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ORIGINAL RESEARCH

Baseline mutational profiles of patients with carcinoma of unknown primary origin enrolled in the CUPISCO study☆

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Background: Patients with unfavorable carcinoma of unknown primary origin (CUP) have an extremely poor prognosis of ∼1 year or less, stressing the need for more tailored treatments, which are currently being tested in clinical trials. CUPISCO (NCT03498521) was a phase II randomized study of targeted therapy/cancer immunotherapy versus platinum-based chemotherapy in patients with previously untreated, unfavorable CUP, defined as per the European Society for Medical Oncology guidelines. We present a preliminary, descriptive molecular analysis of 464 patients with stringently diagnosed, unfavorable CUP enrolled in the CUPISCO study.

Materials and methods: Genomic profiling was carried out on formalin-fixed, paraffin-embedded tissue to detect genomic alterations and assess tumor mutational burden and microsatellite instability.

Results: Overall, ∼32% of patients carried a potentially targetable genomic alteration, including *PIK3CA*, *FGFR2*, *ERBB2*, $BRAF^{V600E}$, *EGFR*, *MET*, *NTRK1*, *ROS1*, and *ALK*. Using hierarchical clustering of co-mutational profiles, 10 clusters were identified with specific genomic alteration co-occurrences, with some mirroring defined tumor entities.

Conclusions: Results reveal the molecular heterogeneity of patients with unfavorable CUP and suggest that genomic profiling may be used as part of informed decision-making to identify the potential primary tumor and targeted treatment options. Whether stringently diagnosed patients with unfavorable CUP benefit from targeted therapies in a similar manner to those with matched known primaries will be a key learning from CUPISCO.

Key words: neoplasms, unknown primary, molecular targeted therapy, precision medicine, genomic profiling

INTRODUCTION

Carcinoma of unknown primary origin (CUP) describes a heterogeneous group of metastatic cancers without an identifiable primary tumor, despite thorough clinical work-up.[1] The incidence of CUP has decreased over the past few decades, likely due to improvements in primary tumor diagnostics.[2,3] Survival remains particularly low among patients with unfavorable CUP, which accounts for 80%-85% of cases and has an extremely poor prognosis of ∼1 year or less.[3,4] As these patients lack specific and effective treatment options, clinical guidelines still recommend empiric platinum-based chemotherapy.[5,6] Yet, many patients with unfavorable CUP rapidly develop resistance to therapy.[1,4] More personalized treatment options, based on particular molecular features, are becoming a consideration and are being tested in clinical trials.[7]

In that regard, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines®),[6] and the recently updated European Society for Medical Oncology (ESMO) guidelines,[8] now

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recommend the consideration of next-generation sequencing (NGS) to identify potentially actionable genomic alterations in patients with CUP, in addition to thorough diagnostic work-up consisting of medical history, complete physical examination, tissue biopsy for immunohistochemistry (IHC) analysis, laboratory tests, and computed tomography or magnetic resonance imaging of the thorax, abdomen, and pelvis. Genomic profiling is an NGS approach that detects variants of all the main classes of genomic alterations in cancer-related genes, as well as genomic signatures, microsatellite instability (MSI), tumor mutational burden (TMB), and genome-wide loss of heterozygosity, to provide prognostic, diagnostic, and predictive insights that inform research or treatment decisions for individual patients across all cancer types.[9] Use of genomic profiling may support appropriate treatment plans in patients with CUP by either narrowing down the potential site of tumor origin or identifying a targeted therapy based on the patient's molecular profile, regardless of the primary site location.[7,10,11] Although recent studies have shown a lack of clinical benefit of site-specific chemotherapy directed by gene expression profiling to determine the tissue of origin,[12,13] targeted therapy irrespective of the primary tumor site may reveal more personalized and effective therapeutic options for patients with unfavorable CUP.

CUPISCO (NCT03498521) was a phase II, randomized study of targeted therapy/cancer immunotherapy versus platinum-based chemotherapy in patients with previously untreated, unfavorable CUP, defined as per the ESMO guidelines.[14] Evidence for this approach was bolstered by a study in 2021,[11] where retrospective NGS of tumoral DNA from 303 CUP specimens found that 31.7% of patients could be matched to an experimental CUPISCO treatment arm, based on their molecular profile. In addition to a lack of effective targeted treatment options, unfavorable CUP remains difficult to diagnose through standardized screening, as demonstrated by a high rate of screening failures (∼55%) seen in the first 628 patients entering screening for the CUPISCO trial.[15] This highlights the current challenges associated with defining CUP, which can impact clinical trial enrollment, timely diagnosis, and subsequent treatment.

We present a preliminary, descriptive molecular analysis of 464 patients with stringently diagnosed, unfavorable CUP designated for enrollment in CUPISCO. Such analysis may help to increase understanding of the heterogeneous population of unfavorable CUP, and thus provide tools to aid diagnosis and potentially tailored treatment in this patient population.

MATERIALS AND METHODS

Patient eligibility

The study design and patient eligibility criteria for CUPISCO are described elsewhere.[15] Briefly, following completion of local diagnostic testing, eligibility was verified by an eligibility review team and patients with CUP underwent a histopathology and clinical review to confirm the diagnosis of unfavorable CUP as per ESMO criteria;[4,14] in cases with incomplete diagnostic testing, additional IHC staining was carried out.[15] Patients were eligible to enroll in CUPISCO if they had a systemic therapy-naive adenocarcinoma or undifferentiated carcinoma, an Eastern Cooperative Oncology Group performance status of 0 or 1, and at least one measurable lesion as per RECIST v1.1.[14,15] In order to exclude carcinomas with a known origin, additional testing was carried out based on histopathology, clinical and radiologic assessment, e.g. specialized physical examination, endoscopy, imaging, laboratory and blood tests, or additional IHC.[15]

Genomic profiling assay

Upon enrollment in CUPISCO, genomic profiling, including determination of TMB and MSI, was carried out on formalin-fixed, paraffin-embedded tissue using the FoundationOne®CDx assay (Foundation Medicine, Inc., Cambridge, MA), as described previously,[16,17] in a Clinical Laboratory Improvement Amendments (CLIA)-certified and College of American Pathologists-accredited laboratory. Genomic profiling was carried out using hybrid-capture, adapter ligation-based libraries to identify genomic alterations [base substitutions, small insertions and deletions, copy number alterations (deep deletions with gene copy number of 0 or amplifications of at least specimen ploidy +4), and gene rearrangements (REs)].

