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Next-Generation Sequencing May Discriminate Extreme Long-term versus Short-term Survival in Patients with Metastatic Small Cell Lung Cancer (SCLC)

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Abstract

BACKGROUND: Molecular underpinnings that may prognosticate survival could increase understanding of small cell lung cancer (SCLC) tumor behavior. Here, we report the clinicopathological characteristics and biomarker profiles of short-term (ST) versus long-term (LT) survival in patients with metastatic SCLC. *METHODS:* Of the 876 consecutive metastatic SCLC patients receiving standard of care therapy, 44 met the definition of LT and 91 for ST, respectively. Available FFPE tumor tissue blocks were analyzed by next-generation sequencing (NGS). Analysis included gene mutations, copy number variations, mRNA expression, and protein expression by immunohistochemistry, followed by correlation with clinicopathological characteristics. *RESULTS:* There were no statistically significant and clinically relevant differences in cases with or without FFPE according to major clinicopathological variables in ST and LT. However, according to NGS, five mutually exclusive gene mutations were identified (E1A binding protein P300 [EP300] p.N217S; p.E152K; human epidermal growth factor receptor 4 [ERBB4] p.E317K; BRCA1, DNA repair associated [BRCA1] p.E1661N, and epidermal growth factor receptor [EGFR] p.V742A). Comparing LT vs. ST survivals, a twofold increase was found in the average predicted number of drugs per patient off compendium. We found high SSTR2 mRNA expressions in all LT patients (vs. two [20%] ST patients), which may reflect more benign neuroendocrine tumor characteristics. *CONCLUSIONS:* Consolidation radiation therapy and higher predicted drug sensitivity for off compendium were associated with LT compared to ST patients in SCLC. NGS profiling of extreme survivals may improve classification of SCLC and possibly identify clinically relevant new targets.

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Introduction

For small cell lung cancer (SCLC), the molecular underpinnings that may increase our understanding of tumor biology is not well characterized. SCLC is a very aggressive neuroendocrine lung cancer subtype which accounts for 15% of all lung cancers [1]. During the course of treatment, variability of therapeutic response and patient survival is frequently observed. Systemic therapy can induce dramatic responses for certain SCLC patients, though explanations for robust outcomes are often not evaluated or reported. Recent clinical studies in different tumor types have reported on tumor genome sequencing of such "outlier" patients and identified molecular alterations that are posited to be the basis of the tumor's biology or therapeutic response. This approach is considered hypothesis generating and should be subsequently validated in preclinical experiments and/or rationally designed clinical trials. Importantly, this strategy could be applied in the increasingly popular basket trials that involve molecularly matched, tumor agnostic entry criteria.

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Exceptional responders are defined as a minority of cancer patients treated with conventional cytotoxic or targeted anticancer drugs that are not effective in a given tumor type but are unexpectedly successful in the given cases [2]. The first study that investigated extreme responders reported that everolimus (a drug targeting the mammalian target of rapamycin, mTOR) is exceptionally effective in bladder cancer patients whose tumors harbor TSC1 somatic mutations [3]. Another exceptional responder was a patient with TSC2 mutant anaplastic thyroid cancer with an 18-month response to everolimus [4]. In other examples, antiangiogenic receptor tyrosine kinase inhibitors such as sunitinib or pazopanib were associated with favorable or poor response in patients with PBRM1 and TP53 mutated metastatic renal cell carcinoma, respectively [5]. There are also studies that highlight the importance of in-depth analysis of exceptional responders to cytotoxic chemotherapy. For example, an unusual curative response to irinotecan and a checkpoint kinase 1 inhibitor in a urothelial small cell cancer patient with a somatic mutation in the RAD50 gene was reported by Al-Ahmadie et al. [6]

Because surgical resection in SCLC is rarely prospectively planned and provides little clinical benefit [7], the majority of SCLC patients are cytologically diagnosed. Accordingly, the limited availability of tumor tissue and the relatively low number of patients treated in each facility hamper the in-depth investigation of genomic and proteomic data in SCLC. Therefore, we aimed to study the clinicopathological characteristics and biomarker profiling of short-term (ST) versus long-term (LT) SCLC patients.

Material and Methods

Study POPULATION

A total of 876 consecutive metastatic SCLC patients receiving standard of care therapy were evaluated between 2000 and 2013 at the National Koranyi Institute of Pulmonology, as described earlier for inclusion in this analysis [8]. LT patients [defined as patients having an overall survival (OS) > 24 months] and ST patients (defined as patients having an OS range 2-8 weeks) with histologically confirmed metastatic SCLC were included. To avoid other competing causes of short OS, using clinical and autopsy reports, ST patients with known additional concurrent life-threatening diseases (e.g., pneumonia, severe hepatic or kidney failure, cardiovascular disease, pulmonary embolism) were excluded from the study. Clinicopathological data collected included gender, age, smoking history, Eastern Cooperative Oncology Group performance status (ECOG PS), chemo- and radiotherapy treatments, and OS. TNM stage according to the Union for International Cancer Control (seventh edition) [9], ECOG PS, and age at the time of diagnosis were recorded.

Patient LT5 and ST15 had 5% and 10% tumor content, respectively. Therefore, NGS biomarker analysis was not performed for these cases, and we excluded them from further NGS biomarker-based therapy option assessment. However, proteomic analysis was possible in case ST15 but not LT5.

Of those with available tumor tissue for NGS analysis, there were 3 LTs (1 man and 2 women) and 10 STs (5 men and 5 women) that met the eligibility criteria for NGS and were further analyzed for potential therapeutic options.

