ABSTRACT

Introduction: Most patients (70%) with limited-stage SCLC (LS-SCLC) who are treated with curative-intent therapy suffer disease relapse and cancer-related death. We evaluated circulating tumor DNA (ctDNA) as a predictor of disease relapse and death after definitive therapy in patients with LS-SCLC.

Methods: In our previous work, we developed a plasma-based ctDNA assay to sequence 14 genes (TP53, RB1, BRAF, KIT, NOTCH1-4, PIK3CA, PTEN, FGFR1, MYC, MYCL1, and MYCN) that are frequently mutated in SCLC. In this work, we evaluated 177 plasma samples from 23 patients with LS-SCLC who completed definitive chemoradiation (n = 21) or surgical resection (n = 2) and had an end-of-treatment blood collection (median 4 d, range 0–40 d from treatment completion) plus monthly surveillance blood sampling. Median overall survival (OS) and progression-free survival (PFS) were compared using a Wilcoxon test.

Results: The median OS among patients in whom we ever detected ctDNA after definitive treatment (n = 15) was 18.2 months compared with a median OS of greater than 48 months among patients in whom we never detected ctDNA after definitive treatment (n = 8; p = 0.081). The median PFS among patients in whom we ever detected ctDNA after definitive treatment was 9.1 months compared with a median PFS of greater than 48 months among patients in whom we never detected ctDNA after definitive treatment (p < 0.001).

Conclusions: Detection of ctDNA in patients with LS-SCLC after curative-intent therapy predicts disease relapse and death. Prospective trials using ctDNA as an integral biomarker for therapeutic selection should be considered in SCLC.

*Corresponding author.

Disclosure: Dr. Lovly has served as a consultant for Pfizer, Novartis, AstraZeneca, Genoptix, Sequenom, ARIAD, Takeda, Foundation Medicine, Blueprints Medicine, Achilles, and Cepheid; has been an invited speaker for Abbott and Qiagen; and has received research funds (to her university) from Novartis, AstraZeneca, and Xcovery. Dr. Horn is a consultant for AstraZeneca, EMD Serono, Genentech-Roche, Tesaro, Pfizer, Incyte AbbVie, Bristol-Myers Squibb, Merck & Co., and Xcovery and has received research support from Xcovery, Bristol-Myers Squibb, and Boehringer Ingelheim. Dr. Iams reports consulting for Genentech, Outcomes Insights, and Defined Health and clinical trial funding from EMD Serono. Ms. Bertucci, Mr. Shaffer, Ms. Hodsdon, Dr. Garg, Dr. Hosseini, and Dr. Lim are employees and shareholders of Resolution Bioscience. The remaining authors declare no conflict of interest.

Address for correspondence: Christine M. Lovly, MD, PhD, Department of Medicine, Division of Hematology and Oncology, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 2220 Pierce Avenue, 777 Preston Research Building, Nashville, TN 37232-6307. E-mail: christine.lovly@vumc.org

Cite this article as: Iams WT, et al. Blood-Based Surveillance Monitoring of Circulating Tumor DNA From Patients With SCLC Detects Disease Relapse and Predicts Death in Patients With Limited-Stage Disease. JTO Clin Res Rep 1:100024

© 2020 The Authors. Published by Elsevier Inc. on behalf of the International Association for the Study of Lung Cancer. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

ISSN: 2666-3643

https://doi.org/10.1016/j.jtocrr.2020.100024
Introduction

Lung cancer is the most common cause of cancer-related death in the United States, with SCLC comprising 15% of cases and accounting for 30,000 deaths annually.\(^1,2\)

Although SCLC is initially responsive to chemotherap\y in most patients,\(^3\) most patients with limited-stage SCLC (LS-SCLC) (70%) have lethal disease recurrence (30% locoregional and 70% distant)\(^4\) with a median overall survival (OS) of 25 to 30 months.\(^5\) Currently, after completion of concurrent chemoradiotherapy or definitive surgery and adjuvant chemotherapy, patients with LS-SCLC are monitored with conventional radiography (typically computed tomography [CT] scans) every 2 to 3 months. There is an unmet need to identify microscopic disease after definitive therapy in patients with LS-SCLC to intervene and attempt to prolong patient survival.

The detection of both circulating tumor cells (CTCs)\(^6-12\) and circulating tumor DNA (ctDNA)\(^14-19\) has been well validated in patients with SCLC. On the basis of our previous work revealing the ability of ctDNA detection to precede radiographic progression in patients with SCLC,\(^20\) we hypothesized that detection of ctDNA in patients with LS-SCLC after definitive therapy would predict disease relapse and death.

