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Circulating Tumor DNA Profiling for Detection, Risk Stratification, and Classification of Brain Lymphomas

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abstract

PURPOSE Clinical outcomes of patients with CNS lymphomas (CNSLs) are remarkably heterogeneous, yet identification of patients at high risk for treatment failure is challenging. Furthermore, CNSL diagnosis often remains unconfirmed because of contraindications for invasive stereotactic biopsies. Therefore, improved biomarkers are needed to better stratify patients into risk groups, predict treatment response, and noninvasively identify CNSL.

PATIENTS AND METHODS We explored the value of circulating tumor DNA (ctDNA) for early outcome prediction, measurable residual disease monitoring, and surgery-free CNSL identification by applying ultrasensitive targeted next-generation sequencing to a total of 306 tumor, plasma, and CSF specimens from 136 patients with brain cancers, including 92 patients with CNSL.

RESULTS Before therapy, ctDNA was detectable in 78% of plasma and 100% of CSF samples. Patients with positive ctDNA in pretreatment plasma had significantly shorter progression-free survival (PFS, $P < .0001$, log-rank test) and overall survival (OS, $P = .0001$, log-rank test). In multivariate analyses including established clinical and radiographic risk factors, pretreatment plasma ctDNA concentrations were independently prognostic of clinical outcomes (PFS HR, 1.4; 95% CI, 1.0 to 1.9; $P = .03$; OS HR, 1.6; 95% CI, 1.1 to 2.2; $P = .006$). Moreover, measurable residual disease detection by plasma ctDNA monitoring during treatment identified patients with particularly poor prognosis following curative-intent immunochemotherapy (PFS, $P = .0002$; OS, $P = .004$, log-rank test). Finally, we developed a proof-of-principle machine learning approach for biopsy-free CNSL identification from ctDNA, showing sensitivities of 59% (CSF) and 25% (plasma) with high positive predictive value.

CONCLUSION We demonstrate robust and ultrasensitive detection of ctDNA at various disease milestones in CNSL. Our findings highlight the role of ctDNA as a noninvasive biomarker and its potential value for personalized risk stratification and treatment guidance in patients with CNSL.

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ASSOCIATED CONTENT

See accompanying editorial on page 1649

Data Supplement

Video Abstract

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

The clinical management of patients with CNS lymphomas (CNSLs) is characterized by two major challenges. First, despite effective therapies for newly diagnosed primary CNSL and isolated secondary CNSL, clinical outcomes remain highly heterogeneous and many patients nevertheless experience lymphoma progression and mortality.[1,2] Widely used clinical risk models, such as the Memorial Sloan Kettering Cancer Center or International Extranodal Lymphoma Study Group scores, have shown insufficient prognostic value in the era of intensified therapies.[3-6] Separately, the role of response assessment by contrast-enhanced magnetic resonance imaging (MRI) for risk stratification remains largely unclear.[7-10] Therefore, novel approaches for improved prediction of CNSL outcomes are needed to facilitate personalized treatment strategies. Second, the diagnosis of CNSL requires invasive neurosurgical procedures that often cannot be safely performed in certain high-risk situations (eg, in elderly/frail patients or when lesions are located in deep brain structures) or are delayed because of concurrent corticosteroid or antiplatelet therapies.[11-15] Conventional analysis of CSF by cytopathology or flow cytometry and diagnostic MRI have demonstrated suboptimal sensitivity and discriminative capacity to allow surgery-free CNSL diagnosis.[7-9,16-21] Thus, improved methods that overcome these limitations and allow reliable noninvasive

CONTEXT

Key Objective

Clinical outcomes of patients with CNS lymphoma (CNSL) are highly heterogeneous, with current clinical tools lacking the ability for accurate risk stratification. Furthermore, the identification of CNSL requires invasive neurosurgical procedures that are often associated with high perioperative risk or delayed because of concurrent corticosteroid treatment. We here optimized an ultrasensitive high-throughput sequencing technology to explore the potential of circulating tumor DNA (ctDNA) as a clinically useful biomarker in CNSL.

Knowledge Generated

We found that ctDNA levels at diagnosis and during treatment were strongly predictive of clinical outcomes in CNSL. Furthermore, ctDNA profiling from CSF and plasma allowed robust biopsy-free identification of CNSL in a subset of cases.

Relevance *(I.K. Mellinghoff)*

Accurate disease monitoring can be challenging in patients with CNS cancer. The current study suggests that determination of ctDNA in plasma and CSF can be helpful in the assessment of treatment response in patients with CNS lymphoma.*

*Relevance section written by *JCO* Associate Editor Ingo K. Mellinghoff, MD.

identification of CNSL would be transformative for the clinical care of patients with suspected brain lymphomas.

Profiling of circulating tumor DNA (ctDNA) from plasma or CSF has emerged as a promising biomarker in solid and hematologic malignancies, including systemic diffuse large B-cell lymphoma (DLBCL).[22-26] Previous studies in CNSL, however, have shown suboptimal ctDNA detection rates, mostly because of soberingly low ctDNA concentrations in blood plasma and limitations intrinsic to single-gene assays.[27-40] Here, we optimized a customized targeted sequencing approach for improved ultrasensitive ctDNA profiling and explored its utility for noninvasive risk stratification and biopsy-free CNSL identification.

