Circulating Tumor DNA Profiling in Small-Cell Lung Cancer Identifies Potentially Targetable Alterations

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Abstract

Purpose: Patients with SCLC rarely undergo biopsies at relapse. When pursued, tissue obtained can be inadequate for molecular testing, posing a challenge in identifying potentially targetable alterations in a clinically meaningful time frame. We examined the feasibility of circulating tumor DNA (ctDNA) testing in identifying potentially targetable alterations in SCLC.

Experimental Design: ctDNA test results were prospectively collected from patients with SCLC between 2014 and 2017 and analyzed. ctDNA profiles of SCLC at diagnosis and relapse were also compared.

Results: A total of 609 samples collected from 564 patients between 2014 and 2017 were analyzed. The median turnaround time for test results was 14 days. Among patients with data on treatment status, there were 61 samples from 59 patients and 219 samples from 206 patients collected at diagnosis and relapse, respectively. The number of mutations or amplifications detected per sample did not differ by treatment status. Potentially targetable alterations in DNA repair, MAPK and PI3K pathways, and genes such as MYC and ARID1A were identifiable through ctDNA testing. Furthermore, our results support that it may be possible to reconstruct the clonal relationship between detected variants through ctDNA testing.

Conclusions: Patients with relapsed SCLC rarely undergo biopsies for molecular testing and often require prompt treatment initiation. ctDNA testing is less invasive and capable of identifying alterations in relapsed disease in a clinically meaningful timeframe. ctDNA testing on an expanded gene panel has the potential to advance our knowledge of the mechanisms underlying treatment resistance in SCLC and aid in the development of novel treatment strategies.

Introduction

Small-cell lung cancer (SCLC) accounts for approximately 13% of all lung cancer diagnoses in the United States and is characterized by a tendency for early metastasis and an aggressive clinical course. Despite initial response to platinum-based chemotherapy, virtually all patients with extensive disease and the majority of those with limited stage disease develop tumor relapse, which is associated with treatment resistance and short survival. Although recent studies have identified potentially targetable alterations in SCLC, patients with relapsed disease generally deteriorate quickly and rarely undergo tissue biopsies at progression to facilitate molecular testing for biomarker-directed therapy. Challenges in obtaining tissue biopsies has also limited the ability to comprehensively characterize mechanisms of treatment resistance, which are of critical importance in aiding the discovery of novel therapies.

Circulating tumor DNA (ctDNA) may detect DNA from cancer cells shed into the bloodstream. Several studies have demonstrated the utility of ctDNA in directing therapy, examining mechanisms of treatment resistance, and prognosis in patients with non-small cell lung cancer (NSCLC; refs. 7–9). As ctDNA analysis can potentially capture spatial and temporal clonal heterogeneity of cancer without necessitating multiple invasive biopsies, it is an attractive modality for studying the genomic landscape of SCLC, especially at relapse (10–13). Here, we report the ctDNA landscape of SCLC using Guardant360, a targeted hybrid capture–based next-generation sequencing (NGS) platform focused on querying mutations in a set of cancer-related genes, and explore its utility and limitations in identifying alterations that potentially inform therapy.

Materials and Methods

Patients

We employed deidentified ctDNA test data from patients with SCLC who underwent ctDNA testing at a clinical laboratory.
Translational Relevance

Patients with relapsed SCLC rarely undergo biopsies for molecular testing and often require prompt treatment initiation. Circulating tumor DNA (ctDNA) testing is less invasive and is capable of identifying alterations in relapsed disease that can be potentially targeted. ctDNA profiles of nearly 600 patients who underwent testing on a commercially available targeted gene panel were analyzed in this study. Our results demonstrate that it is feasible to detect potentially targetable alterations in patients with SCLC in a clinically meaningful time frame. In addition, ctDNA testing has the ability to provide insights into the biology of SCLC at relapse.

While the maximum AFs (mean of 25.8% vs. 16.5%) of amplifications detected per sample did not differ significantly by treatment status (P = 0.2 and 0.1, respectively; Fig. 1). Among the 30 patients who underwent serial testing, nonsynonymous mutations and amplifications were detectable in more than one sample for 24 and 8 patients, respectively, without significant differences in the number of alterations between first and subsequent collections.
Genomic features of samples collected at diagnosis and relapse

Nonsynonymous mutations in TP53 were the most frequent (72%), followed by alterations in RB1 (18%; Supplementary Figs. S1 and S2) across all samples, and this did not considerably differ by treatment status (Supplementary Tables S3 and S4). Following mutations in TP53 and RB1, mutations in ARID1A were the most frequent (14.5%) in samples collected at relapse, with approximately 33% of alterations consisting of nonsense mutations and indels (Fig. 2).

