A prospective national precision medicine trial comparing blood and tissue profiling in patients with Cancer of Unknown Primary (CUPCOMP)

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Abstract

Background

Cancer of Unknown Primary (CUP) is an aggressive malignancy with limited treatment options and poor patient outcomes. In most cases, the primary site remains elusive, limiting therapeutic options to non-specific chemotherapy. The CUPCOMP study assessed the feasibility of integrating a blood-based molecular profiling approach with a National Molecular Tumour Board (MTB) into the treatment pathway of patients with CUP.

Patients and Methods:

Between June 2021 and February 2023, patients with histologically confirmed CUP, as per ESMO 2015 guidelines, were recruited from seven UK sites. Blood- and/or tissue-based genomic profiling, including whole genome sequencing, was performed, and results were reviewed at a virtual MTB. Retrospective genomic analysis was performed to evaluate concordance between sampling types and identify potentially actionable alterations.

Results

A total of 117 patients were recruited, with 113 completing 12 months of follow-up. Molecular profiling was successful in 115 patients, with blood-based profiling more successful than tissue-based (93% vs 61%, respectively). The MTB identified potentially actionable alterations (PAA) in 63% (73/115), with 26% (31/117) treated using a precision medicine (PM) approach. In all, 13% (15/117) received therapies targeting PAAs, 6 entered clinical trials, and 15 received site-directed standard of care therapy. Primary tumours were confirmed in 12 patients and suspected in 26. Median overall survival (OS) was 9.5 months, significantly longer in the PM group vs Doublet Chemotherapy (HR 0.29, $p < 0.0001$). The likelihood of detecting a PAA in blood was impacted by tumour fraction and 33% of patient had a tumour fraction of < 1%.

Conclusion

This study demonstrates the feasibility and clinical utility of liquid biopsies in CUP. While tissue profiling remains the preferred option for patients with low tumour fraction, blood-based profiling offers a viable alternative when tissue is unavailable or inaccessible or when genomic profiling results are required urgently, especially in selected patient subgroups.

Trial Registration:

Prospectively registered via ClinicalTrials.gov (NCT04750109)

INTRODUCTION

Cancer of Unknown Primary (CUP) represents a heterogeneous group of aggressive cancers where the origins of the cancer remain elusive. CUP accounts for 2–3% of all new cancer diagnoses but remains and leading cause of cancer death (1, 2). Previously an under-researched entity, several recent studies have highlighted the importance of adequate diagnostic workup and the use of molecular profiling for diagnosis prediction and/or biomarker treatment stratification, including recently updated European Society for Medical Oncology (ESMO) guidelines (3–6). Improved management pathways attempt to aid the diagnosis of otherwise 'diagnostically challenging' primary tumours that can often be misclassified as CUP and expand the classification of 'favourable' CUP subsets to include those tumours clinically aligned to known tumour types (3). Patients within these 'favourable' subsets (~ 20%) can access the aligned tumour site-directed therapies and achieve better clinical outcomes (7). However, the majority of patients (80–85%) fall within the 'unfavourable' or 'poor-risk' CUP subset where treatment is limited to 'one-size-fits-all' chemotherapy with poor efficacy and resulting dismal prognosis of less than 1 year (2). CUPCOMP was an investigator-initiated multi-centre prospective non-randomised clinical trial comparing the clinical feasibility and utility of molecular profiling from tissue and blood. We sought to evaluate the integration of routine molecular profiling alongside a National Molecular Tumour Board into the CUP management pathway. To overcome the known challenges of accessing good quality tissue for profiling in this population we also compared the clinical utility of tissue and blood-based circulating tumour DNA (ctDNA) profiling.

METHODS

Trial design and ethical approval

CUPCOMP (NCT04750109) was conducted in accordance with Good Clinical Practice and the ethical principles originating from the Declaration of Helsinki. Ethical approval was obtained from the Yorkshire and The Humber–Sheffield Research Ethics Committee (REC: 20/YH/0305) and trial sponsorship was undertaken by The Christie NHS Foundation Trust. All patients provided fully informed written consent for the trial with optional consent regarding genetic result disclosure and acquisition of additional research samples (Supplementary Methods).

Patient recruitment and sample collection

Patients with CUP were recruited between June 2021 and February 2023 through seven United Kingdom sites (Supplementary Methods). Eligibility was determined locally, and inclusion criteria included: appropriate local pathological confirmation of a diagnosis of 'favourable' or 'poor-risk' CUP according to ESMO guidelines 2015 (8), and discussion at a local multi-disciplinary team meeting (MDT). Patients

were required to have an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0–2 and eligible if treatment-naïve or previously treated.

Genomic Analysis

All consenting patients provided baseline blood samples for genomic profiling using the FoundationOne®Liquid CDx (F1LCDx) assay (Foundation Medicine, Inc). Diagnostic formalin fixed paraffin-embedded (FFPE) tissue was profiled using the FoundationOne® CDx (F1CDx) assay (Foundation Medicine, Inc) where suitable residual tissue was available. Where clinically appropriate, patients had additional blood draws on progression following treatment for additional F1LCDx testing. Patients consenting to a fresh tissue biopsy also provided samples for Whole Genome Sequencing (WGS) and an additional blood sample for germline control. DNA extraction of germline blood and fresh frozen tissue was performed by The North West Genomic Laboratory Hub (NW GLH), Manchester and WGS was performed by Source Biosciences UK Ltd (Nottingham, UK) (Molecular profiling details are supplied in Supplementary Methods).

Molecular Tumour Board (MTB)

A monthly virtual CUP MTB, with an integrated digital platform for genomic analysis interpretation (eTARGET) (https://upsmart.digitalecmt.com/etarget-3/), was attended by treating medical oncologists, site-based healthcare professionals, and clinical scientists from the NW GLH. Variant interpretation was undertaken by clinical scientists from NW GLH including: classification for pathogenicity/oncogenecity assigned according to relevant guidelines (9–12), and identification of variants of potential germline origins according to ESMO guidance (13). Variant interpretation results were discussed in the context of clinical cases, including patterns of alterations that indicated a primary tumour origin, and those alterations that may be predictive of response to an existing Standard of Care (SoC) or experimental therapy(potentially actionable alteration; PAA). Recommendations for treatment, trial entry or further confirmatory testing were communicated to the treating team, including recommendations for clinical genetic referrals.

Data collection and analysis

Patients' clinical data was collected at 6- and 12-months following recruitment to study including: receipt of any systemic anti-cancer therapy (SACT), radiotherapy or entry into a clinical trial, if a primary tumour diagnosis was determined/suspected, survival status, date of last clinical contact, and date of death. Scottish Inflammatory Prognostic Score (SIPS) was calculated where baseline blood tests results were available, as previously described (14). MTB outcome data was extracted from electronically recorded outcomes.