Treatment

Patients were treated either with first-line platinum-etoposide doublet regimen or with a combination of cyclophosphamide, epirubicin, and vincristine). Second-line topotecan or radiation therapy (RT) including thoracic RT, prophylactic cranial irradiation, or whole brain radiation therapy was administered to selected patients. All treatments were conducted in accordance with contemporary NCCN guidelines.

Biomarkers were considered clinically actionable if they had a published association with treatment response in earlier publications in humans. In the next-generation sequencing (NGS) report, we classified drugs as "on compendium" (commercially available drug), "off compendium" (clinical trial drug), or drugs with reduced or no efficacy.

Statistical Methods

Patients were grouped according to ST and LT. We then evaluated the associations among the various biomarkers and clinicopathological characteristics (e.g., gender, age, smoking, stage, ECOG PS, chemotherapy [CHT], RT, metastatic site, presenting symptoms, and other diseases). Clinical characteristics, drugs on and off compendium, and predicted drug efficacy (reduced/lack) for ST versus LT were compared. Predicted drug sensitivity and reduced/lack efficacy percentages for LT and ST were calculated (number of patients in a given group [predicted to be sensitive or drugs with reduced/lack efficacy] was divided by the total number of LT or ST patients). Categorical parameters; major clinicopathological factors in ST and LT according to FFPE tissue availability; and the number of available drugs on compendium, off compendium, and predicted drug efficacy with SCLC tumors resistant to different drugs in ST versus LT SCLC patients were analyzed by Fisher's exact and χ^2 tests. Age as a continuous variable was analyzed in the ST and LT groups according to FFPE tissue availability by Student's t test. P values $< .05$ were two-sided and were considered significant. All statistical analyses were performed using the PASW Statistics 18.0 package (SPSS Inc., Chicago, IL, USA).

Molecular Methods

The diagnosis of each case was confirmed on a freshly cut hematoxylin and eosin–stained slide. DNA and RNA were isolated from FFPE tissues as earlier described [10]. A comprehensive NGS test was performed to analyze actionable gene mutations, copy number variations (CNVs), and mRNA expression by using the Paradigm Cancer Diagnostic's (PCDx) NGS platform according to the previously described methodology [11]. The PCDx test is a clinical-grade targeted NGS test run in a Clinical Laboratory Improvement Amendments–certified and College of American Pathologists–accredited laboratory [12]. The platform measures genomic, transcriptomic, and proteomic aberrations linked with 86 unique therapies based on published patient research information for tumors all cancer types. Accordingly, the PCDx test helps to guide treatment especially for tumor types that have potential targeted therapy options, like breast cancer, colorectal cancer, and NSCLC, but also SCLC, mesothelioma, and gastric cancer [12]. The sequencing was performed by using the Ion 318 chip on the Ion PGM sequencer (Thermo Fisher Scientific, Waltham, MA, USA). mRNA was analyzed for elevated expression at $P \leq .001$ [11]. Clinically relevant protein expressions were studied by IHC (PCDx, Paradigm) as earlier described [11]. Multiplex sequencing analysis had coverage >5000*x*. During the study, PCDx interrogated 116 molecular alterations (CNVs, mismatched repair [MMR] abnormalities, DNA point mutations, gene fusions, and mRNA and protein

expression) using the PCDx cancer testing NGS gene panel, and a list of biomarkers was analyzed as described earlier [10,11,13]. The genes included in this gene panel belong to different cancer pathways and are associated with cancer development and progression across the major organs including lung cancer, breast cancer, pancreatic cancer, prostate cancer, bladder cancer, ovarian cancer, thyroid cancer, osteosarcoma, melanoma, and leukemia. All of these are tied to either a level of evidence relative to a treatment or a clinical trial for a treatment. The test is able to detect base substitutions with 4% frequency at 99.9% sensitivity and indels with 7% frequency at 99.4% sensitivity. The specificity of mutation assays was optimized to be 99.99% at the patient level, meaning that less than 0.01% of patient reports will contain a false-positive result as earlier described [10]. The minimum tumor content for successful NGS was 20%. Not applicable (N/A) was indicated when i) RNA or DNA concentration was below the standard limit of detection (<0 ng/μl) and/or ii) when repeated attempts to obtain sufficient coverage to report on all biomarkers including RNA sequencing fail and/or iii) only a small percentage of tumor in the tissue was available (e.g., 5%). The advertised submission guidelines for tumor tissue percentage were a minimum of 20%, and more than 40% was optimal.

Results

Clinicopathological Characteristics

Of the 876 patients, 44 and 91 met the definition of LT and of ST, respectively (Figure 1). The major clinicopathological characteristics of the study population are summarized in Supplementary Table 1 ($n = 135$). Clinicopathological characteristics and treatments of LT and ST patients with available FFPE and molecular analysis are shown in Tables 1 and 2 ($n = 15$).

First-line platinum-etoposide chemotherapy [including treatment with carboplatin (CE) ($n = 3$) and cisplatin (CisE) ($n = 1$)] was given to LT patients. LT patients received second-line chemotherapy [topotecan ($n = 1$) and CAV therapy ($n = 2$)]. All LT patients, but none of the ST patients, received consolidation thoracic RT along with standard-of-care chemotherapy. All 14 patients are now deceased.

There were no statistically significant and clinically relevant differences in cases with or without FFPE according to major clinicopathological variables in ST and LT (Table 3).

