RESEARCH LETTER

blood neoplasia

TO THE EDITOR:

Liquid biopsy for molecular profiling in cutaneous T-cell lymphoma: a pilot feasibility study

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Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of non-Hodgkin lymphomas of skin-homing malignant T cells.[1,2] Molecular profiling increasingly guides diagnosis and treatment, but tissue-based approaches have practical limitations: although tumor tissue is generally accessible via skin biopsy, early-stage lesions may be histologically ambiguous, repeated biopsies can be burdensome in patients requiring longitudinal monitoring, and some specimens yield insufficient tumor content for comprehensive molecular testing.[3,4] Moreover, tissue biopsy inherently samples a single anatomic site, whereas plasma-derived cell-free (cf) nucleic acids integrate molecular signal shed from the entire lymphoid compartment, potentially capturing disease heterogeneity across multiple involved sites that no single biopsy can represent. Clinically, CTCL recurrence or progression can be difficult to distinguish from inflammatory or infectious mimics, particularly in erythrodermic or treatment-altered skin, highlighting the potential value of a minimally invasive molecular assay to support therapeutic decision-making when clinicopathologic findings are equivocal.

Liquid biopsy offers a minimally invasive alternative, but its application in CTCL faces unique challenges.[5,6] Unlike B-cell malignancies where circulating tumor DNA serves as a surrogate for tumor burden, CTCL has low peripheral tumor fraction except in leukemic variants.[7-10] Additionally, clonal hematopoiesis of indeterminate potential (CHIP) confounds interpretation, as CHIP-associated mutations (*DNMT3A*, *TET2*, *ASXL1*) overlap substantially with those mutated in CTCL.[11,12] We evaluated the feasibility of applying a validated next generation sequencing (NGS) platform with parallel DNA and RNA analysis to plasma-derived cf nucleic acids in CTCL, with explicit attention to CHIP confounding and T-cell receptor (TCR)–clonality assessment.

We retrospectively analyzed patients with CTCL who underwent tissue-based molecular profiling (n = 180 submitted, 178 evaluable after 2 quantity-not-sufficient exclusions) and liquid biopsy testing (n = 38, no quantity-not-sufficient failures) at Genomic Testing Cooperative between May 2018 and April 2025. Detection rates were assessed separately in each cohort; concordance was evaluated in the subset of 15 patients with paired tissue and liquid biopsy samples. Testing utilized a validated NGS platform detecting somatic single nucleotide variants and small insertions/deletions copy number variants, gene fusions, and TCR clonality from cfDNA and cfRNA. DNA and RNA were co-extracted from Formalin-Fixed Paraffin-Embedded (FFPE) sections; cfDNA and cfRNA were extracted from plasma. Patients were identified by International Classification of Diseases-10 codes (C84.0, C84.1) or clinical indication of CTCL, mycosis fungoides, or Sézary syndrome. The study was approved by the Western Copernicus Group institutional review board (number 1-1476184-1).

Submitted 17 February 2026; accepted 29 April 2026; prepublished online 13 May 2026. https://doi.org/10.1016/j.bneo.2026.100245.

Deidentified individual participant data, along with the associated data dictionary, will be made available upon reasonable request to the corresponding author, Larisa J. Geskin (ljg2145@cumc.columbia.edu), subject to institutional data-sharing agreements and review board approval.

The full-text version of this article contains a data supplement.