Statistical analysis

Median overall survival (OS), with associated 95% confidence intervals (CI), was estimated using the Kaplan-Meier method, from the date of consent until 12-month follow-up for all patients with complete follow-up data (n = 113). To compare survival by treatment, post-hoc groupings were defined according to interventions received by patients after consent (Supplementary Methods). All patients with follow-up data were included, except patients with a neuroendocrine carcinoma (NEC) histology (n = 2) and one patient treated radically with surgical resection. Differences between the groups were assessed using a pairwise Mantel-Cox Log-rank test to assess significance and hazard ratios, with p-values derived from two-sided tests.

A univariate Cox-proportional hazard model was used to assess the association between comprehensive tumour fraction score (CTFS; see Supplementary Methods) and survival. Clinical characteristics, including CTFS and SIPS, were compared between the three treatment groups using Chi-squared, Fisher's exact two-sided tests, or the Kruskal-Wallis rank sum test. A CTFS of < 1% was used to define low tumour fraction for comparisons. All statistical analyses were performed using R version 2024.04.2, SPSS version 29.0.0.0 and GraphPad Prism version 10. A two-sided significance level of 5% was used throughout.

Retrospective genomic analysis

Retrospective analysis of all genomic data were performed to assess pathogenicity utilising genomic interpretation software (OncoKB) and to compare tumour and blood alteration concordance (Supplementary Methods).

RESULTS

Of 137 patients assessed for eligibility, 117 patients with a histologically confirmed diagnosis of CUP were enrolled (Fig. 1). The baseline demographics of the enrolled participants are shown in Table 1. The median age was 63 years, and 66 (56%) participants were female. At baseline, the ECOG PS was 0 or 1 in 95 (81%) participants and 64 (55%) were never smokers. Histological subtypes included adenocarcinoma (n = 62, 53%), carcinoma (n = 29, 25%) and squamous cell carcinoma (n = 19, 16%). Differentiation was recorded for only 65 (55%) patients with 52 of these recorded as poorly differentiated (n = 50) or undifferentiated (n = 2) (Table 1). Thirty-six patients (31%) had metastatic disease restricted to a single organ site. Two patients had NEC histology and were excluded from downstream survival analysis following the 2023 ESMO CUP guidelines, which excludes NEC as a subtype of CUP. Thirty-five patients (31%) had received at least one prior line of therapy before consent; the remaining 75 patients were treatment naïve.

Table 1
Baseline participant demographics

Characteristic		*n* = 117 (%)
Age at consent		63 (54, 72)
Gender	Female	66 (56%)
	Male	51 (44%)
Ethnicity	African	1 (0.9%)
	Any other Asian background	1 (0.9%)
	Any other white background	8 (6.8%)
	British	90 (77%)
	Chinese	1 (0.9%)
	Missing	1 (0.9%)
	Unknown	15 (13%)
Histological type	Adenocarcinoma	62 (53%)
	Carcinoma	29 (25%)
	Other	4 (3.4%)
	Squamous Carcinoma	19 (16%)
	Unknown	3 (2.6%)
Degree of differentiation	Well	4 (3.4%)
	Moderate	9 (7.7%)
	Poor	50 (43%)
	Undifferentiated	2 (1.7%)
	Not specified	47 (40%)
	Missing	5 (4.3%)
Median number of metastatic sites (IQR)		3 (2, 4)
Metastasis to bone		33 (29%)
Metastasis to the liver		29 (25%)
Metastasis to the lymph nodes		70 (60%)
Weight Loss in last 6 months?	Yes	34 (29%)
	No	74 (63%)

Characteristic		*n* = 117 (%)
	Missing	9 (7.7%)
ECOG PS	**0**	35 (30%)
	1	60 (51%)
	2+	20 (17%)
	Unknown	2 (1.7%)
Smoking Status	**Current**	19 (16%)
	Missing	1 (0.9%)
	Never	64 (55%)
	Past	31 (26%)
	Unknown	2 (1.7%)
Family history of cancer	**No**	31 (26%)
	Yes	49 (42%)
	Unknown	37 (32%)
IQR = Interquartile Range		

Blood-based molecular profiling was more successful than tissue-based molecular profiling

Molecular profiling via tissue and/or blood was successful at some point during trial enrolment in 115/117 patients (Figs. 1&2). In the 2 patients where molecular profiling was not achieved, blood-based profiling failed due to insufficient DNA yield or insufficient blood draw for testing. At baseline, 109/117 (93%) patients had successful blood profiling (Fig. 2A).

Only 21/117 (18%) patients consented to a fresh biopsy sample to be taken, and 17 patients had successful WGS performed. Excluding one patient who withdrew consent for the study prior to tissue testing, the remaining cohort (95/117) had diagnostic FFPE tissue assessed for F1CDx testing resulting in 54/95 (57%) cases with successful tissue profiling (Fig. 1&2; failure reasons detailed in Supplementary Table 1). Including progression blood samples, 68 patients had both tissue and blood molecular profiling performed during study; 17 with WGS and F1LCDx blood and 51 with F1LCDx blood and F1CDx tissue.

Real-time clinical decision-making at the National Molecular Tumour Board

Real-time molecular profiling interpretation and MTB discussions occurred for 111/117 (95%) patients (Fig. 2B). For 2 patients no molecular profiling data was obtained during the study, 1 patient withdrew consent, and for 3 patients' molecular data was not discussed in real-time (Fig. 2B).

MTB-determined PAA were found in 73/115 (63%) patients considering all profiling approaches, including 3 patients discussed retrospectively (Fig. 2B and C). The median number of PAAs per patient was 1 (range 1–6), with 24/73 (33%) patients harbouring more than one PAA. For 38/73 (52%) of these patients, the PAA was found in blood, and 4 patients had PAAs only found in tissue profiling (Fig. 2C).

Across the whole cohort, 31 of 117 (26%) patients were treated with a precision medicine (PM) approach (Group 3, see methods), with 15 of 117 (13%) patients having treatment targeting the PAA identified. Six patients were treated within a clinical trial, 10 patients accessed immunotherapy outside of a clinical trial, and 15 patients had SoC treatment directed to a confirmed/suspected primary tumour site (Fig. 2A and Supplementary Table 2). Nine patients (8%) harboured potential oncogenic germline variants and were discussed at a germline MTB for specialist genetics input.

Clinical Outcomes and Survival Analysis

Of the 117 patients enrolled, 113 completed the 12-month follow-up (*n* = 3 lost to follow-up; *n* = 1 withdrew consent) (Fig. 1). Thirty-eight (34%) patients had a primary tumour diagnosis confirmed or suspected during the study period (12 confirmed; 26 suspected) (Fig. 1 and Supplementary Fig. 1). For 3 patients, the confirmed diagnosis was supported by molecular profiling and for 8 patients, molecular data led to or supported a clinical suspicion of a primary tumour type (Supplementary Fig. 1; Supplementary Table 2). The median OS for the cohort (*n* = 113) was 9.5 months (Fig. 3A).