Genomic Biomarkers in ST vs. LT Patients

Clinically relevant biomarkers and their possible therapeutic associations in our SCLC cohort are listed in Supplementary Table 2. Supplementary Figure 1 shows CNVs, MMR abnormalities, and DNA point mutations according to ST vs. LT. We identified in five patients four mutations in cancer that have not been previously reported (E1A binding protein P300 [EP300]: c.650A > G p.N217S and c.4561G > A p.E152K; human epidermal growth factor receptor 4 [ERBB4]: c.949G > A p.E317K; BRCA1, DNA repair associated [BRCA1]: c.4981G > A p.E1661N). Additionally, we found the epidermal growth factor receptor [EGFR] mutation c.2225T > C p.V742A which was described in NSCLC (COSM13183) but not in SCLC. CNV (CDKN2A) was not identified in any of the samples. ERBB4 belongs to a class of proteins having high homology with EGFR and is widely recognized for its importance in cancer, just as EGFR, while EP300 regulates transcription via chromatin remodeling and is important in the processes of cell proliferation and differentiation. BRCA1 meanwhile is a known tumor suppressor that plays a role in maintaining genomic stability.

Figure 2 shows the heat map analysis of the mRNA genes in our panel according to LT versus ST. Supplementary Figure 2 shows mRNA expression according to individual patients fold change in ST vs. LT. In most of the ST cases, the cellular uptake of nucleosides mediator human equilibrative nucleoside transporter-1 [HENT1 (SLC29A1)] mRNA with 50%-79% protein concordance was high

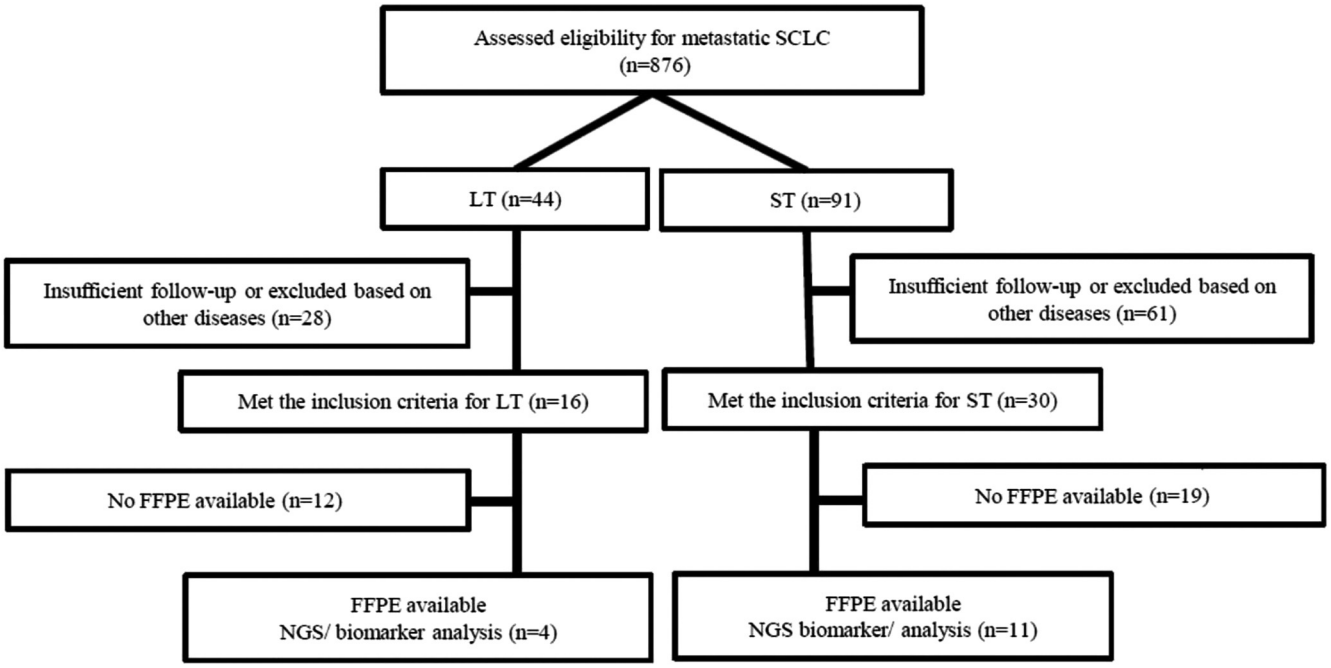

Figure 1. Patient cohort and molecular analysis flow chart according to tissue availability ($n = 876$ patients).

Table 1. Major Clinicopathological Characteristics of Metastatic SCLC Patients with ST and LT Survival ($n = 15$)

ID	Gender	Age	T	N	M	OS (Months)	ECOG PS	Smoking Status	Presenting Symptoms	Other Diseases
LT5	F	58.4	T2	Nx	M1-brain	29.9	1	Current	Neurological symptoms	Hypertension, alcoholism, and anemia
LT7	F	54.6	T1	N0	M1-brain	39.7	0	Current	Left hemiparesis	Asthma
LT6	F	66.1	T2	N2	M1-liver	28.2	1	Former	Cough	Hypertension, diabetes type 2
LT9	M	67.2	T2	Nx	M1-liver	24.0	0	Former	Chest pain	Hypertension, TB, bronchiectasis, Zollinger-Ellison syndrome
ST15	M	71.7	T2	N2	M1-brain	1.07	3	Current	Aphasia	Iliac artery bypass, coronary atherosclerosis
ST17	F	71.6	T4	Nx	M1-adrenal	1.0	2	Current	Dyspnea	DVT, hypertension
ST24	M	49.7	T4	N2	M1-adrenal	0.7	2	Current	Chest pain	Alcoholism
ST21	M	77.2	T3	N1	M1-brain	1.1	2	Current	Aphasia	Hypertension
ST23	M	49.9	T4	Nx	M1-brain	1.0	1	Current	Hemoptysis	No previous diseases
ST9	F	64.5	T2	N2	M1-brain-bone	2.0	1	Current	Cough	TB, bronchitis
ST12	F	59.8	T3	N3	M1-liver	1.0	2	Current	Dyspnea	Hypertension
ST14	F	69.2	T2	Nx	M1-liver	2.1	1	N/A	Chest pain, dyspnea	Hyperthyroidism
ST1	M	66.8	T2A	N2	M1A-pleura	0.3	1	Former	Cough	Cardiac arrhythmia
ST5	F	70.6	T2B	N2	M1-brain	0.7	2	N/A	Ataxia	Hyperthyroidism, hypertension, ischemic heart disease
ST2	M	52.3	T3	Nx	M1-liver	0.3	3	N/A	Hemoptysis	DVT, hypertension, cardiac arrhythmia, COPD, pancreatitis, heart failure

