JNCI: Journal of the National Cancer Institute, 2023, 115(8), 994–997
https://doi.org/10.1093/jnci/djad095
Advance Access Publication Date: May 18, 2023
Brief Communication

Feasibility and value of genomic profiling in cancer of unknown primary: real-world evidence from prospective profiling study

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Abstract

Real-world evidence regarding the value of integrating genomic profiling (GP) in managing cancer of unknown primary (CUP) is limited. We assessed this clinical utility using a prospective trial of 158 patients with CUP (October 2016-September 2019) who underwent GP using next-generation sequencing designed to identify genomic alterations (GAs). Only 61 (38.6%) patients had sufficient tissue for successful profiling. GAs were seen in 55 (90.2%) patients of which GAs with US Food and Drug Administration–approved genomically matched therapy were seen in 25 (40.9%) patients. A change in therapy was recommended and implemented (primary endpoint of the study) in 16 (10.1%) and 4 (2.5%) patients of the entire study cohort, respectively. The most common reason for inability to implement the profiling-guided therapy was worsening of performance status (56.3%). Integrating GP in management of CUP is feasible but challenging because of paucity of tissue and aggressive natural history of the disease and requires innovative precision strategies.

Precision cancer medicine has become synonymous with genomic sequencing or profiling coupled with genomically guided therapy. However, advances have been mostly driven by select drugs, cancers, and genomic alterations (GAs), barring rare tissue-agnostic approvals (1). Although the feasibility and efficacy of this approach has been shown in advanced solid tumors, the few prospective trials have revealed limited improvement in outcomes (2,3).

Cancer of unknown primary (CUP), a metastatic disease without a discernible primary despite adequate standard diagnostic workup, is a rare and aggressive presentation, accounting for 2%-3% of all cancer cases (4). Despite advances in systemic therapy, including targeted therapy, for cancers with known primaries, treatments, and prognosis for patients with CUP are limited (1-year survival <50%) (5). Empiric chemotherapy covering putative primary tumors is suboptimal and argues for integration of genomic profiling (GP) to exploit druggable molecular aberrations. Early retrospective evidence shows clinically relevant GAs in CUP, which can potentially influence and personalize therapy, but prospective, real-world data are lacking (3,6-8). We performed this prospective profiling effort in a real-world setting to determine feasibility and impact of GP on management of CUP patients.

This prospective study included patients with a confirmed CUP diagnosis evaluated at MD Anderson Cancer Center, Houston, Texas, USA, between October 2016 and September 2019. CUP was defined as histologically proven metastatic cancer without a discernable primary despite thorough clinicopathologic and radiologic evaluation as recommended by international guidelines. Tumor tissue was analyzed using clinically validated Jackson Laboratory ActionSeq Plus and FusionSeq next-generation sequencing (NGS) panels. Full details of methodology are available in supplemental materials (Supplementary Methods, available online).

Baseline characteristics of 158 enrolled patients (median duration of follow-up at data cutoff [August 2021] = 50.6 months) are

Received: February 03, 2023. Revised: April 13, 2023. Accepted: May 16, 2023

shown in Supplementary Table 1 (available online). Median age was 60 (range = 21-86) years, 91 (57.6%) were females, and 89 (56.3%) had adenocarcinoma. The median overall survival of the entire cohort was 20.7 (95% confidence interval [CI] = 15.7 to 25.8) months. Eastern Cooperative Oncology Group Performance Status was 0-1 in 96% of patients. The most common biopsied sites were peritoneum (including retroperitoneum and pelvis) (35%), lymph nodes (19%), and liver (15%). Median number of immunohistochemistry stains performed was 9.

Study schema and key study timelines are shown in Figure 1, A. A total of 77 (48.7%) patients had profiling sent prior to starting any therapy. Of 158 consented patients, 97 (61.4%) had insufficient or inadequate tumor tissue for testing, and profiling could not be performed. (Figure 1, B). The median time from consent to tissue procurement and result reporting was 34 and 52 days, respectively. Successful profiling was associated with better performance status and early timing of profiling (Supplementary Table 1, available online).

