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Research article

Lung adenocarcinoma patients with *ROS1*-rearranged tumors by sex and smoking intensity

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ABSTRACT

Background: *ROS1* rearrangements (*ROS1*+) define a distinct molecular subset of lung adenocarcinomas. *ROS1* + tumors are known to occur more in never-smokers, but the frequency and outcome of *ROS1* positivity by sex and smoking intensity are not clearly documented.

Patients and methods: This patient cohort study included all never- (<100 cigarettes lifetime) and light- (100 cigarettes-20 pack-years) smokers, and a sample of heavy-smokers. *ROS1* + rates by sex and smoking intensity were compared within and beyond our study. Survival outcomes were analyzed using Kaplan-Meier curves and Cox proportional hazards models.

Results: Of the 571 total patients, *ROS1* + was detected in 24 (4.2%): 6.4% in men and 3.0% in women; 5.1% in never-, 5.7% in light-, and 1.8% in heavy-smokers (P=0.05). Among the 209 stage IIIB-IV patients, men had much higher *ROS1* + rate (11.1%) not only than women (1.7%, P=0.004) in our study, but also than men (0.4%–1.8%) in 8 published studies (Ps = 0.0019–0.0001). *ROS1*+ rates were similar between never- (9.3%) and light-smokers (8.1%) and significantly lower in heavy-smokers (1.2%, P=0.017), a finding confirmed by 6 published studies (Ps = 0.041–0.0001). Overall survival of *ROS1* + patients were significantly better than the *ROS1*- (P=0.023) mainly due to targeted therapy. Among patients who exhibited resistance to crizotinib, follow-up treatment of entrectinib and lorlatinib showed remarkable survival benefits.

Conclusions: The *ROS1* + rates were higher in men than in women, and similar in never- and light-smokers, more pronounced in stage IIIB-IV patients. Newer-generation *ALK/ROS1*-targeted drugs showed efficacy in a cohort of crizotinib resistant *ROS1* + patients. These results, when validated, could assist efficiently accruing *ROS1* + patients.

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1. Introduction

The incidence and mortality of lung cancer have been decreasing; however, it remains the primary cause of cancer-related deaths in the United States [1]. Most patients are diagnosed with metastatic disease and have a 5-year survival rate of approximately 7% [1]. There have been major advancements in the diagnosis and treatment of advanced non-small cell lung cancer (NSCLC), with one of the most important developments being the characterization of key molecular alterations that drive lung carcinogenesis in a subset of NSCLC. Previous reports suggested that *c-ros oncogene 1* (*ROS1*) rearrangements occurred in 0.9%–1.7% NSCLC patients [2–5] and 1.3%–2.8% advanced-stage patients [6,7].

ROS1 is a receptor of tyrosine kinase of the insulin receptor family and has been identified as a driver gene of NSCLC patients [2]. *ROS1* rearrangements (*ROS1*+) tumors are usually associated with younger age, no smoking history, and adenocarcinomas, and appear a similar frequency in men and women [2]. Genomics Evidence Neoplasia Information Exchange project (GENIE) showed alike *ROS1* + rates in men and women among NSCLC patients [8] while other studies found *ROS1* + rates tended to be prevalent in women [9–11]. The first study by Bergethon and colleagues did not give a clear definition for “light-smoker” that could be differentiated from other smokers regarding *ROS1* + rate [2]; GENIE defined light-smokers as 1–15 pack-years (py) smoking history with *ROS1* + rate at 0.9%, higher than the heavy-smokers as more than 15py (0.08%, P=0.0057) [8]. In a recent real-world population-based study from the Netherlands [12], the prevalence of *ROS1* + was 0.43% among stage-IV NSCLC patients, without information on smoking history.

In 2016, crizotinib was the first tyrosine kinase inhibitor (TKI) approved by the U.S. Food and Drug Administration (FDA) for *ROS1* + NSCLC based on PROFILE 1001 study [13]; entrectinib was approved later [14] and lorlatinib was the newest treatment option [15]. The response to targeted therapy and progression-free survival (PFS) for *ROS1* + NSCLC have improved much with median time to progression of 5.5–20 months [16].

Although *epidermal growth factor receptor* (*EGFR*) gene mutated proportionally more in women than in men [17], the variation of *ROS1* + by sex is still inconclusive. Given the higher incidence of *ROS1* + in never-smokers, the PROFILE 1001 study included a very small population of smokers and former smokers [13], and the distribution of *ROS1* + tumors among patients with different smoking intensity was unavailable. We hypothesized that the distribution of *ROS1*+ tumors varied by sex and smoking status in real-world patients, especially in never- and light-smokers. Therefore, we conducted an observational cohort study combined with a comparison of our findings with the published literature to further elucidate the rate of *ROS1* + according to sex and smoking intensity.

2. Methods

2.1. Patient population

Patients were enrolled from the Mayo Clinic Lung Cancer Cohort, which included approximately 17,000 newly diagnosed primary lung cancer patients between 1997 and 2015. Detailed procedures of patient enrollment, diagnosis, data collection, and follow-up have been previously described [18–20]; the study was approved by the Mayo Foundation Institutional Review Board (IRB# 225-9). Briefly, pathologic diagnoses of primary lung cancer for the study cohort were made at Mayo Clinic, where all patients were treated, prospectively enrolled, and followed through 2022.