Post-hoc analysis based on treatment following consent to trial was performed on 110 patients where survival data was available. 69 of 110 (63%) patients of this cohort went on to have SACT and 41 of 110 (37%) were treated with best supportive care (BSC) (Fig. 1).

OS was significantly longer in patients treated with a PM approach (Group 3) when compared to patients treated with SoC Doublet chemotherapy (Group 1); median OS was not reached in Group 3 vs 8.4 months in Group 1 (HR 0.29 [CI 0.14–0.59], *p*-value < 0.0001) (Fig. 3B). There was no statistical difference in survival between patients treated with Doublet chemotherapy vs those that received BSC (Group 2); median OS was 8.4 and 4.5 months, respectively (HR 1.29 [CI 0.77–2.14], *p*-value 0.3317). Between the 3 treatment groups there was no significant difference in baseline characteristics except ECOG PS and notably no difference in Scottish Inflammatory Prognostic Score (SIPS) (Supplementary Table 3).

Survival remained superior in the PM Group 3 when restricted to those patients with no prior SACT (*n* = 75) (Supplementary Fig. 2A&B). Median OS was not reached in the PM Group 3 compared to 8.5 months in the Doublet chemotherapy Group 1 (HR of 0.24 [CI 0.09–0.64], *p*-value 0.0042). In the treatment naïve setting, survival was significantly improved in the Doublet chemotherapy (Group 1) compared to BSC (Group 2; Median OS of 3.7, HR of 1.87 [CI 1.04–3.35], *p*-value 0.0367).

Retrospective analysis of blood-based molecular profiling to evaluate clinical utility

To further assess the feasibility and clinical utility of blood-based molecular profiling, retrospective analysis was performed. Firstly, to compare the concordance of the blood and tissue approaches, the cohort was restricted to those patients where Foundation Medicine genomic profiling had been performed in both tissue and blood ($n = 51$).

Although the total number of reported alterations in blood and tissue was similar across the cohort (Fig. 4A), 1072 unique alterations, only 487 (45%) were reported in both modalities (Supplementary Fig. 3A). A high proportion of blood-only alterations were Single Nucleotide Variants (SNV), whereas tissue-only alterations were more likely to be Copy Number Alterations (CNA), particularly deletions (Fig. 4B). This remained true when restricting to alterations that were predicted as oncogenic and to PAAs as determined by OncoKB (Fig. 4B&C and Supplementary Fig. 3A). On a patient level, concordance ranged from 9–89% (average 47%), and concordance rate was significantly correlated with CTFS (Pearsons's correlation $R^2 = 0.1139$, *p*-value = 0.0154; Supplementary Fig. 3B).

A Tumour Mutation Burden (TMB) of ≥ 10 Muts/Mb in either blood or tissue was reported in 13 patients: 10 patients in tissue only, 8 patients in blood only, 5 patients in both modalities. Where TMB was ≥ 10 Muts/Mb in tissue but not in blood ($n = 5$), the CTFS in blood was 0%. There was a non-significant correlation between blood-TMB (bTMB) and tissue-TMB (tTMB) across all 51 patients (Pearson correlation coefficient, $R^2 = 0.07526$, *p*-value = 0.0514) but significant correlation when restricted to patients with CTFS of ≥ 1% ($n = 30$, Pearsons correlation, $R^2 = 0.2493$, *p*-value = 0.0050; Fig. 4D&E).

To further explore the clinical utility of blood-based profiling in the absence of tissue, all patients with at least one blood sample were evaluated ($n = 112$). Sixty-five of 112 (58%) patients harboured at least one OncoKB-determined PAA (level 1-3B actionability) (Supplementary Fig. 4A&B). Level 1 evidence of actionability included twenty patients with a bTMB of ≥ 10 and four patients with a *BRAF* V600E mutation (Supplementary Fig. 4B&C). In 33% ($n = 41$) of patients, CTFS was reported as < 1%, with PAAs found in only 11 of these individuals. A reported PAA was significantly less likely in patients with CTFS < 1% as compared to patients with ≥ 1% (Fishers exact two-sided test; 27.5% vs 75.4%; *p*-value < 0.0001). The variant allele frequency (VAF) of alterations demonstrated a bimodal distribution with 442/1642 alterations (27%) having a VAF of < 1% and 394/1642 (24%) alterations with a VAF between 45% and 55% (Supplementary Fig. 5A). The top 10 altered genes with the highest reported VAFs in blood were genes commonly altered in Clonal Haematopoiesis of Indeterminate Potential (CHIP) (Supplementary Fig. 5B).

Finally, to further evaluate clinical utility of blood-based approaches, the CTFS score from baseline blood samples (n = 109) was compared to clinical features to assess which patients may benefit most from blood-based approaches (Fig. 5A&B). Patients with liver or bone metastases were more likely to have a CTFS of ≥ 1% compared to those without (Liver metastases: 36% vs 10%; Pearson's Chi-squared test; *p*-value = 0.003; Bone metastases: 38% vs 15%; Pearson's Chi-squared test; *p*-value = 0.022)

(Supplementary Table 4). CTFS ≥ 1% had no impact on survival (data not shown), and there was no significant difference in the proportion of patients with CTFS ≥ 1% between treatment groups 1–3 (Supplementary Table 3).

DISCUSSION

CUPCOMP is the first multi-site prospective study in CUP assessing the feasibility and potential clinical utility of a blood-based NGS approach as compared to tissue-based profiling. Here, we present data highlighting the ongoing challenges in attempting tissue-based molecular profiling and demonstrate the clinical utility and feasibility of blood-based profiling in a broad 'real-world' population of patients with CUP. Both 'favourable' and 'poor-risk' CUP subtypes were included, with ECOG PS 0–2, and patients were recruited at any point in their treatment journey. Greater than 50% of patients had PAAs reported, consistent with several retrospective single-institution descriptive studies (15–19). In our prospective study, one-third of the cohort received a PM approach to treatment, with significant improvement in survival. This supports the growing evidence of successful integration of PM approaches into CUP treatment pathways to aid diagnosis and treatment decisions (20–22). Most pivotal is the recently published randomised Phase II trial, CUPISCO, demonstrating that a PM approach can improve outcomes in patients diagnosed with 'poor-risk' CUP who responded to induction chemotherapy (4, 23). However, this cohort was restricted to those patients with poor-risk CUP, PS 0–1 and adenocarcinoma. The benefit therefore remains unclear in those patients with poorer performance status, high inflammatory markers, non-adenocarcinoma and 'favourable' subtypes of CUP (14).

