaries did not detect substantial differences. An important limitation of this work was that all CUPs cases were belonging to the favourable subgroups of CUPs [38].

Nevertheless, discrepancies between putative primary lesions found in autopsy studies and through molecular analyses cast doubt on their perfect reliability, even though those studies were not conducted at the same periods of time [33]. Several works failed to identify a unique “molecular CUP signature” [2,32], consequently weakening the “true” CUP hypothesis.

Chromosomal instability as a hallmark of CUPs?

The “true” CUP hypothesis states that CUPs, whatever their cell-of-origin, share similarities and could be addressed as a specific entity. Unfavourable CUPs are definitively sharing clinical characteristics that distinguish them from other cancers: aggressive phenotype with rapid growing metastases, unpredictable metastatic pattern, poor response to chemotherapy and poor clinical outcomes [20]. CUP metastases occur early and subsequently undergo genetic evolution toward a highly complex and unbalanced cytogenetic aberrations independent from the

primary tumour [33,39]. Many of these genome aberrations originate from a persistent rate of chromosome missegregation during cell division, a phenomenon known as Chromosomal Instability (CIN) [40,41], which in turn can lead to whole chromosome losses and gains (aneuploidy) [42] or to other dramatic genome aberrations. It is recognised that CIN drives cancer aggressiveness by increasing tumour heterogeneity and genetic diversity within the tumour cell population, which allows adaptation to unfavourable conditions and therapy resistance [43] not only to conventional chemotherapy, but to immunotherapy as well [44]. Furthermore, CIN has been suggested to directly drive the metastatic process itself [45], and is associated with poor clinical outcomes [39,46,47].

Tumour metastasis is a clonal process driven by CIN, through which metastatic cancer cells acquire chromosome segments that encode genes ending survival benefit and metastasising potential [45,48]. A work conducted by Vikesa and colleagues showed that CUPs, in comparison with metastases of known origin, presented microRNA signatures of CIN [49]. While reviewing details from the 57 cases that are described, we estimate that only 4 of them could have belong to the favourable subgroup of CUP (3 colon cancer and 1 cervical cancer presenting with inguinal lymph node), suggesting that CIN is exclusive to the poor prognosis subgroup of CUP but this should be confirm in other studies. It can therefore be postulated that CIN facilitates independent progression of metastatic sites in CUP following early dissemination and primary tumour regression/dormancy (possibly as the latter are less chromosomally instable). One could hypothesise that this could be a consequence of early CIN increase accumulating already in locally advanced primary tumours, which then could either grow enough in size to be detectable or stay genomically unstable but small in size, challenging identification, or even experience regression while some cells have been able to disseminate to distant organs. This scenario would fit well with the most predominantly mutated gene found in CUPs, the *TP53* gene. Such mutations, if acquired early during tumorigenesis could allow permissive conditions for accumulation of CIN and derived alterations, allowing cancer cells to continue diving and propagating instead of being eliminated [50,51]. The prevalence of *TP53* mutations in CUPs is found to be in the same range as the average *TP53* mutation rate across all tumour types (*TP53* is mutated in approximately 50% of all human cancers [52]).

In favour of fast genetic evolution increasing aggressiveness in CUPs, the role of other CIN-related events such as chromothripsis could be considered. It has been recently postulated that even one single wrong chromosomal segregation in one cell division can lead to a mutational cascade fuelling evolution and subclonal heterogeneity in cancers [53], suggesting that clonal evolution of tumours could in fact start very early.

Although CIN accelerates phenotypic adaptation under selective pressures, very high CIN levels are counter-productive for tumour survival owing to frequent generation of unviable phenotypes. In this context, it is CIN tolerance and attenuation mechanisms that allow an optimal equilibrium and sustainable CIN propagation [43].

It would, therefore, be of interest to investigate CIN levels in CUPs as a potential explanation for their specific nature and behaviour.

Some studies indicate that cytotoxic therapies induce CIN, and combination therapy with taxanes may synergize together [54,55].