An analysis of differentially altered genes between diagnosis and relapse samples, demonstrated a higher frequency of alterations in the androgen receptor gene, AR, (14% vs. 2%, \( P < 0.05 \), Fisher’s exact test) among relapsed samples (Fig. 2; Supplementary Table S4). AR mutations were observed in 26 samples from 25 patients at relapse, of which 21 were collected from females and 5 from males and all AR amplifications were only seen in samples collected from females. Of the nonsynonymous mutations identified in AR across all samples, 44% were in the N-terminal domain Activation Function 1 (NTD AF1) region, 15% in the

| Table 1. Clinical and demographic characteristics of study cohort |

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>( N (%) )</th>
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<tbody>
<tr>
<td>Number of patients</td>
<td>564</td>
</tr>
<tr>
<td>Male</td>
<td>271</td>
</tr>
<tr>
<td>Female</td>
<td>293</td>
</tr>
<tr>
<td>Average age (range)</td>
<td>60.5 years (22–99)</td>
</tr>
<tr>
<td>Number of samples</td>
<td>609</td>
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<tr>
<td>Median turnaround time (range, 95% CI)</td>
<td>14 days (6–32, 14–15 days)</td>
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Test details

- 54-gene panel: 10
- 68-gene panel: 86
- 70-gene panel: 260
- 73-gene panel: 253

Treatment status: samples

- At diagnosis: 61 (59)
- At relapse: 219 (206)

Number of samples with nonsynonymous alterations (patients)

- At diagnosis: 53 (61)
- At relapse: 191 (219)

Number of samples with amplifications (patients)

- At diagnosis: 26 (61)
- At relapse: 85 (219)

Figure 1.

Comparison of the ctDNA mutational profiles of SCLC at diagnosis and relapse. A and B, Maximum AFs and MAFs, respectively, when samples are categorized by treatment status (diagnosis vs. relapse). C and D, The number of mutations and amplifications, respectively, in diagnosis and relapse samples. Error bars, mean ± SEM (*, \( P < 0.05 \); **, \( P < 0.01 \); n.s, not significant).

Figure 2.

Type and frequency of mutations in commonly altered genes in relapsed SCLC. An oncoplot demonstrating distribution of the 10 most frequently altered genes across relapse samples. The right barplot represents the frequency of mutations in each gene. Amp, amplification; Multi-Hit, presence of multiple types of mutation in same gene in a given sample. Each column represents an individual patient sample.
DNA-Binding (DBD) and hinge Domain, and 21% in the Ligand-Binding Domain (LBD). While none of the identified mutations have been previously reported as activating, most activating AR mutations that have been reported, are typically found in these domains (Fig. 3; ref. 19). Mutations in APC, a negative regulator of β-catenin (CTNNB1) and WNT signaling, were also more prevalent among relapse samples (18 patients) compared with those obtained at diagnosis (1 patient; 11% vs. 2% of samples, $P<0.05$, Fisher exact test; Figs. 2 and 3; Supplementary Fig. S3). In addition to alterations in APC, we also detected known hotspot mutations in CTNNB1 in 5 samples. While frequencies of alterations in multiple genes appeared to differ by treatment status, none reached statistical significance after correcting for multiple comparisons, possibly owing to inadequate number of samples in each treatment category.

Identification of potentially targetable genomic alterations

SCLC is characterized by amplifications in SOX2 and MYC family genes including MYC, MYCL1, and MYCN (3, 4). SOX2, which is included in the Guardant360 panel, is located on the long arm of chromosome 3 (3q26) adjacent to PIK3CA. Apart from PIK3CA and MYC, amplifications in several other genes were observed (Supplementary Table S3). MYC amplifications, which may be potentially targetable by aurora kinase inhibitors, were observed in approximately 3.5% of the relapse samples (20). Despite data supporting a role for MYC in treatment-resistant SCLC, the frequency of MYC amplifications did not differ according to treatment status (5% at diagnosis vs. 3.5% at relapse; Supplementary Fig. S4; ref. 21). Notably, copy number assessment in ctDNA may have lower sensitivity than direct tumor analysis, which could explain the differences in frequency of amplification in genes like MYC between this study and other tissue sequencing studies.