TMB and MSI

TMB was calculated as the number of nondriver somatic mutations per megabase (Mb) of genome sequenced; based on the cutoffs used in CUPISCO, samples were classified as TMB-high if they had ≥16 mutations/Mb, and TMB-low if they had <16 mutations/Mb.[18] MSI status was determined by analyzing 114 intronic homopolymer repeat loci for length variability; MSI-high status was defined as described previously.[19]

Genomic alterations and clusters

Genomic alterations prevalent in ≥3% of patients were considered for analysis using multiple correspondence analysis of dichotomous data and subsequent hierarchic clustering to identify co-occurrences. All genomic alterations studied included only those described as functional or pathogenic based on a review of the literature and the Catalogue of Somatic Mutations In Cancer (COSMIC) repository,[20] or those with a likely functional status (frameshift or truncation events in tumor suppressor genes). Variants of unknown significance were not included in the study. Known and likely pathogenic alterations were called down to a mutant allele fraction (MAF) of 1%. Since required tumor purity for samples was 20%, this allowed for the detection of all alterations except for highly subclonal alterations. Alterations below 1% MAF were present in only a very small fraction of tumor cells and were deemed unlikely to be driving the bulk of the tumor biology. 'Targetable

alterations' were defined as those reported by Ross et al.[11] (i.e. a genomic alteration for which a targeted therapy or cancer immunotherapy was available).

Subsequent genomic clusters were associated with genomic, clinical, and pathologic covariates. For each genomic cluster, expression patterns of proteins typically used to diagnose various tumor types were analyzed by IHC. The IHC pattern of the genomic clusters was also compared with typical IHC panels of various tumor types, as defined in Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2023.102035.

Mutational signature calling

Mutational signature calling was carried out as described previously.[21] Predicted germline alterations were excluded, and exon-adjacent noncoding alterations were included. The DNA mismatch repair (MMR) analysis included the COSMIC signatures 1, 6, 15, 20, and 26 and was improved from the published methods by combining the scores from the 'Aging' and 'MMR' signatures. Dominant signatures were called with a threshold of 40%. While examining these data, we observed that 409 of 435 samples were assessable for mutational signatures. Among those 409 samples, 16 had a dominant tobacco signature, 13 had a dominant apolipoprotein B messenger RNA-editing enzyme, catalytic polypeptide (APOBEC) signature, 12 had a dominant MMR signature, and 2 had a dominant ultraviolet signature.

Statistical analyses

All statistical analyses were carried out using R version 4.2.[22]

RESULTS

Baseline demographics/characteristics

CUPISCO enrolled patients with proven primary diagnosis of unfavorable CUP. Overall, 1509 patients were screened at 159 centers in 34 countries (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2023.102035). A total of 498 patients were enrolled into the trial at the time of this analysis and here we report the 464 patients who had molecular data available at the time of analysis. Baseline demographics of the 464 patients enrolled in CUPISCO and included in this analysis are shown in Table 1. Overall, 229/464 (49.4%) samples were from female patients and the median age was 61 years (range 22-84 years).

Table 1. Baseline demographics

Characteristic	Overall (n = 464)
Sex	
Female	229 (49.4)
Male	235 (50.6)
Age, years	
Mean (SD)	60.3 (11.8)
Median (range)	61 (22-84)
BMI, kg/m^2	
Mean (SD)	26.0 (4.95)
Median (range)	25.7 (14.9-48.8)
Missing	7 (1.5)
Ethnicity	
White	356 (76.7)
Asian	39 (8.4)
Black or African American	4 (0.9)
American Indian or Alaska Native	6 (1.3)
Unknown/missing	59 (12.7)
Tobacco usage	
Current	87 (18.8)
Previous	189 (40.7)
Never	188 (40.5)
ECOG PS	
0	175 (37.7)
1	282 (60.8)
2/3	0
Missing	7 (1.5)

Data are presented as n (%) unless otherwise specified.
BMI, body mass index; ECOG PS, Eastern Cooperative Oncology Group performance status; SD, standard deviation.

Overall frequency of genomic alterations

The most frequent genomic alterations are shown in Figure 1A and Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2023.102035; they occurred in: *TP53* [210/464 (45.3%)], *CDKN2A* [142/464 (30.6%)], *KRAS* [102/464 (22.0%); *G12C*: 12/464 (2.6%); *G12D*: 29/464 (6.3%); *G12V*: 22/464 (4.7%); Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.102035], *CDKN2B* [91/464 (19.6%)], *MTAP* [61/464 (13.1%)], *ARID1A* [56/464 (12.1%)], *STK11* [53/464 (11.4%)], *PIK3CA* [45/464 (9.7%)], *MYC* [39/464 (8.4%)], *PBRM1* [37/464 (8.0%)], *FGFR2* [35/464 (7.5%)], *TERT* [34/464 (7.3%)], and *BAP1* [32/464 (6.9%)].

Mean TMB was 6.33 (standard deviation 9.21) and median TMB was 3.78 (range 0-76.9). In this cohort, 8.6% (40/464) and 83.8% (389/464) of samples, respectively, were TMB-high ($\geq$16 mutations/Mb) and TMB-low ($<$16 mutations/Mb) [7.5% (35/464) were missing]. Overall, 87.3% (405/464) and 2.4% (11/464) of samples were microsatellite stable and MSI-high, respectively [10.3% (48/464) were ambiguous].

In our analysis, $\sim$32% of patients carried a potentially targetable genomic alteration (Figure 1B and Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2023.102035), defined as those reported by Ross et al.[11] Targetable genomic alterations were found in *PIK3CA* [45/464 (9.7%)], *FGFR2* [35/464 (7.5%)], *ERBB2* [29/464 (6.3%)], *BRAF* [27/464 (5.8%); *V600E*: 14/464 (3.0%); Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2023.102035], *EGFR* [13/464 (2.8%)], *MET* [12/464 (2.6%)], *NTRK1* [8/464 (1.7%)], *ROS1* [6/464 (1.3%)], and *ALK* [4/464 (0.9%)].