Materials and Methods

Study Design and Patients

Patients with LS-SCLC treated at the Vanderbilt-Ingram Cancer Center were prospectively identified and consented using an institutional review board (IRB #030763)–approved protocol for collection of blood up to once per month plus medical record review. All samples were de-identified, and protected health information was reviewed according to the Health Insurance Portability and Accountability Act guidelines. Similar to analogous work, predominantly in patients with NSCLC,\(^21\) patients in whom blood was collected within 2 months of completion of definitive chemoradiation or surgical resection were included (Fig. 1). For eligible patients, all additional blood samples available were analyzed, resulting in the following breakup: 13 samples from treatment-naive patients, 15 samples from patients on definitive treatment, and 23 patients with analyzable end-of-treatment samples (Supplementary Table 1).

Blood Samples and Cell-Free DNA Isolation

Blood samples (7.5 mL per tube) were collected in Streck tubes (Streck Inc., Omaha, NE) at up to monthly intervals at time points before, during, and after therapy. Blood was centrifuged at 1200g for 30 minutes. Plasma was removed and recentrifuged at 500g for 10 minutes and immediately aliquoted and stored at -80°C. DNA was extracted from the patients’ plasma samples using circulating nucleic acid extraction kits following the manufacturer’s instructions except for samples that were incubated with proteinase K for 1 hour rather than 30 minutes (Qiagen, Hilden, Germany). The yield of the double-strand DNA was quantified using a Qubit fluorometer (Thermo Fisher, Waltham, MA) and the corresponding double-strand DNA quantification kit. Approximately 40 to 100 ng of ctDNA, depending on the yield of ctDNA from the sample, was used for the library construction.

Targeted Next-Generation Sequencing

A detailed description of the platform used for ctDNA sequencing has been provided in a previous publication.\(^20\) Briefly, the panel contains 1608 probes that target all coding exons of BRAF, KIT, NOTCH1-4, PIK3CA, PTEN, RB1, and TP53. The panel also contains probes for copy variation detection in the genes FGFR1, MYC, MYCII, and MYCN, and control probes that target select regions in all 22 autosomes. Full next-generation sequencing results for all patients and time points are included in Supplementary Table 1.

Statistical Analysis

Patient demographics and clinical information were summarized with median and range for continuous variables and frequency and percentage for categorical variables. The primary study end points were progression-free survival (PFS) and OS. PFS was defined as the time from the first treatment start date to the date of radiographic relapse, previous follow-up without progression, or death. OS was defined as the date of disease diagnosis to the date of all-cause death or previous follow-up. The Kaplan-Meier method, log-rank test, and Cox proportional hazard models were used to investigate the associations between PFS and OS and ctDNA status. Estimated hazard ratios (HRs) and 95% confidence intervals (CIs) were provided to measure the effect of the association between ctDNA clearance with

Keywords: Small cell lung cancer; Circulating tumor DNA; Minimal residual disease; Liquid biopsy; Next-generation sequencing
All statistical inferences were assessed using a two-sided 5% significance level, and all summary statistics, graphics, and survival models were generated using R version 3.6 statistical software.

Results

Patient Demographics and Treatment

We prospectively enrolled 23 participants with a median age of 70 years (Table 1) over a period of 42 months. For subsequent analyses, we divided the cohort of patients into the following two groups: patients in whom we never detected tumor-associated cell-free DNA (circulating tumor DNA [ctDNA]; n = 8) after definitive therapy and (2) patients in whom we ever detected circulating tumor DNA after definitive therapy (n = 15). CT, computed tomography.

PFS and OS. All statistical inferences were assessed using a two-sided 5% significance level, and all summary statistics, graphics, and survival models were generated using R version 3.6 statistical software.

PFS and OS by ctDNA Detection After Definitive Treatment

The median PFS among patients in whom we ever detected ctDNA was 9.1 months compared with a median PFS of greater than 48 months among patients in whom we never detected ctDNA (p < 0.001) (Fig. 2A). The median OS among patients in whom we ever detected ctDNA was 18.2 months compared with a current median OS of greater than 48 months among patients in whom we never detected ctDNA (p = 0.081) (Fig. 2B).