In 61 (38.6%) patients who had adequate tumor tissue for testing, 11 (18.0%), 5 (8.2%), and 45 (73.8%) had successful ActionSeq, FusionSeq, and both components performed, respectively. A total of 524 GAs were found (Figure 1, C) in 55 (90%) of all profiled patients. Group 1 (for genomically matched US Food and Drug Administration–approved therapies), group 2, and group 3 GAs were seen in 25 (40.9%), 40 (65.6%), and 55 (90.2%) patients, respectively (Figure 1, D; Supplementary Table 2, available online).

A change in therapy from preprofiling plan to a profiling-guided therapy (PGT) was recommended in 16 patients, comprising 26.2% of patients who underwent successful testing and 10.1% of the entire study cohort (Supplementary Table 3, available online). A subsequent treatment plan with a PGT was executed in 4 patients (6.6% and 2.5% of all profiled and enrolled patients). The most common reason for the inability to undergo PGT was decline in performance status (9 [56.3%]) (Figure 1).

Widespread adoption of genome-driven cancer therapy without robust prospective data has raised questions regarding genetic reductionism and cost-effectiveness in cancer care, considering modest benefits and financial toxicity (1). However, the challenge of the true application of profiling in CUP is compounded by absence of tissue context, as targeted therapy response rates vary considerably based on cancer type (9). Although approval of tissue agnostic therapies such as pembrolizumab (high microsatellite instability or high tumor mutational burden) and larotrectinib (*tropomyosin receptor kinase* fusions) presents new opportunities for select CUP patients to receive targeted therapies, it is critical to understand the tissue of origin context in CUP before application of targeted therapies to this populace.

This cohort represents the largest, prospective, real-world experience of GP in patients with CUP reported to date (3,6-8). Our study demonstrates feasibility of this approach but highlights significant challenges with its use in this patient population. The adequacy and quality of tissue specimens is a major limitation, as patients with CUP often undergo extensive testing with immunohistochemistry, so remaining tissue for profiling can be limited; this was cited as the reason for screen failure in 14.1% of CUP patients consented for the international CUPISCO (A Clinical Trial to Compare Targeted Therapy or Cancer Immunotherapy with Chemotherapy in Patients with Cancer of Unknown Primary) trial (10). Our cohort had a higher failure rate, with 61.4% of patients having unsuccessful testing. It is possible a referral bias led to more extensive pathologic workup, increasing the likelihood of tissue exhaustion, though this phenomenon has been well-described in CUP. Repeat biopsy may be necessary for comprehensive testing in a substantial number of patients. Analysis of circulating tumor DNA (ctDNA) could help preserve tissue and is feasible in CUP (6). However, questions regarding ctDNA remain, including consistency of results compared with tissue-based NGS, and the reliability of results in a CUP population. Some patients with CUP may have low shedding of DNA into the blood like other malignancies, which may limit the utility of ctDNA analysis. Additionally, few patients (<3%) currently receive and benefit from PGT because of delayed testing, declining performance status, absence of a larger drug portfolio with proven benefit, and coverage issues. National Comprehensive Cancer Network Guidelines now state that molecular profiling can be considered for CUP; given the aggressive disease biology, we advocate for early molecular profiling in this patient population.

Study limitations include the use and scope of a single NGS assay with higher DNA and RNA inputs, as other assays may have different requirements for tissue processing and genomic coverage. However, a panel with wide coverage of GAs is important given recent histology agnostic drug approvals. In addition, variants of unknown significance may become clinically relevant in the future as more genomic data are available, so the number of actionable mutations may increase. Therefore, our data may underestimate the percentage of patients who could potentially benefit from PGT. Concerted efforts with dedicated biomarker-driven trials (eg, CUPISCO study) and real-world registries to annotate the use and benefit of off-label therapies can help enrich an evidence-based approach for patients with CUP. Another limitation included the 52-day delay in reporting time. Turnaround time remains paramount for optimizing clinical workflow and for bringing maximum value to patients. Efforts should be made to streamline sample transport and processing time.