Although molecular profiling is an increasingly integrated requirement across the advanced cancer spectrum, routine use in CUP is variable internationally and not currently reimbursed or approved in several countries. Where approval is granted, this is mostly for tissue-based NGS or single-gene testing. Tumour tissue in CUP, with appropriate cellularity and quality for NGS testing, is limited in supply. Therefore, patients require repeat tumour biopsies to obtain adequate tissue, limiting accessibility to testing as biopsies may be technically unfeasible or unsafe. This study demonstrates the challenges of obtaining adequate tissue for molecular profiling in CUP with only a 57% success rate using archival tissue; comparable to other reported studies where success was reported at 40% in one 'real-world' setting (24–26). Biopsy acquisition is invasive, adds further delays to testing and treating, increases costs and requires extra resources, all of which are factors that likely contributed to the low (18%) rate of repeated biopsy in this study. Blood-based profiling, however, was quick, highly feasible and can overcome the issue of tissue scarcity in a population that has often already had significant delays in the diagnostic process.

This study highlights the value of real-time MTB interpretation in patients with CUP; molecular profiling may not only identify PAAs but may also suggest a potential primary tumour (Fig. 5B) (5, 27, 28). Indeed, in the CUPISCO dataset, co-occurring mutation patterns clustered patients with molecular features reminiscent of known tumour types despite the strict eligibility criteria of this study (28), and molecular features of a colorectal cancer-like CUP favourable subtype have been defined within ESMO clinical

guidelines for some time (8). In our cohort, 38 patients had a primary tumour diagnosis confirmed or suspected, and in 29% of these cases, molecular profiling contributed to the diagnosis. With the increasing availability of mutation profiling from large cohorts of patients with CUP and known tumours, there is a clinical need for guidelines to guide the interpretation of co-mutation patterns to aid diagnosis (5).

Just over a quarter of the CUPCOMP cohort went on to have a PM treatment, either through the determination of a primary tumour, entry into a clinical trial or compassionate access. For 12% of the total cohort, the PM approach involved targeting molecular results, which is less than reported in the molecularly guided arm of CUPISCO (26%) but similar to larger pan-cancer PM trials (29, 30). Of note, 37% of the CUPCOMP cohort had no SACT (BSC), with a higher proportion of patients with ECOG PS 2 in this group, and median survival of only 4.5 months (3.7 months if treatment naïve). This highlights the need for appropriate consideration of genomic testing and reserving testing for patients who are most likely to benefit from treatment. A PM approach was associated with an improved OS in this all-comers real-world data. Survival comparison data suggested that the treatment naïve group are more likely to benefit from this approach and upfront molecular profiling can support a primary tumour diagnosis, which will directly impact first-line treatment.

Retrospective comparative analysis and evaluation of oncogenicity across blood-based profiling has identified key considerations for interpretation when using blood-based profiling for clinical decision-making in CUP (Fig. 5B). Blood-based profiling can miss potentially informative CNAs that are present in tissue and detect potential actionable SNVs at low VAF in genes commonly associated with CHIP or germline mutations. It does, however, have the advantage of detecting pathogenic germline mutations (8% in this cohort) for onward referral to clinical genetics. Our data showed that CTFS of < 1% (present in 36% of cohort) impacts the likelihood of a PAA being reported and under-reporting of high-TMB. Therefore, the most appropriate molecular profiling for an individual should be considered before requesting (Fig. 5A). We know that burden and sites of disease can impact ctDNA content as well as tumour type-dependent factors (31). In our cohort, the presence of liver metastases or bone metastases significantly impacted the likelihood of a CTFS ≥ 1%.

Real-time MTB discussions considered broad actionability criteria, including early-phase clinical trial entry, resulting in a much higher actionability than reported in other studies (63%) (4, 28, 32). Restricting actionability criteria to OncoKB levels 1-3b for analysis of the blood-based cohort resulted in 58% of the cohort with PAAs; 19% with level 1 evidence of actionability (tumour-agnostic indication). However, 15% of the level 1 evidence is applying a bTMB cut-off the same as tissue (≥ 10 Muts/Mb). The predictive ability of bTMB-high for immunotherapy response varies across different tumour types and immunotherapies, and tests vary in their calculation. There is debate as to the appropriate cut-off to employ for ctDNA, especially in the context of low tumour fraction. In the CUPISCO trial, more than two-thirds of the patients in the MGT group (without an actionable alteration) received immunotherapy with chemotherapy. In our study more than two-thirds of the patients in the PM group received immunotherapy either within or outside of clinical trials. Despite the debate over reliability of predictive

immunotherapy biomarkers, this signal calls for properly designed clinical trials to explore the efficacy of immunotherapy in this group of patients.

Four patients harboured a *BRAF* V600E mutation which was recently deemed targetable with BRAF inhibitors in the tumour-agnostic setting (33). Interestingly of these 4 patients, 1 had a suspected melanoma following molecular profiling and another had a suspected GI malignancy. The two remaining patients had no primary tumour suspected or confirmed. Despite tumour agnostic approvals for BRAF inhibitors in this setting, it is known that the context of the tumour origins plays a pivotal role in selecting combination treatment and predicting response rates (33). The remaining patients that harboured PAAs constituted level 3a or 3b evidence of therapeutic actionability. Most cases were level 3b – the biomarker is predictive of response but in a different tumour type indication. Without a primary tumour diagnosis, the true actionability of the biomarker remains uncertain. For some patients, the presence of such a biomarker is highly indicative of the primary site. For example, 3 patients harboured *FGFR2* fusions and subsequently had a confirmed or highly suspected diagnosis of intrahepatic cholangiocarcinoma (iCCA). FGFR inhibitors are SoC and approved in the UK and USA for iCCA, and therefore the molecular pattern and primary diagnosis changed SoC therapeutic options significantly (34, 35). Another patient with suspected breast cancer harboured both *ERBB2* amplification and *PIK3CA* mutation, both level 1 evidence in breast cancer for response to HER2 antagonists and alpelisib, respectively (36).

There were several limitations to this study. This study was not randomised, so treatment groupings were not matched for clinical characteristics such as ECOG PS or prognostic biomarkers, and therefore potential bias may impact results. Although ECOG PS was significantly higher in the BSC group, SIPS prognostic scores were not different between treatment groups, supporting the independent benefit on survival of the PM approach compared to chemotherapy. In addition, tissue profiling was not routinely performed at baseline, although at the outset, repeat tissue biopsy was mandated. This meant that a comparison of the clinical utility of blood and tissue, including turnaround time and success of profiling, was not feasible. An amendment was made early in the trial to include FFPE profiling if blood-based testing failed, as it was apparent that timely repeat biopsies were limiting recruitment and were difficult to obtain. This does, however, support the clinical utility of offering blood-based profiling over tissue in some patients with CUP.

We recognise that this cohort of patients with CUP represents a selective and heterogeneous group of patients, reflected in the relatively good 12-month median OS of 9.4 months. A significant proportion of patients could be categorised as favourable prognosis group (single site, lymph node limited, primary found/highly suspected) likely due to more permissive eligibility criteria but representing a more “real world” cohort. Given the disparity in organisation and provision of CUP services globally we demonstrate the feasibility of incorporating NGS early in the CUP pathway ensuring timely stratification of patients to potential PM approaches.