The evaluated cohort consisted of patients with an adenocarcinoma according to the World Health Organization (WHO) classification [21]. All patients who had available slides or tumor tissue for pathologic review were screened and categorized into light-, never- and heavy-smokers. The design was to maximize patients with *ROS1* + in never- and light-smokers, with a random sample of heavy-smokers as a comparator; total sample size was restricted by funding limit. The cigarette smoking intensity was classified by self-report smoking history at the time of diagnosis recorded in patient medical records: never-smokers (none to fewer than 100 cigarettes during their lifetime), light-smokers (100 cigarettes-20py), or moderate-heavy smokers (>20py, abbreviated as heavy-smokers in this study).

2.2. ROS1 diagnosis and co-occurring mutations

ROS1 + was detected using a laboratory-developed dual-color break-apart probe (Vysis; Abbott Molecular, Abbott Park, IL) [22]. Hematoxylin and eosin (HE)-staining was performed on 5-μm sections. Using HE slide as a reference; target areas were etched with a diamond-tipped etcher on the back of the unstained slide to be assayed by a pathologist. Each probe was hybridized to the appropriate target area and 2 technologists each analyzed 50 interphase nuclei (100 total) with the results recorded as percentage of abnormal nuclei. The probe set was independently validated in a blinded study on 20 paraffin-embedded lung adenocarcinoma tissue samples and 25 noncancerous controls. *ROS1* + was verified in samples previously identified using reverse-transcriptase-polymerase-chain reaction. Normal controls were used to determine a cutoff value for this assay.

Fluorescence in situ hybridization (FISH) analysis of rearrangement was performed on cytology or tissue samples using probes for 3′ and 5′ regions of the *ROS1* gene at 6q22 in 100 nuclei [3]. FISH scoring was performed by experienced technicians in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory, and the results were reviewed by expert pathologists. According to the ratios of separated signals, FISH results were dichotomized into *ROS1* + and *ROS1*-. Cases with either >9% of the tumor cells showing an isolated 3′ signal or >5% of the tumor cells showing an isolated 3′ signal plus other types of isolated signals in >3% of the tumor cells were ruled as *ROS1*+; all other cases were considered *ROS1* negative (*ROS1*-). The 3′ signal was used, since the kinase domain is theoretically within this region, and if lost, there likely is no kinase domain; an isolated 5′ domain is clinically equivocal.

The co-occurring mutations of *EGFR* and *ALK* were detected when the patients were diagnosed with lung adenocarcinoma by pathologists. A polymerase chain reaction (PCR) based assay employing allele specific amplification was used to test for the presence of 29 mutations within exons 18–21 of the *EGFR* gene (G719A, G719S, G719C in exon 18; small deletions in exon 19; T790 M, S768I, and small insertions in exon 20; and L858R and L861Q in exon 21). FISH was used for testing rearrangement of the *ALK* gene locus using FDA approved probe kit for the 3′ and 5′ regions of the *ALK* gene at 2p23 in 50 nuclei.

2.3. Data extraction and outcomes

Data retrospectively collected on identified patients included clinical characteristics, tobacco consumption, outcomes, and all information on therapeutic strategies. Clinicopathologic stage was assigned according to the seventh edition of the TNM staging system [23], which was published in 2009. In TNM system, the letter T refers to size and extent of the main tumor, usually called primary tumor; the letter N represents the number and location of regional lymph nodes involvement; the letter M represents distant metastasis [24]. Responses were defined as the best response from the start of treatment until disease progression, according to Response Evaluation Criteria In Solid Tumors (RECIST) guidelines version 1.1 [25]. Overall response rate (ORR) was defined as the proportion of patients who achieved a complete response (CR) or partial response (PR). The disease control rate (DCR) was defined as the proportion of patients with a best overall response of CR, PR or stable disease (SD); CR, PR, and SD were defined in accordance with RECIST [25].

Table 1
Descriptive characteristics of the population.

Characteristic	Total (N = 571)	*ROS1* status		
		ROS1 + (n = 24)	*ROS1*-(n = 547)	*P*-value
Age at diagnosis				
Mean (SD)	66.1 (11.5)	58.4 (13.5)	66.4 (11.4)	**0.003**
Median (range)	68 (26–93)	60 (33–85)	68 (26–93)	
Gender, n (%)				**0.052**
Male	203 (35.6)	13 (54.2)	190 (34.7)	
Female	368 (64.4)	11 (45.8)	357 (65.3)	
Race, n (%)				1.000
Caucasian	517 (90.5)	22 (91.7)	495 (90.5)	
Other	54 (9.5)	2 (8.3)	52 (9.5)	
Cigarette smoking status, n (%)				0.344
Never	334 (58.5)	17 (70.8)	317 (58.0)	
Former	140 (24.5)	3 (12.5)	137 (25.0)	
Current	97 (17.0)	4 (16.7)	93 (17.0)	
Smoking intensity among all smokers (n = 237), n (%)				0.200
Light (100 cigarettes-20 pack-years)	70 (29.5)	4 (57.1)	66 (28.7)	
Heavy (>20 pack-years)	167 (70.5)	3 (42.9)	164 (71.3)	
Smoking Intensity (all patients)				0.065
Never-light (0–20 pack-years)	404 (70.8)	21 (87.5)	383 (70.0)	
Heavy (>20 pack-years)	167 (29.2)	3 (12.5)	164 (30.0)	
Tumor grade, n (%)				0.287
Well	177 (31.0)	5 (20.8)	172 (31.4)	
Moderate	192 (33.6)	7 (29.2)	185 (33.8)	
Poor	202 (35.4)	12 (50.0)	190 (34.7)	
Stage, n (%)[c]				0.195
I	213 (37.3)	6 (25.0)	207 (37.8)	
II	59 (10.3)	1 (4.2)	58 (10.6)	
IIIA	90 (15.8)	5 (20.8)	85 (15.5)	
IIIB	26 (4.6)	3 (12.5)	23 (4.2)	
IV	183 (32.0)	9 (37.5)	174 (31.8)	
ALK rearrangement				0.445
Positive	13 (2.3)	0	13 (2.4)	
Negative	558 (97.7)	24 (100)	534 (97.6)	
EGFR mutation, n (%)				0.711
Positive	47 (8.2)	1 (4.2)	46 (8.4)	
Negative	524 (91.8)	23 (95.8)	501 (91.6)	
Treatment[a]				0.018
Drug or drug-related therapy	170 (29.8)	12 (50.0)	158 (28.9)	
Surgery and drug with/without radiation	124 (21.7)	7 (29.2)	117 (21.4)	
Surgery only or other therapy	277 (48.5)	5 (20.8)	272 (49.7)	
Targeted therapy[b]				**< 0.001**
ALK/ROS1 drug – Yes	20 (3.5)	9 (37.5)	11 (2.0)	
ALK/ROS1 drug – No	551 (96.5)	15 (62.5)	536 (98.0)	

