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BRIEF REPORT: SMALL CELL LUNG CANCERS IN PATIENTS WHO NEVER SMOKED CIGARETTES

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INTRODUCTION

While tobacco smoking remains the most important risk factor for the development of small cell lung cancer (SCLC), an epidemiological study reported that 3% of patients with SCLCs are never-smokers.[1] Several reports describe the rare, but well-documented, phenomenon of transformation to SCLC as a mechanism of acquired resistance (AR) to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in 4–14% of patients initially treated for *EGFR*-mutant lung adenocarcinomas, including in some patients who are never-smokers.[2–5]

Scant literature exists describing *de novo* SCLCs among patients who are never-smokers. We report on 23 patients with SCLCs who classify themselves as never-smokers.

MATERIALS AND METHODS

Patient identification and smoking history documentation

We performed a retrospective review of 1040 SCLC patients evaluated at Memorial Sloan-Kettering Cancer Center (MSKCC) between 2005 and 2012. Patients with lung cancers assessed at MSKCC complete a prospective, self-administered smoking questionnaire at the initial visit. Never-smokers are defined as those patients who report having smoked ≤ 100 cigarettes in their lifetime.

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Pathologic review of small cell lung cancer

The diagnosis of SCLC was confirmed through histologic and immunohistochemical (IHC) testing (chromogranin, synaptophysin, CD56, and MIB1) by a thoracic pathologist.

KRAS, EGFR, ALK, and RB testing

Patients with sufficient tissue underwent testing for *EGFR* and *KRAS* mutations and *ALK* rearrangements. *EGFR* mutations (exon 19 deletions and exon 21 L858R amino acid substitutions) were identified by mutation-specific PCR-based methods.[6–8] *KRAS* codon 12 and 13 mutation identification was performed by both mass-spectrometry (Sequenom, Inc., San Diego, CA)-based genotyping or direct sequencing. *ALK* rearrangements were tested using either fluorescence in situ hybridization (dual-color break-apart ALK probe, Abbott Molecular) or IHC (ALK-01 Ventana 790-2918). RB expression was analyzed using IHC (clone 1F8, Leica Biosystems).

Comprehensive, integrated mutation analysis of actionable cancer genes using next generation sequencing (NGS)

DNA was extracted from biopsied tissue and cytology specimens (and patient-matched normal tissue) using Qiagen nucleic acid extraction kits. Using our MSK-IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets), bar-coded sequence libraries were prepared (Illumina TruSeq), and exon capture was performed on bar-coded pools by hybridization (Agilent SureSelect Target Enrichment) using custom oligonucleotides to capture all exons and select introns of 279 cancer genes. DNA was sequenced on an Illumina HiSeq 2000 to maximize sensitivity for detecting mutations. This strategy enables the identification of mutations, indels, and copy number alterations involving these 279 genes.

RESULTS

Incidence

Two percent of patients (23/1040) with SCLCs were never-smokers. Among never-smokers with SCLCs, 83% (19/23) had *de novo* SCLCs, and 17% (4/23) of patients had small cell transformation as a mechanism of AR to EGFR TKIs in *EGFR*-mutant lung adenocarcinoma. Baseline characteristics are listed in Table 1. Clinical and pathologic characteristics of each patient are listed in Table 2.

De novo Small Cell Lung Cancers

Pathologic Characteristics—Pathologic re-review confirmed the following: 15 pure SCLC; one mixed SCLC and large cell neuroendocrine carcinoma; one SCLC and spindle and giant cell carcinoma (Figures 1A and 1B); and two mixed SCLC and adenocarcinoma.

Clinical Characteristics—Five of 19 patients with *de novo* SCLCs were either lost to followup or had inadequate information available regarding treatment course and response. Of the 14 cases with available treatment history, all received first-line etoposide/platinum doublets. Fifty-seven percent of these patients (8/14) had a response to chemotherapy that

lasted ≥3 months from completion of first-line therapy. Median time to progression was 11 months (95%CI: 5–13 months). Median overall survival (OS) from the time of SCLC diagnosis was 23 months (95%CI: 10–27 months).

