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Detection of *MEN1* resistance mutations in cell-free DNA from acute leukemia patients treated with menin inhibitors

Blood Cancer Journal (2026)16:94 ; https://doi.org/10.1038/s41408-026-01545-4

Dear Editor,

Menin inhibitors have emerged as a targeted therapeutic strategy for patients with acute leukemias driven by *KMT2A* gene rearrangements (*KMT2Ar*) and *NPM1* mutations, where aberrant menin-KMT2A interactions sustain *HOX-MEIS1*-dependent transcriptional programs that are critical for leukemogenesis [1–4]. Positive early-phase studies led to regulatory approval of both revumenib and ziftomenib for these leukemia subtypes [5, 6]. Despite these advances, acquired resistance frequently limits durability of menin inhibitor treatment response [7].

We previously characterized somatic *MEN1* mutations as a recurrent genetic mechanism of on-target resistance to menin inhibition [7]. These mutations impair drug binding while preserving the ability of menin to scaffold leukemogenic chromatin complexes, thereby maintaining *HOX* gene expression. Currently, detection of *MEN1* resistance mutations relies on next-generation sequencing (NGS) of genomic DNA (gDNA) derived from bone marrow aspirates. Although informative, marrow-based testing is invasive and logistically challenging for serial monitoring, particularly in patients with low circulating blast counts or in morphologic remission. Plasma-derived cell-free DNA (cfDNA) analysis offers a complementary strategy that enables minimally invasive, repeated sampling and may better capture these mutations across disease compartments. This is especially advantageous in the pediatric setting, where bone marrow aspiration generally necessitates sedation. High-sensitivity cfDNA assays can detect low-frequency variants, enabling the possibility of identifying emergent resistance mutations prior to morphologic relapse or in the absence of circulating disease. Whether cfDNA can reliably detect *MEN1* resistance mutations in patients treated with menin inhibitors remains undefined.

In this study, we describe the feasibility and utility of an NGS assay called MSK-ACCESS MEN1 for the detection of *MEN1* resistance mutations from plasma-derived cfDNA samples from patients receiving menin inhibitors. We compare these findings to marrow samples sequenced by MSK-IMPACT, a CLIA-certified hybridization capture-based NGS assay developed for comprehensive molecular profiling of hematologic malignancies [8]. The MSK-ACCESS Heme assay is a CLIA-compliant targeted hybrid capture NGS assay designed to identify somatic genomic alterations from plasma cfDNA across 115 genes [9, 10]. Capture probes targeting all 9 coding exons of the MEN1 gene were spiked into the existing MSK-ACCESS Heme design, hereafter referred to as "MSK-ACCESS MEN1". This approach leveraged the validated MSK-ACCESS Heme assay as a backbone rather than developing a separate assay, thereby maintaining established CLIA-compliant performance characteristics. Integration of *MEN1* probes into the MSK-ACCESS Heme backbone did not compromise performance as per-gene depth and uniformity for the original MSK-ACCESS Heme targets were preserved following probe addition (Supplementary Figs. 1 and 2).

Established clinical thresholds were employed for *MEN1* mutation variant calling. Detailed methods on variant calling can be found in the Supplemental Methods. All samples and clinical sequencing data were collected with informed consent on an IRB-approved research protocol (NCT01775072).

To benchmark MSK-ACCESS MEN1 against standard marrow-based molecular profiling, we assessed concordance between MSK-ACCESS MEN1 and MSK-IMPACT Heme by testing marrow-derived gDNA samples with known MEN1 mutations and comparing specific variant calls and variant allele frequencies (VAFs). Across 4 marrow samples with 8 total MEN1 mutations, VAFs determined by MSK-ACCESS MEN1 closely tracked those determined by MSK-IMPACT Heme, with a strong linear relationship (Pearson correlation coefficient 0.979) across the full range of observed allele frequencies (Supplementary Fig. 3).