[a] 294 patients received drug therapy, including chemo-, targeted-, immune- and other pharmacologic agents; "other therapy" consisted of radiation only, surgery with adjuvant radiation, other treatment, and unspecified treatment.
[b] Targeted therapy included crizotinib or ceritinib.
[c] Because of low number of patients, stages I + II and IIIA + IIIB were also combined, and not reach significance (*P*=0.128).

Annual verification of patients' vital status was accomplished through the Mayo Clinic's electronic medical notes and registration database, next-of-kin reports, death certificates, and obituary documents filed in the patient's medical records, as well as through the Mayo Clinic Tumor Registry and Social Security Death Index website. Follow-up was used for determination of OS, defined as the time from diagnosis to date of last follow-up or patient death, and disease-free survival (DFS), defined as the time from diagnosis to tumor recurrence, progression, or death from lung cancer. Data on these events were collected in a prospective manner during follow-up that was up to 20 years from the date of diagnosis.

2.4. Literature review and comparison

Published literature from 2007 to 2023 was searched and retrieved from PubMed with a strategy of ("ros1"[All Fields] AND ("sex"[MeSH Terms] OR "sex"[All Fields])) OR ("ros1"[All Fields] AND ("smoke"[MeSH Terms] OR "smoke"[All Fields] OR "smoked"[All Fields] OR "smokes"[All Fields] OR "smoking"[MeSH Terms] OR "smoking"[All Fields] OR "smokings"[All Fields])). Three inclusion criteria for eligible articles were as follows: (a) observational studies for NSCLC in real-world setting or cohort; (b) *ROS1* status among sex or smoking history was involved; and (c) race of the participants was comparable to our study. Studies specifically on Asian population, case reports and guidelines of *ROS1* + in NSCLC were excluded. *ROS1* + rates by sex and smoking intensity, measured as pack-years, were compared within our study sample and to what were reported in studies selected from the online search.

2.5. Statistical analysis

Descriptive statistics characterized means and proportions, and *t*-test and Fisher's exact test were used to evaluate proportions for sparse data [26]. Kaplan-Meier survival analysis was used to evaluate OS and DFS, with comparisons performed using log-rank tests [27]. Patients still alive at the last visit were censored at the date of last follow-up, as were patients without documented evidence of an event. To evaluate the independent role of each variable on survival outcome, univariate and multivariate Cox proportional hazards (CoxPH) models were applied separately for OS and DFS [27]; one model adjusted for age, sex, smoking status, and treatment (drug or

Table 2
Descriptive Characteristics of Stage IIIB-IV patients (N = 209).

Characteristic	Total, N = 209	*ROS1* status		
		ROS1+	*ROS1-*	*P*-value
		(n = 12)	(n = 197)	
Age at diagnosis				**0.009**
Mean (SD)	63.4 (12.1)	53.3 (15.2)	64.0 (11.7)	
Median (range)	64 (26.0–93.0)	49.5 (33.0–85.0)	64 (26.0–93.0)	
Gender, n (%)				**0.004**
Male	90 (43.1)	10 (83.3)	80 (40.6)	
Female	119 (56.9)	2 (16.7)	117 (59.4)	
Race, n (%)				0.605
Caucasian	184 (88.0)	10 (83.3)	174 (88.3)	
Others	25 (12.0)	2 (16.7)	23 (11.7)	
Cigarette smoking status, n (%)				0.180
Never	86 (41.1)	8 (66.7)	78 (39.6)	
Former	63 (30.1)	2 (16.7)	61 (31.0)	
Current	60 (28.7)	2 (16.7)	58 (29.4)	
Smoking intensity among all smokers (n = 119), n (%)				0.046
Light (100 cigarettes-20 pack-years)	37 (30.1)	3 (75.0)	34 (28.6)	
Heavy (>20 pack-years)	86 (69.9)	1 (25.0)	85 (71.4)	
Smoking Intensity (all patients), n (%)				0.017
Never-light (0–20 pack-years)	123 (58.9)	11 (91.7)	112 (56.9)	
Heavy (>20 pack-years)	86 (41.1)	1 (8.3)	85 (43.1)	
Tumor grade, n (%)				0.679
Well	12 (5.7)	0 (0.0)	12 (6.1)	
Moderate	66 (31.6)	4 (33.3)	62 (31.5)	
Poor	131 (62.7)	8 (66.7)	123 (62.4)	
ALK rearrangement, n (%)				0.477
Positive	8 (3.8)	0 (0.0)	8 (4.1)	
Negative	201 (96.2)	12 (100.0)	189 (95.9)	
EGFR mutation, n (%)				0.889
Positive	38 (18.2)	2 (16.7)	36 (18.3)	
Negative	171 (81.8)	10 (83.3)	161 (81.7)	
Treatment, n (%)				0.131
drug or drug-radiation therapy	143 (68.4)	11 (91.7)	132 (67.0)	
surgery and drug with/without radiation	16 (7.7)	1 (8.3)	15 (7.6)	
surgery only or other therapy	50 (23.9)	0 (0.0)	50 (25.4)	
Targeted therapy, n (%)				**<0.001**
ALK/ROS1 drug – Yes	15 (7.2)	8 (66.7)	7 (3.6)	
ALK/ROS1 drug – No	194 (92.8)	4 (33.3)	190 (96.4)	