Due to the nature of the study, it is likely and appropriate that only those patients with better prognostic features were selected for the study, and survival is broadly similar to other reported studies evaluating PM approaches in CUP cohorts. Recently reported outcomes from an Australian study demonstrated a site-specific approach improved OS compared to treatment with chemotherapy alone (Median OS 20.2 vs 10.9 months, respectively) (22). CUPISCO interim OS data has demonstrated a median OS of 14.7 months for chemotherapy responders and 14.0 months in non-responders for the PM group compared to 11.0 months for responders and 5.2 for non-responders, in those patients who continued chemotherapy alone (4).

CONCLUSIONS

In conclusion, this study highlights the feasibility and clinical utility of liquid biopsies in patients diagnosed with CUP. In a disease type with limited treatment options, implementing a PM approach can dramatically alter potential therapeutic choices either through changing primary tumour diagnosis and/or highlighting potential PM options. In this cohort, blood testing was highly successful, but there remains a proportion of patients where tumour fraction was low, risking inaccurate results and for whom tissue profiling would be the preferred option, if available. Where tissue is lacking, or where there may be delays in accessing tissue, opting for blood-based profiling is recommended (see Fig. 5A&B).

Abbreviations

CUP, Cancer of Unknown Primary; ESMO, European Society for Medical Oncology; BSC, Best Supportive Care; bTMB, blood-Tumour Mutation Burden; CHIP, Clonal Haemopoiesis of Indeterminate Potential; CI, Confidence Intervals; ctDNA , circulating tumour DNA; CTFS, Comprehensive Tumour Fraction Score; ECOG PS, Eastern Cooperative Oncology Group Performance Status; F1CDx, FoundationOne®CDx ; F1LCDx, FoundationOne®Liquid CDx ; FM, Foundation Medicine; MDT, Multi-Disciplinary Team meeting; MTB, Molecular Tumour Board; NEC, Neuroendocrine Carcinoma; NW GLH, North West Genomic Laboratory Hub; OS, Overall Survival; PAA, Potentially Actionable Alteration; PM, Precision Medicine; SACT, Systemic Anti-Cancer Therapy; SIPS, Scottish Inflammatory Prognostic Score; SoC, Standard of Care; TMB, Tumour Mutation Burden; tTMB, tissue-Tumour Mutation Burden; VAF, Variant Allele Frequency; WGS, Whole Genome Sequencing.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the Yorkshire and The Humber–Sheffield Research Ethics Committee (REC: 20/YH/0305) and trial sponsorship was undertaken by The Christie NHS Foundation Trust. All patients provided fully informed written consent for the trial.

Consent for publication

Not applicable.

Availability of data and materials

All the data generated or analysed during this study are included in this published article or are available from the corresponding author upon reasonable request. Genome data has been deposited at the European Genome-phenome Archive (EGA) which is hosted at the EBI and the CRG, under accession number EGAXXXXXXXXXXX.

Competing interests

NC has received (1) research grants from Roche Products Limited, (2) consulting fees from Roche Products Limited and REDX Pharmaceuticals, (3) payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Roche Products Limited Pharmaceuticals, (4) support for attending meetings and/or travel from Roche Pharmaceuticals, (5) research funding to research team from AstraZeneca, Orion, Roche Products Limited, Taiho, GSK, Novartis, Starpharma, Bayer, Eisai, UCB, RedX Pharmaceuticals, Stemline Therapeutics, LOXO-oncology, Avacta, Boehringer Ingelheim, Merck and Tarveda Therapeutics, (6) been the named investigator at the patent registered by “Cancer Research Technology Ltd,” (7) participated in data safety monitoring board or advisory board at Roche Products Limited and Cancer Research UK, (8) had the leadership or fiduciary role in CUP Foundation. KK has received (1) payment or honoraria for lectures, presentations, speaker’s bureaus, manuscript writing, or educational events from Roche Products Limited, (2) support for attending meetings and/or travel from Roche Products Limited. AMC has received (1) support for attending meetings and/or travel from Roche Products Limited and (2) been the named investigator at the patent registered by “Cancer Research Technology Ltd,”. KW is employed by Roche Products Limited (UK) and receive shares in Roche None of the other authors have declared any disclosures related to this manuscript.

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Author Contributions

AMC and MR contributed equally to this project including the curation, analysis and interpretation of data, drafting of original and revised manuscript. MC, SC, TT, KKS, LM, SD, EA, UM, ZZ, TG, AD, JAS, AT, MS, CM, PO, GJB contributed to study execution, data acquisition and interpretation and reviewed and approved the final manuscript. KW and NC conceived, designed, executed and obtained funding for the trial, contributed to data acquisition and interpretation and revised the final manuscript.