To evaluate whether plasma-derived cfDNA can support the detection of MEN1 resistance mutations in the setting of minimal or absent circulating disease, we analyzed banked plasma samples from patients with *MEN1* resistance mutations identified in contemporaneous bone marrow aspirates by MSK-IMPACT Heme at the time of overt progression. Figure 1A illustrates this scenario in a patient with relapsed *KMT2Ar* T-cell acute lymphoblastic leukemia who had previously received revumenib monotherapy with an initial response followed by disease progression requiring treatment with subsequent salvage chemotherapy. At the time of disease reassessment after salvage therapy, a marrow aspirate showed refractory disease with 56% blasts, and MSK-IMPACT Heme performed on bone marrow identified three MEN1 resistance mutations, $MEN1^{G331D}$, $MEN1^{G331R}$, and $MEN1^{M327I}$. Peripheral blood testing drawn on the same day demonstrated profound leukopenia (WBC 0.16 k/μL) with no reported circulating blasts. Despite the absence of detectable peripheral blasts, MSK-ACCESS MEN1 analysis of cfDNA identified all three MEN1 resistance mutations (Fig. 1A). These findings demonstrate that MSK-ACCESS MEN1 can sensitively detect polyclonal *MEN1* resistance mutations from plasma cfDNA in the setting of marked leukopenia and absent circulating blasts.

In a second illustrative case, we assessed whether cfDNA profiling could identify emergent *MEN1* resistance mutations during early clonal evolution, when disease burden in peripheral blood remained low and marrow-directed clinical sequencing had not yet revealed overt resistance. The patient had relapsed *KMT2A-MLLT4*-rearranged AML and initiated revumenib therapy with high marrow disease burden (79% blasts). After two cycles of therapy, marrow blasts decreased to 6%, and *MEN1* resistance mutations were not detected in paired bone marrow samples by MSK-IMPACT Heme or in contemporaneous plasma cfDNA

Received: 25 March 2026 Revised: 1 June 2026 Accepted: 11 June 2026
Published online: 16 June 2026