PATIENTS AND METHODS

Patient Enrollment and Sample Collection

We collected 282 tumor, CSF, and plasma samples from 92 patients with primary CNSL or isolated secondary CNSL and 44 patients with other CNS malignancies or inflammatory diseases (non-CNSL), as well as 24 plasma samples from healthy individuals (Data Supplement, online only [Appendix Fig A1, Supplemental Tables S1 and S2]). All patients provided informed consent approved by the local ethics committee in accordance with the Declaration of Helsinki (DRKS00015307) and were continuously enrolled between January 2016 and April 2021 at the University Medical Center Freiburg. Patients were divided into two cohorts: The main CNSL cohort consisted of 67 CNSL cases with available tumor genotyping and matched tumor-plasma/CSF samples (Data Supplement [Appendix Fig A1]). This cohort was used for tumor genotyping, tumor-informed ctDNA quantification/monitoring (Data Supplement [Appendix Fig A1, red part]), and CNSL classification (Data Supplement [Appendix Fig A1, blue part). Median follow-up of this cohort was 14.0 months. The extended cohort consisted of patients with CNSL without matched tumor genotyping specimens and additional non-CNSL patients, and was used for biopsy-free CNSL identification (ie, the classification approach) and tumor-agnostic CSF-ctDNA genotyping (Data Supplement [Appendix Fig A1, blue part]). All patients underwent routine diagnostic procedures and treatment according to study protocols where applicable (ClinicalTrials.gov identifier: NCT02531841 or DRKS00011932), or institutional standards and national/international guidelines. Further details are provided in the Data Supplement.

Sequencing and ctDNA Quantification

Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) was performed as previously described, with detailed information provided in the Data Supplement.[42,43] Targeted genomic regions covered by the customized panel are summarized in the Data Supplement (Supplemental Table S3).[22,41,43] For CSF samples, cell-free and sonicated cellular DNA were pooled before library preparation, with all tumor-derived molecules denoted as CSF-ctDNA throughout the manuscript. For genotyping purposes, somatic alterations were called by paired analysis of either tumor, CSF, or pretreatment plasma with germline DNA, as described before.[42-44] We used Phased Variant Enrichment and Detection Sequencing to quantify ctDNA and monitor phased reporters identified from tumor genotyping samples in plasma or CSF.[44] To determine whether a given sample was positive for ctDNA exceeding the background signal, we applied a previously described Monte Carlo framework.[43,44] Specificity was assessed by monitoring for tumor-derived reporters in plasma samples of healthy donors or in unmatched CSF samples (Data Supplement). Finally, levels of ctDNA were quantified as haploid genome equivalents per milliliter plasma or CSF, determined as the product of total cell-free DNA concentration and the mean allele fraction of somatic reporters.[22,41]

Noninvasive Brain Lymphoma Classification

To noninvasively identify CNSL using ctDNA from plasma and CSF, we built a machine learning algorithm (ensemble

of Kernel Bayesian models) leveraging mutation calls from tumor samples from a large training data set to distinguish between CNSL and non-CNSL lesions. The development of the classifier is detailed in the Data Supplement. The following features were included in the model: total mutation count within the target space, presence of *MYD88* and *CD79B* hotspot mutations, sample assignment to match one of six mutational clusters, as well as mutation counts of the top three defining regions of each cluster (Data Supplement [Supplemental Table S4]). The final model was then applied to an independent withheld set of 183 specimens from both patients with CNSL (n = 62) and patients with other brain cancers or inflammatory processes (ie, non-CNSL patients, n = 44), all of whom underwent invasive neurosurgery as diagnostic workup for a contrast-enhancing brain lesion by MRI (Data Supplement [Appendix Fig A1, blue part, and Supplemental Tables S1 and S5]). Importantly, all patients of the validation cohort had no sign of systemic disease at the time of CSF/plasma analysis. The classifier results were compared with the histopathologic diagnosis as the gold standard.

Statistical Analysis

We used the non-parametric Mann-Whitney U test to compare continuous variables and the Fisher's exact test for categorical variables. Linear relationships were determined using Spearman's correlation coefficient (*r*) or Pearson correlations. Time-to-event variables were visualized using the Kaplan-Meier method. Log-rank test was used to evaluate survival differences. Survival associations were analyzed using Cox proportional-hazards regression. Normalized hazard ratios (z-scores) are reported throughout the manuscript. Progression-free survival (PFS) and overall survival (OS) were estimated according to the revised response criteria for malignant lymphoma.[45] PFS and OS were calculated from start of therapy, or the respective landmark as noted.

RESULTS

Improved ctDNA Detection in Pretreatment Samples

We identified somatic alterations in 100% of tumor genotyping specimens by CAPP-Seq, including recurrent mutations in known driver genes such as *MYD88* (82% of patients; *MYD88* L265P: 73%), *PIM1* (70%), and *CD79B* (61%; Data Supplement [Appendix Fig A2]). The median number of detected mutations per patient was 288 (range, 31-536), with most variants located within the immunoglobulin loci (median, 61%; range, 24-88; Data Supplement [Supplemental Table S6]). We then applied Phased Variant Enrichment and Detection Sequencing to matched pretreatment samples obtained before treatment initiation, either at initial diagnosis or at disease progression, to assess tumor-informed ctDNA detection rates in both plasma and CSF. We identified ctDNA in 61/78 (78%) plasma samples and 24/24 (100%) CSF specimens, with specificities of 96% and 97%, respectively (Fig 1A).

In general, ctDNA levels were highly variable, with significantly higher concentrations observed in CSF compared with plasma (median of 4.16 *v* 0.55 haploid genome equivalents per milliliter, $P = .0004$, Fig 1B and Data Supplement [Appendix Fig A3A]). We further found that CNSL plasma ctDNA concentrations were substantially lower than those in systemic DLBCL (Data Supplement [Appendix Fig A3B and A3C]), even when normalized to radiographic tumor volumes ($P < .0001$, Fig 1C and Data Supplement [Appendix Fig A3D]). By contrast, CSF-ctDNA levels in CNSL were similar to plasma ctDNA concentrations in DLBCL after adjusting for total radiographic tumor volumes (TRTV; Fig 1C).