In our view, early dissemination in the parallel progression, along with early chromosomal instability in metastatic clones or at the level of locally advanced disease, has biological robustness and could be hallmarks of CUP tumorigenesis. We provide an illustration of this notion in Fig. 2. The existence of other pro-metastatic hallmarks/signatures that could transcend tissue of origin signature in CUPs is not excluded and could lie in other “-omics” levels (DNA methylation, histone acetylation, proteomics, non-coding regions) [38,56]. Even in the absence of final answers on the biology of CUP, recognising the possibility of the “true CUP hypothesis” could be important for clinicians in the direction of avoiding endless diagnostic work-up (in order to find the primary) in patients requiring rapid treatment initiation. Furthermore, considering the relevant role of CIN in the aggressiveness of CUPs, it could serve in

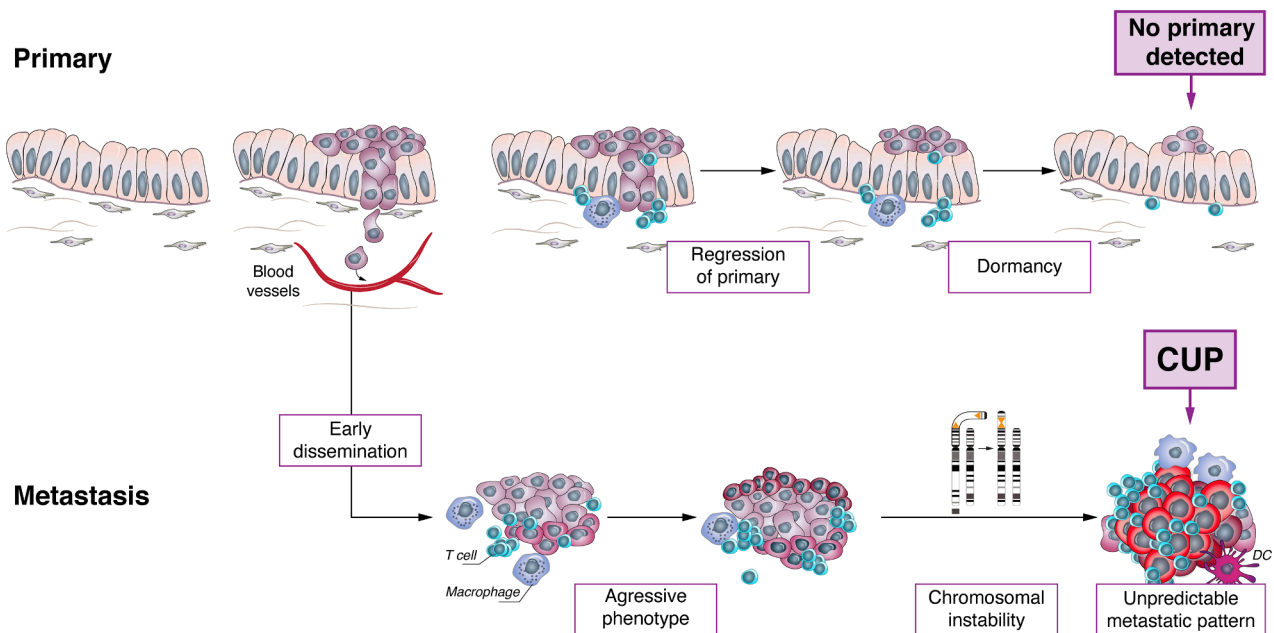


Fig. 2.

the future as an important diagnostic marker as well as a targetable axis in these types of tumours.

Genomic alteration in CUPs

Several studies retrospectively examined the genomic landscape of CUPs through DNA profiling (Table 2).

In a large retrospective cohort (1806 patients), Gatalica and colleagues found *EGFR* and *ERBB2* amplification as the most common amplifications. *TP53* (38%) and *KRAS* (18%) were the most common mutated genes [57]. A retrospective analysis of 303 CUP patients whose archival tumour specimens underwent next-generation sequencing revealed that 32% would have been potentially eligible for the available molecularly guided targeted or immunotherapy treatment options [58]. This technology is the basis for target identification and management strategy in the ongoing CUPISCO trial (NCT03498521, see below). Among 333 patients with CUPs evaluated in Memorial Sloan Kettering Cancer Center (MSKCC), 150 of them had a genomic analysis through the inclusion in MSK-IMPACT [59]. The most commonly mutated genes were *TP53*, *KRAS*, *CDKN2A*, *KEAP1*, and *SMARCA4*. Authors identified 45 (30%) patients with potentially targetable alterations and 15 (10%) actually received targeted therapies. Among the latter, time to treatment failure ranged from less than 1 month to 14 months [60].