drug-related therapy, surgery and drug with/without radiation, surgery only or other therapy), while the other model adjusted for smoking status and *ALK/ROS1*-targeted drug (yes = crizotinib or ceritinib, no = no such drug), with results expressed as hazard ratios (HR) and 95% confidence intervals (95% CI). *P*-values <0.05 were considered statistically significant. The descriptive and Kaplan-Meyer analyses were performed using SAS version 9.0 (SAS Institute, Cary, NC) and for the CoxPH models, the survival R package for the CoxPH Models was applied [28,29]. The CoxPH curves were truncated at 10 years for all patients and, separately at 5 years for stage IIIB-IV patients, adjusting for the smoking history, treatment, and sex.

3. Results

3.1. Frequency and clinical characteristics of the patients in the study

Among a total of 571 patients with adenocarcinoma, 203 (35.6%) were men and 368 (64.4%) women; 334 (59%) were never smokers, 70 (12%) light and 167 (29%) heavy smokers. *ROS1* + tumors were identified in 24 (4.2%) patients; mean (±SD) age at diagnosis of *ROS1* + patients were 58.4 (±13.5), significantly younger than the *ROS1*- patients at 66.4 (±11.4) years. More detailed information of all stage patients was provided in Table 1. There was no significant difference in tumor grade and stage between *ROS1* + and *ROS1*- groups. Table 2 described the 209 patients diagnosed with stage IIIB-IV disease; 183 (87.6%) were stage IV and 12 (5.7%) were *ROS1*+. In addition to significant difference in age, *ROS1* + and *ROS1*- groups differed for receiving *ALK/ROS1*-targeted therapies as expected.

None of the *ROS1* + patients had *ALK* mutations. Two *ROS1* + patients had co-occurring *EGFR* mutations (exon19): one was a woman who smoked and had stage IB disease, and another was a man who never-smoked and had stage IV disease. Further analysis of the EGFR + patients showed no preponderance either by sex, or by smoking status (Supplementary Table 1).

3.2. *ROS1* + rates by sex and smoking intensity

ROS1 + tumors were detected in 13 (6.4%) of 203 men and 11 (3.0%) of 368 women, *P*=0.052. As shown in Supplementary Figure 1, 17 (5.1%) of 334 "never smokers" and 7 (2.9%) of 237 "smokers" were *ROS1* + (*P*=0.210). The "smoker" patients, categorized by smoking intensity, showed *ROS1* + rates at 5.7% in light-smokers and 1.8% in heavy-smokers (*P*=0.200). Stratifying *ROS1* + rate by sex and smoking intensity, we found *ROS1* + rates for never-, light- and heavy-smokers were 9.5%, 8.8%, 2.4% in men (*P*=0.113), and 3.6%, 2.8%, 1.2% in women (*P*=0.545), respectively. Never-smokers had significantly higher *ROS1* + rate in men (9.5%) than in women (3.6%, Table 3).

When focusing on stage IIIB-IV patients, as shown in Table 2, the frequency of *ROS1* + in men (11.1%) was much higher than in women (1.7%); in never-smokers, men had higher *ROS1* + rate than women (20.7% vs. 3.5%, Table 3). Furthermore, the *ROS1* + rate in light-smokers (8.1%) was much higher than in heavy-smokers (1.2%); more specifically, we found 9.3%, 8.1% and 1.2% were *ROS1*+, respectively in never-, light-, and heavy smokers, supporting a similar *ROS1*+ rates between never- and light-smokers; the *ROS1* + rate in never- or light-smokers (8.9%) was significantly higher than that in heavy-smokers (1.2%).

3.3. *ROS1*+ rates comparison with published studies

Two hundred and two articles were identified and reviewed; eight studies were included for comparison of *ROS1* + (Supplementary Figure 2). Of the eight studies, one had all Whites, one 79.4% Whites, one 88% non-Asian, one 89.8% Whites, and four were from European countries without information on racial distribution. All eight studies were included for the sex comparison, and seven of eight studies with available smoking history were for smoking status comparison. Comparing to the 8 published studies with *ROS1* + by sex, in which 6 included all-stage patients and two advanced-stage, the frequencies of *ROS1* + were 0.3%–1.8% in men and 0.7%–10.2% in women; women were found comparable or higher than men in *ROS1* + rates (*Ps*=0.5809–0.0001). As shown in Supplementary Table 2, the *ROS1* + rate of women in our study was similar to what were reported; however, men had much higher *ROS1* +

Table 3
The distributions of *ROS1*+ by sex and smoking status and intensity.