Clinical Correlates of Pretreatment ctDNA

We next explored whether pretreatment ctDNA detection in plasma and CSF was associated with clinical risk indices or radiographic tumor characteristics. Notably, we did not observe a significant difference in plasma ctDNA levels between patients who received corticosteroids and those without steroid treatment before ctDNA analysis (Fig 1D). In both compartments, ctDNA concentrations at initial diagnosis were not significantly associated with conventional Memorial Sloan Kettering Cancer Center or International Extranodal Lymphoma Study Group scores, the number of lymphoma lesions, and infratentorial, bihemispherical, or deep brain involvement (Data Supplement [Appendix Fig A4A-M]). However, patients with detectable ctDNA in pretreatment plasma had higher tumor volumes ($P = .006$, Fig 1E and Data Supplement [Appendix Fig A5A]) and ctDNA concentrations were strongly correlated with TRTV ($r = 0.53$, $P < .0001$; Fig 1F). Consequently, when monitoring individual single-nucleotide variant alleles identified in tumor biopsies, plasma ctDNA detection rates were markedly higher in patients with high tumor volumes (mean of 37% *v* 12%, $P < .0001$, unpaired t-test), including for key *MYD88* and *CD79B* driver mutations (55% *v* 18%; Data Supplement [Appendix Fig A5B and A5C]).

By contrast, ctDNA concentrations in CSF did not correlate with TRTV (Data Supplement [Appendix Fig A5D]). Here, we found significantly higher ctDNA yields in patients with periventricular involvement compared with those with lymphomas located in other parts of the brain ($P = .005$; Fig 1G and Data Supplement [Appendix Fig A4I]). As a result, significantly more genetic variants were recovered by CSF-ctDNA monitoring in patients with periventricular involvement ($P = .01$, unpaired t-test; Fig 1H and Data Supplement [Appendix Fig A5E]).

Noninvasive Genotyping From CSF

Having demonstrated that normalized CSF-ctDNA yields in CNSL were comparable with those in DLBCL plasma, we hypothesized that CSF-ctDNA could also be used for tumor-agnostic genotyping by CAPP-Seq.[22,24] Indeed, we identified at least one somatic mutation in 76% of CSF samples, with a median of 47 aberrations per patient (Fig 1I). Yet, the ability to detect aberrations was strongly associated with periventricular involvement and CSF DNA input, achieving a 100% detection rate above a threshold of 33 ng (Fig 1I