The means through which these alterations could be detected is a major subject of research. Liquid biopsy techniques offer many advantages, notably in cases of no or minimal archival tissue availability [2]. At the 2020 annual meeting of the American Society of Clinical Oncology (ASCO) the results of cell-free DNA analysis from the largest cohort to date of in CUP patients were presented [61]. In their work, 90% of patients had at least one cell-free DNA (cfDNA) alteration. When classifying alterations according to the OncoKB annotation [62], the authors found that 46% of patients with at least one alteration were presenting with level 1, level 2B or resistance alteration (level R1). Importantly, 22% of the patients with at least 1 cfDNA alteration presented at least one Level 1 alteration (FDA-recognised biomarker predictive of response to FDA-approved drug in this indication), such as *PIK3CA* oncogenic mutations (22%), *ERBB2* amplification (13%), *BRCA1* (7%) and *BRCA2* (9%) oncogenic mutations, *BRAF* V600E mutation (8%) and *ALK* fusion (2%), amongst others [61].

Interpretation regarding whether these mutations could be

targetable shows wide discrepancy according to studies, varying from 15% to 85% [57,60,63–69]. Such analyses highlight the need to keep a high level of stringency in defining a “clinically relevant” alteration, which should go beyond bio-plausibility, and ideally rely on improved clinical outcomes associated with targeting such alterations, but also access to relevant approved targeted agents in diverse healthcare settings.

The NOMINATOR study assessed the feasibility of genomic testing in rare cancers [70], also assessing actionability as per the OncoKB annotation [62]. This work optimistically reports 56% of rare tumours with at least one actionable finding. The most commonly found aberrant genes included *TP53* (44%), *CDKN2A/B* (14%), *RBI* (14%), *PTEN* (13%) and *NFI* (12%). However, in order to obtain this high percentage of actionable findings, the authors included OncoKB level 1 to 4; if the analysis was to be restricted to biomarkers with at least compelling clinical evidence of utility (rather than including Level 4 alterations for which only compelling biological evidence exists), the respective percentage would be 27%. The authors also exclude from their analysis the patients in whom the analysis could not be done (initial attrition rate of 18%), introducing therefore an overestimation in the estimation of percentage of “clinically relevant” alterations. Furthermore, only 13 out of 121 (11%) patients ultimately had access to a matched drug, and outcomes are not reported, hence the real impact strength was not accurately assessed.

Results are awaited for the RP-1843 Arcagen collaborative project of the EORTC-SPECTA cohorts [71], aiming to perform comprehensive molecular analysis in a 2100-strong cohort of rare cancer patients. An initial report of 87 patients with sarcoma, thymic cancer, rare ovarian and head or neck cancers reported a 47% incidence of clinically relevant genomic alteration, with a 14% analysis failure rate [72].

Immune microenvironment and immune biomarkers in CUPs

Few data are available on tumour immune microenvironment in CUPs. One study reported no prognostic value of the presence of CD8 positive tumour-infiltrating lymphocytes (TILs) by IHC on a cohort of 92 CUPs. Transcriptional analysis on 71 cases revealed two sub-groups: inflamed and non-inflamed. Interestingly, there was inverse association between levels of VEGF-A gene and inflamed phenotype, suggesting that VEGF blockade may enhance anti-PD1/PD-L1 in CUPs [73].

Table 2
Genomic alterations identified within CUP tissue analysis.