Patient clusters based on co-occurrence of genomic alterations

In an exploratory analysis, we applied hierarchical clustering of co-mutational profiles to identify 10 clusters (1-10) with specific genomic alteration co-occurrences (Figure 2 and Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2023.102035). These clusters were also

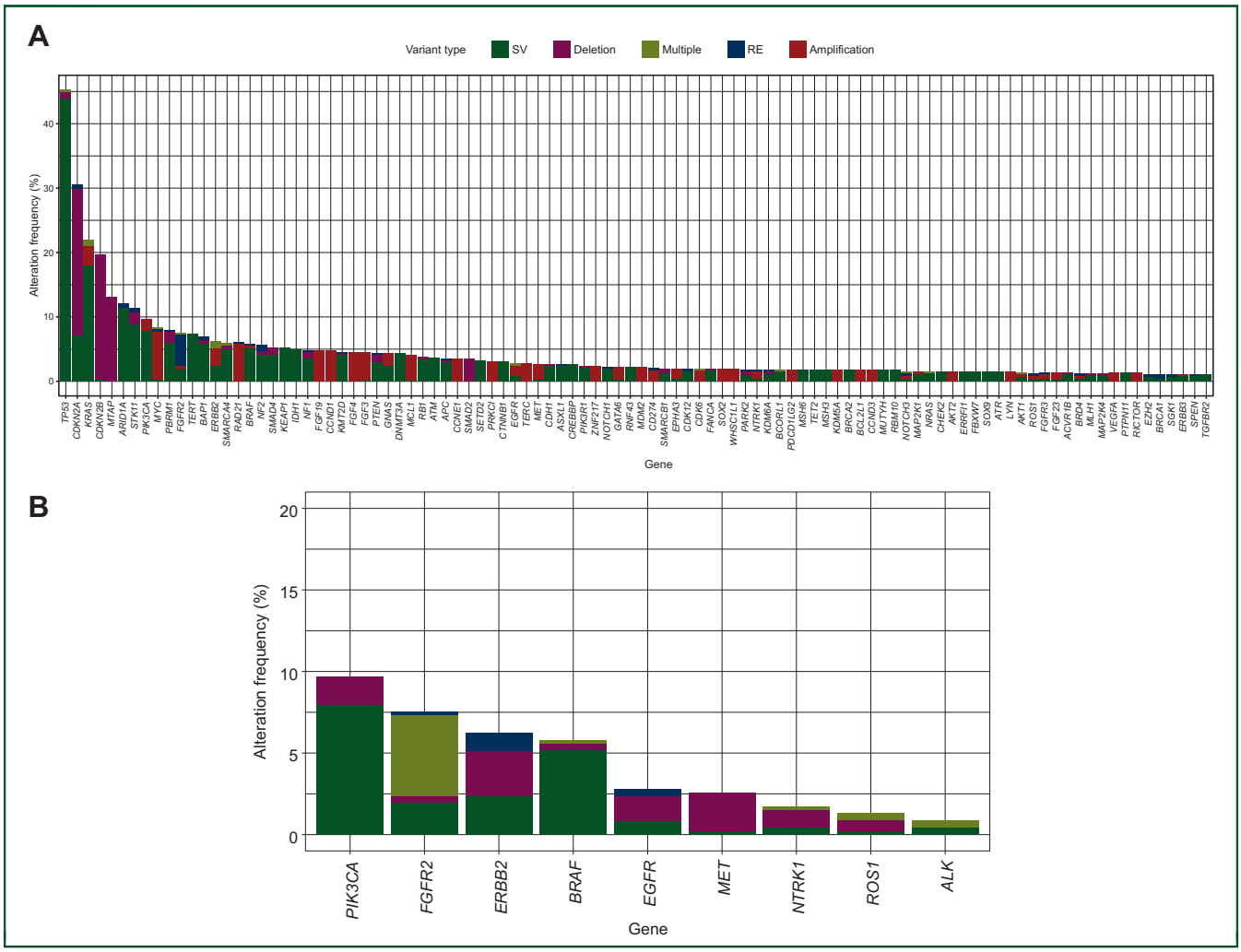

Figure 1. Genomic landscape of patients enrolled in CUPISCO. Overall frequency of (A) all genomic alterations and (B) targetable genomic alterations.
RE, gene rearrangement; SV, short variant.

found after analyzing a similar population with the same methodology (FoundationCORE™ dataset;[11] Supplementary Figure S2, available at https://doi.org/10.1016/j.esmoop.2023.102035).

Cluster 2 showed a high prevalence of *FGFR2* REs [21/40 (52.5%)] and *BAP1* short variants [SVs; 19/40 (47.5%)], whereas cluster 6 demonstrated a high frequency of *TP53* [30/80 (37.5%)], *ARID1A* [29/80 (36.3%)], and *IDH1* [17/80 (21.3%); particularly $IDH1^{R132C}$ (9/17; 52.9%)] SVs, all typical of intrahepatic cholangiocarcinoma.[23]

Cluster 3 showed co-occurrence and a high prevalence of *NF2* and *SETD2* SVs [each 11/26 (42.3%)], typically observed in renal cell carcinomas (RCCs).[24] Among the most common genomic alterations in cluster 7 were *STK11* and *KEAP1* SVs [33/54 (61.1%) and 23/54 (42.6%), respectively], as described in non-small cell lung carcinomas.[25-27]