Stage	Sex	Number (%) of patients	Smoking status and intensity								
			Never		*P*-value	Light		*P*-value	Heavy		*P*-value
			Total (%)	*ROS1* + (%)		Total (%)	*ROS1* + (%)		Total (%)	*ROS1* + (%)	
All stage	Male	203 (35.6)	84 (25.1)	8 (9.5)	–	34 (48.6)	3 (8.8)	–	85 (50.9)	2 (2.4)	–
	Female	368 (64.4)	250 (74.9)	9 (3.6)	**0.044**	36 (51.4)	1 (2.8)	0.350	82 (49.1)	1 (1.2)	1.000
Stage IIIB-IV	Male	90 (43.1)	29 (33.7)	6 (20.7)	–	20 (50.1)	3 (15.0)	–	41 (47.7)	1 (2.4)	–
	Female	119 (56.9)	57 (66.3)	2 (3.5)	0.016	17 (45.9)	0		45 (52.3)	0	

rate in our study than that previously reported ($P < 0.002$).

Additionally, our results were compared to the seven studies that ascertained *ROS1* + rates by smoking history (Supplementary Table 3), where we found five studies involved all-stage patients and two focused on advanced stage [2,6,8,10,30–32]. The *ROS1* + rates were 2.4%–6.1% in never-smokers and 0.3%–1.8% in smokers for all-stage patients [2,8,30–32], and 3.4%–12.5% in never-smokers and 0.5%–1.9% in all smokers for advanced-stage patients [6,10]. One study [2] subgrouped ever smokers as “light smoker (n = 62)” and “smoker (n = 695)” with the *ROS1* + rates respectively at 1.6% and 0.3% (P=0.112), but without the definition of “light smoker”; authors reported similar *ROS1* + rates at 5.9% in never-smokers and 1.6% in light-smokers (P=0.171), both higher than the 0.3% in smokers ($P < 0.001$). One study [8] defined never-, light-, heavy-smokers as <1py, 1-15py, >15py, demonstrating *ROS1* + tumors in 22/600 (3.7%) never-smokers and 4/465 (0.9%) light-smokers, both of which were higher than 1/1330 (0.07%) in heavy-smokers (P=0.0001, 0.0057). The frequency of *ROS1* + in never- and heavy-smokers in our study were comparable to the reported rates [6,10,30–32]; the light-smokers in our study was similar to the never-smokers, i.e., much higher than ever smokers reported in literature (Supplementary Table 3).

3.4. Treatment response

A total of 9 *ROS1* + patients with 67% in stage IIIB-IV received *ALK/ROS1*-targeted drugs, including crizotinib, ceritinib, entrectinib and lorlatinib; 4 late-stage *ROS1* + patients did not receive *ALK/ROS1*-targeted therapy since they were diagnosed and treated prior to the development of such therapy. Five of the 9 patients received crizotinib as first-line monotherapy; one as post-operative adjuvant therapy and another with unknown response were excluded from the analysis (Supplementary Figure 3). The ORR was 43% and the DCR was 86%, and for first line therapy, these rates were 60% and 100%, respectively. However, the ORR and DCR decreased with second- or third-line therapy (25% and 25%, respectively). Two patients had early progression on first-line crizotinib (<3 months) followed by pemetrexed and ceritinib as second- and third-line therapy.

Change in tumor burden, the best indicator of response, was described for each of the 7 evaluable stage IIIB-IV patients as shown in Fig. 1; three patients had stable disease, and three had partial responses, with reductions in tumor burden that ranged from 55.6% to 71.4%. Median DFS among these 7 patients was 7.9 months, and the DFS at 12 months was 42.9%.

Moreover, the newer generation of *ALK/ROS1* targeted drugs were observed during the long-term, routine patient follow-up. Follow-up treatment of four patients (Supplementary Table 4), whose disease progressed after crizotinib (drug 1) when newer *ALK/ROS1*-targeted therapy (drug 2–4) became available, showed remarkable benefit in prolonged survival time. All four patients were no more than 65 years old, never- or light-smokers, and diagnosed stage IV disease. Cases 1 and 2, who were treated by entrectinib, survived more than five years; both had intracranial metastases and were treated with radiotherapy before the use of entrectinib. Cases 3 and 4 were on lorlatinib and survived more than six and seven years, with response duration of 63 months and 12 months, respectively.

3.5. Recurrence/progression

For patients with lung cancer recurrence, progression, or both that occurred after initial treatment and during follow-up, there was no significant difference in clinical characteristics between *ROS1* + and *ROS1*- groups.