At the time of analysis, four of the 15 patients (26%) in whom ctDNA was ever detected remain alive, whereas seven of the eight patients (88%) in whom we never detected ctDNA remain alive.

Clinical Sequelae and Genomic Sequencing Results at ctDNA Detection

A summary of all patient cases with salient genomic changes is provided in Table 2. There was one lethal relapse in a patient in whom we never detected ctDNA. This individual withdrew consent for longitudinal blood collections 5 months and 18 days before disease relapse. The patient's disease relapsed locally (in the hilar area).
and was identified during hospitalization for an acute cerebrovascular accident with substantial debility. The patient enrolled in hospice care without receiving second-line systemic therapy and died 18 months after the diagnosis.

Among the 15 patients in whom we ever detected ctDNA, two have not had disease recurrence (11 and 20 mo after completion of definitive chemoradiation; patient IDs 21 and 19, respectively, in Table 2). Three patients in whom we ever detected ctDNA died without clear radiographic progression (with the most recent CT imaging 1, 2, and 7 mo before death; patient IDs 7, 12, and 16, respectively, in Table 2). In the remaining 10 patients, CT recurrence was first identified at an intrathoracic site in four patients, only at an extrathoracic site in three patients (including one patient with brain-only recurrence), and at both intra- and extrathoracic sites in three patients (Fig. 3). Among the 10 patients in whom radiographic relapse was identified, three did not receive further systemic therapy; three received nivolumab plus ipilimumab with best responses of stable disease in one patient and progression of disease at first evaluation in two patients; two patients with platinum-sensitive disease received platinum rechallenge and both initially responded; one patient enrolled on a clinical trial of a CHK1 inhibitor (LY2606368; NCT02735980)23 and responded; and one patient received paclitaxel and progressed at first disease evaluation. Only patients who clinically responded to second-line systemic therapy experienced bloodstream clearance of their ctDNA (three of seven, 43%).

At the first time of detection of ctDNA in the ever detected cohort, the median variant allele frequency (VAF) was 0.4%, with a range of 0.15% to 15.05%. In 11 of the 15 patients in whom we ever detected ctDNA, there was a TP53 variant at greater than or equal to first ctDNA Table 1. Baseline Demographics of Patients With Limited-Stage SCLC in Our Study Cohort

|                        | Overall (N = 23) | ctDNA Never Detected After Definitive Treatment (n = 8) | ctDNA Ever Detected After Definitive Treatment (n = 15) |
|------------------------|-----------------|--------------------------------------------------------|--------------------------------------------------------|
| Median age, y (range)  | 70 (43-82)      | 66 (43-75)                                             | 70 (53-82)                                             |
| Median follow-up, d (range) | 524 (70-1474)  | 748 (331-1474)                                         | 510 (70-860)                                          |
| Sex, no. (%)           |                 |                                                        |                                                        |
| Female                 | 16 (70)         | 5 (63)                                                 | 11 (73)                                                |
| Male                   | 7 (30)          | 3 (37)                                                 | 4 (27)                                                 |
| Ethnicity, no. (%)     |                 |                                                        |                                                        |
| White                  | 20 (88)         | 8 (100)                                                | 12 (80)                                                |
| Black                  | 2 (8)           | 2 (13)                                                 | 1 (7)                                                  |
| Asian                  | 1 (4)           | 1 (4)                                                  | 1 (7)                                                  |
| Year of diagnosis      |                 |                                                        |                                                        |
| 2015                   | 6               | 1                                                      | 5                                                      |
| 2016                   | 5               | 3                                                      | 2                                                      |
| 2017                   | 8               | 2                                                      | 6                                                      |
| 2018                   | 4               | 2                                                      | 2                                                      |
| 2019                   | 0               | 0                                                      | 0                                                      |
| TNM stage at diagnosis |                 |                                                        |                                                        |
| IA2                    | 2               | 1                                                      | 1                                                      |
| IA3                    | 1               | 1                                                      | 0                                                      |
| IB                     | 1               | 1                                                      | 0                                                      |
| IIA                    | 0               | 0                                                      | 0                                                      |
| IIB                    | 2               | 1                                                      | 1                                                      |
| IIIA                   | 11              | 2                                                      | 9                                                      |
| IIIB                   | 3               | 1                                                      | 2                                                      |
| IIIC                   | 3               | 1                                                      | 2                                                      |
| First-line treatment, no. (%) |     |                                                        |                                                        |
| Platinum/etoposide with radiation | 21 (92) | 6 (75)                                                 | 15 (100)                                               |
| Surgical resection     | 1 (4)           | 1 (12.5)                                               |                                                        |
| Surgical resection with chemotherapy | 1 (4) | 1 (12.5)                                               |                                                        |
| Prophylactic cranial irradiation, no. (%) | 10 (43) | 4 (50)                                                 | 6 (40)                                                 |
| Clinical treatment response, no. (%) |            |                                                        |                                                        |
| Partial response       | 21 (91)         | 6 (75)                                                 | 15 (100)                                               |
| Complete response      | 2 (9)           | 2 (25)                                                 | 0 (0)                                                 |