The clusters were assessed for specific clinical features (Figure 3 and Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2023.102035). Cluster 1 demonstrated a greater frequency of non-smoking, younger females with TMB-high and MSI-high tumor samples. Patients in cluster 1 also showed the greatest frequency of alterations in MMR (*MSH2*, *MSH3*, *MSH6*, *MLH1*, and *PMS2*) or other DNA repair genes (*MUTYH*, *PARP1*, *ERCC4*, *RAD51B*, *XRCC2*, *RAD54L*, *BRCA1*, *MRE11A*, *NBN*, *FANCC*, *BRCA2*, *FANCG*, *BRIP1*, *FANCL*, *PALB2*, *RAD51C*, *POLE*, *ATRX*, *ATM*, *ATR*, and *CHEK1*) [6/25 (24.0%) and 13/25 (52.0%), respectively], and had a mutational signature consistent with MMR [6/23 (26.1%); Figure 4 and Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2023.102035]. Compared with those in other clusters, patients in clusters 7 and 9 demonstrated a greater frequency of current/previous smoking [41/54 (75.9%) and 26/37 (70.3%), respectively]; patients in cluster 7 also had a dominant tobacco mutational signature [6/50 (12.0%); Figure 4 and Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2023.102035], suggestive of a diagnosis of non-small cell lung carcinoma. Regarding biopsy location, cluster 2 was detected at a higher prevalence in liver samples versus samples from other locations [16/40 (40.0%)].

Cluster 3, whose genomic alterations were typical of RCC, demonstrated a protein expression pattern suggestive of clear-cell renal carcinoma (CK7 negative, CK20 negative, PAX8 positive, PAX2 positive, racemase positive, CD10

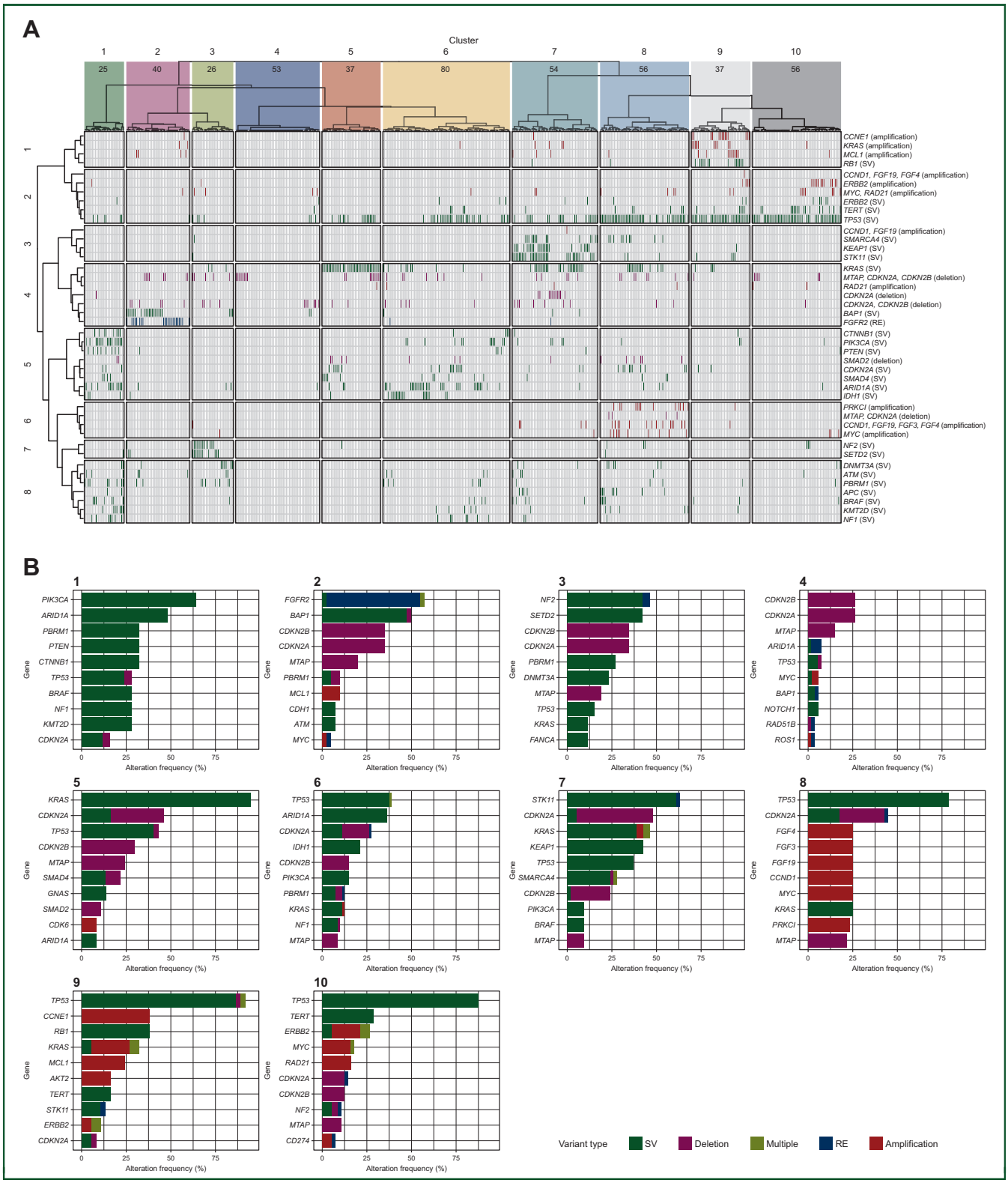

Figure 2. Mutational clustering of patients enrolled in CUPISCO. (A) Overview of patient clusters based on mutational profiles and (B) most prevalent genomic alterations by cluster.

RE, gene rearrangement; SV, short variant.

positive, RCC positive; Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2023.102035). All clusters showed varying levels of human epidermal growth factor receptor 2 expression (Supplementary Figure S3, available at https://doi.org/10.1016/j.esmoop.2023.102035).