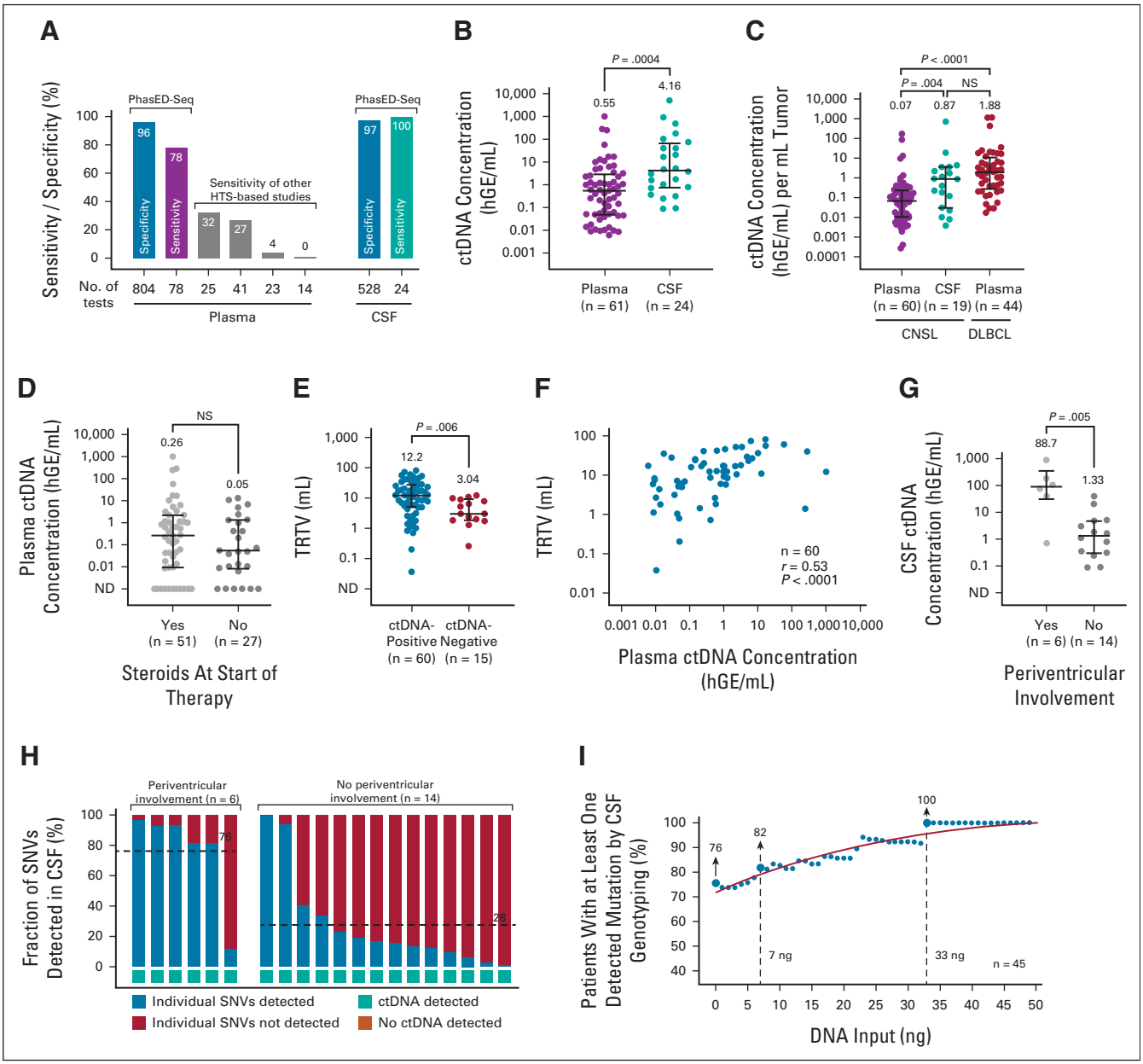

FIG 1. Tumor-informed ctDNA detection/quantification and CSF-ctDNA genotyping in patients with CNSL. (A) ctDNA monitoring in pretreatment plasma and CSF using PhasED-seq. Left: specificity (blue) and sensitivity (purple) of ctDNA monitoring in pretreatment plasma, compared with previous reports using high-throughput sequencing technologies (gray).[29,30,36,37] Right: specificity (blue) and sensitivity (teal) of ctDNA monitoring in pretreatment. (B) Comparison of ctDNA concentrations (in hGE/mL) between pretreatment plasma and CSF in ctDNA-positive cases. (C) Comparison of pretreatment plasma and CSF ctDNA concentrations in CNSL with pretreatment plasma ctDNA concentrations in patients with DLBCL, normalized to TRTV. TRTVs and ctDNA concentrations in patients with DLBCL were derived from a previously published study reported by Kurtz et al.[41] (D) Comparison of ctDNA levels in pretreatment plasma samples between patients receiving corticosteroid treatment and patients without corticosteroid treatment before sample collection. (E) Comparison of TRTV between ctDNA-positive and ctDNA-negative pretreatment plasma samples. (F) Correlation between TRTV and ctDNA concentrations in ctDNA-positive pretreatment plasma samples. (G) Comparison of ctDNA concentrations in pretreatment CSF between patients with periventricular involvement and patients with no sign of periventricular lymphoma localization. Statistical comparisons in (B), (C), (D), (E), and (G) were performed using the Mann-Whitney *U* test. Medians and ranges are indicated. (H) Bar plots depicting the monitoring performance of individual SNVs in pretreatment CSF samples, contrasting patients with (left) or without periventricular involvement (right). Dotted lines show the mean fraction of SNVs detected. Squares below the bar plots show whether ctDNA was detected (teal) or not detected (orange). (I) Biopsy-free genotyping from pretreatment CSF. Individual dots represent the percentage of patients with at least one SNV detected by tumor-agnostic genotyping from pretreatment CSF, ordered by increasing DNA input mass. Dotted lines indicate thresholds at 7 ng and 33 ng DNA input. ng, nanogram. CNSL, CNS lymphoma; ctDNA, circulating tumor DNA; DLBCL, diffuse large B-cell lymphoma; hGE/mL, haploid genome equivalents per milliliter; HTS, high-throughput sequencing; ND, not detected; NS, not significant; PhasED-seq, Phased Variant Enrichment and Detection Sequencing; *r*, Spearman correlation coefficient; SNV, single-nucleotide variant; TRTV, total radiographic tumor volumes.

and Data Supplement [Appendix Fig A5F]). Importantly, we observed highly concordant plasma ctDNA allelic fractions regardless of whether estimated either from tumor- or CSF-informed genotypes ($r = 0.99$, $P = .0002$; Data Supplement [Appendix Fig A5G]). Thus, although tumor genotyping remains the gold standard for molecular profiling in CNSL, our results indicate that mutation detection and monitoring from CSF can be a valid alternative in selected cases (Data Supplement [Appendix Fig A6]).

Prognostic Value of Pretreatment Plasma ctDNA

We next investigated whether pretreatment plasma ctDNA assessed before treatment initiation at initial diagnosis and at lymphoma progression could enable identification of patients at highest risk for unfavorable clinical outcomes. Eighty-two percent of ctDNA-positive cases experienced lymphoma progression within 1 year and 78% of such patients died within 2 years of plasma profiling. By contrast, when ctDNA was undetectable, 68% of patients remained disease-free and 90% were alive at these same landmarks (Figs 2A and 2B). In log-rank analyses, ctDNA-positive patients had significantly shorter PFS and OS, with corresponding hazard ratios of 4.6 (95% CI, 2.7 to 8.0; $P < .0001$) and 9.6 (95% CI, 5.0 to 18.3; $P = .0001$; Figs 2C and 2D).

In multivariate Cox regression analyses accounting for key clinical and radiographic features known to carry prognostic value, higher continuous ctDNA levels (HR, 1.4; 95% CI, 1.0 to 1.9; $P = .03$; HR, 1.6; 95% CI, 1.1 to 2.2; $P = .006$) and lower Karnofsky performance status (HR, 1.6; 95% CI, 1.1 to 2.2; $P = .005$; HR, 1.7; 95% CI, 1.1 to 2.5; $P = .004$) were significantly and independently associated with inferior PFS and OS (Figs 2E and 2F). Importantly, the prognostic value of ctDNA was maintained when restricting analyses to frontline assessment of treatment-naive patients (Data Supplement [Appendix Fig A7]).