F: female, *M:* male, *N/A:* not available, *COPD:* chronic obstructive pulmonary disease, *DVT:* deep vein thrombosis, *TB:* tuberculosis.

[ST, 5 (50%) vs. LT, 0%], just as the survivin (baculoviral IAP repeat containing 5 [BIRC5]) mRNAs (ST, 7 (70%) vs. LT, 0%), which encodes negative regulatory proteins that prevent apoptotic cell death. All LT vs. two (20%) ST patients had high somatostatin receptor 2 (SSTR2) mRNA, which encodes an endogenous cyclic polypeptide that inhibits the release of many hormones, such as growth hormone, and other secretory proteins. In first-line platinum-etoposide—treated patients ($n = 8$), SSTR2 mRNA expressions were high in all LT patients [vs. 2 (20%) ST patients]. Molecular testing revealed that three ST patients treated with CAV were not predicted to be sensitive to doxorubicin or epirubicin [significantly high topoisomerase (DNA) II alpha [TOPO IIa] mRNA expression with high (>80%) protein concordance was present]. TOPO IIa encodes an enzyme that controls and alters the topologic states of DNA during transcription. In contrast, all three LT patients that were treated with CAV were predicted to be sensitive to doxorubicin or epirubicin (low TOPO IIa mRNA expression was found). Most patients ($n = 9$) had tumor suppressor BRCA1 mRNA overexpression, without a significant difference in survival. Two ST patients had high mRNA expression. As part of the gene panel, the IHC for hormone receptors did not identify overexpression of progesterone receptor (PR), estrogen receptor (ER), androgen receptor (AR), inflammation, wound healing, oocyte maturation, and cell proliferation mediator epiregulin (EREG), carcinoma cell line inhibitor amphiregulin (AREG), and DNA replication and repair mediator thymidylate synthetase (TS [TYMS]) in any of the cases. Of note, PR and ER IHC was not done. Additional potential therapeutically relevant biomarkers and their therapeutic associations are listed in Supplementary Table 2.

Table 2. Treatment of Metastatic SCLC Patients with ST and LT Survival ($n = 15$)

ID	Cycles of CHT	1st Line CHT	2nd Line CHT	3rd Line CHT	WBRT	PCI	Thoracic RT	Other RT
LT5	4	CE	No	No	Yes	No	Yes	No
LT7	4	CE	Topo	CAV	Yes	No	Yes	Stereotactic brain
LT6	2×4	CE	CAV	Topo	No	Yes	Yes	No
LT9	4	CisE	CAV	CisE	Yes	Yes	Yes	No
ST15	2	Platinum E	No	No	Yes	No	No	No
ST17	2	CE	No	No	No	No	No	No
ST24	2	CisE	No	No	No	No	No	No
ST21	2	CE	No	No	No	No	No	No
ST23	1	CE	No	No	Yes	No	No	No
ST9	1	CAV	No	No	Yes	No	No	No
ST12	1	CAV	No	No	No	No	No	Vertebral
ST14	3	CAV	No	No	No	No	No	No
ST1	0	None	No	No	No	No	No	No
ST5	0	None	No	No	No	No	No	No
ST2	0	None	No	No	No	No	No	No

CE: carboplatin, etoposide; *E:* etoposide; *CisE*: cisplatin, etoposide; *CAV*: cyclophosphamide, epirubicin, vincristine; *Topo*: topotecan; *PCI*: prophylactic cranial irradiation; *WBRT*: whole brain radiation therapy.

Proteomic Biomarkers in ST vs. LT Patients

Supplementary Figure 3 shows the list of IHC biomarkers available and utilized on FFPE sections. Supplementary Table 3 shows the protein expression intensity thresholds. Topoisomerase (DNA) I [TOP1], a protein that alters the topologic states of DNA during transcription, was expressed in all patients (except for one ST). Tumor-infiltrating lymphocytes (TILs) or tumor cells did not express in any of the samples ($n = 13$) the immune checkpoint programmed cell death ligand 1 (PD-L1) or O-6-methylguanine-DNA methyltransferase (MGMT), a protein that catalyzes transfer of methyl groups from the DNA to its own molecule, which repairs the toxic lesions. Tumor suppressor and DNA mismatch repair genes MutL homolog 1 (MLH1), MutS homolog 2 (MSH2), and MutS homolog 6 (MSH6) were expressed in the cases that could be analyzed, indicating there was no evidence for MMR abnormalities (3 LT and 9 ST patients).