DISCUSSION

This descriptive analysis of 464 patients with stringently diagnosed, unfavorable CUP enrolled into CUPISCO provides a robust overview of their heterogeneous molecular landscape. The overall distribution and co-occurrence of

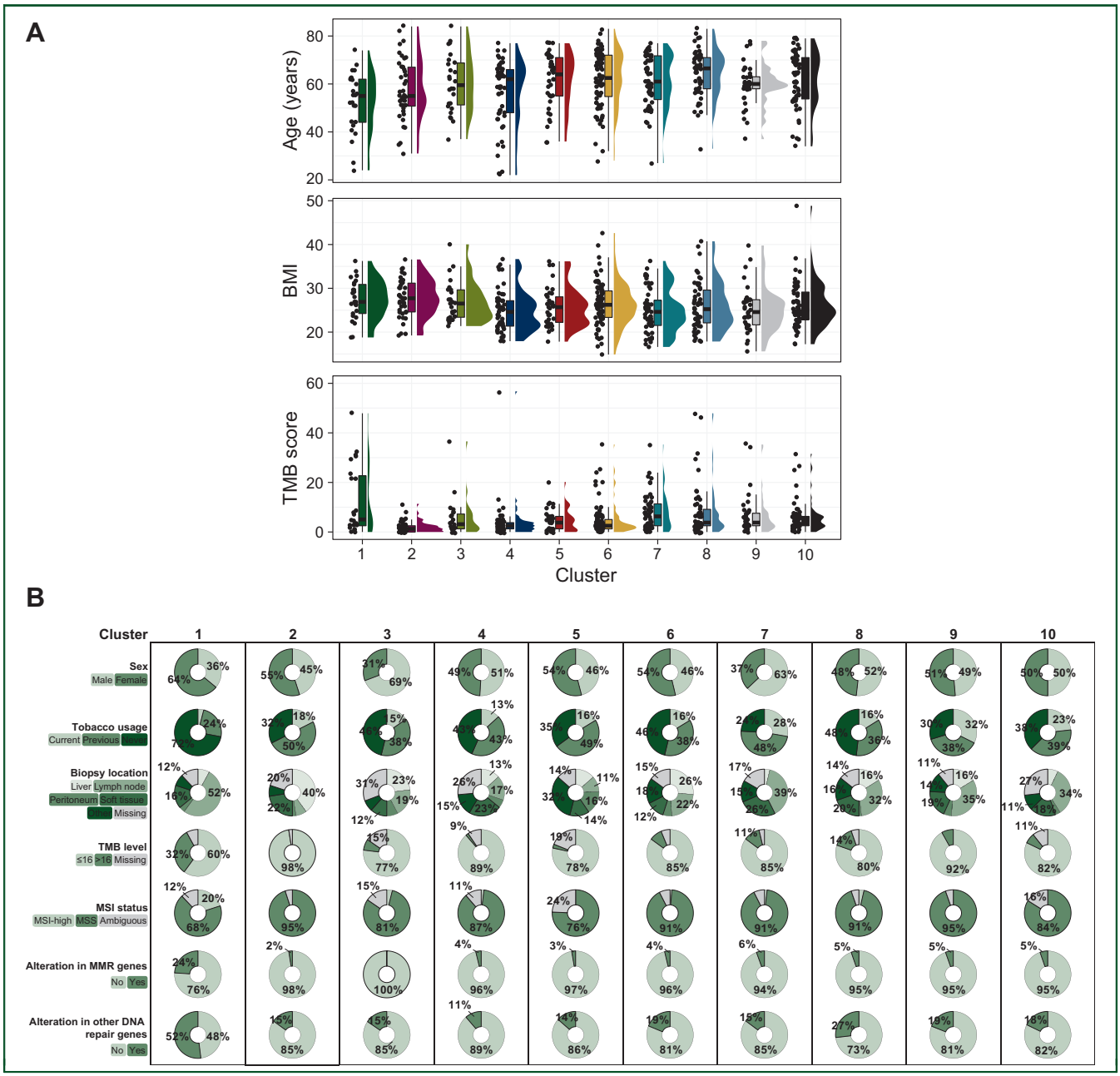

Figure 3. Distribution of clinical features by cluster.
(A) Age, BMI, and TMB score; (B) Sex, tobacco usage, biopsy location, TMB level, MSI status, alterations in MMR genes,[a] and alterations in other DNA repair genes.[b] BMI, body mass index; MMR, DNA mismatch repair; MSI, microsatellite instability; MSS, microsatellite stable; TMB, tumor mutational burden.
[a]*MSH2*, *MSH3*, *MSH6*, *MLH1*, and *PMS2*.
[b]*MUTYH*, *PARP1*, *ERCC4*, *RAD51B*, *XRCC2*, *RAD54L*, *BRCA1*, *MRE11A*, *NBN*, *FANCC*, *BRCA2*, *FANCG*, *BRIP1*, *FANCL*, *PALB2*, *RAD51C*, *POLE*, *ATRX*, *ATM*, *ATR*, and *CHEK1*.

genomic alterations from patients enrolled in CUPISCO, including in targetable genes, was comparable with data from a similar, independent CUP population.[11] Key potentially targetable genomic alterations included *PIK3CA*, *FGFR2*, *ERBB2*, *BRAF*^{V600E}, *EGFR*, *MET*, *NTRK1*, *ROS1*, and *ALK*. In line with previous studies,[10,11] our results from the first 464 patients included in CUPISCO suggest that genomic profiling of CUP samples allows for the identification of therapeutically relevant genomic alterations in a significant proportion of patients and can thus guide personalized treatment of these tumors. This may extend to germline testing, given that ∼8.6% of patients with CUP have been shown to have pathogenic germline variants, according to a recent analysis of the MASTER trial.[28]

Using the data obtained from genomic profiling we carried out further exploratory analyses to investigate whether CUP cases within CUPISCO could be clustered based on their molecular profiles. Without clinical outcome data and prospective evaluation of these clusters, the data presented here must be considered hypotheses generating. Based on genomic sequencing and after subsequent hierarchic clustering, these clusters may provide evidence for informed decision making regarding primary tumor identification. In fact, some of these exploratory clusters were reminiscent of