Prognostic Value of On-Treatment Plasma ctDNA

Evaluation of ctDNA early during treatment has emerged as a promising biomarker in diverse tumor types.[23,41,46-48] Therefore, we investigated the performance of on-treatment measurable residual disease monitoring for outcome prediction by profiling ctDNA in plasma samples collected during curative-intent induction immunochemotherapy from 28 CNSL cases (Fig 3A). Although ctDNA was identified in 76% of patients experiencing lymphoma progression or death, it was not detectable during treatment in 91% of cases who were disease-free after therapy completion (Data Supplement [Appendix Fig A8A]). Consequently, we found that patients with positive ctDNA at any time point during treatment had significantly inferior PFS (HR, 6.2; 95% CI, 2.3 to 16.7; $P = .0002$) and OS (HR, 7.9; 95% CI, 2.6 to 23.9; $P = .004$) than ctDNA-negative cases (Fig 3B and Data Supplement [Appendix A8B]). To control for guarantee-time bias, we explored whether this association remained significant in a landmark analysis, assessing ctDNA within the first two cycles of induction. Indeed, ctDNA profiling in blood plasma within this 3-week window reflected clinical outcomes, with ctDNA positivity strongly predicting unfavorable PFS (HR, 4.7; 95% CI, 1.5 to 15.1; $P = .003$) and OS (HR, 6.5; 95% CI, 1.8 to 23.8; $P = .001$; Fig 3C). However, three on-treatment plasma samples were ctDNA-negative in patients with overt radiographic CNSL, representing false-negative results (Fig 3A). Collectively, our data suggest that serial ctDNA monitoring early during therapy could provide key advantages for CNSL response assessment in individual patients (Data Supplement [Appendix Fig A9]).

Biopsy-Free Diagnosis of CNSL by ctDNA Profiling

Distinguishing CNSL from other primary brain tumors or brain metastases through surgery-free procedures remains challenging.[8,17] Thus, we next sought to develop a method that facilitates noninvasive diagnosis of CNSL on the basis of ctDNA mutational patterns and burden in CSF or plasma. We first trained statistical models to distinguish CNSL from non-CNSL malignancies informed by the distribution and frequency of mutations in tumor tissue, and summarized these models into a classifier score. For this purpose, we combined CNSL tumor sequencing profiles from this study (n = 30) with tumor mutational landscapes of non-CNSL malignancies retrieved from public databases (n = 2,647; Data Supplement [Appendix Fig A1, Supplemental Table S7]). We then benchmarked the classifier's performance in 53 withheld tumor specimens, where it correctly identified CNSL in 34/35 (97%) cases and non-CNSL in 18/18 (100%) patients (Fig 4A). Finally, we validated the classifier in an independent withheld set of 130 CSF and plasma samples profiled by CAPP-Seq. Our approach achieved a sensitivity of 59% (27/46) from CSF and 25% (13/52) from plasma for correctly identifying CNSL, reflecting the difference in ctDNA concentrations and allelic fractions between the two compartments (Fig 1B). Of note, the classifier maintained 100% specificity and positive predictive value (PPV) in our study for single diagnostic plasma (n = 16) and CSF samples (n = 16) obtained from non-CNSL patients (Figs 4A and 4B). Conventional CSF analyses by flow cytometry and cytopathology identified CNSL in 22% of patients (Fig 4B).

DISCUSSION

We here describe the efficacy of an improved high-throughput molecular profiling approach for noninvasive ctDNA assessment in patients with CNSL.[29-31,36,37] By using single-gene assays covering clonotypic immunoglobulin V(D)J rearrangements or the *MYD88* L265P hotspot mutation, several previous studies have shown in principle that ctDNA is present in plasma and CSF of patients with CNSL.[27-37] However, plasma ctDNA detection rates in these studies were suboptimal even before initiation of therapy, mostly because of minute amounts of ctDNA in patients with