ctDNA, circulating tumor DNA.
detection after definitive therapy, the detected variants were in \textit{BRAF}, \textit{NOTCH1}, \textit{NOTCH3}, and \textit{PIK3CA} (patient IDs 6, 5, 19, and 11, respectively, in Table 2).

\textbf{Exemplary Patient Cases}

As a case demonstrative of the use of ctDNA after definitive therapy for patients with LS-SCLC, patient ID 14 (Table 2; Fig. 4A and B) was diagnosed with stage IIIA disease with a 7-mm upper lobe lung primary and biopsy-confirmed ipsilateral hilar and mediastinal lymph node involvement. She was 74 years at diagnosis, a former smoker with a 50 pack-year history, and Eastern Cooperative Oncology Group performance status score of 1. She had a ctDNA assessment at diagnosis with multiple findings: \textit{TP53} E298* at 44.4%, \textit{RB1} E313* at 40.86%, \textit{PIK3CA} E726K at 13.05%, \textit{KIT} N655H at 2.36%, a \textit{PIK3CA} amplification, and a \textit{PTEN} deletion. She was treated with concurrent chemoradiation with carboplatin plus etoposide, and she had a complicated treatment course with febrile neutropenia and pancytopenia limiting her chemotherapy to two cycles. Four days before her final first-line chemotherapy dose (day 50), she continued to have detectable ctDNA: \textit{TP53} E298* at 0.96%, \textit{RB1} E313* at 0.96%, \textit{PIK3CA} E726K at 0.4%, and \textit{KIT} N655H at 0.1%. She completed 68 gray thoracic radiation 6 weeks after her previous dose of chemotherapy, and at that time, she had no detectable ctDNA in her end-of-treatment blood sample (day 96). At her follow-up with medical oncology four weeks after completion of radiation (day 117), she again had detectable ctDNA (\textit{TP53} E298* at 0.26%, \textit{RB1} E313* at 0.17%, \textit{PIK3CA} E726K at 0.11%), but a partial response on chest CT, with expected radiation-related changes, was observed. She proceeded to receive prophylactic cranial irradiation, and at her follow-up 3 months after therapy completion, she had multiple sites of disease recurrence (a fluorodeoxyglucose avid 1.5 cm paratracheal lymph node, an avid 3.4 cm adrenal metastasis, and an avid 1.7 cm renal metastasis) with a significant rise in her ctDNA: \textit{TP53} E298* at 8.63%, \textit{RB1} E313* at 8.17%, and \textit{PIK3CA} E726K at 3.06%. She was treated with nivolumab plus ipilimumab, but she had significant disease progression at first evaluation with both enlargement of her preexisting adrenal lesion and new areas of disease (retroperitoneal, supraclavicular, and para-aortic lymph nodes, contralateral adrenal lesion) prompting a change of therapy to paclitaxel. At the time of progression on nivolumab plus ipilimumab, her ctDNA was notably increasing: \textit{TP53} E298* at 45.28%, \textit{RB1} E313* at 45.88%, \textit{PIK3CA} E726K at 12.2%, \textit{NOTCH4} C205* at 0.24%, \textit{NOTCH1} P711P at 0.05%, a \textit{PIK3CA} amplification, and a \textit{PTEN} deletion. Her disease continued to progress on paclitaxel, with initial ctDNA stabilization, followed by a notable rise on a blood draw 1 week before her death (she transitioned to hospice care 1 week before death): \textit{TP53} E298* at 56.55%, \textit{RB1} E313* at 59.27%, \textit{PIK3CA} E726K at 16.51%, \textit{NOTCH1} P711P at 1.05%, a \textit{PIK3CA} amplification, and a \textit{PTEN} deletion.