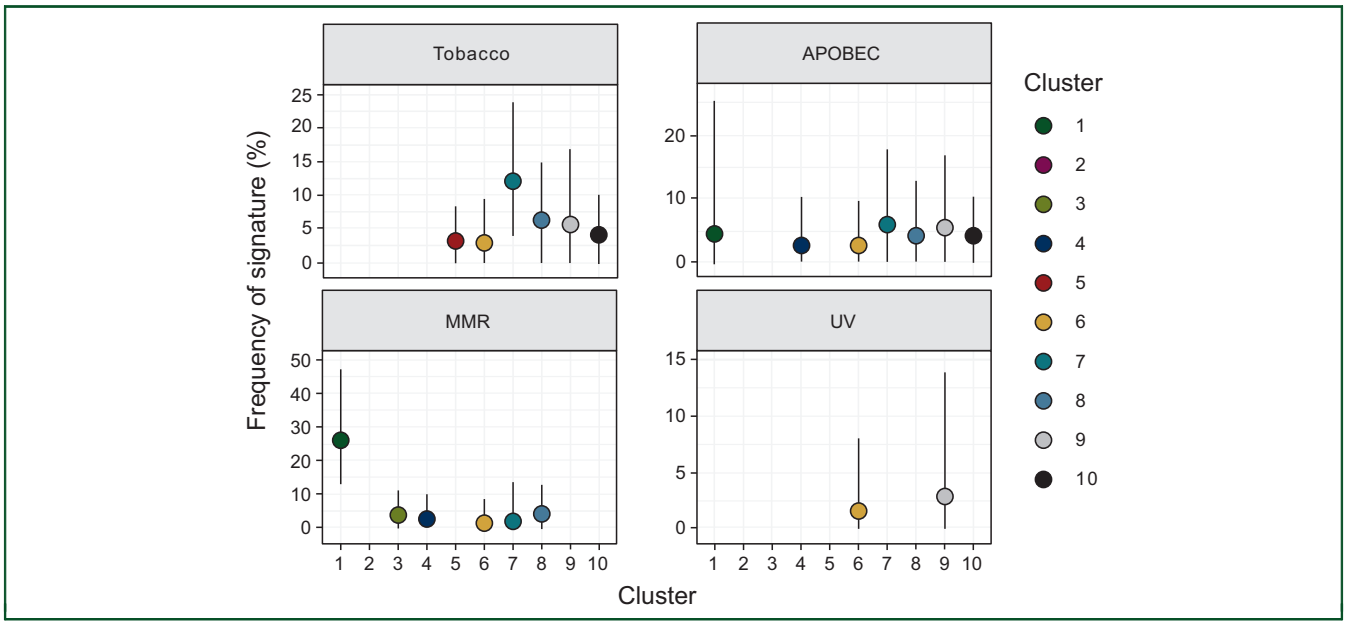

Figure 4. Mutational signatures by cluster.
Mutational signatures were calculated for each sample. Overall, 409 samples were assessable for mutational signatures. APOBEC, apolipoprotein B messenger RNA-editing enzyme, catalytic polypeptide; MMR, DNA mismatch repair.

specific tumor types. Cluster 3 demonstrated a high frequency of *NF2* and *SETD2* SVs, typically seen in RCCs.[24] This finding was consistent with the IHC analysis, which showed cluster 3 to have the greatest protein expression of the RCC markers CD10, racemase, RCC, PAX2, and PAX8, demonstrating the ability of genomic profiling to provide additional information to IHC regarding tissue-of-origin identification. While *SETD2* is a frequent finding in clear-cell RCC, genetic alterations involving *NF2* occur at low frequencies in RCC across all of the major histologic subtypes. Previously, *NF2* and *SETD2* alterations have been seen in high abundance in 'unclassified' or 'not otherwise specified' RCC, a subtype of RCC that exhibits non-clear-cell histology, has no standard therapy, and presents formidable diagnostic and management challenges.[29] Furthermore, clusters 2 and 6 were suggestive of intrahepatic cholangiocarcinoma, showing a high frequency of *FGFR2* REs, and *BAP1*, *TP53*, *ARID1A*, and *IDH1* (particularly $IDH1^{R132C}$) SVs.[23] Multiple targeted therapies for both RCC and intrahepatic cholangiocarcinoma exist, but clinical data from CUPISCO are needed to determine their applicability in the CUP setting.[30,31] Finally, patients in cluster 7 demonstrated a high frequency of *STK11* and *KEAP1* alterations, which co-occurred with *KRAS* and *SMARCA4* SVs, commonly seen in non-small cell lung carcinomas,[25-27] and these patients were frequently current/previous all tobacco with a dominant smoking mutational signature. In non-small cell lung carcinoma, the presence of such alterations has been shown to predict differing biologic and immune profiles, and varying responses to cancer immunotherapy, showing the ability of genomic profiling to differentiate this group of patients with CUP based on their likelihood of therapeutic response.[25,26,32,33] *SMARCA4*-deficient lung cancers also classically exhibit aberrant negativity for TTF-1 and thus may be inaccurately diagnosed as CUP based on conventional histopathologic evaluation.[34]

Defining the value of genomic profiling for patients with CUP is challenging, as this type of testing encompasses a variety of potential benefits. Based on preliminary learnings from CUPISCO, at least 32% of enrolled patients carried a potentially targetable genomic alteration. Additionally, while not the purpose of genomic profiling, when used in the care of patients with unfavorable CUP, these results may help establish the primary tumor site, e.g. *TMPRSS2-ERG* or *-ETV1* fusions are diagnostic of metastatic prostate cancer,[35,36] or provide increased evidence for primary tumor site identification, e.g. an *FGFR2* fusion highly enriches the likelihood that a CUP case is a primary intrahepatic cholangiocarcinoma[31] and UV mutational signatures enrich for cutaneous origin.[37] The diagnostic value of comprehensive DNA panel sequencing was also shown in a retrospective analysis of DNA and RNA tests across 215 patients with CUP.[38] DNA features provided additional diagnostic evidence in 31% of true CUPs (including features such as gene fusions, cancer-type enriched mutation drivers, and mutational signatures) when this information was combined with other clinicopathologic evidence.[38]

At present, our study is limited by the lack of clinical outcome data, meaning that the clusters cannot be associated with patient prognosis to further elucidate their clinical relevance in CUP. Furthermore, CUPISCO had not completed enrollment at the time of this analysis, so the complete cohort has not been included. However, overall, our results reveal the molecular heterogeneity of patients with unfavorable CUP and suggest that clusters of CUP may share features with defined tumor entities; these clusters are hypothesis-generating and not yet validated, and therefore the clinical utility is unproven. Accordingly, our

results indicate that genomic profiling may be used as part of informed decision-making regarding primary tumor identification, even though respective primaries were not found following a thorough clinicoradiologic work-up in the unfavorable cohort. Despite the lack of clinical benefit of site-specific chemotherapy directed by gene expression profiling to determine the tissue of origin,[12,13] primary tumor identification might still be critical to inform prognosis and potentially tailored treatment. Whether stringently diagnosed patients with unfavorable CUP benefit from targeted therapies to a similar extent as do their counterparts with matched known primaries will be one of the key learnings from the CUPISCO study.