Biomarkers Identified in the SCLC Superpath

Next, we compared our NGS biomarker data to the PathCards database SCLC superpath analysis [14]. PathCards is an integrated database of human biological pathways and their annotations. Human pathways were clustered into SuperPaths based on gene content similarity. PathCards provides information on one SuperPath which represents more human pathways relevant in SCLC. According to the SCLC SuperPath, in our study, we found high mRNA expression in apoptosis regulator BCL2 (BCL2) [one LT and two STs], and BIRC5 [seven STs], transcription factor inhibitor conserved helix-loop-helix ubiquitous kinase (CHUK) [one LT], and tumor suppressor PTEN [one LT and one ST]. In contrast, DNA mutations and CNVs were not present in any relevant genes according to the SCLC SuperPath in our study.

Table 3. Comparison of Major Clinicopathological Characteristics of Metastatic SCLC Patients with ST and LT Survival According to Available FFPE Tissue for This Study

		Group ST				*P* Value	Group LT				*P* Value
		ST with FFPE		ST without FFPE			LT with FFPE		LT without FFPE		
		Value	Column *N* %	Value	Column *N* %		Value	Column *N* %	Value	Column *N* %	
Age		63.95 (±9.63)	–	64.41 (±9.6)	–	.54	61.57 (±6.5)	–	59.3 (±9.2)	–	.88
Gender	Male	6	54.50	50	62.50	.74	1	25.00	19	47.50	.61
	Female	5	45.50	30	37.50		3	75.00	21	52.50	
Smoking	Smoker	8	100.00	40	100.00	N/A	4	100.00	20	100.00	N/A
CHT	No CHT	3	27.30	12	15.00	.38	0	0.00	2	5.00	NS
	Received CHT	8	72.70	68	85.00		4	100.00	38	95.00	
WBRT	No WBRT	9	81.80	78	97.50	.07	1	25.00	29	72.50	.089
	WBRT	2	18.20	2	2.50		3	75.00	11	27.50	
PCI	No PCI	11	100.00	80	100.00	N/A	2	50.00	32	80.00	.218
	PCI	0	0.00	0	0.00		2	50.00	8	20.00	
Thoracic RT	No thoracic RT	11	100.00	76	95.00	NS	0	0.00	19	47.50	.12
	Thoracic RT	0	0.00	4	5.00		4	100.00	21	52.50	
Other RT	No other RT	11	100.00	80	100.00	N/A	3	75.00	37	92.50	.32
	Other RT	0	0.00	0	0.00		1	25.00	3	7.50	

Age (±confidence interval).
χ^2 test was used to analyze categorical parameters, and Student's *t* test was used for comparing age means between given groups.
N/A: statistics not available due to 0 case number; *NS*: *P* value is very close to 1.

Therapeutic Associations

Figure 3, *A-C* shows the predicted drug efficacy ratio according to LT vs. ST patients based on biomarkers identified and reported in different tumor types (DTT) (Supplementary Table 2). We identified EGFR V742A mutation. Of note, for this mutation, off compendium gefitinib in DTT was reported. Comparing LT vs. ST patients, a

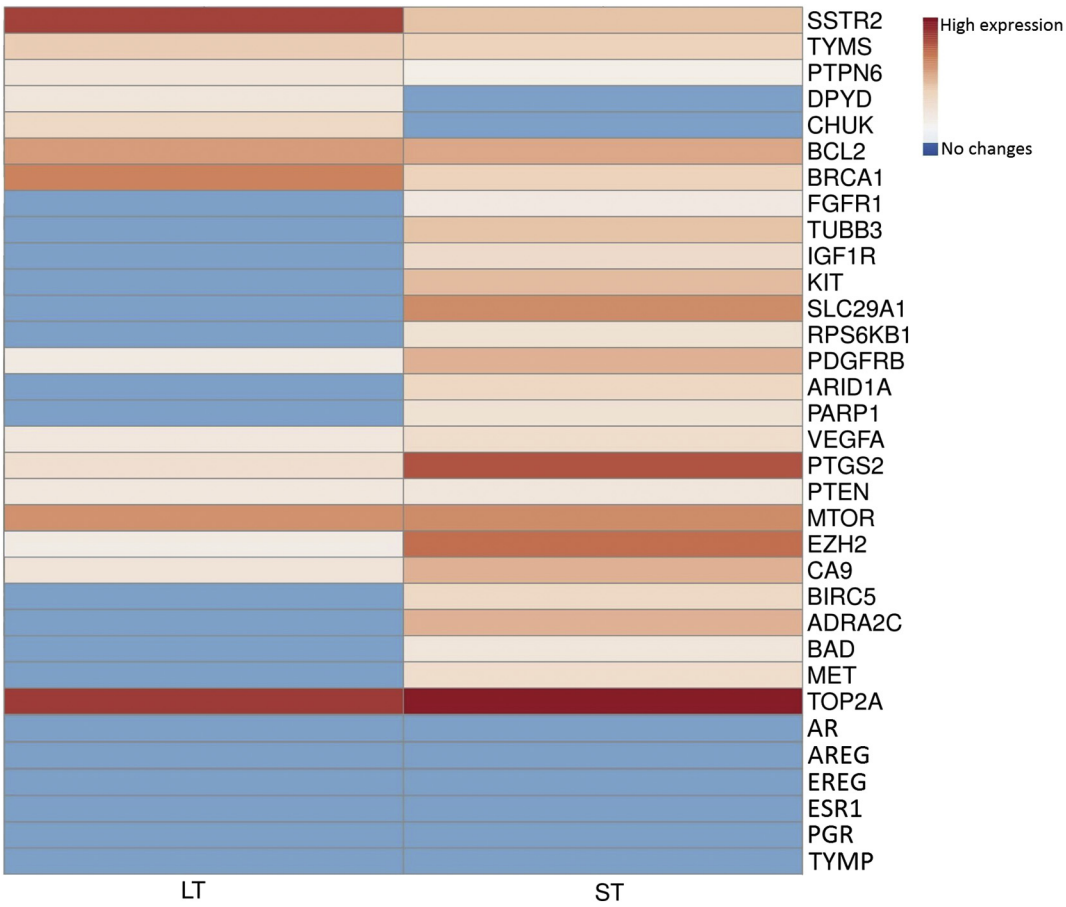

Figure 2. Heat map analysis of the mRNA expression profile of the top SCLC associated genes in our panel according to LT versus ST survival in patients with metastatic SCLC.Each row represents a single gene, while the columns indicate the two groups of patients according to survival time. The color blue indicates that there were no changes in expression, while the color red and its shades represent higher expression.