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Figures

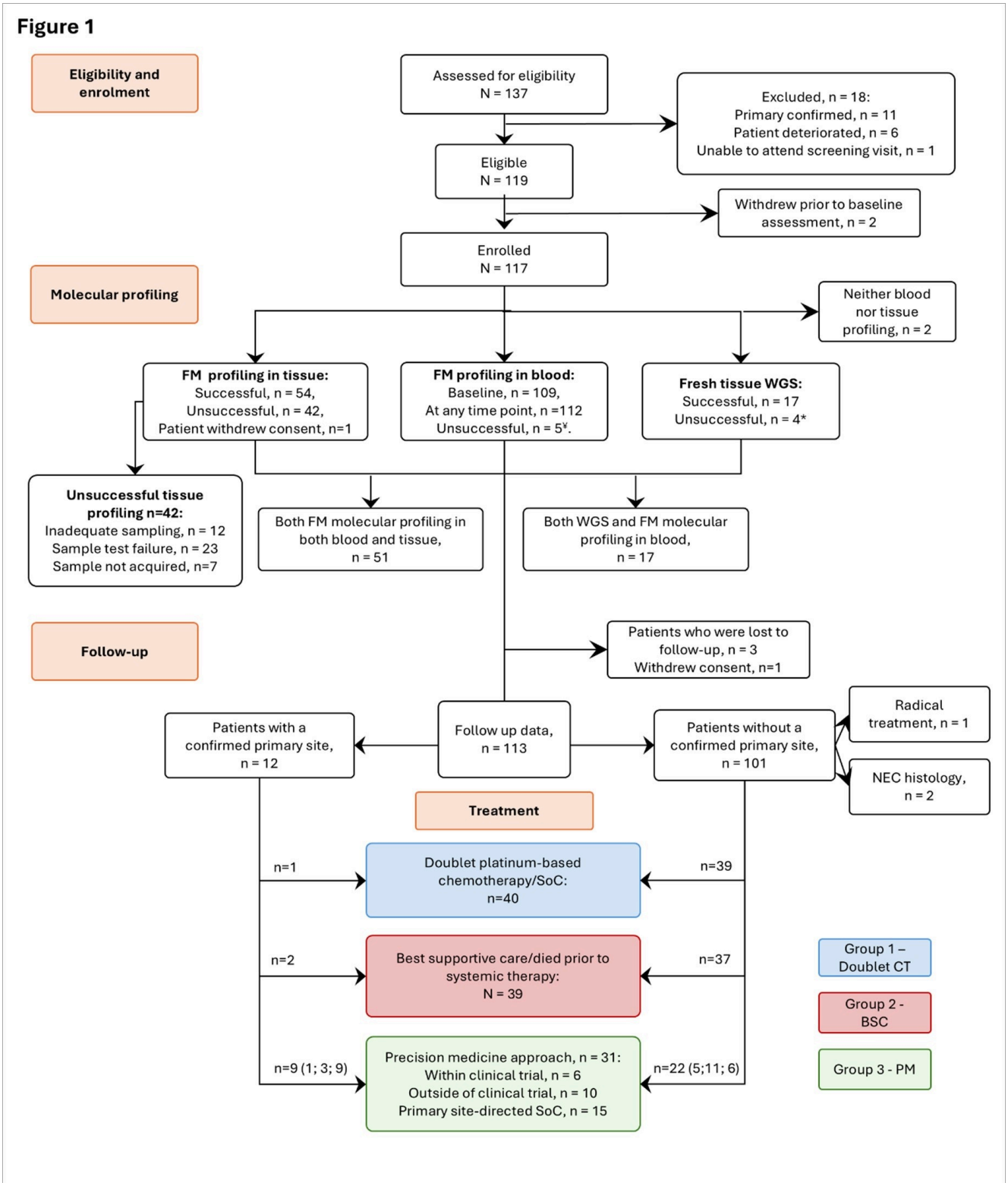

Figure 1

Consort Diagram: Final treatment groupings shown in coloured box; blue=Group 1 received standard of care (SoC) doublet platinum-based chemotherapy (Doublet CT); red=Group 2 patients that died prior to systemic therapy or received best supportive care (BSC); green=Group 3 patients that received a precision medicine (PM) approach. ¥ Insufficient blood draw/DNA yield/patient already commenced chemotherapy *sample mishandling.

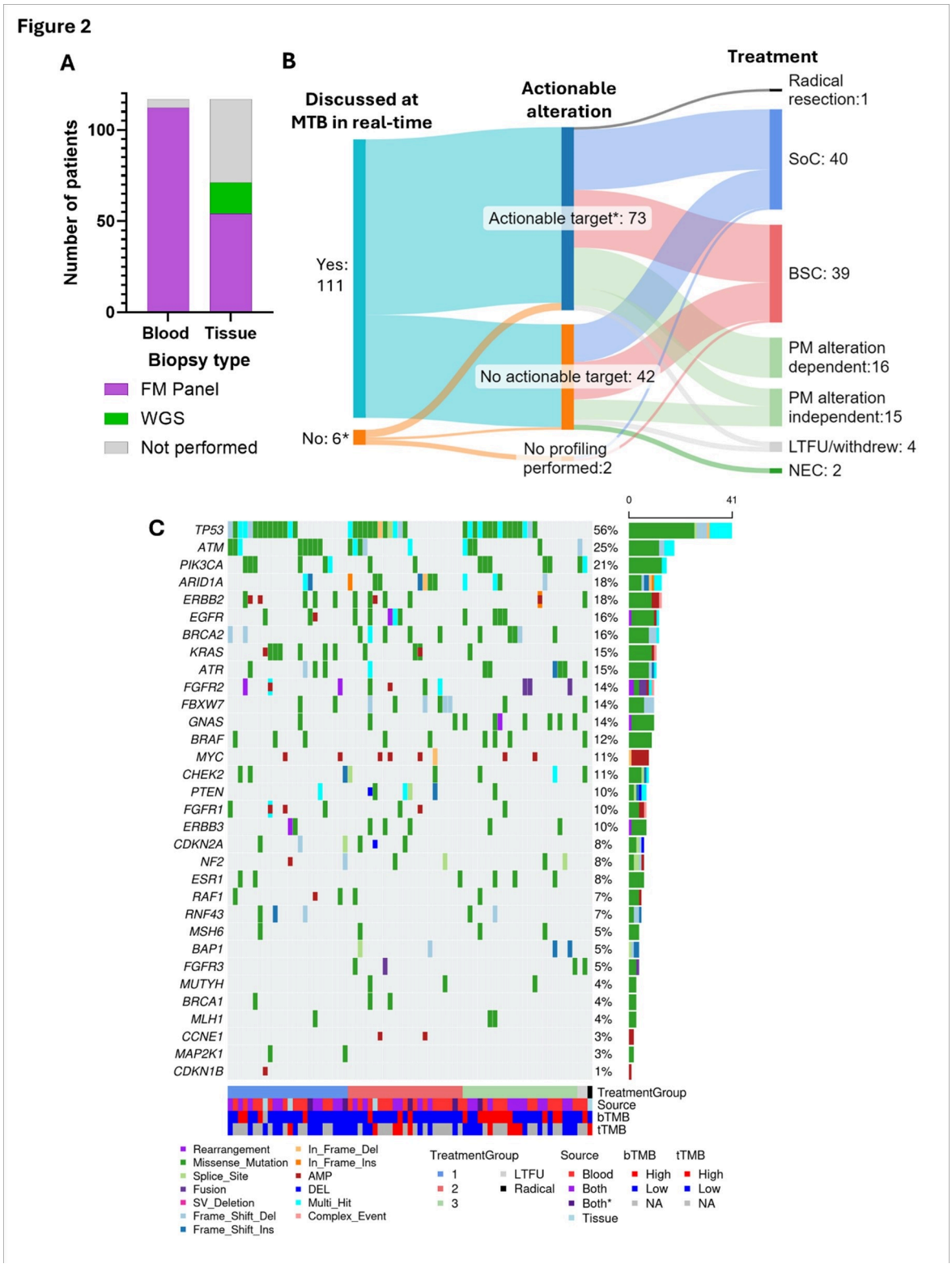

Figure 2

Real-time molecular data interpretation and summary. (A) Successful molecular profiling in blood and tissue; *two patients had neither blood nor tissue profiling performed. (B) Sankey plot of MTB outcome discussions linked to treatment outcomes. Colour codes of treatment represent final treatment groupings. (Blue= Group 1: Standard of Care Chemotherapy (SoC); Coral=Group 2: Best supportive care (BSC)/No treatment; Green = Group 3: Precision Medicine (PM) approach *4 patients results reviewed in

retrospective MTB and 3 determined potentially actionable alterations. (C) Oncoplot of select genes highlighted in MTB discussions as potentially actionable in 73 patients. Blood and tissue tumour mutation burden (bTMB and tTMB) status annotated, and source corresponds to which profiling source the alteration was found in. Both * means partial concordance where more than one potentially actionable alteration found. FM=Foundation Medicine; WGS=Whole genome sequencing; NEC=Neuroendocrine Carcinoma; LTFU=Lost to follow up.