In contrast to the aforementioned case, patient ID 4, who we described in our previous publication\textsuperscript{20} and

\begin{figure}
\centering
\includegraphics[width=\linewidth]{figure2.png}
\caption{Progression-free and overall survival for study cohort of patients with limited-stage SCLC. (A) Progression-free survival of the cohort of patients in whom we ever detected circulating tumor DNA (ctDNA) after definitive therapy (n = 15, blue line) versus never detected ctDNA after definitive therapy (n = 8, red line). (B) Overall survival of the cohort of patients in whom we ever detected ctDNA after definitive therapy (n = 15, blue line) versus never detected ctDNA after definitive therapy (n = 8, red line). LS-SCLC, limited-stage SCLC.}
\end{figure}
| Patient ID | Age at Dx (y) | Sex | TNM Stage at Dx | Treatment- Naive Specimen | TP53 VAF (%) in Treatment-naive Specimen | Treatment Completion Specimen Peak Mutation VAF (%) | TP53 VAF (%) in First Posttreatment Specimen | Months Between ctDNA Detection and Radiographic Relapse or Death | Site(s) of Relapse | PFS (mo) | OS (mo) |
|-----------|--------------|-----|-----------------|----------------------------|----------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|------------------|---------|-------|
| 1         | 65           | Female | IIIA            | No tx-naive sample         | No detectable ctDNA                   | R175H 0.67%                                  | 0                                             | Brain                                              | 6                | 18      |       |
| 2         | 54           | Female | IIIC            | Yes | Q192 38.48%        | No detectable ctDNA                   | Q192 12.6%                                  | 0                                             | Adrenal, thoracic LN                                 | 6                | 23      |       |
| 3         | 74           | Male   | IIIA            | Yes | R65 32.2%          | No detectable ctDNA                   | R65 1.73%                                   | 3                                             | Bone marrow                                          | 6                | 9       |       |
| 4         | 39           | Female | IIIA            | Yes | None               | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 48                | 50      |       |
| 5         | 74           | Male   | IIIA            | No  | No tx-naive sample | No detectable ctDNA                   | None                                         | 0                                             | Pulmonary nodules                                     | 9                | 23      |       |
| 6         | 65           | Female | IIIB            | No  | No tx-naive sample | No detectable ctDNA                   | BRAF K601E 0.65%                            | None                                         | 12                                           | Lung, hilar LN                                      | 12                | 29      |       |
| 7         | 72           | Male   | IIIA            | Yes | No tx-naive sample | No detectable ctDNA                   | TP53 K351 0.22%                             | K351 0.22%                                     | Unknown                                             | 1                | 3       |       |
| 8         | 65           | Female | IIIC            | No  | No tx-naive sample | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 40                | 42      |       |
| 9         | 55           | Male   | IIIB            | Yes | E204 26.08%        | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 32                | 40      |       |
| 10        | 61           | Female | IIIB            | Yes | E258K 2.1%         | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | Lung                                             | 14                | 18      |       |
| 11        | 79           | Female | IIIB            | Yes | No detectable ctDNA | No detectable ctDNA                   | None                                         | 6                                             | Lung                                             | 4                 | 16      |       |
| 12        | 67           | Female | IA2             | No  | No tx-naive sample | TP53 V272L 0.4%                       | V272L 0.4%                                  | 4                                             | Unknown (clinical POD)                              | 4                 | 7       |       |
| 13        | 69           | Male   | IB              | Yes | No detectable ctDNA | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 26                | 31      |       |
| 14        | 74           | Female | IIIA            | Yes | E298 44.4%         | No detectable ctDNA                   | E298 0.96%                                  | 2                                             | Paratracheal LN, renal, adrenal                     | 4                 | 11      |       |
| 15        | 66           | Male   | IIIC            | No  | No tx-naive sample | No detectable ctDNA                   | C176F 15.05%                                | 0                                             | Liver, bone                                        | 3                | 17      |       |
| 16        | 65           | Female | IIIB            | Yes | Y205S 2.63%        | No detectable ctDNA                   | Y205S 0.24%                                 | 7                                             | Unknown (clinical POD)                              | 12                | 15      |       |
| 17        | 67           | Female | IIIA            | No  | No tx-naive sample | TP53 frameshift 0.39%                 | Frameshift 0.39%                            | 7                                             | Pleural, supraclav LN                               | 7                 | 24      |       |
| 18        | 72           | Female | IIIA            | No  | No tx-naive sample | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 18                | 22      |       |
| 19        | 76           | Female | IIIA            | No  | No tx-naive sample | NOTCH3 T272M 0.37%                    | None                                         | 20 (ongoing)                                      | None                                             | 20                | 23      |       |
| 20        | 52           | Female | IIIA            | Yes | G245C 19.89%       | No detectable ctDNA                   | G245C 0.51%                                 | 3                                             | Lung                                              | 11                | 18      |       |
| 21        | 78           | Female | IIIA            | Yes | Y234C 33.61%       | No detectable ctDNA                   | R248W 0.15%                                 | 11 (ongoing)                                     | None                                             | 12                | 17      |       |
| 22        | 73           | Female | IA3             | Yes | No detectable ctDNA | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 12                | 14      |       |
| 23        | 59           | Male   | IA2             | Yes | No detectable ctDNA | No detectable ctDNA                   | NA                                           | No detectable ctDNA                               | NA                                              | 12                | 13      |       |