two-fold increase was found in the average predicted number of drugs per patient off compendium (6.0 vs. 3.0) when compared to drugs on compendium (3.3 vs. 3.3) or with reduced/lack of efficacy (4.6 vs. 4.5) [Figure 3, *A-C*]. The overall drug sensitivity percent (drugs on and off compendium pooled together) was more than 60 for 8 drugs in LT patients (vs. 1 ST patient, $P = .0197$; dashed line in Figure 3, *A-C* indicates that at least 60 of the patients are sensitive to a given drug). This ratio appeared to be more than 60 for drugs on compendium including doxorubicin, irinotecan, and topotecan, and drugs off compendium including epirubicin, lanreotide, octreotide, and ipilimumab plus nivolumab. The same ratio for reduced efficacy drugs included fluorouracil, tamoxifen, capecitabine, CisE, and trabectidin. Of note, we also found a two-fold increase in LT/ST drug sensitivity ratio for on compendium drugs irinotecan and topotecan, and off compendium drugs gefitinib, lanreotide, and octreotide.

Discussion

Solid tumors have historically been treated based on tumor histology. However, recently, basket trials can enroll in a tumor tissue agnostic fashion based on the molecular characteristics of a tumor rather than the tissue of origin. In our study, we used targeted NGS to identify new potential therapeutic targets in SCLC. While the number of new agents and treatment options has markedly increased in other cancers, for SCLC, CHT and RT remain the main component of care with no new class of systemic therapy entering clinical practice in the past three decades [15]. SCLC patients treated with CHT often experience highly varying treatment responses. Currently, in SCLC, there are no reliable clinical or molecular predictors available for identifying those with rapid versus slow progression. Therefore, many patients are aggressively treated with CHT with low, minimal, or no benefit. It remains unclear how SCLC biology and the lack of prolonged response to therapy are responsible for rapid tumor progression. Others have used NGS analysis of "outlier" patients to identify molecular alterations that are posited to be the basis of their biology or drug response [3,4]. In previous SCLC studies using whole-genome sequencing, extremely high transversion mutation rates were reported, which were considered to be predominantly smoking related [16]. In our study, we did not find a high transversion mutation rate, though in contrast to previous studies, we used targeted NGS.

We found a number of potential new targets and therapeutic associations for SCLC patients. Another group has reported that EGFR tyrosine kinase inhibitors (TKI) resistant tumors transformed from NSCLC into SCLC and were sensitive to standard SCLC treatments [17]. Genetic mechanisms of resistance were lost in the absence of the continued selective pressure of TKI, and such cancers