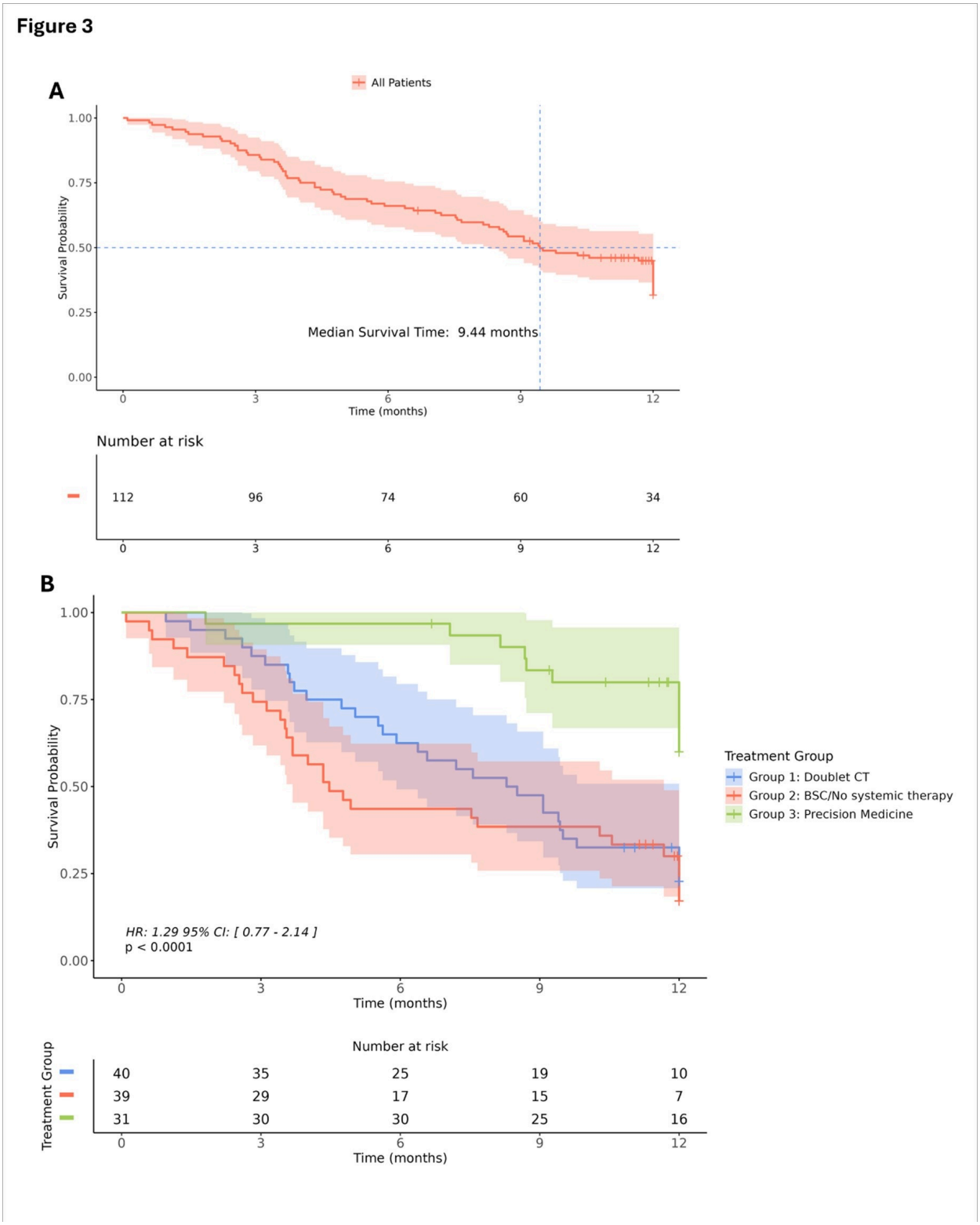

Figure 3

Survival analysis. (A) Whole cohort with sufficient follow up data (n=113). (B) Survival analysis split by post-hoc treatment categorisation (n=110); excluding patients with neuroendocrine histology (n=2) and patient who underwent radical resection of oligometastasis (n=1). HR=hazard Ratio; CI=Confidence Intervals; Doublet CT=Doublet chemotherapy; BSC=Best Supportive Care; Precision Medicine (PM).