Author (year) (number of patients)	Gene mutations	Chromosomal abnormalities	
		Amplifications	Deletions
Ross et al. (2020) ⁵⁸ (n = 303)	TP53 (55%); KRAS (27%); MYC (23%); CDKN2A (19%); ARID1A (11%); MCL1 (10%); PTEN (7%); PIK3CA (9%); ERBB2 (8%); BRAF (6%); NFI (4%)	CDKN2A; CDKN2B; MTAB; MYC; RAD21; ERBB2	FGFR2; CDKN2A; STK11; SMARCA4
Galatica et al. (2018) ⁶⁶ (n = 389)	TP53 (54%); KRAS (22%); ARID1A (13%); PIK3CA (9%); CDKN2A (8%)	CCND1 (5%); FGF (3%); ERBB2 (3%); MYC (3%)	ND
Clynick et al. (2018) ⁶⁸ (n = 21)	TP53 (47%); KRAS (12%); MET (12%)	MYC (12%); CCND1 (6%); FGFR1 (6%)	ND
Varghese et al. (2017) ⁶⁰ (n = 150)	TP53 (~50%); KRAS (~25%); KEAP1 (15%)	KRAS (2%); TERT (2%)	CDKN2A/B (12%)
Subbiah et al. (2017) ⁶⁹ (n = 17)	TP53 (29%); ARID1A (18%); PIK3CA (18%)	SOX2 (18%); CCND1 (12%)	CDKN2A/B (17%)
Löffler et al. (2016) ⁶⁷ (n = 128)	TP53 (55%); CDKN2A (9%); KRAS (16%); BRAF (5%); EGFR (4%)	FGFR3 (5%); NRAS (5%); ERBB2 (4%); MET (4%); EGFR (2%); KRAS (2%)	CDKN2A (15%); RB1 (7%)
Ross et al. (2015) ⁶³ (n = 200)	TP53 (55%); KRAS (19%); ARID1A (11%); PIK3CA (7%); BRAF (6%); ERBB2 (4%)	MYC (12%); MCL1 (11%); ERBB2 (4%)	CKN2A/B (11%)
Pentheroudakis et al. (2014) * ⁶⁵ (n = 87)	CTNNB1 (20%); KRAS (12%); PIK3CA (9%); MET (7%); BRAF (6%)	ND	ND
Galatica et al. (2014) ⁵⁷ (n = 1806)	TP53 (38%); KRAS (18%); BRCA2 (~11%); PIK3CA (~9%); STK11 (~6%); cKIT (1%); EGFR (less than 1%)	EGFR (17%); PIK3CA (14%); ERBB2 (5%); cMET (1%)	ND
Tothill et al. (2013) ⁶⁴ (n = 16)	TP53 (62%); GNAS (25%); NOTCH1 (18%); PIK3CA (18%); CDKN2A (12%); KRAS (12%)	JAK2 (6%); CCDN1 (6%); VHL (6%)	CDKN2A (6%); BRCA1 (6%); STK11 (6%)

ND, not documented, *CTNNB1, MET, PIK3CA; KRAS, BRAF targeted sequencing

Another study analysed 592 genes of 389 CUPs cases, showing that 28% of them had at least one potential predictive biomarker to CPI response, such as PD-L1 overexpression, MSI-H profile, or high TMB [66].

The incidence of patients with MSI-H profile CUPs is globally low, with some variation depending on analysis technique: 1.6% with next-generation sequencing (NGS) analysis and immunohistochemistry [66] and 2.4% via cfDNA analysis [61].

Table 3 illustrate data of potential predictive biomarkers of response to immune checkpoint inhibitors in CUP.

How Effective Is The Current Standard Of Care?

One-size fits all approach

With the exception of the specific subsets of CUPs of favourable prognosis (15%-20% of CUPs) that require specific treatments, doublet combination chemotherapies, including platinum and taxanes, or other

Table 3
Investigational predictive biomarkers of response to immune checkpoint inhibitors in CUP.

Ross et al. (2021) ⁵⁸ (n = 303)	PD-L1 expression \geq 50%: 14% TMB high: 11.6% MSI-high: 1% TMB High : 11.8% MSI-H : 1.8% PD-L1 expression \geq 5%: 22.5%
Galatica et al. (2018) ⁶⁶ (n = 389)	

TMB high: Tumor mutational burden \geq 16 mutations/megabase.

MSI-H : microsatellite instability high

doublet chemotherapies with new compounds, are accepted standard “empiric” chemotherapy regimens in international guidelines [3]. They should be selected according to local expertise and facilities and considering toxicity profiles and patient comorbidities.

Table 1 presents all existing randomized trials specifically studying CUP patients, with the regimens and their respective median overall survival and response rates when available [11,74–89]. As described, the prognostic classification (based on performance status and LDH levels) separates a favourable prognosis subgroup (median survival of 12 months) from a poor prognosis subgroup (median survival of 4 months); for the latter, best supportive care should also be considered upfront in the management algorithm [3,13].

A systematic review of randomised trials, excluding data from the specific favourable subsets of CUPs, showed no significant benefit for any treatment group over others (including platinum compounds, taxanes, gemcitabine, vinca-alkaloid and irinotecan). Hazard ratios for death of combination treatments containing taxanes, platinum or both showed a favourable trend over monotherapy with agent other than platinum or taxanes [90]. Another meta-analysis based on 32 studies showed a trend toward better survival outcome with platinum or taxane treatment. After adjustment for prognostic factors this trend was no longer significant for platinum vs non-platinum based regimen, while taxane-based regimens remained significant [91].