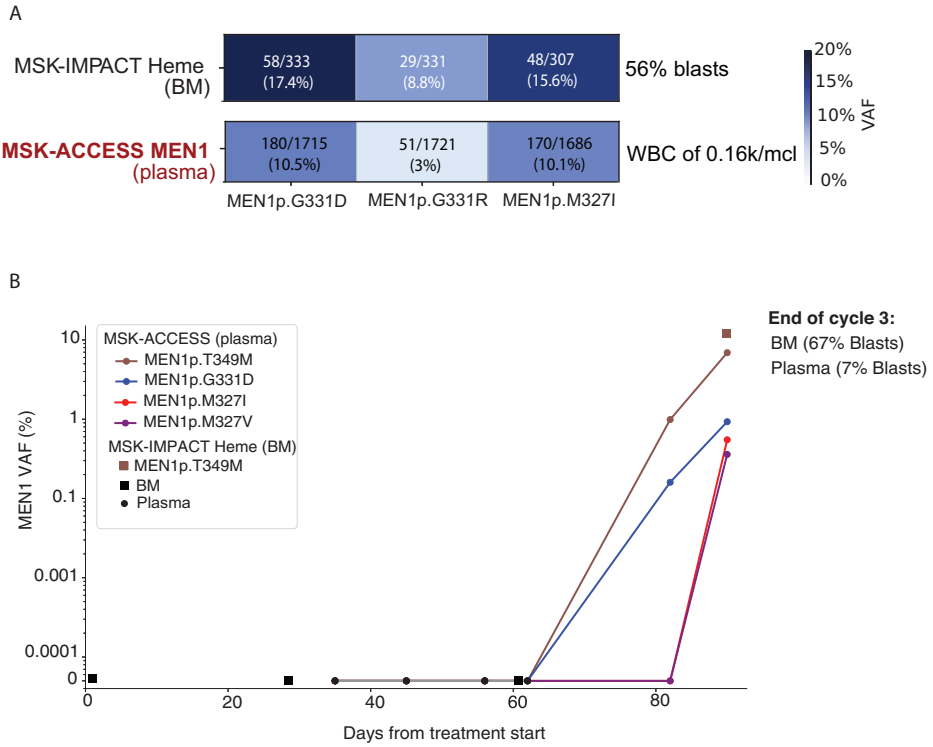

Fig. 1 Plasma-derived cfDNA detection of *MEN1* resistance mutations from patients with absent or low-level circulating blasts. **A** Comparison of MSK-IMPACT Heme from marrow-derived gDNA with MSK-ACCESS Heme from plasma-derived cfDNA in a revumenib-treated patient with *KMT2A-rearranged* T-ALL with active marrow disease but marked leukopenia. **B** Serial samples from a revumenib-treated patient with *KMT2A-MLLT4* AML comparing results of marrow-derived gDNA with MSK-IMPACT Heme and plasma-derived cfDNA with MSK-ACCESS MEN1 to identify low VAF polyclonal resistance patterns. BM Bone Marrow.

samples by MSK-ACCESS MEN1. Three weeks into cycle 3, the patient had limited circulating disease (3% blasts). MSK-ACCESS MEN1 performed on a banked plasma cfDNA specimen from this time point identified two emergent *MEN1* resistance mutations at low VAFs: *MEN1*^{T349M} (VAF 1.0%) and *MEN1*^{G331D} (VAF 0.16%) (Fig. 1B). Eight days later, at end-of-cycle evaluation, the patient demonstrated frank disease progression with 67% marrow blasts. MSK-IMPACT Heme performed on the bone marrow aspirate at this time point detected *MEN1*^{T349M} at a VAF of 11% but did not identify additional *MEN1* variants. In contrast, MSK-ACCESS MEN1 performed on same-day plasma cfDNA detected *MEN1*^{T349M} and additionally identified three other *de novo MEN1* resistance mutations at VAFs below 1% (Fig. 1B). The longitudinal pattern, with low-level detection in cfDNA preceding and then expanding in parallel with clinical progression, supports the premise that high-sensitivity cfDNA profiling can [1] identify emerging *MEN1* resistance mutations before they are captured by standard marrow sequencing, and [2] more sensitively resolve low-level polyclonal resistance at the time of relapse, particularly for subclones present below the effective detection threshold of conventional tumor-content-dependent assays.

To determine whether cfDNA profiling could identify emerging *MEN1* resistance mutations prior to overt clinical disease progression, we examined longitudinally banked plasma samples from two patients who ultimately developed confirmed *MEN1*-mediated resistance and for whom serial specimens were available for multiple cycles preceding relapse. Figure 2A illustrates the case of a patient with relapsed/refractory *NPM1*-mutant AML treated with revumenib who exhibited stable disease through the first three cycles of therapy, with marrow blast percentages remaining in the 7-15% range. Consistent with the absence of overt genetic escape mutants early in treatment, MSK-ACCESS MEN1 analysis of cfDNA obtained prior to cycles 1–3 did not capture any *MEN1* variants. At the start of cycle 4, a routine bone marrow assessment continued to demonstrate stable disease with 11% blasts. MSK-IMPACT Heme performed on the marrow aspirate was negative for *MEN1* mutations, indicating no detectable resistance variants by standard marrow-based clinical sequencing. In contrast, MSK-ACCESS MEN1 performed on a contemporaneous cfDNA specimen collected the same day identified an emerging *MEN1*^{M327V} mutation at a low VAF (1.6%; 9/562 reads) (Fig. 2A, left). Notably, peripheral blood parameters at that time were not suggestive of circulating disease, underscoring that detection reflected cfDNA shedding rather than readily detectable peripheral leukemia burden. The patient received an additional cycle of revumenib and experienced rapid clinical progression by end of cycle 4. Repeat marrow evaluation at that time demonstrated marked leukemic expansion with 89% blasts. MSK-IMPACT Heme performed on this bone marrow specimen identified *MEN1* resistance mutations, including the expanded *MEN1*^{M327V} mutation at a higher VAF, and a second *MEN1*^{M327I} mutation, consistent with polyclonal resistance emerging alongside overt relapse.