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The sponsor, F. Hoffmann-La Roche Ltd, contributed to the design of this analysis. Data collected by the authors were analyzed by employees at Foundation Medicine, Inc. and F. Hoffmann-La Roche Ltd. The authors employed by the study sponsor contributed to the conduct of the analysis; to the collection, management, analysis, and interpretation of the data; and to the preparation, review, and approval of the manuscript, as well as to the decision to submit the manuscript for publication.

DISCLOSURE

CBW reports honoraria from Bayer, Celgene, Ipsen, Servier, Taiho, and F. Hoffmann-La Roche Ltd, has participated in advisory boards for Celgene, Shire/Baxalta, Rafael Pharmaceuticals, RedHill BioPharma, and F. Hoffmann-La Roche Ltd, and has received travel/accommodation expenses from Bayer, Celgene, RedHill BioPharma, F. Hoffmann-La Roche Ltd, Servier, and Taiho. JFG is an employee of and has stocks/shares in F. Hoffmann-La Roche Ltd. CP has received an institutional research grant from F. Hoffmann-La Roche Ltd, works as a study pathologist for the CUPISCO trial, and has received travel coverage and remuneration for study-related work such as histopathology reviews for patients in screening and in molecular tumor boards, for the benefit of her employer. ARK, NCh, and GDP are employees of and hold stocks/shares in F. Hoffmann-La Roche Ltd. AB was an employee of F. Hoffmann-La Roche Ltd. TB has received an institutional research grant from F. Hoffmann-La Roche Ltd, works as a study oncologist for the CUPISCO trial, and has received coverage for study-related travel and remuneration for study-related work in a molecular tumor board for the benefit of his employer. NCo has participated in an advisory board for RedX Pharmaceuticals and has received institutional research funding from AstraZeneca, Orion, F. Hoffmann-La Roche Ltd, Taiho, GSK, Novartis, Starpharma, Bayer, Eisai, UCB, RedX Pharmaceuticals, Stemline Therapeutics, Boehringer Ingelheim, Merck, Avacta Pharmaceuticals, and Tarveda Therapeutics. EH is an employee of and holds stocks/shares in F. Hoffmann-La Roche Ltd. DXJ is an employee of Foundation Medicine, Inc. and holds stocks/shares in F. Hoffmann-La Roche Ltd. FL has received institutional research funding from F. Hoffmann-La Roche Ltd, Amgen, and Merck, has received travel/accommodation expenses from F. Hoffmann-La Roche Ltd and Merck, has participated in an advisory board for F. Hoffmann-La Roche Ltd, Amgen, Merck, Sanofi, and Servier, and has participated in a speaker bureau/expert testimony for F. Hoffmann-La Roche Ltd and Sanofi. LM has received travel/accommodation expenses from F. Hoffmann-La Roche Ltd and BeiGene. HM has received honoraria from or has participated in advisory boards for F. Hoffmann-La Roche Ltd, Ventana, Definiens, Merck, BMS, Astellas, Johnson & Johnson, Bayer, Ipsen, and Amgen, has received travel/accommodation expenses from F. Hoffmann-La Roche Ltd and Definiens, and has received institutional research funding from F. Hoffmann-La Roche Ltd. JSR has received honoraria from, holds stocks/shares in, and has a leadership role in Foundation Medicine, Inc. ESS is an employee of Foundation Medicine, Inc. and holds stocks/shares in F. Hoffmann-La Roche Ltd. RWT has received honoraria from Merck Serono Australia. AK has received honoraria from F. Hoffmann-La Roche Ltd, Daiichi Sankyo, and AbbVie, honoraria to his institution from F. Hoffmann-La Roche Ltd and Bayer, has a leadership role in F. Hoffmann-La Roche Ltd, has received institutional research funding from Merck and Bayer, has received travel/accommodation expenses from F. Hoffmann-La Roche Ltd, Celgene, and Daiichi Sankyo, and has acted as an advisory consultant for Daiichi Sankyo, BMS, and AbbVie. All authors received research support (medical writing support) from F. Hoffmann-La Roche Ltd.

DATA SHARING

All relevant data are provided as supplementary information with this manuscript. Due to HIPAA requirements, we cannot share individualized patient genomic data from the FoundationCore dataset, which contains potentially identifying or sensitive patient information. Academic researchers can request access to aggregated data from Foundation Medicine, Inc. data by filling out a study review committee form. You and your institution will be required to execute a data transfer agreement. For further questions please reach out to Foundation Medicine, Inc., Cambridge, MA's

compliance department (compliance@foundationmedicine.com).

