Smoking-Related Genomic Mutation Patterns in Patients With Small Cell Lung Cancer Treated in the ASTRUM-005 Study

FPN: 1796P

Ying Cheng[1], Liang Han[2], Lin Wu[3], Jun Chen[4], Hongmei Sun[5], Guilan Wen[6], Yinghua Ji[7], Mikhail Dvorkin[8], Jianhua Shi[9], Mingming Jia[10], Xinyi Yang[10], Stanislav Lechpammer[11], Mengkai Chen[10], Fang Yang[10], Chen Hu[10], Qingyu Wang[10], Chen Ling[10], Jing Li[10], Yongqiang Shan[10], Jun Zhu[10]

[1]Jilin Cancer Hospital, Changchun, China; [2]Xuzhou Central Hospital/The Fourth People's Hospital of Xuzhou, Xuzhou, China; [3]Thoracic Medical Oncology Department, Hunan Cancer Hospital, Changsha, China; [4]Tianjin Medical University General Hospital, Tianjin, China; [5]Jiamusi Cancer and Tuberculosis Hospital, Jiamusi, China; [6]The First Affiliated Hospital of Nanchang University, Nanchang, China; [7]The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, China; [8]Budgetary Healthcare Institution of Omsk Region "Clinical Oncology Dispensary", Omsk, Russian Federation; [9]Linyi Cancer Hospital, Linyi, China; [10]Shanghai Henlius Biotech, Inc., Shanghai, China; [11]Fosun Pharma USA Inc., Princeton, USA

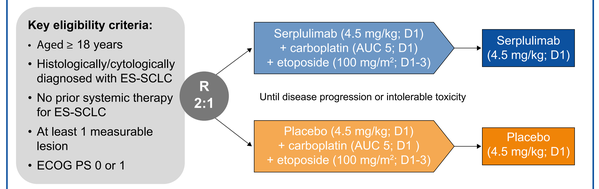
Background

- Transversion mutations (interchanges between purine and pyrimidine) occur predominately in tobacco smokers, whereas transition mutations (interchanges within purine or pyrimidine) are more frequent in non-smokers in smoking-associated cancers.[1]
- In non-small cell lung cancer (NSCLC), several studies have consistently drawn an association between tobacco-smoking history and genetic alterations in cancer-related pathways.[1,2]
- Although small cell lung cancer (SCLC) is strongly associated with tobacco smoking, only a few comparison studies on tobacco-smoking–related mutation signatures were performed with inconsistent results due to the lack of non-smokers in SCLC cohorts.[3,4]
- Here, we report the results of mutation signature analyses for patients with SCLC in the ASTRUM-005 trial and the relation of their tobacco-smoking history.

Methods

- ASTRUM-005 was a randomized, double-blind, placebo-controlled, global, phase 3 trial in patients with extensive-stage SCLC (**Figure 1**).

Figure 1. Study design



AUC, area under curve; D, day; ECOG PS, Eastern Cooperative Oncology Group performance status; ES-SCLC, extensive-stage small cell lung cancer; R, randomization.

Smoking signature analysis

- Genomic mutations in 302 patients with available baseline tumor samples were assessed by the Med1CDx panel, which included exon regions of 601 genes.
- Two bioinformatic methods, the transversion/transition ratio (TTR) method and the Catalogue of Somatic Mutations in Cancer (COSMIC) Signature 4 method, were applied to analyze tobacco-smoking–related signature.
- For the TTR method:
  - An R Bioconductor package, Maftools, was used to calculate the fraction of transversion and transition in each sample. TTR value was then defined as the transversion fraction divided by the transition fraction in each sample.[3,5]
  - Specifically, Maftools classified single nucleotide variants (SNVs) into 6 different transition and transversion events (C>A:G>T, C>G:G>C, C>T:G>A, T>A:A>T, T>C:A>G, T>G:A>C).
  - Synonymous SNVs were included in these analyses.
- For the Signature 4 method:
  - Mutational signatures were extracted and the contributions of tobacco-smoking–related signatures (Signature 4), annotated by the COSMIC, from the genomic mutation panels of each patient were estimated.[6]
  - R package "deconstructSigs" was used to calculate the contribution of the mutation.[7]
  - Both methods were validated on targeted panel sequencing from a published NSCLC data set. Both methods were able to distinguish smokers from non-smokers in NSCLC.[8]

Statistical analysis

- For progression-free survival (PFS) and overall survival (OS), the median was calculated from product-limit (Kaplan–Meier) estimates, while n was the number of patients in each subgroup category. The hazard ratio (HR) and its 95% confidence interval (CI) were estimated using an unstratified Cox proportional hazards model; Efron's method was used to handle ties.
- For the analyses of genomic-based smoking signatures, the Wilcoxon test was used to calculate the difference among patients with different smoking histories. The clinical data cutoff date was June 13, 2022.