In summary, GP represents an important method of determining future treatment options for patients with CUP, but few patients received genomic PGT in our real-world patient population because of high rates of insufficient tissue and low rates of actionable alterations. Implementing early NGS in CUP patients (eg, at time of initial CUP diagnosis) may lead to earlier implementation of genome-targeted therapy. In the same vein, further assessment of the utility of ctDNA is warranted. As the armamentarium of genome-targeted or genome-informed therapies grows, the importance of implementing an integrated precision strategy in CUP will only increase. Therefore, adoption of strategies that can overcome challenges associated with this approach is needed for CUP patients.

Data availability

Due to the nature of this research, which was primarily clinical practice based, participants of this study did not agree for their data to be shared publicly as a part of their informed consent process. Due to potential for identifiability, patient level data are not available. However, de-identified data can be made available under a data transfer agreement and upon MD Anderson Cancer Center institutional review board approval. We encourage investigators interested in data access and collaboration to request them by emailing MD Anderson institutional review board at IRB_Help@mdanderson.org.

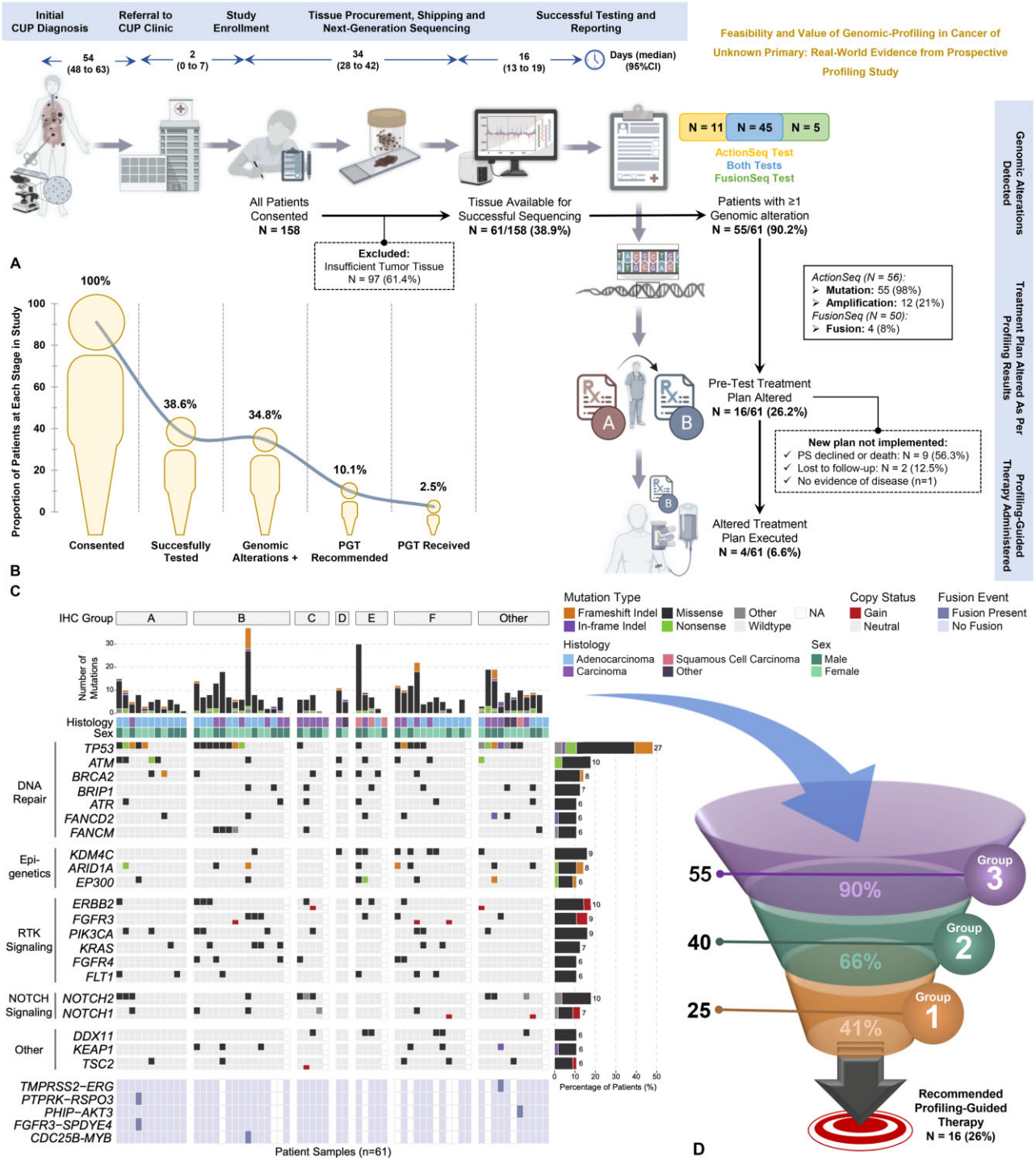

Figure 1. Study schema, genomic landscape, and clinical actionability of genomic alterations identified on profiling patients with cancer of unknown primary (CUP). **A)** Shows the study schema, describing flow of patients with CUP through current study and key study-specific milestones with timelines. Although genomic alterations are seen in a substantial subset of patients able to achieve successful profiling, only a minority can undergo profiling-guided therapy (PGT). **B)** Shows attrition of patients seen at each landmark stage of the study with the highest magnitude of breakdown in clinical profiling of CUP occurring in tissue procurement. Only 38.9% patients appear to have adequate tumor tissue for profiling. **C)** Shows the genomic landscape of CUP patients with oncoplot displaying genes altered in at least 10% of patients. Each column represents a patient and each row a gene. Alterations are grouped by pathway (**left**) and in descending order by frequency. Patients are arranged by immunohistochemical (IHC) grouping (**top**) (Supplementary Methods, available online): group A: strongly or diffusely positive for CDX2 and CK20 (lower gastrointestinal [GI] profile); group B: CDX2 positive and CK20 negative or weakly positive or CDX2 weakly positive (upper GI profile); group C: GATA3 positive; group D: PAX8 positive; group E: CK5/6, p63 or p16 positive; group F: CK7 positive but not included in any of the groups above. **Colored squares** show variant, and **grey and white squares** show no mutation and unsuccessful mutation testing. Fusions are shown along the bottom grid. **Upper bars** indicate total mutations identified in each patient. **Horizontal bars** (far right) indicate percentage of patients harboring a variant. **D)** Shows proportion of patients with genomic alterations classified for clinical actionability (Supplementary Methods, available online). Group 1 genomic alterations, those with biomarker-specific genomically matched US Food and Drug Administration–approved drugs in any solid tumor indication, were seen in 40% cases. CI = confidence interval; seq = sequence. PS = Performance Status; RTK = Receptor Tyrosine Kinase.

Author contributions

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Funding

This work was supported in part by Painter Research Funds, The Jackson Laboratory and CCSG (Cancer Center Support Grant) Award from the National Institutes of Health (NIH) (P30 CA016672).

Conflicts of interest

HVR, LC, GO, KK, and JR are all employees of Jackson Laboratory (Farmington, CT, USA). All other authors report no conflicts of interest.

Acknowledgements

This manuscript is dedicated to the memory of Dr (Professor) Gauri Varadhachary (1968-2021) who is included here as a coauthor for her significant contributions to this research work. We thank the patients, their families, and caregivers for participating in the trial and all site personnel and clinical staff for clinical trial support in this trial. This study was supported in part by the NIH CCSG Award (CA016672) and The University of Texas MD Anderson Cancer Center through the Molecular Evaluation and/or Biopsy Related Support Program (used for performing biopsy) and by The Jackson Laboratory, Director's Innovation Fund, award number 19000-17-02. The funders had no role in the design of the study; collection, analysis or interpretation of the data; writing of the manuscript or decision to submit it for publication.

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