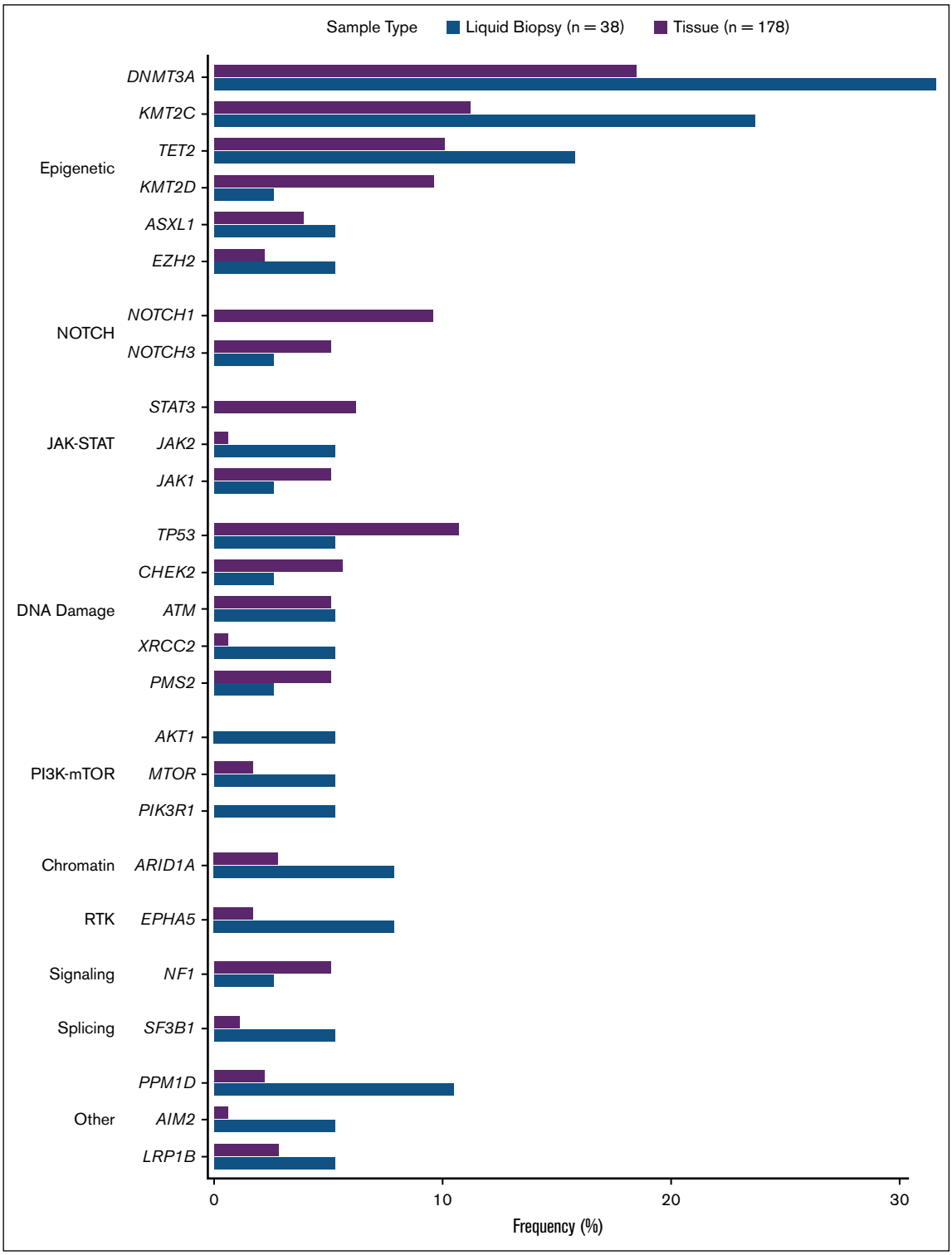

Figure 1. Mutation frequencies in CTCL by sample type, grouped by biological pathways. Horizontal bar chart showing mutation frequencies (%) for genes detected at ≥5% frequency in either the liquid biopsy (n = 38, blue) or tissue biopsy (n = 178, purple) cohorts. Genes are grouped by biological pathways.