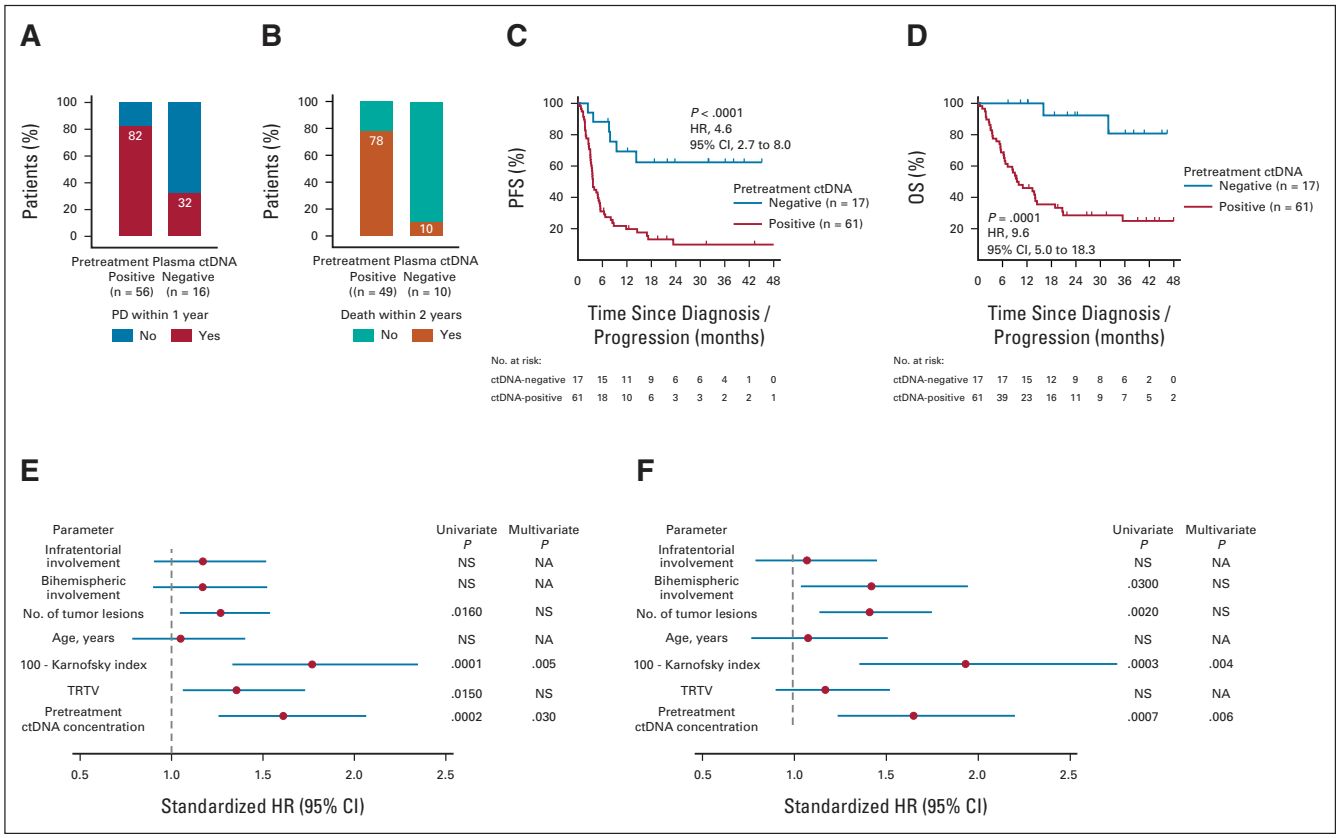

FIG 2. Prognostic value of ctDNA in pretreatment plasma samples. Bar graphs showing the percentage of cases with (A) progressive disease within 1 year or (B) death within 2 years after ctDNA analysis, stratified by positive and negative pretreatment ctDNA. Cases censored before 1 year in (A) or 2 years in (B) were not considered for this analysis. Kaplan-Meier analysis of (C) PFS and (D) OS in patients with detectable (red) and undetectable pretreatment plasma ctDNA (blue). Forest plots showing standardized hazard ratios for (E) PFS and (F) OS estimated by univariate and multivariate Cox proportional-hazards regression outcome analyses, incorporating ctDNA concentrations, TRTV, and key clinical and radiographic indices. ctDNA, circulating tumor DNA; HR, hazard ratio; NA, not assessed (ie, not considered for multivariate analysis); NS, not significant; OS, overall survival; PD, progressive disease; PFS, progression-free survival; TRTV, total radiographic tumor volumes.

brain cancer and limited applicability of those single-gene assays.[27-32,38-40] Thus, ctDNA has not yet emerged as a useful clinical biomarker for risk stratification, outcome prediction, disease detection, or assessment of clonal heterogeneity in CNSL.[31] Here, we overcome some of these obstacles and demonstrate substantial improvements in ctDNA detection and profiling through novel methodologic advances of our high-throughput sequencing–based technologies.[44] Despite being in part limited by small samples sizes, this allowed us to unveil ctDNA characteristics in both plasma and CSF in relation to radiographic features assessed by MRI and other clinical properties.[41] For example, we revealed that plasma ctDNA accurately mirrors tumor burden in the CNS, while CSF-ctDNA levels are largely reflective of disease localization. Furthermore, although we found numerically higher ctDNA levels in plasma after corticosteroid therapy that were not statistically significant, these observations require further examinations in larger patient cohorts also adjusting for dose and duration of such exposure. Most importantly, to the best of our knowledge, this study is the first to demonstrate potential utility of ctDNA at various disease milestones in CNSL.

One major finding of our study was the association of ctDNA detection with clinical outcomes in CNSL. We observed ctDNA detection in plasma before therapy and early during treatment to be a strikingly strong and independent prognostic biomarker. These results may help to overcome limitations of radiographic response assessment,[8-10] and might also have significant implications for future risk-adapted treatment strategies. The identification of patients at exceptionally low or high risk for disease progression could provide opportunities for early interventions and adjustments to current standard therapies. Although high-risk patients might benefit from early treatment intensification or alternative innovative strategies such as CNS bioavailable small molecules, immunomodulatory drugs, or chimeric antigen receptor T-cell therapies, patients with predicted favorable outcomes on the basis of ctDNA could do just as well with treatment de-escalation.[49-53] Interestingly, in contrast to other recent reports,[54-57] age was not a prognostic factor in our study, which may reflect selective inclusion of elderly patients with good performance status.