A phase III trial in 198 patients compared a triplet combination (paclitaxel/carboplatin/etoposide) with a gemcitabine/irinotecan combination: the triplet regimen was not superior and more toxic [85]. Doublet chemotherapy seems better than monotherapy regarding objective response rate (ORR) and is the first choice when feasible [92]. However, this has not been prospectively proved to be significant [86].

In further lines, chemotherapy is for a very selected population, with no trials featuring best supportive care as a comparative arm. Response rates are usually around 10% with median OS ranging from 3 to 9.7 months [92].

Tissue of origin-based chemotherapy

If CUPs share a “biological signature”, they would behave similarly whatever their tissue of origin. On the contrary, if CUPs are rather a subgroup of their corresponding primary, patients would benefit more from a tissue of origin-tailored treatment. In favour of the last hypothesis is the subgroup of specific subset of favourable CUPs, mainly composed of subtypes of entities that are closed to their known primary.

A substantial proportion of CUPs present with relatively chemo-resistant tissue of origin tumours (pancreas, biliary tract) [20]. Other CUPs, presenting with chemosensitive tumours (ovarian, breast), will be likely to respond to “empiric” chemotherapy whatever their tissue of origin. Treatment strategies for certain primaries have considerably changed during the last decades, as for lung cancer, with checkpoint inhibitors (CPI) and tyrosine kinase Inhibitors (TKI) being incorporated into standard-of-care. Hence, a randomised trial comparing the efficacy of “empiric” chemotherapy to a “classifier-directed treatment” was required.

A large prospective non-randomized phase II trial studied outcomes of 194 patients CUP patients receiving assay-directed site-specific

treatment, showing a median overall survival of 12.5 months, which compares favourably with a historical control of 396 patients of CUPs patients treated with empiric regimens in four other trials (9.1 months). Methodological bias does not allow for highly reliable conclusions on the basis of such an indirect comparison. Another non-randomised trial assessing the combination of a platinum/taxane regimen with everolimus described a higher ORR (53% versus 26%, $P = 0.0097$) and a better median overall survival (17.8 versus 8.3 months, $P = 0.0052$) in the group of patients with a platinum/taxane-sensitive tissue of origin-predicted tumours [93].

A randomised phase II trial, conducted by Hayashi and colleagues, compared carboplatin/paclitaxel with site-specific treatment, resulting in no benefit in the “empirical” arm [88], with definitive conclusions difficult to draw because of limitations already described (out-of-date standard arm for some primaries, low proportion of lung and breast tissue of origin cancers due to environmental and ethnic differences).

The results of the prospective randomised phase III GEFCAPI 04 trial, precisely addressing this question, were presented at the 2019 ESMO Congress. The trial used a 92-gene real-time RT-PCR mRNA profiling assay essentially providing a more sophisticated phenotype of the unknown primary tissue of origin based on a gene expression profile algorithm. Based on this profiling, annotated against a large reference database, the study randomised between a control arm of empiric chemotherapy (gemcitabine/cisplatin doublet) and a personalised treatment arm (standard treatment of the suspected primary). The study excluded specific subsets with favourable prognosis. It aimed to improve median PFS by 3 months (from 5 to 8 months), and stratified for geographic site, PS and LDH. With an accrual of 243 patients, this is the largest CUP trial so far. GEFCAPI 04 failed to demonstrate an improvement in either PFS (HR: 0.95 by central review) or OS (HR: 0.92). There was a trend for improved OS in patients with cancers whose tissue of origin was unlikely to respond to gemcitabine/cisplatin (such as melanoma, colorectal and kidney cancer), nevertheless non-significant (HR: 0.74, $p = 0.3347$), owing to the small sub-cohort sizes. Overall, outcomes of GEFCAPI 04 confirmed that the prognosis of CUPs (excluding specific subsets) remains poor (median PFS of 5 months, median OS of 10 months). A plausible explanation of this negative result could be that a significant percentage of identified tissue of origin were tumours for which a platinum-based regimen is standard-of-care treatment, such as pancreatico-biliary cancer and squamous cell carcinoma. This could “annul” the effect of randomisation, as those patients received a platinum-based doublet independently of the randomisation arm. Moreover, a quarter of patients did not receive tailored treatment for multiple reasons (urgent initiation of treatment, dramatic clinical deterioration).