Among the 24 *ROS1* + patients, significant differences were observed based on presence of metastases; the 12 patients with metastases were predominantly men (83% vs. 25%; P=0.004), younger (mean age 53 vs. 64 years; P=0.046) and had more aggressive

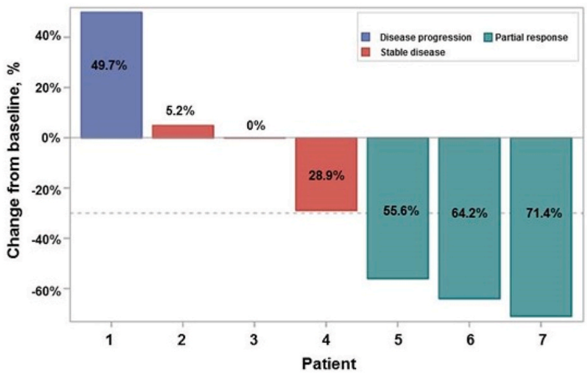

Fig. 1. Percent change in tumor burden among patients who received *ALK/ROS1* targeted drugs (n = 7). The panel showed the best response of each patient with *ALK/ROS1* targeted drugs. The bar indicated the best percentage of tumor shrinkage from baseline size. One patient had progressive disease with a 49.7% tumor increase, indicating the drug was not effective in this patient (purple). Three patients were evaluated as stable disease; one of them had a tumor increased at 5.2%; one had no change of tumor size and the other one had a tumor decrease at 28.9% (red). Three patients notably benefited from the treatment and showed partial response to the drugs with tumor shrinkages at 55.6%, 64.2%, 71.4%, respectively (green). Response definitions according to RECIST guidelines[1]; broken line reflects the 30% minimum decrease required for a partial response. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tumors (100% vs. 58%; P=0.039) compared to non-metastatic patients. Metastatic regions included within the lung (n = 4), parietal pleura (n = 3), and distant metastases (n = 5) of brain, vertebra, ribs, and liver.

3.6. Survival

While being a smoker was consistently associated with poorer OS (HR, 1.62; 95% CI, 1.25 to 2.0; $P < 0.001$) and DFS (HR, 2.70; 95%CI, 1.86–3.94; $P < 0.001$), late-stage disease was a significant risk factor only for OS (HR, 5.38; 95% CI, 3.83 to 7.55; $P < 0.001$). After adjusting for smoking and tumor stage, OS had no difference between *ROS1* + and *ROS1*- (Fig. 2A), and median survival was 6.3 and 5.1 years, respectively. Like OS, *ROS1* status was not associated with DFS after adjusting for smoking and tumor stage (P=0.921, Fig. 2B). The covariates without statistical significance were not adjusted in Fig. 2.

In patients with stage IIIB-IV, *ROS1* + patients had better OS than *ROS1*- patients in the univariate analysis (median, 4.5 vs. 1.3 years, P=0.029). In the multivariate model, *ROS1* status remained significant when adjusted for age, smoking status, and treatment (Table 4 and Fig. 2C). However, when adjusted for smoking history and *ALK/ROS1*-targeted drug, *ROS1* status was not significant (Table 4). For DFS, there was no significant difference between *ROS1* + and *ROS1*- in patients with late-stage tumors (Fig. 2D) when adjusting for smoking, treatment and sex truncated at 5 years.

4. Discussion

Our study observed a higher frequency of *ROS1* + tumor in men than in women, particularly in stage IIIB-IV patients, which has not been consistently or adequately reported; specifically, through a literature review and comparison, we found female preponderance [9,10,30,32] or no sex disparity [2,6,8,31]. Two studies having patient population and gender proportion comparable to our study demonstrated a similar *ROS1* + rate in men and women among 3115 all-stage patients (1.1% vs. 0.9%; P=0.581) [8] and a male preponderance in 550 advanced-stage patients (1.8% vs. 0.7; P=0.246) [6], respectively. These two studies included large sample sizes from multi-center registries but did not focus analysis on patients with *ROS1* alterations. In contrast, our study was designed to have complete sampling of never- and light-smokers, who were known to harbor higher rate of *ROS1* + tumors yet severely