Figure 3.

Altering the emerging role of investigational agents targeting DNA repair pathways, genomic instability, and immunotherapies in relapsed SCLC, we examined alterations in DNA repair and damage response pathway genes (22, 23). Alterations in BRCA1 (9%), BRCA2 (5.5%), ATM (3%), and MLH1 (1%) were detectable in a subset of relapse samples, despite limited coverage of ATM and MLH1 exons. BRCA1, CCNE1, and FBXW7 alterations, which drive genome-wide instability (tandem-duplicator phenotype), were observed in 9%, 8%, and 5% of relapse samples, respectively (Fig. 4), and mutations in ARID1A, associated with responsiveness to PARP and EZH2 inhibitors, were observed in nearly 15% of patients at relapse (24).

PI3K/mTOR and RAS/RTK pathway alterations

Alterations in PI3K/mTOR signaling genes were observed in approximately a third of all SCLC samples (4). Although SCLC is not characterized by RAS/RTK pathway alterations, ctDNA profiling showed an unexpectedly high frequency of these alterations. Activating mutations in RAS/RAF pathway genes EGFR, KRAS, NRAS, HRAK, BRAF, MAP2K1, and ERBB2 were observed in 96 samples (18%) collected from 77 patients. Mutations in KRAS were the most frequent (n = 36), followed by EGFR (n = 34) and BRAF (n = 9). Amplifications in ERBB2 and MET were detectable in nearly 30% of patients at relapse. All samples demonstrating known activating EGFR mutations were collected at relapse, suggesting that these samples were possibly collected from patients with...
transformed SCLC following EGFR-directed therapy (25). We also observed oncogenic ALK and RET rearrangements in one sample each, but the treatment status of these samples was unknown.

Characteristics of TP53 mutations in SCLC

TP53 alterations are essentially universal and typically clonal in SCLC (4). We analyzed the characteristics of TP53 mutations to obtain a better insight into the ability of ctDNA testing to facilitate disease monitoring. TP53 mutations were detectable in 72.5% of samples with detectable alterations, of which 31% had multiple TP53 mutations. Of the samples collected at relapse, 40% showed multiple TP53 mutations (vs. 31% at diagnosis, \( P = 0.35 \), Fisher exact test). TP53 mutations predominantly clustered in the DNA binding domain irrespective of their AF (Fig. 5; Supplementary Fig. S5). When only variants with the highest AFs in a sample were considered for each gene, mutations in TP53 and RB1 appeared to be clonal, as expected for SCLC (Fig. 5). Among samples with multiple TP53 mutations, the average ctDNA clonality value of TP53 mutations was approximately 4.5-fold higher for mutations with the highest AFs compared with mutations with lower AFs (0.89 vs. 0.22), suggesting a subclonal or hematopoietic source for low AF variants.

Discussion

We examined the ctDNA profiles of nearly 600 patients with SCLC, making this the largest study to examine the genomic features of SCLC to date, whether in tissue or in plasma. This large sample size enabled us to make several important observations and investigate the utility of ctDNA in identifying potentially targetable alterations in SCLC. TP53 and RB1 alterations play an essential role in SCLC tumorigenesis, and are also retained at relapse, a feature that is consistently reproducible through ctDNA analysis (4, 26). The frequency of RB1 mutations was notably lower compared with their nearly universal presence in tumor biopsies. Of note, the test used herein cannot easily identify biallelic loss or loss of heterozygosity. Notably, the frequency of RB1 mutations in our data (12%) was comparable with previously published studies when only missense and nonsense variants were considered (18% to 35% in tissue sequencing studies), suggesting that these differences could be explained by differences in RB1 coverage (for type of mutation) between tissue sequencing in previous studies and earlier versions of the ctDNA test (3, 4, 17, 18).
The number of mutations detectable per sample, a surrogate for tumor mutation burden, did not differ by treatment status. The MAF of detected variants was significantly higher across samples collected at diagnosis compared with those collected at relapse. Previous studies suggest a correlation between MAF of variants detected on ctDNA and radiographic disease burden (27). Therefore, our observation may be explained by the fact that patients with SCLC often present with a higher burden of disease at diagnosis.