The second longitudinal case with banked plasma specimens preceding clinical progression was from a patient with relapsed *KMT2A-MLLT4*-rearranged AML who achieved a deep response to revumenib, with an MRD-negative bone marrow documented at the start of cycle 8 (Fig. 2B). Despite ongoing clinical stability,

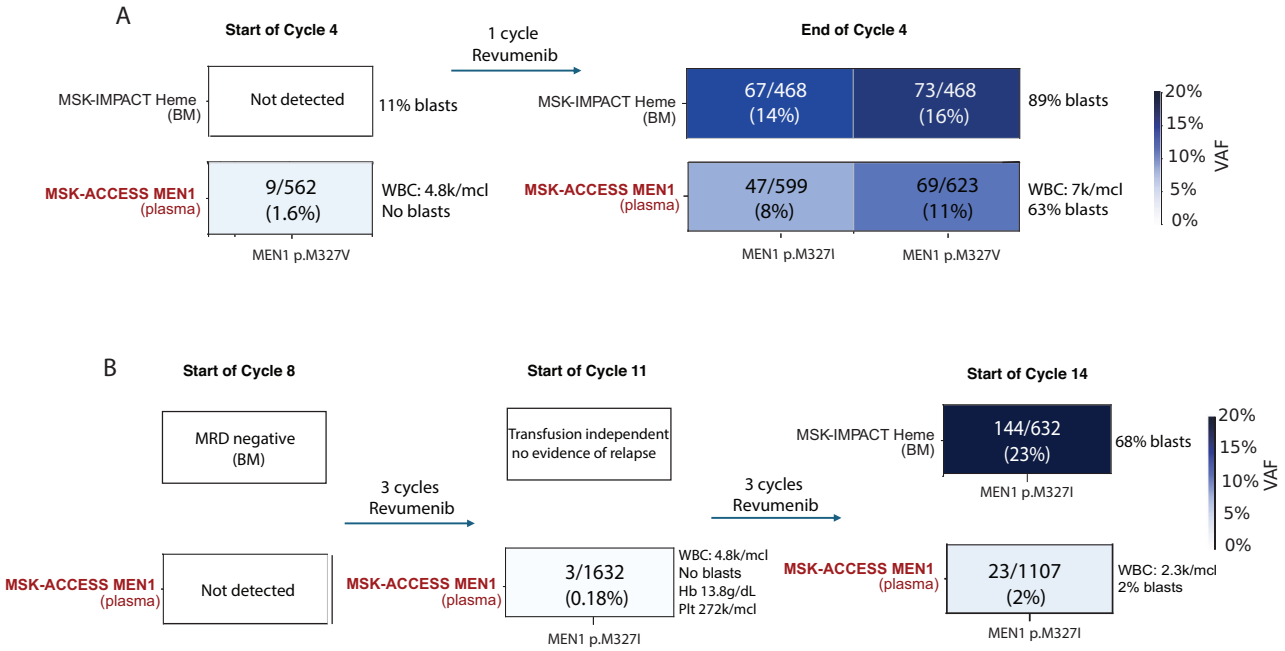

Fig. 2 Early identification of *MEN1* resistance mutations enabled by MSK-ACCESS MEN1. Comparison of longitudinal detection of *MEN1* mutations between MSK-IMPACT Heme from marrow-derived gDNA with MSK-ACCESS MEN1 from plasma-derived cfDNA in a revumenib-treated patient with **A** *NPM1 mutant* AML and **B** *KMT2A-MLLT4* AML.