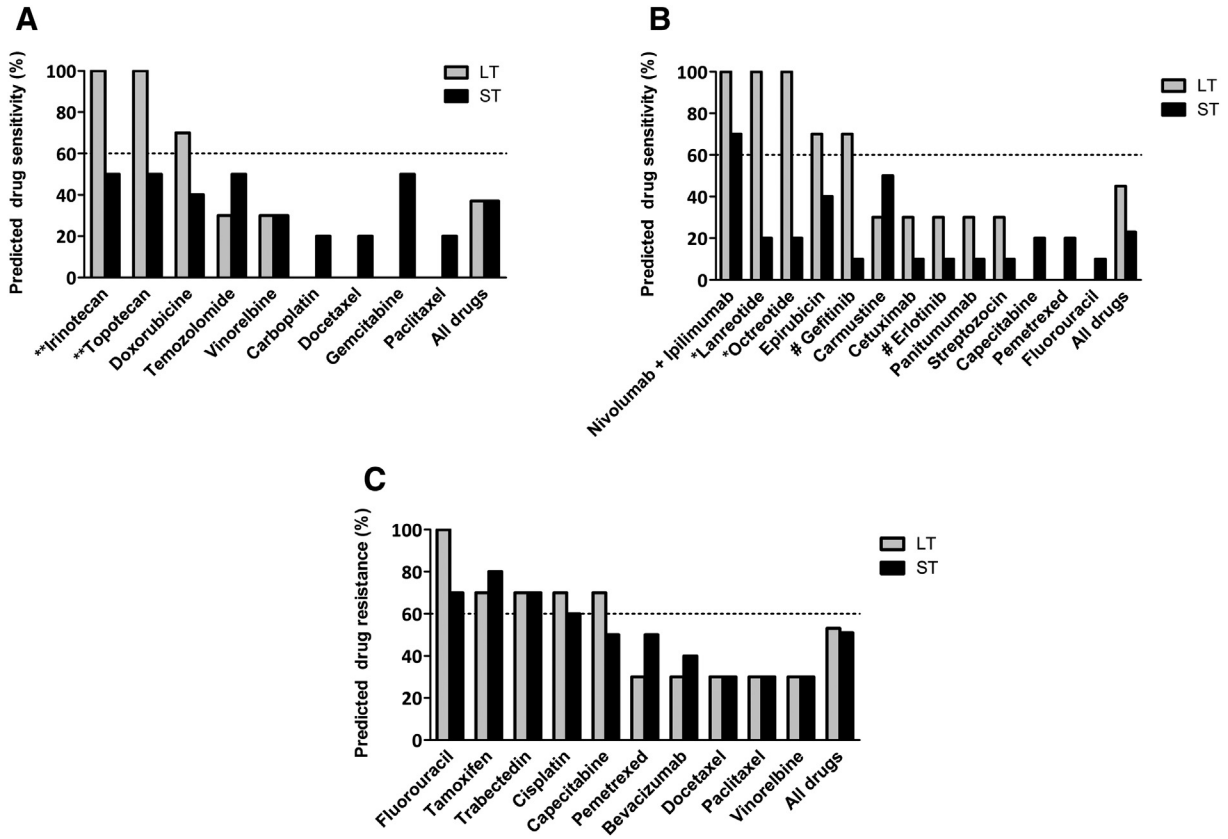

Figure 3. Comparison and availability of drugs on compendium (A), off compendium (B), and predicted drug efficacy with SCLC tumors resistant to different drugs (C) for ST versus LT SCLC patients ($n = 13$).Drug sensitivity percent (drugs on and off compendium pooled together) was more than 60% for eight drugs in LT survivors (vs. one ST survivor, $P = .0197$; Fisher's exact test; dashed line in Figure 2, *A-C* indicates that at least 60% of the patients are sensitive to a given drug). This ratio appeared to be more than 60% for drugs on compendium including doxorubicin, irinotecan, and topotecan, and off compendium drugs including epirubicin, lanreotide, octreotide, ipilimumab plus nivolumab, and gefitinib. The same ratio was found for reduced-efficacy drugs included fluorouracil, tamoxifen, capecitabine, CisE, and trabectidin. Of note, we found a two-fold increase in LT/ST drug sensitivity ratio for on compendium drugs irinotecan and topotecan, and off compendium drugs gefitinib, lanreotide, and octreotide.[#, *, **] Differences in predicted drug sensitivity are based on studies on the same biomarker in different tumor types.Dashed line indicates at least 60% drug sensitivity.

were sensitive to a second round of treatment with EGFR inhibitors. Interestingly, in our study, we found a baseline, pretreatment EGFR mutation in an LT patient.

Furthermore, we found a significantly increased number of drugs and also a two-fold increase in off compendium drugs available for LT vs. ST patients. Our data suggest the potential for clinical benefit using this panel of biomarkers that would need to be validated in large SCLC cohorts. We also identified four cancer mutations that have not been previously reported in SCLC. ERBB4, EGFR, and EP300 belong to the same HER2 signal transduction pathway, and although the exact clinical relevance of the aforementioned genes is still partly unknown, the activation of ERBB3 and PI3K signaling contributes to acquired resistance to tyrosine kinase inhibitors targeting EGFR and HER2 in lung and breast cancer [18]. According to a recent study on lung adenocarcinoma patients, a positive correlation might exist between EGFR and BRCA1 methylation but not EGFR mutation, and epigenetic modifications of BRCA1 are independent events against EGFR mutation [19]. However, no data are available regarding the association between the aforementioned mutations in SCLC. Meanwhile, BRCA1 might also serve as a potential target in BRCA-related breast cancer treated with Olaparib [20]. Of note, TP53 point mutation was not detected in our analysis possibly due to poor sample quality, while RB1 was not included in the gene panel provided by Paradigm at the time of the analysis because it was not yet targetable with any drugs available on or off compendium.

As mentioned before, no CNV was identified either in any of the samples. To our knowledge, to date, no comprehensive study was performed on CNVs according to the top SCLC associated genes. However, others reported that CNVs in SCLC are greatly influenced by the administered treatment and CHT lines as well, and constant CNVs are rare [21].

Using this biomarker panel, we did not find mRNA overexpression in PR or ER in any of the patients. However, PR or ER IHC was not done, as hormone therapy does not have clinical relevance in SCLC. Transcription analysis of HENT1 (SLC29A1) predicts survival in pancreatic cancer patients treated with gemcitabine [22,23]. In our study, HENT1 (SLC29A1) mRNA with 50%-79% protein concordance revealed gemcitabine as a potentially actionable drug in SCLC. Gemcitabine as a single agent has been studied in SCLC; however, a modest activity was shown in previously treated, resistant SCLC patients [24,25].

Diseases associated with SSTR2 include thymoma type C and neuroendocrine tumor [26]. Octreotide and lanreotide were reported as an effective drug for those with high SSTR2 expression [27,28]. We found high SSTR2 mRNA expressions in all LT patients versus two of ST patients, which may reflect more benign neuroendocrine tumor characteristics in the ST group. In this scenario, octreotide and lanreotide could be an effective drug for those with high SSTR2 expression [27,28].

TOPO IIa mRNA expression could predict response to commonly used chemotherapeutic agents, as the protein product of this gene represents the molecular targets of anthracycline drugs [29−31]. Moreover, in a variety of human cancers, cell lines data suggest that tumors with high TOPO IIa mRNA expression might be highly sensitive for TOPO IIa inhibitor aclarubicin [32]. In our study, in contrast to three LTs, molecular testing revealed that three ST patients treated with CAV were not predicted to be sensitive to doxorubicin or epirubicin.

Loss of AT-rich interaction domain 1A (ARID1A) tumor suppressor gene in ovarian clear cell carcinoma is a negative prognostic factor in patients treated with platinum-based chemotherapy [33]. In rectal and bladder cancer, the expression of the inhibitor-of-apoptosis (IAP) protein survivin was evaluated and identified as a strong independent prognostic factor for response and survival after CisE-containing chemotherapy [34−36]. Decreased BRCA1 expression may identify subsets of triple-negative breast cancers that are CisE sensitive [37,38]. Furthermore, survivin is also an important target for cancer vaccines and immunotherapy as well [39]. In our study, most patients' tumors would be predicted to be resistant to CisE (BRCA1 and/or survivin (BIRC5) mRNA overexpression was observed), and only two STs were sensitive to CE (ARID1A mRNA expression was high), though all patients received combination platinum therapy.