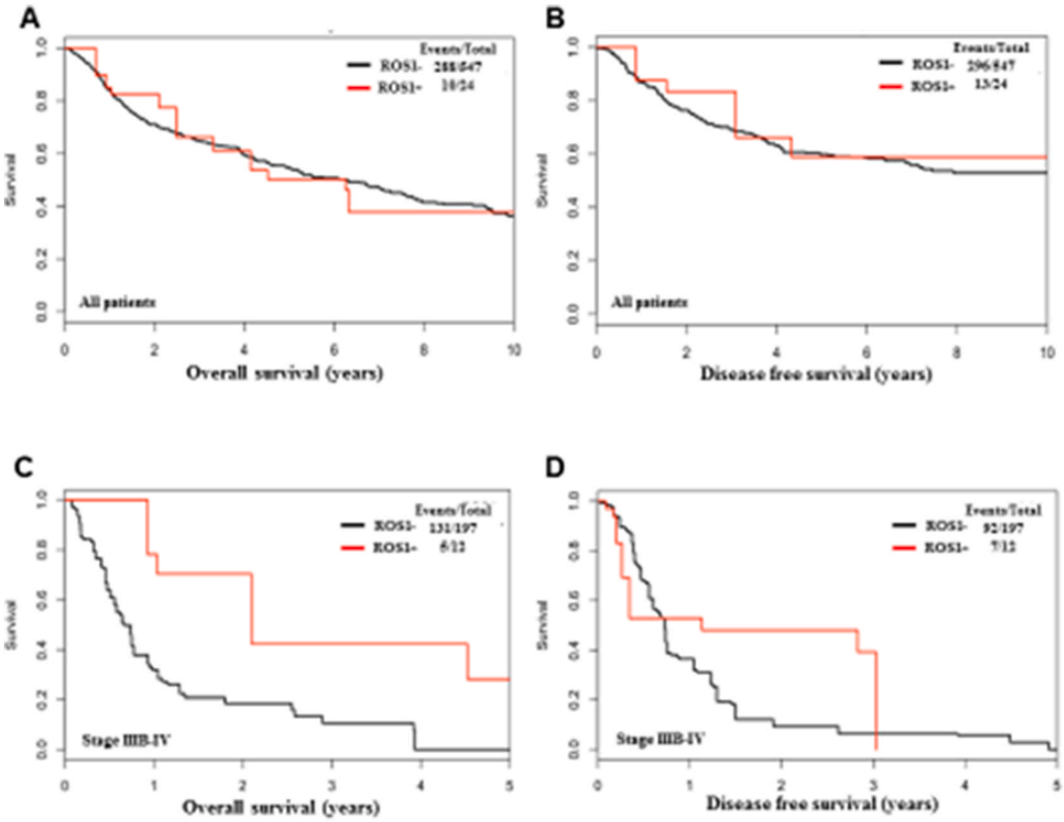

Fig. 2. CoxPH curves in *ROS1*+ and *ROS1*- patients. (A) Overall survival in all patients adjusted for smoking and stage truncated at 10 years. (B) Disease-free survival in all patients adjusted for smoking and stage truncated at 10 years. (C) Overall survival among patients with stage IIIB-IV adjusted for smoking and treatment truncated at 5 years. (D) Disease-free survival among patients with stage IIIB-IV adjusted for smoking, treatment and sex truncated at 5 years.

Table 4
Overall survival of patients with stage IIIB-IV lung cancer (n = 209).

Variable	Hazard ratio (95% CI)	*P-value*
Cox model 1		
ROS1 status		**0.026**
Positive	0.51 (0.29–0.91)	
Negative	1.0	
Treatment		
Drug or drug-related therapy	0.48 (0.33–0.70)	<0.001
Surgery and drug with/without radiation	0.25 (0.12–0.52)	<0.001
Surgery only or other therapy	1.0	
Age		
≤55	0.81 (0.53–1.24)	0.321
56-76	1.0	
≥77	0.74 (0.44–1.25)	0.256
Smoking status		0.008
Ever smoker	1.60 (1.13–2.28)	
Never-smoker	1.0	
Cox model 2		
ROS1 status		**0.265**
Positive	0.57 (0.20–1.62)	
Negative	1.0	
ALK/ROS1 drug		0.311
Yes	0.60 (0.22–1.69)	
No	1.0	
Smoking status		0.044
Ever smokers	1.432 (1.0–2.04)	
Never-smokers	1.0	

underrepresented in non-selected or randomly or conveniently sampled NSCLC patients of European ancestry.

Few studies analyzed the distributions of *ROS1* + tumors in men and women varied by smoking history as our study has done. We found men had higher *ROS1* + rates in never-, light-, and heavy-smokers than women; this sex difference in never-smokers reached statistical significance (P=0.044) for all-stage patients and was more pronounced for stage III-IV patients (P=0.016).

It is known that a prominently higher incidence of *ROS1* + tumors occurred in never-smokers [2], but we found studies reporting the relationship of *ROS1* + and smoking intensity were insufficient. Light-smokers were categorized separately from other smokers and shown to have a higher frequency of *ROS1* + at 1.6% [2]; however, the definition of smoking intensity was not provided and patients with unknown smoking history had a higher *ROS1* + rate than light-smokers (7.2% vs. 5.8%) in that study. The GENIE project defined light-smokers as 15py or less, and reported light-smokers had a higher *ROS1* + rate than heavy-smokers but much lower than never-smokers [8]. Although the widely used definition of a never-smoker was a person who consumed fewer than 100 cigarettes lifetime, per the U.S. Centers for Disease Control and Prevention [33], there was no consensus on the cut-off point for light- and heavy-smokers in the published literature; most studies used 10py [34–36], 20py [37–42], or 30py [43–45] of cigarettes, and others defined light-smokers at 15py [46,47], 21.5py [48], 33py [49], 40py [50] and 60py [51]. Only a few studies had definite start point of cigarette consumption for light-smokers (1-15py, 0< py < 10) [8,52]. Noted, 1-py of a light smoker equals 7300 cigarettes, much greater than a never smoker.

We investigated the frequency of *ROS1*+ among patients with complete smoking history and intensity information, using a definition of 100 cigarettes-20py for light-smokers. Our results indicated that light-smokers had much higher incidence of *ROS1* + than ever smokers reported in comparable studies. Six published studies included for comparison defined never-smokers as "no history of smoking" without specific counts of cigarettes, among which the highest incidence of *ROS1* + rate was in a French population for all-stage patients [31] and in a Italy population for advanced-stage patients [10]. In the French study, all patients were Caucasians; among them, 14 (2.5%) were *ROS1* + with 6 never-smokers (6.1% *ROS1*+), 4 light-smokers (5-20py) and 4 heavy-smokers (25-80py); unfortunately, the denominator of light- and heavy-smokers were not provided. In the Italy study, 12.5% "nonsmokers" and 1.9% "smokers" harbored *ROS1* + tumors, supporting our findings of the *ROS1* + rates in never-light smokers versus heavy-smokers among stage IIIB-IV patients. Although the mechanism why *ROS1* + being more prevalent in light-smokers is unknown, knowing the high-risk subpopulation could assist future studies designed to efficiently accrue *ROS1* + patients. We excluded studies specifically in Asian population in our literature comparison; this was because their race of the participants was not comparable to our study; therefore, our results predominantly supported the frequency of *ROS1* + in Caucasian populations.