We also observed a higher percentage of alterations in APC and AR in relapse samples. Although identification of APC alterations in our study is consistent with a recent study highlighting the role of WNT activation in chemoresistant SCLC, a more systematic evaluation of the impact of these alterations in SCLC is needed (5). The identification of AR alterations is provocative because it may be potentially targetable. In SCLC, both testosterone and dihydrotestosterone stimulated growth of AR-positive SCLC cell lines, which was reversed by the addition of antiandrogens, including flutamide (28). In addition, AR manipulation has an established role in patients with AR-positive tumors, and there are anecdotal cases describing responses with this approach in other solid tumors (29, 30). Response to anti-AR therapy may also depend on whether AR copy number gain is focal or a result of aneuploidy. Although not addressable in this study, it may be possible to adopt approaches such as comparing the number of copies of ARAF and AR genes, as both reside on the X chromosome, to filter out aneuploidy. Further studies are warranted to explore the role of AR signaling in SCLC, especially considering the role this pathway plays in driving hormone resistance in prostate cancer (31).

The role of RTK/RAS signaling in SCLC is controversial (32). We observed known RTK/RAS pathway activating mutations in nearly 18% of our patients, including therapeutically targetable alterations in EGFRL, BRAF, ERBB2, and MET. While it is possible that these findings are a consequence of misclassification of NSCLC or large-cell neuroendocrine cancers as SCLC, presence of mixed histology tumors, or identification of hematopoietic variants by ctDNA testing, alterations in RAS pathway genes, and/or pathway activation in SCLC have been previously reported (33, 34). Cancer initiation and progression are complex processes, and preclinical studies in murine models suggest that the final histology of lung cancer is likely to be influenced by the cell of origin and the combination and sequence in which these cells acquire specific mutations (35, 36). It is therefore possible that these findings reflect heterogeneity in the cell of origin, tumorigenesis, and inter- or intratumoral heterogeneity (37, 38). The higher ctDNA clonality values of some of the KRAS mutations support this hypothesis. The ability to target RAS signaling has been reported in SCLC with combination therapies (39). We also observed amplifications in ERBB2 and MET in 12% of the samples. Although there is no data in SCLC, studies have reported benefit from targeting these alterations in other solid tumors (40–45).

Mutations in genes involved in DNA repair and damage signaling were observed in a subset of relapse samples, and potentially predict for responsiveness to therapeutic strategies targeting genomic instability and immunotherapies (22, 24). In particular, we observed alterations in BRCA1, FBXW7, CCNE1, and ARID1A in relapse samples. Alterations in these genes may predict response to DNA-damaging therapies, PARP inhibitors, and/or immunotherapies raising the possibility for more targeted therapies for a subset of patients with SCLC (46–48).

Finally, nearly a third of samples in this analysis demonstrated multiple TP53 mutations. In many instances, these mutations were detectable at very low percentages in plasma, raising the possibility of them being subclonal or hematopoietic in origin. Recent data have shown the ability of ctDNA and tissue molecular testing to pick up variants from clonal hematopoiesis (CH; refs. 49, 50). Incorporation of strict quality control measures, a high level of clinician suspicion, and/or bioinformatic filtering is critical when calling mutations to ensure that they are related to the tumor of interest and not CH. Our results suggest that CH identified on ctDNA testing could potentially lead to the identification of spurious variants, incorrect estimation of tumor mutation burdens, or pose a challenge in interpreting presence of minimal residual disease. Delineating potential hematopoietic variants from ctDNA variants may require sequencing of matched PBMCs as shown by some groups (27). Utilizing ctDNA clonality values may aid in the identification of variants that are likely to have originated from the tumor compartment. Nevertheless, this approach will require further validation.

Given the novelty of ctDNA analysis in SCLC, it is important to recognize the limitations of our study. First, we were unable to perform independent verification of the histology or treatment status of a significant number of samples analyzed in this study. Second, samples collected at diagnosis and relapse were also not typically patient-matched. Therefore, we cannot rule out the possibility that the observed variant frequencies are a consequence of sample misclassification or lack of pairing. Third, samples in this study were sequenced using different versions of a commercial assay that differed in their abilities to identify mutations and amplifications in different genes and the extent to which some genes were sequenced (limited exons vs. full coding sequence). Fourth, treatment status of several samples was inferred on the basis of date of diagnosis and sample collection, which can be inaccurate. Some or all of these factors could have altered the frequency at which various mutations were identified in samples collected at diagnosis and at relapse. Fifth, information related to disease burden at time of collection, specific treatment received by individual patients and response to treatment were not available for a more informed clinical-correlate analysis. In addition, hematopoietic variants may have been misclassified as tumor derived in our analysis. However, this limitation is not exclusive to ctDNA analyses, because leukocyte infiltration of solid tumors can also lead to the detection of these variants with tissue-based sequencing (50).