Can Precision Medicine Change The Paradigm?

Despite celebrated examples of targeted therapy, the initial promise of a complete transformation of oncology based on genomics has not materialised [94]. Trials exploring the benefit of implementing molecular profiling in advanced cancers showed rather disappointing results, leading authors to advocate for innovative approaches in precision oncology [95,96]. In the ProfILER trial, which included 2,579 adult and paediatric patients with previously treated metastatic cancer, molecular-based treatment has been recommended in 27% of patients, with only 6% receiving it, leading to a response in 0.9% of the whole-population [97]. The deceiving results of molecular-based recommended therapy trials [96] are not weakening the principles of precision oncology *per se*; they do, nevertheless, bring to light that beyond the thus far approved targeted therapies, the magnitude of benefit stemming from detailed molecular profiling remains, for now, small.

Targeted therapy based on molecular analysis

Targeting specific alteration

Case-reports describe successful treatment with targeted therapy in CUP patients [98–106], yet the reporting bias does not allow for evaluation of their real impact.

Targeted treatment used in an unselected fashion

The phase II single arm trial that assessed the triple combination of everolimus and carboplatin/paclitaxel in untreated CUP patients showed promising anti-tumoral activity (ORR: 36% in 45 assessable patients); however, without a control arm, the benefit of the addition of everolimus is unknown [93]. Furthermore, although the influence of tissue of origin identification on outcome was assessed in this study, the choice of everolimus was not based on molecular analysis.

A bevacizumab/erlotinib combination was tested in 51 poor prognosis patients, as first or second line, with only 10% of partial response but an overall 71% disease control rate [107]. This led to test the addition of bevacizumab/erlotinib to a carboplatin/paclitaxel regimen, yielding interesting outcomes with a median overall survival of 12.6 months and an ORR of 53%, although a non-controlled study [108]. Another phase II randomised trial showed that the addition of belinostat (a histone deacetylase inhibitor) to a carboplatin/paclitaxel combination did not lead to improved PFS as first line treatment [74]. Table 4 is detailing data from phase II trials investigating targeted therapies in CUP.

A paradigm shift recently occurred in oncology with the agnostic-histology approval of biomarker-based tissue-agnostic treatments, such as NTRK and RET inhibitors targeting the respective fusion-positive cancers [109,110]. This could open a possible successful treatment option for the subset of CUPs that features the above characteristics,

Table 4
Targeted therapies in CUP.

Author (number of patients)	Trial design & Patients	Intervention	Outcomes	
			Primary	Secondary
Ross <i>et al.</i> (2021) ⁵⁸ (n = 303)	Single arm phase II Newly diagnosed CUP	Everolimus 30 mg weekly Carboplatine AUC 6 and paclitaxel 200 mg/m ²	Response rate: 36%	Median PFS: 4.1 months Median OS: 10.1 months
Ross <i>et al.</i> (2021) ⁵⁸ (n = 303)	Single arm phase II CUP previously treated or untreated with poor- prognosis clinical features	Bevacizumab 10 mg/kg biweekly and erlotinib 150 mg daily	Response rate: 10%	Median PFS: 3.9 months Median OS: 7.4 months
Hainsworth <i>et al.</i> ¹⁰⁸ (n = 60)	Single arm phase II Newly diagnosed CUP	Carboplatine AUC 6 + paclitaxel 175 mg/m ² + bevacizumab 15 mg/kg on day 1 every 21 days and erlotinib 150 mg daily	Response rate: 53%	Median PFS: 8 months Median OS: 12.6 months
Hainsworth <i>et al.</i> ⁷⁴ (n = 89)	Randomized phase II Newly diagnosed CUP	Belinostat plus paclitaxel/ carboplatine <i>versus</i> paclitaxel/ carboplatine	PFS ; 5.4 vs 5.3 months, p = 0.85	Median OS: 12.4 vs 9.1 months, p = 0.20 Investigator- assessed response rate: 45% vs 21%; $p = 0.02$

PFS = progression free survival, OS = overall survival

effectively bypassing tissue of origin. Nevertheless, these tumour-agnostic biomarkers currently remain exceptions, and their real-life impact is not yet clear [111,112]. The tissue of origin, even in the emerging era of new tumour-agnostic biomarkers, remains of pivotal importance in tumour response. The efficacy of biomarker-driven therapy varies greatly amongst tumour types. For instance, ORR to anti-PD1 pembrolizumab in MSI-H tumours varied from 57% in endometrial cancers to 18% in pancreatic cancers and 0% in brain tumours [113]. BRAF inhibition is a striking illustration among others: the efficacy of vemurafenib in targeting the *BRAF* V600E mutation observed in melanoma patients [114] is not reproducible in colon cancers [115,116]. The Cancer Genome Atlas analysis of 11,286 specimens confirmed that cell-of-origin pattern is the dominating classification of cancers [117].