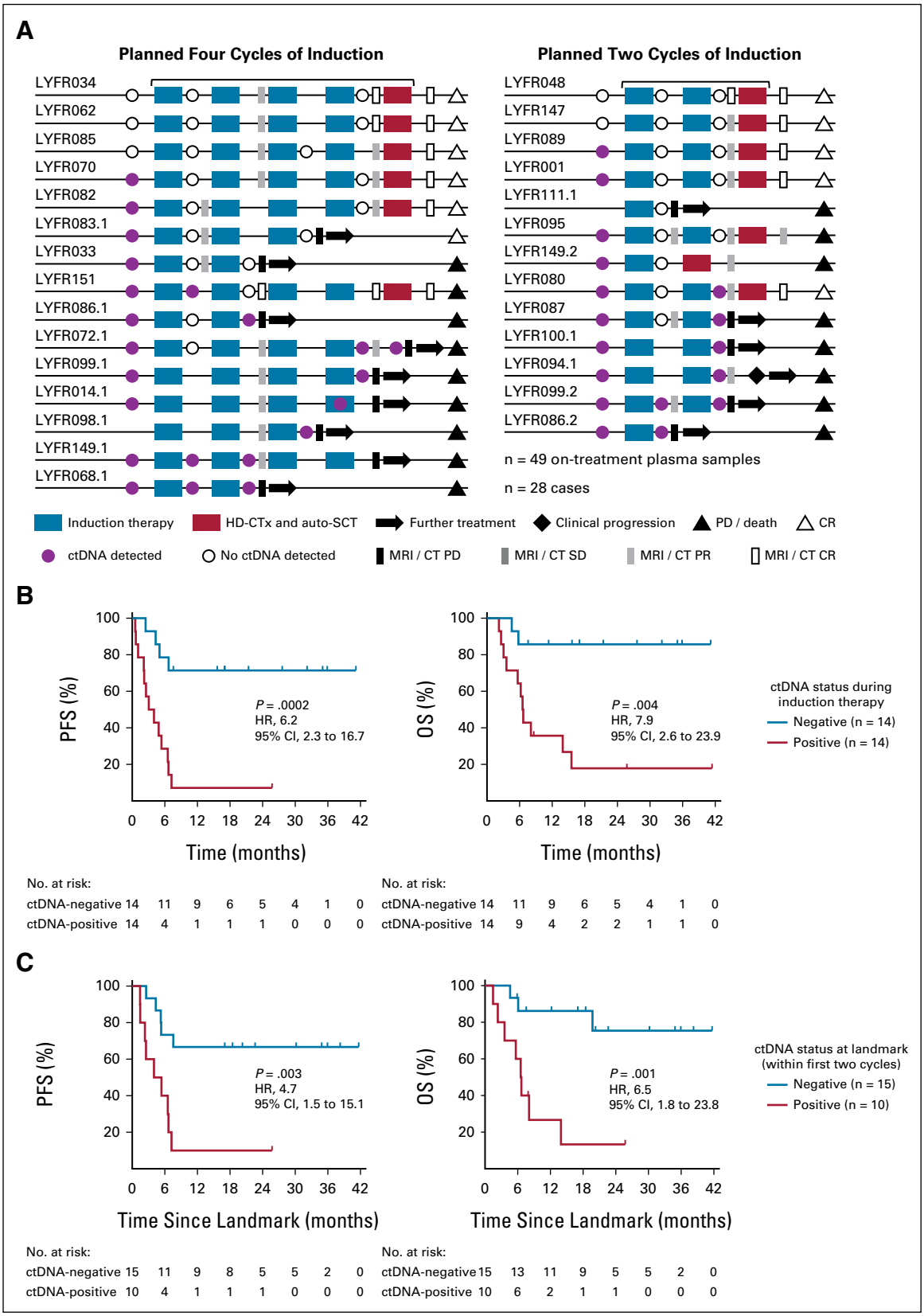

FIG 3. On-treatment plasma ctDNA as a prognostic biomarker. (A) Detection of ctDNA during curative-intent treatment as a function of time. Patient-level data demonstrating ctDNA detection during either intended four-cycle (left) or two-cycle induction therapy (right, blue horizontal bars). Relapses/progression were confirmed either clinically (black rhombus) or radiographically (black rectangle). (continued on following page)

FIG 3. (Continued). Black arrows indicate further treatment following disease progression during treatment. Triangles show the ultimate clinical outcome, either progression/death (black triangle, always accompanied by radiographic or clinical disease progression) or CR (open triangle). Red bars represent HD-CTx followed by auto-SCT. Purple circle, ctDNA detected; open circle, ctDNA not detected; black rectangle, PD by MRI/CT scan; dark gray rectangle, SD by MRI/CT scan; light gray rectangle, PR by MRI/CT scan; open rectangle, CR by MRI/CT scan. (B) Kaplan-Meier analysis of PFS (left) and OS (right) in patients with at least one ctDNA-positive plasma sample at any time point during induction therapy (red) compared with patients without detectable ctDNA during induction therapy (blue). (C) Kaplan-Meier analysis of PFS (left) and OS (right) in patients with positive ctDNA within the first two cycles of induction therapy (red) compared with patients without detectable ctDNA during the first two cycles of induction treatment (blue). Significance in (B) and (C) was assessed using the log-rank test. auto-SCT, autologous stem-cell transplantation; CR, complete remission; CT, computed tomography; ctDNA, circulating tumor DNA; HD-CTx, high-dose chemotherapy; HR, hazard ratio; MRI, magnetic resonance imaging; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial remission; SD, stable disease.

Histopathologic tumor assessment following invasive stereotactic biopsy is the gold standard procedure to diagnose brain lymphomas. However, in high-risk situations or when patients obtain concurrent corticosteroids or antiplatelet treatment, stereotactic biopsies are often delayed, or a final diagnosis remains unconfirmed. In these situations, noninvasive disease classification could have transformative impact on the clinical management of patients with suspected intracranial lymphoma, particularly in the light of low CNSL identification rates by conventional CSF analyses or diagnostic imaging.[8,9,16,17,19-21,58-60] Previous seminal studies have shown in principle that assessment of CSF-ctDNA allows noninvasive identification of brain cancers and metastases.[19,38,59,61-64] In CNSL specifically, prior work explored strategies leveraging specific proteins, microRNAs, or genetic aberrations for biopsy-free identification of CNSL