High TOP1 protein expression was associated with irinotecan and topotecan sensitivity based on previous reports of CRC and ovarian carcinoma studies [40,41]. In our study, in most of the cases, the presence of TOP1 supports the current guidelines that camptothecins can be effective drugs in SCLC.

In our study, TIL and tumor cells were negative for PD-L1 in all 13 analyzable cases. In contrast, two recent studies have reported PD-L1 positivity in 16.5%-28.6% of SCLCs [42, 43]. A possible explanation is the different antibody and/or methodology (in contrast to our study, similar threshold but KEYNOTE-028 study considered PD-L1−positive patients that had membranous PD-L1 expression in $\geq$1% of tumor and associated inflammatory cells or positive staining in stroma) used in the different studies.

Temozolomide has modest antitumor activity in glioblastoma, and expression of MGMT correlates with response to temozolomide. MGMT expression was not detected by IHC in any of the samples, so it would exclude temozolomide [44] or carmustine [45] as a possible therapeutic option in our cohort of SCLC. A phase II trial in patients with platinum sensitive or refractory SCLC treated with veliparib and temozolomide suggests that temozolomide has activity in relapsed SCLC and response to temozolomide may correlate with MGMT methylation in SCLC [46].

We found two patients (one ST and one LT) with COX2 (PTGS2) overexpression, a potential target for aspirin (however, these two patients were not treated with aspirin). Additionally, three ST patients received aspirin with no COX2 (PTGS2) overexpression [47]. Based on these results, we cannot confirm the relevance of aspirin in SCLC. Furthermore, our previous study showed no survival benefit with patients on standard of care therapy and aspirin [8].

When we compared our biomarker data to the PathCards database SCLC superpath analysis, there was no clear association to any pathways already detected. Interestingly, MYC overexpression, which is associated to a variant neuroendocrine subtype, a potential target and negative prognostic factor, was not found among ST cases [48].

Thoracic RT was coincidentally linked to improved OS in this cohort of patients [8]. RT was eventually offered when the local control of the extrathoracic disease could be controlled by chemotherapy or when organ metastases were treated successfully either with surgery or RT. Consequently, in our study, besides the reported clinically relevant biomarkers identified, local control of the organ metastasis along with consolidation thoracic RT may have been a contributor to patients experience LT survival.

Finally, according to our findings regarding the therapeutic associations at the time of this analysis, there would have been potential clinical trials available for two patients: LT9 patient could have been a potential candidate for a trial with treatment including lapatinib, erlotinib, and sunitinib for primary tumor type based on EGFR [49] mutation and also for trials conducted in cancer outside of primary tumor type based on BCL-2 [50] and EGFR [51–53] mutations, while ST2 patient could have been a potential candidate for other trials only outside of the primary tumor type based on high BRCA1 [54,55] and mTOR [51] mRNA expression.

There are several limitations of this study. There were no matched tumor and normal DNA pairs. Therefore, in contrast to extreme responders in recent studies using whole-genome sequencing, our study used comprehensive targeted NGS on tumor tissue. Another limitation of this study was the small number of eligible patients with tumor tissue available. As is often the cases in routine practice, most cases of SCLC had only cytological samples and/or the entire FFPE block was cut and used in the routine diagnostic pathological processing. Unfortunately, these common practices (cytology and exhaustion of sample in pathology processing) leave far fewer remaining samples for potential retrospective research evaluation in SCLC than in other cancer types (e.g., colorectal and breast cancer, even NSCLC). Therefore, only descriptive statistics were used to summarize the findings of this study. It is somewhat reassuring that for the clinicopathologic characteristics between patients that had tumor NGS and those that did not, there were no significant differences. Also, we cannot fully discriminate that RT is a consequence or influencing longer OS. Targeted genomic sequencing might serve as a prognostic marker rather than a predictive marker. It may be that patients who do not have actionable targets have more biologically aggressive or resistant disease.

Conclusions

In conclusion, we found a two-fold increase in off compendium drugs available for LT vs. ST. Furthermore, our study demonstrated that consolidation RT and higher predicted drug sensitivity for off compendium were associated with LT compared to ST patients in SCLC.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2019.08.004.

Ethics Statement

The study was directed in accordance with the guidelines of the Helsinki Declaration of the World Medical Association. The national level ethics committee (Hungarian Scientific and Research Ethics Committee of the Medical Research Council, ETTTUKEB-7214-1/2016/EKU) approved the study. The need for individual informed consent for this retrospective study was waived. After clinical information was collected, patient identifiers were removed, and subsequently, patients cannot be identified either directly or indirectly.

Competing Interests

G.J. W. is an employee of Unum Therapeutics, outside of this work; reports personal fees from MiRanostics Consulting, Paradigm, Angiex, IBEX Medical Analytics, Spring Bank Pharmaceuticals, Pfizer, IDEA Pharma, GLG Council, Guidepoint Global, Ignyta, and Circulogene, all outside this work; has received travel reimbursement from Cambridge HealthTech Institute and Tesaro; has ownership interest in MiRanostics Consulting, Unum Therapeutics, and Circulogene, outside the submitted work; and has a patent for methods and kits to predict prognostic and therapeutic outcome in small cell lung cancer issued, outside the submitted work. Other authors declare no potential conflicts of interest.

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