Targeted NGS utilized a high-depth panel (302 genes at the DNA level; >1600 genes at the RNA level).[13] Sequencing depth was ~2500× for tissue and 25 000× for liquid biopsy. Analytical sensitivity was 1% variant allele frequency (VAF) for hot spots and 10% for non–hot spots. TCR clonality was assessed by RNA sequencing of 383 IMGT TCR genes across 4 chains (TRA, TRB, TRG, and TRD); dominant clones had ≥10-fold enrichment over the next most abundant clonotype.

Table 1. Patient-level concordance in paired samples

Pair	Shared	Liquid only	Tissue only	Concordant genes
1	0	0	6	–
2	2	3	2	POT1, TNFAIP3
3	0	0	0	–
4	1	1	2	MTOR
5	0	3	0	–
6	3	3	0	TET2, BTK, DNMT3A
7	0	0	0	–
8	4	1	15	SMC1A, NOTCH3, TET2, BRAF
9	3	2	1	NOTCH2, KMT2C, KMT2D
10	4	0	1	ASXL1, ERBB3, IRF4, PDGFRA
11	4	10	0	TET2, DNMT3A, RHOA, SF3B1
12	2	5	10	DICER1, LRP1B
13	2	1	2	RHOA, H3F3A
14	2	2	17	MLH1, EZH2
15	3	0	0	MUTYH, KMT2C, CD36

Distribution of concordance categories among 15 evaluable paired tissue/liquid biopsy samples. Per-variant concordance was calculated as shared mutations/total unique mutations detected in either compartment. Median gene-level concordance was 23.1% (range, 0%-100%).

Cohorts were nonoverlapping; paired samples were defined as tissue and liquid biopsies from the same patient. Concordance was quantified as a per-variant metric: the number of shared mutations divided by the total number of unique mutations detected in either compartment, calculated at gene and variant levels. All analyses used R (version 4.4.2). Detailed methods are provided in supplemental Methods.

Somatic mutations were detected in 30 of 38 patients who underwent liquid biopsy (79%), with 129 mutations across 60 unique genes (supplemental Table 1). A nonoverlapping tissue cohort (176 evaluable) demonstrated a 75.0% detection rate (132/176; 74.2% intent-to-test). The liquid biopsy detection rate exceeds prior CTCL reports (as low as 17%), although cross-study comparison is limited.[14]

The mutational landscape recapitulated known CTCL biology (Figure 1).[12] Epigenetic modifiers predominated, with *DNMT3A* being the most frequently mutated gene (tissue 19%, liquid 32%), followed by *KMT2C* (tissue 11%, liquid 24%) and *TET2* (tissue 10%, liquid 16%); however, the increased frequency of *DNMT3A* and *TET2* in liquid biopsy likely reflects, at least in part, CHIP. Certain genes were detected exclusively in tissues (*NOTCH1*, *PLCG1*, and *STAT3*), consistent with low circulating tumor fraction.

CHIP-associated genes (*DNMT3A*, *TET2*, *ASXL1*, *TP53*, and *PPM1D*) were detected at lower VAF (median, 1.01%) compared to CTCL-specific genes (*NOTCH1*, *PLCG1*, *RHOA*, and *STAT3*; median 2.25%), and both categories showed substantially lower VAF in liquid biopsy than in tissue (supplemental Table 2). Critically, allele fraction alone is insufficient to distinguish age-related clonal hematopoiesis from tumor-derived mutations in plasma, necessitating integration of gene identity, tissue findings, and clinical context.[15]

Among the 15 paired samples, median gene-level concordance was 23.1% (range, 0%-100%) and variant-level concordance was 20.0% (range, 0%-100%), reflecting the limited sensitivity of cfDNA for low-frequency tissue mutations. *TET2* (3 pairs), *DNMT3A* (2 pairs), and *KMT2C* (2 pairs). CTCL-associated genes including *RHOA, NOTCH2, and NOTCH3* also showed concordance (Table 1). Collection timing between tissue and liquid biopsy (median, 21 days; range, 15-608 days) was not associated with concordance (Mann-Whitney *U* test, $P > .05$).