MSK-ACCESS MEN1 detected an emerging *MEN1*^{M327I} resistance mutation in cfDNA obtained at the beginning of cycle 11, at a very low allele fraction (VAF 0.18%, 3/1632 reads) (Fig. 2B). Importantly, this molecular signal was observed in the setting of continued transfusion independence and no clinical evidence of relapse, with a normal complete blood count at the time of collection and no reported circulating blasts. The patient continued revumenib monotherapy for multiple additional cycles. However, toward the end of cycle 13, peripheral blood counts became abnormal with the emergence of circulating blasts. Bone marrow aspiration confirmed frank progression with a markedly increased blast burden (68% blasts). MSK-IMPACT Heme performed on the progression marrow identified the *MEN1*^{M327I} mutation (Fig. 2B), corresponding to the same resistance clone that had been detectable in plasma cfDNA approximately three cycles earlier.

In this study, we demonstrate that plasma cfDNA molecular profiling enables accurate detection of *MEN1* resistance mutations in patients treated with menin inhibitors. Using established CLIA-compliant assays for cross-validation in longitudinally banked biospecimens, cfDNA profiling showed complete concordance with marrow-based testing, detected resistance mutations in the absence of circulating leukemic blasts, resolved low-level subclones at relapse, and provided a measurable lead time before marrow-confirmed progression. These findings align with a growing body of literature supporting cfDNA-based monitoring of resistance mutations in hematologic malignancies [11–15]. Moreover, panel cfDNA testing with assays such as MSK-ACCESS Heme readily allows for the addition of newly discovered genetic resistance mechanisms to menin inhibition. A key limitation to this study is the modest sample size of longitudinally banked plasma samples from a single institution. Prospective evaluation of serial cfDNA collections in ongoing and future clinical trials of menin inhibitors are required to properly incorporate high-sensitivity molecular surveillance and develop adaptive treatment strategies across menin inhibitors.

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ACKNOWLEDGEMENTS

The authors acknowledge support from the NIH Cancer Center Support grant P30 CA008748.

AUTHOR CONTRIBUTIONS

NS and SFC: Conceptualization of the study and original draft writing. NS, KC, SL, KHK, HC, EB, CC, RP, RS, SFC: Data curation and analysis. NS, KC, ARB, MDE, MLS, WLC, MEA, NM, MB, RLL, EMS, RS, SFC: Data interpretation, manuscript drafting, and revisions. All authors reviewed and provided final approval of the manuscript.

COMPETING INTERESTS

NS reports serving on advisory boards of Syndax and Kura Oncology. ARB reports intellectual property rights from SOPHiA Genetics. MDE reports speaking fees from Illumina, consulting for Pillar Biosciences, and advisory board membership for Arima Genomics. MEA reports speaking fees from Biocartis, i3health, consulting for Janssen Global Services, Bristol-Myers Squibb, AstraZeneca, Roche, Biocartis, Sanofi, and intellectual property rights from SOPHiA Genetics. Michael Berger reports personal fees from AstraZeneca, Paige.AI and intellectual property rights from SOPHiA Genetics, and a provisional patent for Systems and methods for detecting cancer via cfDNA screening. RLL is a former member of the Supervisory Board and current SAB member for Qiagen (compensation/equity), a co-founder at Ajax (equity), a board member of the Mark Foundation for Cancer Research, and a former scientific advisor to Mission Bio, Kurome, Syndax, Scorpion, Zentalis, Jubilant, Auron, Prelude, and C4 Therapeutics. For each of these entities, he received equity/compensation. He has received research support from the Cure Breast Cancer Foundation (with IP rights), Calico, Zentalis, and Ajax, and has consulted/provided professional services for ECOG-ACRIN, Genome Canada, Goldman Sachs, and AstraZeneca. EMS reports advisory/consulting services for Syndax, Kura Oncology, Johnson and Johnson, Charm Therapeutics. RS reports royalties received from SOPHiA Genetics for MSK-IMPACT and MSK-ACCESS. SFC is a consultant and/or shareholder for Daiichi-Sankyo, Stelexis BioSciences, and Ursamin, none of which are directly related to the content of this paper; he received research support from Syndax, Trio, and Actinium and is an inventor on a patent related to MEN1 mutations and uses thereof (WO2024112819A1). All other authors report no related conflicts of interest.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41408-026-01545-4.

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