*Ongoing.
*bMYCL1 amplification.
*cNOTCH1 R2272H at 0.18%.
*dBRAF K601E at 0.18%.
*eWithdraw consent 6 months before relapse.
*fPIK3CA E542K at 0.24%.
*gFirst ctDNA detection 6 months after radiographic relapse.
*hSurgical resection with adjuvant platinum/etoposide.
*iNOTCH3 T272M at 0.37%.

Dx, diagnosis; VAF, variant allele frequency; NA, not applicable; PFS, progression-free survival from completion of first-line therapy; OS, overall survival from date of diagnosis; POD, progression of disease; ctDNA, circulating tumor DNA; Tx, treatment; LN, lymph node.
Figure 3. Sites of relapse in patients in whom circulating tumor DNA (ctDNA) was ever detected after definitive therapy. Clinical outcomes with radiographic sites of progression among the 15 patients in whom we ever detected circulating tumor DNA after definitive therapy. CT, computed tomography.

have had subsequent follow-up, (Table 2; Fig. 4C and D) was diagnosed with stage IIIA LS-SCLC with a 3.2-cm right lower lobe primary and bulky mediastinal lymph node involvement. She was 39 years at diagnosis and had a 10 pack-year history of smoking and Eastern Cooperative Oncology Group performance status score of 0. She had a MYCL1 amplification detected in a treatment-naive blood sample but cleared this finding after her first cycle of cisplatin plus etoposide. She completed four cycles of chemotherapy plus thoracic radiation and prophylactic cranial irradiation, and has had 11 peripheral blood assessments since her end-of-treatment draw (up to 36 mo after treatment completion) with no ctDNA findings and no evidence of disease recurrence.

**Prognostic Value of Treatment-Naive VAF and ctDNA Clearance on First-Line Treatment**

Of the 23 patients included in the full analysis, 13 had treatment-naive samples available for analysis. Among these 13 patients, we did not observe any prognostic significance for progression or death on the basis of maximum diagnostic ctDNA VAF (HR for progression: 1.01, CI: 0.97–1.05; HR for death: 1, CI: 0.95–1.04) or mean diagnostic ctDNA VAF (HR for progression: 1.01, CI: 0.94–1.08; HR for death: 0.99, CI: 0.91–1.07). Nevertheless, of the patients with clearance of ctDNA during first-line therapy (n = 9), delayed time to ctDNA clearance was a significant predictor of progression (HR for progression 1.1, CI: 1.01–1.19) and death (HR for death 1.07, CI: 1.01–1.15), with a median time to clearance of 63 days (range 29–92 d) among all patients. Notably, three patients had cleared ctDNA at their first on-treatment draw at approximately 30 days (29 d, 29 d, and 32 d, respectively). These three patients have had no evidence of relapse and all remain alive at greater than 1 year (median 965 d) since the start of their first-line treatment. Of the patients that had disease recurrence, the median time to clearance on first-line therapy was 65 days and median time to progression was 249 days, with all but one patient having died of their disease (median time to death 437 d).