Among concordant variants in paired samples, known CHIP genes showed higher VAF in liquid biopsy than tissue (median liquid biopsyx/tissue ratio >1), whereas CTCL-associated genes showed the opposite (median ratio < 0.5); using this framework, 17 of 37 concordant variant-gene observations (45.9%) were classified as tumor-derived, 14 (37.8%) as possible or likely CHIP, and 6 (16.2%) as likely germ line (supplemental Table 3).

TCR clonality was assessed in 28 evaluable liquid biopsy samples; dominant clones were identified in 7 (25%), with somatic mutations co-detected in all 7 cases, supporting a tumor-derived origin for at least some detected mutations.

Gene fusions were detected in 35 of 176 tissue specimens (19.9%) (supplemental Table 4), with *JAK2-STAT3* via t(9;17)(p24.1;q21.2) the most recurrent (5 cases). In liquid biopsy, only one fusion was detected (*USP34-XPO1*, 1/38), notably in a sample with no somatic single nucleotide variants. Among 4 paired patients with tissue fusions, none were detected in matched liquid biopsy (0/4 events), indicating fusion transcripts from skin-homing tumor cells fall below plasma cfRNA detection thresholds (supplemental Table 5). This discrepancy (tissue 19.9% vs liquid 2.6%) reinforces that tissue biopsy remains essential for actionable kinase fusions.

Distinguishing tumor-derived from CHIP mutations remains the central challenge in CTCL liquid biopsy.[16] No single metric resolves this: tissue concordance is confounded by CHIP-derived leukocytes in tissue specimens, TCR clonality confirms the presence of a malignant T-cell clone but cannot attribute individual mutations to it, and VAF-ratio patterns are inferential.[17] This challenge is compounded by the observation that clonal hematopoiesis is not restricted to the myeloid compartment; CHIP-associated mutations in DNMT3A and TET2 can arise in lymphoid progenitors or in multipotent cells contributing to both lineages, blurring the boundary between tumor-derived and hematopoietic variants.[16] Additionally, spatially distinct CTCL lesions may harbor different mutational profiles, such that a single tissue biopsy cannot serve as a definitive reference standard for classifying plasma-derived variants as concordant or discordant.

This study is limited by its retrospective design and small paired-sample size (n = 15). However, the data demonstrate technical feasibility and support a possible multimodal interpretive framework integrating mutation profiling, TCR clonality, and VAF-ratio analysis. Tissue biopsy remains essential for comprehensive genomic profiling, particularly for fusion detection; however, liquid biopsy may serve as a complement in clinical scenarios including longitudinal circulating tumor DNA monitoring, early relapse detection, molecular profiling in Sézary syndrome where peripheral blood is already the primary diagnostic specimen, and integration of molecular signal across anatomically distributed disease not captured by biopsy of a single site. Future studies should evaluate the role of liquid biopsy in

clinically ambiguous settings, such as differentiating CTCL recurrence from inflammatory or infectious dermatoses in erythrodermic or treatment-altered skin, where molecular clarification could directly influence therapeutic decision-making.

Contribution: All authors conducted the investigation and contributed to writing (review and editing) the manuscript; and Y.S., A.S.E., and M.A. wrote the original draft.