In known primary tumours, mechanisms for primary failure to targeted approaches, or secondary resistance, has been described: high clonal variability and heterogeneity with clonal selection of resistant clones [118], concomitant resistance mutations, redundancy of oncogenic pathways [119]. CIN is known to confer multidrug resistance [120]. In which manner these known resistance mechanisms are important specifically in CUPs is not perfectly understood.

Role of immune checkpoint inhibitors (CPI)

A greater number of patients presenting with CUPs are treated with immune checkpoint inhibitors (CPI): either inside clinical trials, through the FDA approval in case of for microsatellite instability-high (MSI-H) or mismatch repair deficient tumours (MMRd) [121], or off-label. Efficacy biomarkers are needed across cancer types for CPI, and the same applies for CUP. Overall, PD-L1 expression alone is not a sufficient biomarker to predict response to CPI across cancer types [122–124]. Tumour mutational burden (TMB) as a predictive biomarker to CPI treatment has shown clinical impact in some studies [125–128]. However, the real predictive value of TMB remains unclear and needs further investigation [129].

Anecdotal cases showed clinical activity of CPI in CUPs irrespective of the presumed tissue of origin [130,131]. NivoCUP, an open-label phase II trial, is the first trial in this setting to be reported. The efficacy of nivolumab in the unfavourable subset of CUP patients was assessed, with ORR as the primary endpoint [132]. Most of patients (80.3%) were previously treated. In this population (n = 45), the ORR was 22%, with two complete response (4.4%), a disease-control rate of 53.3% and a median duration of response of 12.4 months. Median PFS and OS in this group were 4.0 months and 15.9 months respectively. The reported median PFS was rather short without a plateau that would indicate long term responders. Of note, among the 45 patients previously treated, 20% had 2 prior lines of therapy and 22% has 3 prior lines, which obviously represents a very highly selected population, thus making cross-trial comparison meaningless. In the same work, a very low number of patients were treated upfront with nivolumab with 18.2% ORR. There is indeed signal of efficacy, with the caveat of highly selected patients. The need for predictive biomarkers in order to identify the 20% of CUP patients that will indeed respond to CPI treatment is crucial.

Other trials addressing the role of CPI in CUPs are ongoing. Selection of patients for treatment, based on clinical features and biomarkers, are critical. The ongoing CUPISCO trial includes an atezolizumab monotherapy arm for the TMB-high patients, and a combination chemotherapy/atezolizumab arm for patients with TMB-low or unknown tumours.

While awaiting these results, we must be aware of the specific phenotypic and biologic pattern of the unfavourable prognostic CUPs tumour: how chromosomal instability, a hallmark of unfavourable CUPs, could impact response to CPI remains an open question [43,44].

What if we use precision medicine as a means to an end?

It is very likely that a subset of CUP patients in the unfavourable subgroup would derive more benefit from molecular-driven treatment than classical chemotherapy. Only a prospective study could identify the real proportion of these patients. When such data are available, they could bring an argument for incorporating molecular analyses, such as NGS, into the initial diagnostic work-up of CUP; this was recently advocated for patients for whom a dominant tissue of origin has not been identified [133].