**Discussion**

Using a custom 14-gene SCLC next-generation sequencing panel, we have reported that detection of ctDNA at any time point after curative-intent therapy is a poor prognostic finding. We have reported that residual ctDNA can be detected before radiographic relapse, and it presages relapse at both isolated intrathoracic and extrathoracic sites. These findings are consistent with those of reports in patients with NSCLC and stage III colorectal cancer. We have also reported that in a cohort of patients with LS-SCLC, time to ctDNA clearance during first-line therapy is a significant predictor of PFS and OS. This finding is consistent with those of previous studies revealing the negative prognostic significance of delayed CTC clearance in patients with SCLC. Our finding that there was no association between peak or median diagnostic VAF and clinical outcomes (PFS, OS) among 13 of our patients is not consistent with that of a previous publication demonstrating that patients with SCLC with a higher-than-median VAF at diagnosis (0.18%) have inferior PFS and OS. Importantly, our analysis cohort was smaller (n = 14) and not powered specifically for a diagnostic ctDNA VAF analysis, and the comparator study analyzed a larger cohort of patients (n = 22) with approximately half LS-SCLC and half extensive-stage SCLC. It has also been reported that in only patients with LS-SCLC, detection of 15 or more CTCs per 7.5 mL at diagnosis is a poor prognostic finding independent of therapy. The prognostic significance of ctDNA VAF requires further study to draw definitive conclusions.

The limitations of the current study include its single-center accrual and moderate number of patients. A larger cohort of patients exclusively managed with concurrent chemoradiation is the ideal cohort in which to further validate these findings.

Although the field of SCLC CTC and ctDNA analysis has progressed rapidly from assay validation and characterization of the disease’s dynamic genomic evolution throughout a patient’s treatment course to identification of potential targetable mutations, there remains a significant unmet need in evaluating the clinical utility of the integration of ctDNA into routine patient care, such as for residual disease monitoring.
clinical trial that evaluates the initiation of second-line systemic therapy at the time of detection of ctDNA rather than measurable lesions on conventional imaging in patients with LS-SCLC should be considered.

Acknowledgments

Dr. Lovly was supported in part by a Vanderbilt-Ingram Cancer Center Young Ambassadors Award, a Lung Cancer Foundation of America/International Association for the Study of Lung Cancer Lori Monroe Scholarship, P30-CA086485, UG1CA233259, U54CA217450-01, and U01CA224276-01. Dr. Iams was supported by the National Institutes of Health (NIH) and National Cancer Institute (NCI) Vanderbilt Clinical Oncology Research Career Development Award 2K12CA090625-17 and an American Society of Clinical Oncology/Conquer Cancer Foundation Young Investigator Award. Mr. Zhao was supported in part by CCSG NCI/NIH 2P30CA068485-19. All data storage for this research project utilized Vanderbilt’s Redcap data storage infrastructure, funded by the National Center for Advancing Translational Sciences/NIH grant UL1TR000445. The authors would like to thank the patients and their families. The authors would also like to thank all the members of the Lovly Laboratory (authors Kopparapu, Yan, Brandon Williams, Yunkai Zhang, Huan Qiao, Henry Henderson, and Portia Thomas) and the Cancer Systems Biology Consortium U54 Research Team at Vanderbilt for valuable project discussions. Dr. Iams, Dr. Kopparapu, Ms. Yan, Dr. Lim,
and Dr. Lovly designed the experiments. Dr. Kopparapu and Ms. Yan performed the experiments. Dr. Iams, Mr. Zhao, Dr. Chen, Dr. Cann, Ms. Bertucci, Mr. Shaffer, Dr. Horn, Dr. Garg, Dr. Hosseini, Dr. Lim, and Dr. Lovly generated and analyzed the data. Dr. Horn, Dr. York, Dr. Ancell, and Dr. Wyman provided direct patient care. Dr. Iams and Dr. Lovly wrote the manuscript. Zhao and Dr. Chen performed the statistical analysis. All the authors reviewed the data and final manuscript.

### Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology Clinical and Research Reports* at [www.jtocrr.org](http://www.jtocrr.org) and at https://doi.org/10.1016/j.jtocrr.2020.100024.