EGFR, KRAS, ALK, and RB testing—Eight of the 19 *de novo* SCLC cases underwent testing for *EGFR* Exon 19 deletion and Exon 21 L858R mutation. An *EGFR* L858R mutation was found in one patient, whose tumor contained SCLC and adenocarcinoma components. This patient initially received erlotinib, carboplatin, and etoposide, with progression of disease within three months of completing chemotherapy. While he continued erlotinib throughout his subsequent chemotherapeutic regimens, his disease rapidly progressed. The tumor of one patient with pure SCLC harbored an *EGFR* Exon 19 deletion and *PIK3CA* E545K mutation. This patient had progressive disease after four cycles of etoposide/platinum. He was then initiated on erlotinib, with progression of disease after just four weeks of therapy.

There were no *KRAS* mutations or *ALK* rearrangements identified in the cases tested for these alterations. Six of seven cases tested for RB expression demonstrated RB loss (Table 3).

Acquired Resistance Small Cell Lung Cancer

Pathologic characteristics—Of the four patients with SCLC as a mechanism of AR to EGFR TKIs, two had pure SCLC, one had mixed histology of SCLC and adenocarcinoma, and one had SCLC, adenocarcinoma, and large cell neuroendocrine carcinoma components.

Clinical Characteristics—All four patients were women [median 48 years; range 40–75 years] at the diagnosis of SCLC transformation from *EGFR*-mutant lung adenocarcinoma. Once the diagnosis of SCLC was made, two patients received platinum/etoposide. One of these patients underwent local therapy with surgical resection of a lung nodule that demonstrated recurrent growth while all other sites of disease had resolved.[9] Pathology review of this specimen with acquired resistance demonstrated SCLC, adenocarcinoma, and large cell neuroendocrine carcinoma components. After receiving 6 cycles of adjuvant carboplatin, etoposide, and erlotinib therapy, this patient had a nine month disease-free interval after treatment. The two patients who did not receive platinum/etoposide therapy had varied treatments and clinical courses.

EGFR, KRAS, ALK, and Rb testing—The patients with SCLC as a mechanism of AR to EGFR TKI had persistence of the original *EGFR* mutation in their tumors confirmed on biopsy taken at the time of resistance. Of three cases also tested for *EGFR* Exon 20 T790M mutation at the time of AR, two had the second-site *EGFR* Exon 20 T790M mutation, in addition to SCLC. Insufficient tissue was available for RB testing in these samples.

Comprehensive mutation analysis of actionable cancer genes using NGS

Two patients had adequate tissue available for NGS. In one patient, analysis revealed five mutations in four genes: *PHOX2B, NOTCH1*, *RB1*, and *TP53.* Additionally, amplification was seen in *TERT*. NGS analysis of the second patient's sample revealed two mutations in

the two genes *CBL* and *GNAS*, with amplification seen in *MYCL1*. Neither patients' samples had smoking-associated G -->T transversions (Table 4).

DISCUSSION

Two percent of SCLCs at our institution occurred in never-smokers. This represents the largest cohort of clinically and pathologically described never-smokers with SCLC. 83% of these diagnoses were made *de novo*.

Pathologic review of these SCLC cases confirmed the diagnosis. Immunohistochemistry for RB loss was performed on seven samples, and six of these revealed Rb loss, consistent with lung cancer of SCLC lineage.[10] Of eight patients with *de novo* SCLCs tested for *EGFR* mutations, one patient with mixed SCLC and adenocarcinoma had an *EGFR* L858R mutation, and a second patient with pure SCLC had a tumor with mutations in both *EGFR* (exon 19 deletion) and *PIK3CA* (E545K). Unlike patients with *EGFR*-mutant lung adenocarcinomas and the majority of patients with SCLC, these patients suffered rapid progression of disease that did not respond to therapy with EGFR TKIs or platinum/etoposide. Median OS in SCLC never-smokers was 23 months, which is longer than generally seen in SCLC.[11]

Of the four cases with SCLC as a mechanism of AR to EGFR TKIs in *EGFR*-mutant lung adenocarcinoma, two received cisplatin and etoposide. One patient had a partial response with a disease-free survival of 9 months, and the other did not respond. This small group represents a heterogeneous population in which the disease biology and clinical course can mirror both *EGFR*-mutant lung adenocarcinoma and SCLC.

The presence of *EGFR* mutations in tumors from patients with SCLCs remains an area of controversy. While an *EGFR* mutation in the setting of a mixed diagnosis of SCLC and adenocarcinoma can be seen with the *EGFR* mutation arising from the adenocarcinoma component, the etiology of the *EGFR* mutations in a single biopsy of SCLC is unclear. Prior work from our group has demonstrated that *EGFR* mutations do not exist in pure SCLC or squamous cell lung cancer.[12,13] One interpretation is that this finding of pure SCLC represents sampling error in the setting of tumor heterogeneity rather than two oncogenic mechanisms in a single cell.