To date, molecular analyses for detecting druggable genes, e.g., *EGFR*, *ALK*, *ROS1*, *Kirsten rat sarcoma virus (KRAS)*, *V-raf murine sarcoma oncogene homolog B1* (*BRAF*), *MET* and *human epidermal growth factor receptor* (*HER2*), have become part of standard care in advanced-stage non-squamous NSCLC [53–55]. *ROS1* + appeared to be mutually exclusive of *ALK*, and two *EGFR* mutations were observed in the *ROS1* + patients in our study. While this could be due to chance, previous studies have suggested that *ROS1* + presents in a greater proportion of lung tumors that lack other genetic changes. Among 25 *ROS1* + cases [56], 17 were mutually exclusive of other mutations, while six had *EGFR* and two had *KRAS* mutations. Concomitant *ROS1* and *ALK* rearrangement was extremely rare but found in *EGFR* wild-type patients [57]. As far as could be ascertained in the literature, only several case reports mentioned that patients with oncogene-associated NSCLC, including *ROS1*, may present a particular pattern of metastasis and associated with unusual site of metastases [58,59]; no unusual site of metastases was observed in the *ROS1* + patients in our study.

As recommended by National Comprehensive Cancer Network (NCCN) guideline [60], the first-line standard care for *ROS1*+ advanced-stage NSCLC should be the *ALK/ROS1*-targeted drugs. In stage IIIB-IV patients with *ROS1* + had better OS than *ROS1*- after adjusting for age, sex, smoking status, and treatment because most of them were treated by *ALK/ROS1*-targeted drugs. Since the crizotinib was approved as the first-line therapy for *ROS1*+ NSCLC patients, increasing *ALK/ROS1*-targeted drugs were developed to against resistance with better brain penetration. While the ORR of 43% was substantially lower than what has been reported in a clinical trial of crizotinib (72%) [13], the DCR of 86% was only slightly lower than the 100% reported in that trial. These lower rates may not be surprising given the real-world clinical cohort of our study. Nevertheless, one patient in our study has benefited more than 65 months from the first-line crizotinib, which was longer than the 51.4 months median OS from the updated results of PROFILE 1001 study [61], validating crizotinib was effective in patients with *ROS1*+, although resistance to crizotinib may still occur.

Furthermore, newer molecular targeted drugs, such as entrectinib and lorlatinib, showed striking OS benefits in our study, ranging between 87 and 61 months, relative to the OS using only crizotinib (7–65 months). Entrectinib was designed to penetrate the blood-brain barrier and found to prolong survival in *ROS1* + NSCLC patients with brain metastasis; the overall response of patients with baseline central nervous system disease was 55% [14]. A recent study of 67 stage-IV non-squamous NSCLC patients with *ROS1* + showed disappointing OS (5.2 months) and PFS (4.3 months) due to brain metastasis after receiving *ALK/ROS1*-targeted drug [12]. To date in phase I-II trials, patients using lorlatinib experienced 21.1 months overall median duration of response in advanced-stage *ROS1* + NSCLC patients; whereas for the patients only treated by crizotinib previously, the reported median duration of response was 13.9 months, with a 35% ORR [15]. In our extended follow-up of surviving patients with *ROS1* + tumors, those treated by lorlatinib showed significant advantage in response duration (12–63 months) compared to the reported results. Thus, the follow-up treatment with the newer generation of *ALK/ROS1*-targeted drugs after failure to crizotinib tended to be more effective, which need to be validated by prospective study with larger sample size.

4.1. Study limitations

Limitations include the retrospective nature of the study, as well as potential bias from misclassification resulting from the use of self-report information and arbitrary definition of smoking status and intensity. Additionally, even though we have carefully adjusted potential confounding factors, because of the relatively small *ROS1* + sample size for heavy smokers given the lowest *ROS1* + rate in this group, prudence should be used when interpreting the results and implications of this study.

5. Conclusion

This study found men had higher frequency of *ROS1* + than women and confirmed *ROS1* + had a similar frequency among never- and light-smokers, and a lower frequency in heavy-smokers, especially among stage IIIB-IV patients. Use of newer-generation *ALK/ROS1*-targeted drugs entrectinib and lorlatinib demonstrated prolonged efficacy in *ROS1* + patients resistant to crizotinib. These results call for investigations to confirm and mechanistically explain the prevalence of *ROS1* + tumors in men and light-smokers, and once validated, would assist future studies and trials designed to efficiently accrue *ROS1* + patients.

Ethics statement

The authors declared that informed consent was granted by Mayo Foundation IRB (# 225-99) and signed by all enrolled patients. This study critically complied with all regulations of IRB.

Data availability statement

The patients' original data are confidential. The deidentified or aggregated data will be made available on request.

CRediT authorship contribution statement

Yanmei Peng: Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vinicius Ernani:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Dan Liu:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Qian Guo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Markay Hopps:** Writing – review & editing, Funding acquisition. **Joseph C. Cappelleri:** Writing – review & editing, Funding acquisition. **Ruchi Gupta:** Software, Methodology, Formal analysis. **Mariza de Andrade:** Software, Methodology, Formal analysis. **Jun Chen:** Visualization, Validation, Investigation. **Eunhee S. Yi:** Software, Methodology, Formal analysis. **Ping Yang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28285.

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