Conflict-of-interest disclosure: M.A. works and owns stocks in a diagnostic company that offers tissue and liquid biopsy testing. T.F. has served as an advisory board member for Bristol Myers Squibb (BMS), Seagen (acquired by Pfizer in December 2023), Genmab, and AstraZeneca; is employed at Hackensack University Medical and John Theurer Cancer Center at Hackensack Meridian Health; reports travel expenses from AbbVie, Kite Pharma, Seagen (acquired by Pfizer in December 2023), and Takeda; has provided consultancy to AstraZeneca, BMS, MorphoSys, and Seagen (acquired by Pfizer in December 2023); has participated on speakers' bureaus for AbbVie and Seagen (acquired by Pfizer in December 2023); reports research funding/grants from Amgen, BMS, Celgene, Cell Medica, Corvus, Eisai, Kyowa Hakko Kirin, Pfizer, Portola Pharma, Roche, Seagen (acquired by Pfizer in December 2023), Trillium, and Viracta; and honoraria from AbbVie, BMS, Kite Pharma, Pharmacyclics, Seagen (acquired by Pfizer in December 2023), Takeda, and Genmab. L.J.G. has served as an investigator for Johnson & Johnson, Mallinckrodt, Kyowa Kirin, Soligenix, Innate, Incyte, Trillium, Merck, BMS, and Stratpharma; and on the scientific advisory board for SciTech and Citius. The remaining authors declare no competing financial interests.

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References

1. Willemze R, Cerroni L, Kempf W, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. *Blood*. 2019;133(16):1703-1714.
2. Girardi M, Heald PW, Wilson LD. The pathogenesis of mycosis fungoides. *N Engl J Med*. 2004;350(19):1978-1988.
3. Scarisbrick JJ, Quaglino P, Prince HM, et al. The PROCLIPI international registry of early-stage mycosis fungoides identifies substantial diagnostic delay in most patients. *Br J Dermatol*. 2019;181(2):350-357.
4. Skov AG, Gniadecki R. Delay in the histopathologic diagnosis of mycosis fungoides. *Acta Derm Venereol*. 2015;95(4):472-475.
5. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer*. 2017;17(4):223-238.
6. Colmenares R, Álvarez N, Barrio S, Martínez-López J, Ayala R. The minimal residual disease using liquid biopsies in hematological malignancies. *Cancers (Basel)*. 2022;14(5):1310.
7. da Silva Almeida AC, Abate F, Khiabanian H, et al. The mutational landscape of cutaneous T cell lymphoma and Sézary syndrome. *Nat Genet*. 2015;47(12):1465-1470.
8. McGirt LY, Jia P, Baerenwald DA, et al. Whole-genome sequencing reveals oncogenic mutations in mycosis fungoides. *Blood*. 2015;126(4):508-519.
9. Roschewski M, Rossi D, Kurtz DM, Alizadeh AA, Wilson WH. Circulating tumor DNA in lymphoma: principles and future directions. *Blood Cancer Discov*. 2022;3(1):5-15.
10. Roschewski M, Dunleavy K, Pittaluga S, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol*. 2015;16(5):541-549.
11. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26):2488-2498.
12. Choi J, Goh G, Walradt T, et al. Genomic landscape of cutaneous T cell lymphoma. *Nat Genet*. 2015;47(9):1011-1019.
13. Ip A, Della Pia A, Kim GY, et al. Reliability of cell-free DNA and targeted NGS in predicting chromosomal abnormalities of patients with myeloid neoplasms. *Front Oncol*. 2022;12:923809.
14. Kim SJ, Kim YJ, Yoon SE, et al. Circulating tumor DNA-based genotyping and monitoring for predicting disease relapses of patients with peripheral T-cell lymphomas. *Cancer Res Treat*. 2023;55(1):291-303.
15. Chan HT, Chin YM, Nakamura Y, Low SK. Clonal hematopoiesis in liquid biopsy: from biological noise to valuable clinical implications. *Cancers (Basel)*. 2020;12(8):2277.
16. Buscarlet M, Provost S, Zada YF, et al. *DNMT3A* and *TET2* dominate clonal hematopoiesis and demonstrate benign phenotypes and different genetic predispositions. *Blood*. 2017;130(6):753-762.
17. Menon A, Sukhanova M, Nocito KL, Gao J, Jennings LJ, Vormittag-Nocito ER. Detection and interpretation of clonal hematopoiesis variants during routine solid tumor next-generation sequencing: a single-institution experience. *J Mol Diagn*. 2024;26(12):1149-1158.