### References

1. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. *Adv Exp Med Biol*. 2016;893:1-19.
2. Bernhardt EB, Jalal SI. Small cell lung cancer. *Cancer Treat Res*. 2016;170:301-322.
3. Blackhall F, Frese KK, Simpson K, Kilgour E, Brady G, Dive C. Will liquid biopsies improve outcomes for patients with small-cell lung cancer? *Lancet Oncol*. 2018;19:e470-e481.
4. Salem A, Mistry H, Hatton M, et al. Association of chemoradiotherapy with outcomes among patients with stage I to II vs stage III small cell lung cancer: secondary analysis of a randomized clinical trial. *JAMA Oncol*. 2019;5:e185335.
5. Faiivre-Finn C, Snee M, Ashcroft L, et al. Concurrent once-daily versus twice-daily chemoradiotherapy in patients with limited-stage small-cell lung cancer (CONVERT): an open-label, phase 3, randomised, superiority trial. *Lancet Oncol*. 2017;18:1116-1125.
6. Carter L, Rothwell DG, Mesquita B, et al. Molecular analysis of circulating tumor cells identifies distinct copy-number profiles in patients with chemosensitive and chemorefractory small-cell lung cancer. *Nat Med*. 2017;23:114-119.
7. Hou JM, Krebs MG, Lancashire L, et al. Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. *J Clin Oncol*. 2012;30:525-532.
8. Mohan S, Foy V, Ayub M, et al. Profiling of circulating free DNA using targeted and genome-wide sequencing in patients with SCLC. *J Thorac Oncol*. 2020;15:216-230.
9. Zhang J, Wang HT, Li BG. Prognostic significance of circulating tumor cells in small-cell lung cancer patients: a meta-analysis. *Asian Pac J Cancer Prev*. 2014;15:8429-8433.
10. Naito T, Tanaka F, Ono A, et al. Prognostic impact of circulating tumor cells in patients with small cell lung cancer. J Thorac Oncol. 2012;7:512-519.

11. Cheng Y, Liu XQ, Fan Y, et al. Circulating tumor cell counts/change for outcome prediction in patients with extensive-stage small cell lung cancer. Future Oncol. 2016;12:789-799.

12. Hiltermann TJ, Pore MM, van den Berg A, et al. Circulating tumor cells in small-cell lung cancer: a predictive and prognostic factor. Ann Oncol. 2012;23:2937-2942.

13. Normanno N, Rossi A, Morabito A, et al. Prognostic value of circulating tumor cells’ reduction in patients with extensive small-cell lung cancer. Lung Cancer. 2014;85:314-319.

14. Fernandez-Cuesta L, Perdomo S, Avogbe PH, et al. Identification of circulating tumor DNA for the early detection of small-cell lung cancer. EBioMedicine. 2016;10:117-123.

15. Du M, Thompson J, Fisher H, Zhang P, Huang CC, Wang L. Genomic alterations of plasma cell-free DNAs in small cell lung cancer and their clinical relevance. Lung Cancer. 2018;120:113-121.

16. Nong J, Gong Y, Guan Y, et al. Circulating tumor DNA analysis depicts subclonal architecture and genomic evolution of small cell lung cancer. Nat Commun. 2018;9:3114.

17. Board RE, Williams VS, Knight L, et al. Isolation and extraction of circulating tumor DNA from patients with small cell lung cancer. Ann N Y Acad Sci. 2008;1137:98-107.

18. Devarakonda S, Sankararaman S, Herzog BH, et al. Circulating tumor DNA profiling in small-cell lung cancer identifies potentially targetable alterations. Clin Cancer Res. 2019;25:6119-6126.

19. Morgensztern D, Devarakonda SH, Masood A, et al. Circulating cell-free tumor DNA (cfDNA) testing in small cell lung cancer. J Clin Oncol. 2016;34(suppl 15):e23077.

20. Almodovar K, Iams WT, Meador CB, et al. Longitudinal cell-free DNA analysis in patients with small cell lung cancer reveals dynamic insights into treatment efficacy and disease relapse. J Thorac Oncol. 2018;13:112-123.

21. Chaudhuri AA, Chabon JJ, Lovejoy AF, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov. 2017;7:1394-1403.

22. R Development Core Team. R: A Language and Environment for Statistical Computing: Reference Index. Vienna, Austria: R Foundation for Statistical Computing; 2010.

23. Byers LA, Golden L, Zhang W, Lin AB, Forster M. P2.06-028 A phase 2 study of prexasertib in patients with extensive stage small cell lung cancer: topic mesothelioma and SCLC. J Thorac Oncol. 2017;12(suppl):S1088-1089.

24. Tie J, Cohen JD, Wang Y, et al. Circulating tumor DNA analyses as markers of recurrence risk and benefit of adjuvant therapy for stage III colon cancer. JAMA Oncol. 2019;5:1710-1717.

25. Tay RY, Fernandez-Gutierrez F, Foy V, et al. Prognostic value of circulating tumour cells in limited-stage small-cell lung cancer: analysis of the concurrent once-daily versus twice-daily radiotherapy (CONVERT) randomised controlled trial. Ann Oncol. 2019;30:1114-1120.