Further molecular analysis using NGS of exons of 279 cancer genes revealed mutations in *TP53*, *Notch*, and *RB1*, consistent with prior reports regarding the molecular basis of SCLC.[14,15,16] Interestingly, no smoking-associated G -->T transversions were observed in the two samples tested.

Our analysis has several limitations. This is a retrospective study. Given the rarity of patients with SCLCs who are never-smokers, prospective identification and analysis of these patients is difficult. The available tissue for molecular analysis was limited, and molecular testing could only be performed on a minority of the patients identified. The patients in this series were identified as never-smokers based on self-report and direct questioning by the medical staff.

Patients with SCLCs who are never-smokers represent a clinically and pathologically distinct population because they bear little similarity to non-SCLCs among never-smokers. Their molecular characteristics are unique compared to never-smoker patients with adenocarcinomas. The transformation of *EGFR*-mutant lung adenocarcinomas to a SCLC phenotype co-incident with the emergence of EGFR TKI AR is an area of much interest and research relative to its rarity. This study further supports the need for comprehensive, multiplexed genotyping to improve our ability to provide optimal care and facilitate research in these unique populations.

Acknowledgments

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Figure 1A

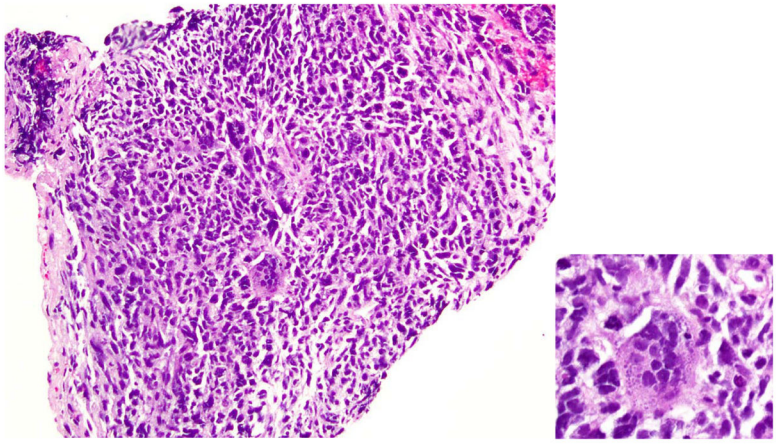

Figure 1B

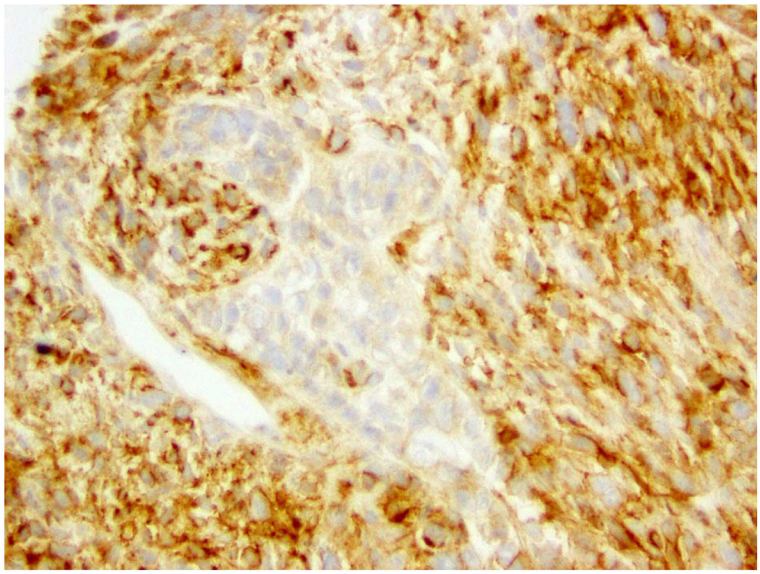

Figure 1.

TABLE 1

CLINICAL CHARACTERISTICS OF PATIENTS WITH SMALL CELL LUNG CANCER WHO ARE NEVER SMOKERS

	SCLC as Acquired Resistance (n = 4)‡	*De novo* SCLC (n = 19)
Sex		
Male	0	9
Female	4	10
Median age at diagnosis(Range)	48 (42–75)*	65 (35–94)
Race		
White	2	13
Black	0	3
Asian	2	3
Stage at diagnosis		
Limited	0	5
Extensive	4	14
Brain Metastases at Presentation		
Yes	2	4
No	2	15

‡All four of these patients had been treated with erlotinib.