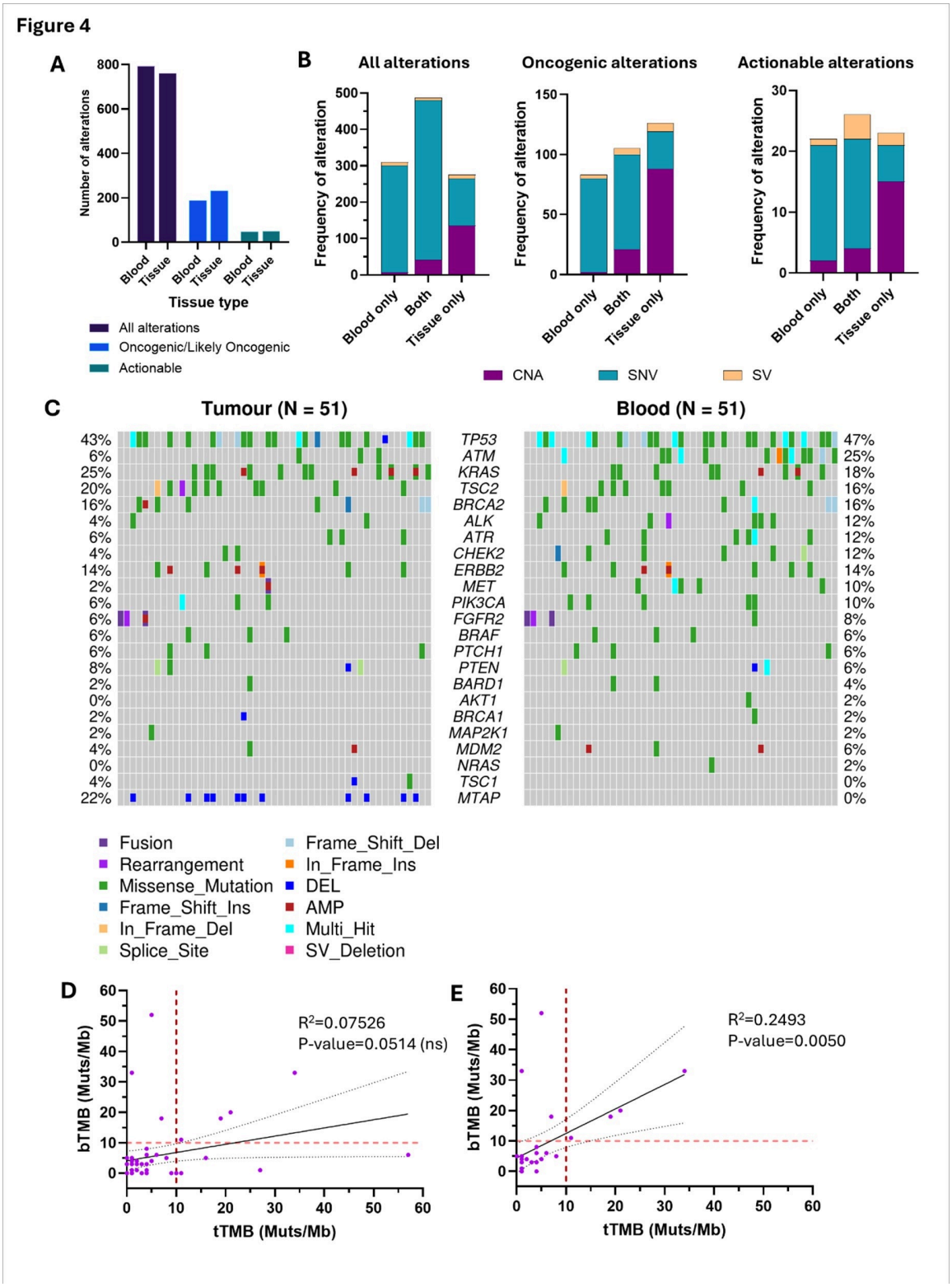

Figure 4

Retrospective comparison of tissue and blood molecular profiling (n=51).(A) Number of alterations reported in blood and tissue: total number within cohort; number determined to be ‘Oncogenic’ or ‘Likely Oncogenic’ by OncoKB analysis; and number deemed actionable to level 3 evidence and above. (B) Frequency of alterations in blood and tissue by alteration type, colour coded by alteration type. (C) Co-Oncoplot of alterations in genes determined to be potentially actionable by OncoKB analysis, plots split by profiling type (tissue or blood), column order is preserved across plots. (D) Comparison of Tumour Mutation Burden (TMB) in blood (bTMB) and tumour (tTMB) (n=51). (E) Comparison of Tumour Mutation Burden (TMB) in blood (bTMB) and tumour (tTMB) excluding those samples with Comprehensive Tumour Fraction Score of (CTFS) <1% (n=30). Red dotted line at 10 Muts/Mb. CNA=Copy Number Alterations; SNV=Single Nucleotide Variants; SV=Structural Variants.

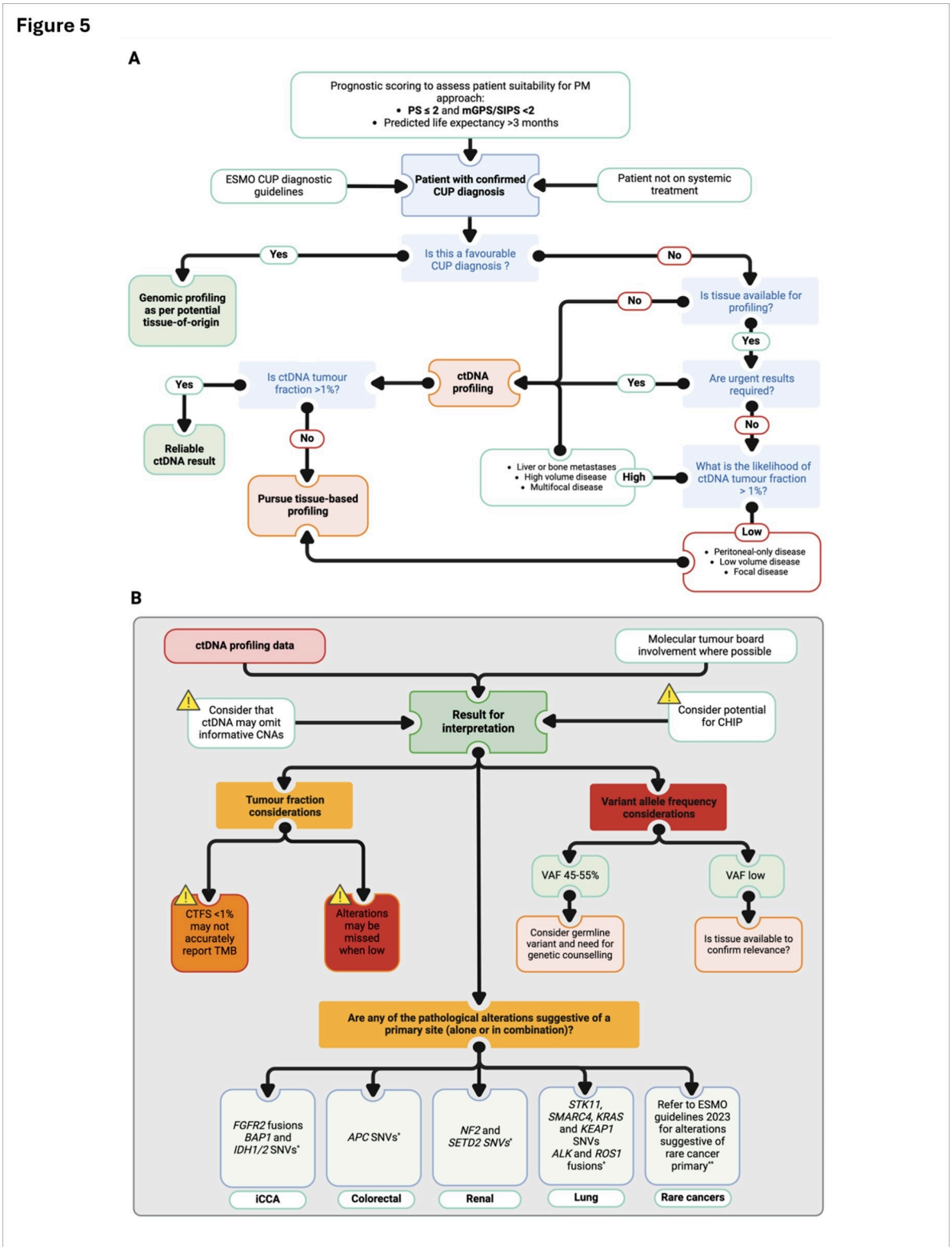

Figure 5

Utilisation and interpretation considerations for molecular profiling in CUP. (A) CUPCOMP decision tree to guide decision-making regarding blood- and tissue-based genomic profiling in patients with a confirmed CUP diagnosis. (B) ctDNA profiling result flow diagram to aid interpretation of genomic profiling data. Note low VAF is dependent on assay, for this study was considered at <1%. CHIP = clonal haematopoiesis of indeterminate potential; CNA = copy number alteration; CTFS = comprehensive

tumour fraction score; ctDNA = circulating tumour DNA; CUP = cancer of unknown primary; iCCA = intrahepatic cholangiocarcinoma; mGPS = Modified Glasgow Prognostic Score; PS = performance status; SIPS = The Scottish Inflammatory Prognostic Score; TMB = tumour mutational burden; VAF = variant allele frequency. *Commonly reported mutation patterns in CUP literature (5, 28) **see ESMO guidelines(3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial1.pdf
- SupplementaryTables14.xlsx