Despite the noted limitations, our findings demonstrate that ctDNA profiling holds promise for examining mechanisms of treatment resistance and directing clinical care in SCLC by facilitating the rapid identification of potentially targetable alterations. Furthermore, unlike prior studies that predominantly sequenced surgically resected early-stage SCLC samples, xenografts, or cell lines, our study included samples collected from a large cohort of patients who most likely received treatment for advanced stage disease, shedding light on alterations that could play important roles in SCLC progression and serve as potential new candidates for therapy. Further studies of ctDNA in patients with SCLC may help accelerate the discovery and development of new drugs against this relentless disease.

Disclosure of Potential Conflicts of Interest

S. Devarakonda is a consultant/advisory board member for Guardant 360.
K.A. Gold is a consultant/advisory board member for AstraZeneca, Takeda, and...
Regeneron. R.B. Lanman is an employee of Biolase, Inc.; has ownership interests (including patents) at Guardant Health, Inc., Biolase, Inc., and Forward Medical, Inc.; and is a consultant/advisory board member for Forward Medical, Inc. A.A. Chaudhuri is an employee of Roche Sequencing, Genescopy, and Tempus Labs; reports receiving speakers bureau honoraria from Foundation Medicine. Roche Sequencing, and Varian Medical Systems; is a consultant/advisory board member for Roche Sequencing, Genescopy, and Tempus Labs; and reports receiving commercial research support from Roche Sequencing. T.K. Owo- nikonko has ownership interests (including patents) in Cambium Oncology; is a consultant/advisory board member for Novartis, Celgene, Lilly, Sandoz, AbbVie, Essex, G1 Therapeutics, Takeda, Seattle Genetics, Bristol-Myers Squibb, MedImmune, BerGenBio, Lilly, Amgen, AstraZeneca, PharmaMar, Roerhinger Ingelheim, EMD Serono, Xcovery, Bayer, Heron Pharmaceutical, and ARMO Biosciences; reports receiving commercial research grants in the form of payments of institution from Novartis, Astellas Pharma, Celgene, Bayer, Stem CentRx, Regeneron, AstraZeneca/MedImmune Abbvie, G1 Therapeutics, Bristol-Myers, Corvus Pharmaceuticals, United Therapeutics, Amgen, Luxo/Lilly, Fuji Film, Pfiear, Aeglea Biotherapeutics, Inelsey, and Merck; and reports being a paid consultant as part of the independent data review committee for EMD Serono. B.T. Li is a consultant/advisory board member for Guardant Health and Merck. S. Devarakonda and reports receiving commercial research grants from Guardant Health and Genentech. C.M. Rudin is a consultant/advisory board member for AbbVie, Ascentage, AstraZeneca, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Genentech/Roche, Ipsen, Luxo, Pharmamar, Varvest, Bridge Medicine, Elucida Oncology, and Harpoison Therapeutics. D. Morgensztern is a consultant/advisory board member for Bristol-Myers Squibb, Takeda, PharmaMar, and AbbVie. No potential conflicts of interest were disclosed by the other authors.

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** S. Devarakonda, K.A. Gold, J.P. Ward, V.M. Raymond, R.B. Lanman, T.K. Owo- nikonko, C.M. Rudin, R. Govindan, D. Morgensztern

**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** S. Devarakonda, S. Sankaranaram, B.H. Herzog, S.N. Waqar, J.P. Ward, V.M. Raymond, R.B. Lanman, A.A. Chaudhuri, T.K. Owo- nikonko, B.T. Li, J.T. Poirier, C.M. Rudin, D. Morgensztern

**Writing, review, and/or revision of the manuscript:** S. Devarakonda, B.H. Herzog, K.A. Gold, S.N. Waqar, J.P. Ward, V.M. Raymond, R.B. Lanman, A.A. Chaudhuri, T.K. Owo- nikonko, B.T. Li, J.T. Poirier, C.M. Rudin, D. Morgensztern

**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** B.H. Herzog, V.M. Raymond, R. Govindan, D. Morgensztern

**Study supervision:** S. Devarakonda, R. Govindan, D. Morgensztern

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