*For patients with SCLC transformation as a mechanism of acquired resistance to EGFR TKIs, median age at diagnosis is the age at which SCLC transformation was diagnosed.

TABLE 2

CLINICAL AND PATHOLOGIC CHARACTERISTICS OF SMALL CELL LUNG CANCERS AMONG NEVER SMOKERS

PATIENT #	SEX	DE NOVO OR ACQUIRED RESISTANCE	STAGE	PATHOLOGY	MUTATIONS IDENTIFIED
1†	M	DE NOVO	EXTENSIVE	SCLC	
2	F	DE NOVO	LIMITED	SCLC / SPINDLE CELL	
3†	F	DE NOVO	LIMITED	SCLC	
4	F	DE NOVO	LIMITED	SCLC	*EGFR* WT / *KRAS* WT
5	F	DE NOVO	EXTENSIVE	SCLC	*EGFR* WT / *KRAS* WT
6	F	DE NOVO	EXTENSIVE	SCLC	*EGFR* WT / *KRAS* WT
7	M	DE NOVO	EXTENSIVE	SCLC	*EGFR* WT / *KRAS* WT
8	F	DE NOVO	EXTENSIVE	SCLC	*EGFR* WT / *KRAS* WT
9	M	DE NOVO	EXTENSIVE	SCLC / ADC	*EGFR* L858R / *KRAS* WT
10	M	DE NOVO	LIMITED	SCLC / ADC	
11	F	DE NOVO	EXTENSIVE	SCLC / Large Cell neuroendocrine carcinoma	
12	M	DE NOVO	EXTENSIVE	SCLC	
13	M	DE NOVO	EXTENSIVE	SCLC	*EGFR* Exon 19 del*
14	F	AR	EXTENSIVE	SCLC	*EGFR* Exon 19 del
15	F	AR	EXTENSIVE	SCLC / NEUROENDOCR INE CA	*EGFR* Exon 19 del
16	F	AR	EXTENSIVE	SCLC / ADC	*EGFR* Exon 19 del
17	M	DE NOVO	EXTENSIVE	SCLC	
18	F	DE NOVO	LIMITED	SCLC	
19	F	DE NOVO	EXTENSIVE	SCLC	
20	F	DE NOVO	EXTENSIVE	SCLC	
21	M	DE NOVO	EXTENSIVE	SCLC	
22	F	AR	EXTENSIVE	SCLC	*EGFR* Exon 19 del
23	M	DE NOVO	EXTENSIVE	SCLC	*EGFR* WT / *KRAS* WT

*This patient's tumor also harbored a *PIK3CA* E545K mutation.

†Please see Table 4 for next generation sequencing results.

M – male

F – female

AR – acquired resistance

SCLC – small cell lung cancer

ADC – adenocarcinoma

NSCLC – non-small cell lung cancer

TABLE 3

PATHOLOGIC CHARACTERISTICS OF SMALL CELL LUNG CANCERS AMONG NEVER SMOKERS

Pathologic confirmation of SCLC	**SCLC as Acquired Resistance (n = 4)**	***De novo* SCLC(n = 19)**
Pure SCLC	2	15
Mixed Histology	2	4
EGFR mutations found / *EGFR* testing performed	4*/4	2/8
KRAS mutations found / *KRAS* testing performed	0/2	0/8
ALK rearrangements found / *ALK* testing performed	0/0	0/5
RB loss found / *RB* testing performed	0/0	6/7

* All 4 patients with *EGFR* mutations had *EGFR* Exon 19 deletions present at biopsies taken at baseline and at the time of acquired resistance to EGFR TKIs.

TABLE 4

NEXT GENERATION SEQUENCING OF ALL EXONS AND SELECTED INTRONS OF 279 CANCER TARGET GENES

Sample	Gene altered	Alteration present	Protein alteration	Base Pair alteration
Pt 1	PHOX2B	Missense Mutation	P82L	G → A
	NOTCH1	Frame Shift Insertion	P2485fs	
	RB1	Splice Site	R500_splice	G → A
	TP53	Frame Shift Deletion	V218fs	
	TP53	Frame Shift Deletion	V73fs	
	TERT	Amplification		
Pt 2	CBL	Missense Mutation	C401S	G → C
	GNAS	Missense Mutation	M102V	A → G
	MYCL1	Amplification		