The reality is that guiding treatment based on optimisation of tissue of origin identification has thus far failed to improve outcomes. As an alternative approach, instead of aiming to identify tissue of origin (phenotypic similarities), we could aim to identify the driver changes behind these aggressive CUP lesions (genotypic driver changes) that can be therapeutically targeted, eventually ignoring the tissue of origin. A integrated approach could combine the two, generating both a detailed tissue of origin -phenotypical analysis and a comprehensive genomic profiling in the aim of identifying targetable alterations, which approximately one-third of CUPs seem to harbour [58]. CUPISCO is an ongoing phase II randomised trial that aims to explore molecularly-guided therapy for CUPs, using a platinum-based chemotherapy comparator arm and a number of targeted therapies globally covering for all targetable alterations with a proven benefit in oncology (NCT03498521). CUPISCO is designed to show whether this approach can improve outcomes. After 3 cycles of platinum-based chemotherapy, non-progressing patients are randomised between the investigational arm of maintenance therapy based on NGS analysis and a control arm (further chemotherapy). This “switch maintenance” design selects for patients responding to standard chemotherapy and positively primes the study for better results, effectively excluding the poor responder patients with very poor prognosis; per CUPISCO protocol, the latter patients will receive second line molecularly guided therapy, and results will be analysed on an exploratory basis. Results are highly anticipated. Should CUPISCO attain its objective, coupled with the continuously enlarging range of targeted therapies, it could bring about a meaningful change in the outcomes of the disease, for which there has been little success so far. Nonetheless, whether an achieved PFS benefit -the study primary endpoint- would translate in either overall survival benefit or better quality of life in this lethal disease remains the main clinically meaningful questions.

However, the CUPISCO investigators have already reported on barriers in enrolling patients. Reporting on the first 157 patients, 58% failed the screening process for several reasons such as issues in identifying this subset of CUP patients, insufficient quality or quantity of tissue available for screening molecular analysis and the declining performance status of prospective candidates [134].

There is a clear need to identify driver genomic abnormalities out of the potential list of pathogenic aberrations identified through molecular profiling. It might actually be more pertinent to spot the differences instead of the similarities with the presumed tissue of origin. In this process, the critical assessment of profiling-identified changes, the interpretation of variants of uncertain significance and the identification of potential germline variants are challenges that require multidisciplinary review and the linking to clinical trials networks [135]. This should be made within molecular tumour boards.

Accrual is a major issue in running specific CUPs trials, explaining why only four of them are actually recruiting (Table 5).

Conclusion

CUPs could be viewed as the quintessence of oncology, with many transversal biological issues, challenges that are found across oncology in general, but crucially with urgently needed answers to improve patient’s clinical outcomes. Although a highly heterogeneous group of cancers, CUPs can be categorised in two very distinct groups, at levels of

Table 5
Ongoing recruiting trials specifically designed for CUP patients.

Trial name	NTC	Title	Phase	Arms	Setting
CUPSICO	NCT03498521	A Phase II Randomized Study Comparing the Efficacy and Safety of Targeted Therapy or Cancer Immunotherapy Versus Platinum-Based Chemotherapy in Patients With Cancer of Unknown Primary Site	II	Multiples arms : - Platinum-based-molecularly guided therapy	First line poor prognosis CUP
CheCUP	NCT04131621	Nivolumab/Ipilimumab in Second Line CUP-syndrome	II	Single arm	Second line poor prognosis CUP
CUPem	NCT03752333	Trial of Pembrolizumab in Cancer of Unknown Primary Site	II	Single arm	2 cohorts: first line and second line settings
CUP	NCT03391973	Pembrolizumab in Patients With Poor-Prognosis Carcinoma of Unknown Primary Site	II	Single arm	First line poor prognosis CUP

biology, clinical presentation, treatment strategies and prognosis. The first group of specific subset CUPs behave closely like their counterpart known primaries, and benefit from same treatment strategies leading to same outcomes. Whether molecular profiling can enlarge this group of good-prognosis, specific-subset CUPs is an interesting point that remains to be seen.

The vast majority of CUP patients belongs to the unfavourable, non-specific subset CUP group. They present with very poorly differentiated tumours, high chromosomal instability, aggressive and unpredictable metastatic pattern, and poor prognosis. A true “molecular signature” has not yet been identified, yet chromosomal instability seems to be a hallmark of unfavourable CUPs. The main issue pertains to this unfavourable subgroup of CUP patients, where almost no improvement has been achieved for decades. Recent randomised trials have shown that tissue of origin classifiers used to guide treatment do not modify outcomes. Molecular analysis in order to find matched targeted therapies could probably select a proportion of patients that could benefit from these treatments, but prospective evidence is awaited. Within this context, whether immune checkpoint inhibitors could be beneficial remains an ongoing question.

A promising area of research focus could be the possibility of identifying a “feature signature” within the unfavourable group of CUPs that could be found in an “-omics” levels and could transcend the tissue of origin pattern (metabolism, microenvironment, non-coding DNA region, epigenetics). How to target chromosomal instability is also of major interest, as well as how to combine treatment to prevent acquired resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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