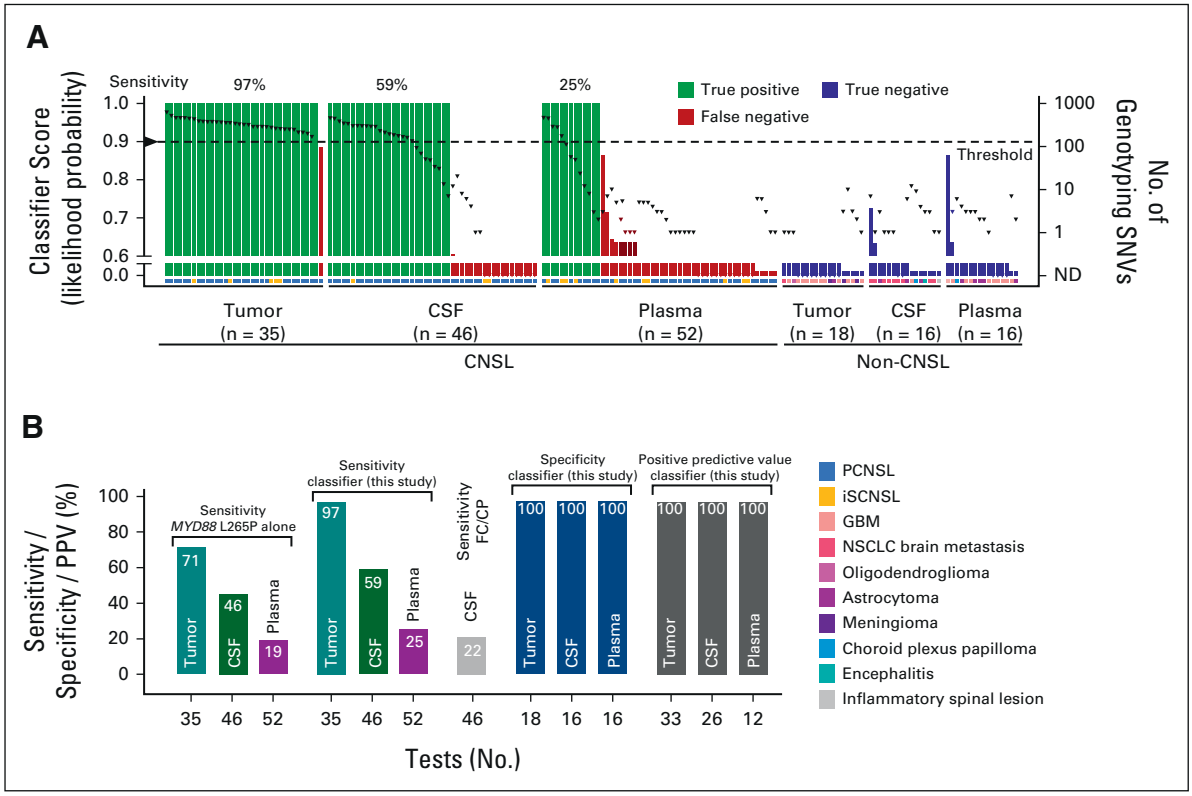

FIG 4. Noninvasive brain lymphoma classification. (A) Classifier scores are shown for each tumor, CSF, and plasma sample of the independent validation cohort (CNSL, left; non-CNSL, right), ordered by decreasing scores within each group (left *y*-axis). Brain cancer entities or diseases are depicted in different colors at the bottom. True-positive cases are shown in green, false-negative cases in red, and true-negative cases in blue. Black triangles represent the number of SNVs identified by Cancer Personalized Profiling by Deep Sequencing genotyping (right *y*-axis). The dashed line highlights the threshold for CNSL classification. (B) Sensitivities of correct CNSL diagnosis from either tumor (teal), CSF (green), or plasma samples (purple) by detection of *MYD88* L265P hotspot mutation alone or by the classification algorithm developed in this study. In addition, sensitivity for CNSL detection by CSF FC and CP is shown in light gray; and specificities (blue) and PPV (dark gray) of the classification algorithm are displayed for tumor, CSF, and plasma specimens. CNSL, CNS lymphoma; CP, cytopathology; FC, flow cytometry; GBM, glioblastoma; iSCNSL, isolated secondary CNSL; ND, not detected; NSCLC, non–small-cell lung cancer; PCNSL, primary CNSL; PPV, positive predictive values; SNV, single-nucleotide variant.

from CSF.[16,17,19,39,61,65-68] However, these studies are in part limited by low samples sizes and lack of independent validation, which introduces the risk of overfitting. This is particularly relevant for protein biomarkers such as interleukin 10 and interleukin 6, for which various and unvalidated thresholds have been used.[17,65,66,68-70] Furthermore, most methods have shown suboptimal specificity, which would lead to inappropriate treatment of non-CNSL cases that are misclassified as CNSL.[17] Finally, single-gene assays do not capture the genetic complexity of the disease, limiting the applicability of these approaches.[17,19,39,69] Here, we propose a novel machine learning model leveraging the genomic pattern and burden of somatic mutations for biopsy-free CNSL identification from ctDNA. We demonstrated robust performance of our assay with 59% sensitivity from CSF-ctDNA, maintaining high specificity and PPV in our cohort.

Although the results presented here are highly promising, hurdles remain to be overcome. First, the value of ctDNA as a biomarker for CNSL identification and monitoring needs to be validated in additional prospective studies, including detailed comparisons with state-of-the-art imaging techniques and assessing the role of concomitant corticosteroid therapy. Specifically, our noninvasive classifier requires further testing on larger cohorts of nonlymphoma patients comprising a wide range of malignant and nonmalignant entities to confirm its high PPV and to further assess the role of certain parameters such as tumor mutational burden. Furthermore, the classifier needs to be vetted in clinical settings that include the necessity for real-time sample collection and processing, and applied in clinically challenging situations that often delay or prohibit biopsies (eg, corticosteroid or antiplatelet treatment, inaccessible location of suspected lesions). Finally, additional technical advances are required to further enhance the sensitivity of our approach and reduce the rate of false-negative ctDNA analyses.

In summary, we have developed a promising noninvasive approach to identify and monitor CNSL that, despite current limitations, might allow to complement clinical standard procedures in the future.

AFFILIATIONS

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Circulating Tumor DNA Profiling for Detection, Risk Stratification, and Classification of Brain Lymphomas

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