REFERENCES

1. Stella GM, Senetta R, Cassenti A, et al. Cancers of unknown primary origin: current perspectives and future therapeutic strategies. *J Transl Med*. 2012;10:12.
2. Rassy E, Pavlidis N. The currently declining incidence of cancer of unknown primary. *Cancer Epidemiol*. 2019;61:139-141.
3. Binder C, Matthes KL, Korol D, et al. Cancer of unknown primary-epidemiological trends and relevance of comprehensive genomic profiling. *Cancer Med*. 2018;7:4814-4824.
4. Fizazi K, Greco FA, Pavlidis N, et al. Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26:v133-v138.
5. Losa F, Soler G, Casado A, et al. SEOM clinical guideline on unknown primary cancer (2017). *Clin Transl Oncol*. 2018;20:89-96.
6. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®): Occult Primary (Cancer of Unknown Primary [CUP]). Version 2.2023. 2023. Available at https://www.nccn.org/professionals/physician_gls/pdf/occult.pdf. Accessed January 19, 2023.
7. Kato S, Alsafar A, Walavalkar V, et al. Cancer of unknown primary in the molecular era. *Trends Cancer*. 2021;7:465-477.
8. Kramer A, Bochtler T, Pauli C, et al. Cancer of unknown primary: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34:228-246.
9. Singh AP, Shum E, Rajdev L, et al. Impact and diagnostic gaps of comprehensive genomic profiling in real-world clinical practice. *Cancers*. 2020;12:1156.
10. Ross JS, Wang K, Gay L, et al. Comprehensive genomic profiling of carcinoma of unknown primary site: new routes to targeted therapies. *JAMA Oncol*. 2015;1:40-49.
11. Ross JS, Sokol ES, Moch H, et al. Comprehensive genomic profiling of carcinoma of unknown primary origin: retrospective molecular classification considering the CUPISCO study design. *Oncologist*. 2021;26:e394-e402.
12. Fizazi K, Maillard A, Penel N, et al. A phase III trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression analysis in patients with carcinomas of an unknown primary (CUP) site (GEFCAPI 04). *Ann Oncol*. 2019;30:v851-v934.
13. Hayashi H, Kurata T, Takiguchi Y, et al. Randomized phase II trial comparing site-specific treatment based on gene expression profiling with carboplatin and paclitaxel for patients with cancer of unknown primary site. *J Clin Oncol*. 2019;37:570-579.
14. Krämer A, Losa F, Gay LM, et al. Comprehensive profiling and molecularly guided therapy (MGT) for carcinomas of unknown primary (CUP): CUPISCO: a phase II, randomised, multicentre study comparing targeted therapy or immunotherapy with standard platinum-based chemotherapy. *Ann Oncol*. 2018;29. Abstract 445TiP.
15. Pauli C, Bochtler T, Mileshkin L, et al. A challenging task: identifying patients with cancer of unknown primary (CUP) according to ESMO guidelines: the CUPISCO trial experience. *Oncologist*. 2021;26:e769-e779.
16. US Food and Drug Administration (FDA). PMA P170019/S014: FDA Summary of Safety and Effectiveness Data — FoundationOne®CDx (F1CDx). 2022. Available at https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S014B.pdf. Accessed January 19, 2023.
17. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol*. 2013;31:1023-1031.
18. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9:34.
19. Trabucco SE, Gowen K, Maund SL, et al. A novel next-generation sequencing approach to detecting microsatellite instability and pan-tumor characterization of 1000 microsatellite instability—high cases in 67,000 patient samples. *J Mol Diagn*. 2019;21:1053-1066.
20. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the Catalogue Of Somatic Mutations In Cancer. *Nucleic Acids Res*. 2019;47:D941-D947.
21. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Deciphering signatures of mutational processes operative in human cancer. *Cell Rep*. 2013;3:246-259.
22. The R Foundation. The R Project for Statistical Computing. 2022. Available at https://www.r-project.org/. Accessed January 19, 2023.
23. Silverman IM, Hollebecque A, Friboulet L, et al. Clinicogenomic analysis of *FGFR2*-rearranged cholangiocarcinoma identifies correlates of response and mechanisms of resistance to pemigatinib. *Cancer Discov*. 2021;11:326-339.
24. Yakirevich E, Perrino C, Necchi A, et al. *NF2* mutation-driven renal cell carcinomas (RCC): a comprehensive genomic profiling (CGP) study. *J Clin Oncol*. 2020;38:Abstract 726.
25. Schoenfeld AJ, Bandlamudi C, Lavery JA, et al. The genomic landscape of *SMARCA4* alterations and associations with outcomes in patients with lung cancer. *Clin Cancer Res*. 2020;26:5701-5708.
26. Arbour KC, Jordan E, Kim HR, et al. Effects of co-occurring genomic alterations on outcomes in patients with KRAS-mutant non-small cell lung cancer. *Clin Cancer Res*. 2018;24:334-340.
27. Gleeson FC, Kipp BR, Levy MJ, et al. Somatic STK11 and concomitant STK11/KRAS mutational frequency in stage IV lung adenocarcinoma adrenal metastases. *J Thorac Oncol*. 2015;10:531-534.
28. Jahn A, Rump A, Widmann TJ, et al. Comprehensive cancer predisposition testing within the prospective MASTER trial identifies hereditary cancer patients and supports treatment decisions for rare cancers. *Ann Oncol*. 2022;33:1186-1199.
29. Chen YB, Xu J, Skanderup AJ, et al. Molecular analysis of aggressive renal cell carcinoma with unclassified histology reveals distinct subsets. *Nat Commun*. 2016;7:13131.
30. QED Therapeutics Inc. TRUSELTIQ (infigratinib). Prescribing Information. 2021. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214622s000lbl.pdf. Accessed January 19, 2023.
31. Incyte Corporation. PEMAZYRE® (pemigatinib). Prescribing Information. 2022. Available at https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/213736s002lbl.pdf. Accessed January 19, 2023.
32. Papillon-Cavanagh S, Doshi P, Dobrin R, et al. STK11 and KEAP1 mutations as prognostic biomarkers in an observational real-world lung adenocarcinoma cohort. *ESMO Open*. 2020;5:e000706.
33. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol*. 2018;36:633-641.
34. Agaimy A, Fuchs F, Moskalev EA, et al. SMARCA4-deficient pulmonary adenocarcinoma: clinicopathological, immunohistochemical, and molecular characteristics of a novel aggressive neoplasm with a consistent $TTF1^{neg}$/$CK7^{pos}$/HepPar-1^{pos} immunophenotype. *Virchows Archiv*. 2017;471:599-609.
35. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005;310:644-648.
36. Adamo P, Ladomery M. The oncogene ERG: a key factor in prostate cancer. *Oncogene*. 2016;35:403-414.
37. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature*. 2013;500:415-421.
38. Posner A, Prall OW, Sivakumaran T, et al. A comparison of DNA sequencing and gene expression profiling to assist tissue of origin diagnosis in cancer of unknown primary. *J Pathol*. 2023;259:81-92.