Results

Baseline characteristics in smoking groups

- Patients in the SCLC cohort (N = 302) were grouped based on their smoking history; 23% were current smokers, 55% former smokers, and 22% never smokers.
- Baseline patient characteristics are summarized in **Table 1**.

Table 1. Baseline characteristics in smoking groups from ASTRUM-005

	Current (n = 69)	Former (n = 166)	Never (n = 67)	All (N = 302)
Age in years, mean (SD)	60 (9.4)	62 (8.3)	60 (9.5)	61 (8.9)
Sex, n (%)				
Male	67 (97)	153 (92)	23 (34)	243 (81)
Female	2 (3)	13 (8)	44 (66)	59 (20)
Race, n (%)				
Asian	37 (54)	138 (83)	62 (93)	237 (79)
White	32 (46)	28 (17)	5 (8)	65 (22)

SD, standard deviation.

- Mutation analysis demonstrated that the most frequently mutated genes were *TP53* (90% and 91%), *RB1* (68% and 69%), and *LRP1B* (31% and 28%) in current and former smokers versus never smokers, respectively (**Figure 2**).
- Differential analysis on the mutated genes demonstrated that smokers and non-smokers have similar top mutated genes, with never smokers having more mutation in *EGFR*, *DMD*, *MED12*, *MTOR*, *NOTCH1*, *REL*, *PGR*, *DNMT1*, *GRM3*, *KMT2A*, and *CD22* (**Figure 2**).

Figure 2. Mutation differences by smoking history

Top 20 genes in different groups

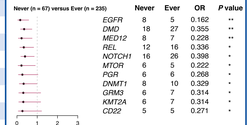
Ever (n = 235, 78%)

Gene name	Mutated samples	Mutation rate (%)
TP53	211	89.79
RB1	160	68.09
LRP1B	72	30.64
KMT2D	50	21.28
SPTA1	39	16.6
CREBBP	37	15.74
FAT1	36	15.32
KMT2C	33	14.04
TP73	29	12.34
DMD	29	12.34
NOTCH1	28	11.91
AR	26	11.06
PTEN	24	10.21
NOTCH3	22	9.36
NOTCH2	21	8.94
PTPRD	20	8.51
GRIN2A	20	8.51
PREX2	20	8.51
PRKDC	19	8.09
EPHA5	19	8.09

Never (n = 67, 22%)

Gene name	Mutated samples	Mutation rate (%)
TP53	61	91.04
RB1	46	68.66
LRP1B	19	28.36
DMD	18	26.87
KMT2D	18	26.87
NOTCH1	16	23.88
SPTA1	14	20.9
KMT2C	13	19.4
REL	12	17.91
TP73	12	17.91
FAT1	10	14.93
ROS1	9	13.43
DNMT1	8	11.94
EGFR	8	11.94
PTPRD	8	11.94
MED12	8	11.94
PRKDC	8	11.94
ZFHX3	7	10.45
ARID1A	6	8.96
GRIN2A	6	8.96

Differential mutated genes of ever/never



	Never	Ever	OR	*P* value
EGFR	8	5	0.162	**
DMD	18	27	0.355	**
MED12	8	7	0.228	**
REL	12	16	0.336	*
NOTCH1	16	26	0.398	*
MTOR	6	5	0.222	*
PGR	6	6	0.268	*
DNMT1	8	10	0.329	*
GRM3	6	7	0.314	*
KMT2A	6	7	0.314	*
CD22	5	5	0.271	*

Odds ratio with 95% CI
(1 = no effect, < 1 Never has more mutants)

OR, odds ratio. *P < 0.05; **P < 0.01.
OR < 1 means more mutants are observed in never smokers.
OR > 1 means more mutants are observed in ever smokers.

Smoking signatures in patients with SCLC

- No significant differences in smoking signatures were found between groups with different smoking histories using both methods (TTR: P = 0.54; Signature 4: P = 0.38) (**Figure 3**).
- Findings were validated by applying the same analyses on 2 cohorts' data sets profiled with whole exome sequencing from published studies.[8,9]
  - In 120 samples from 40 patients with SCLC, no correlation between mutation pattern and smoking history in either method was observed (TTR: P = 0.91; Signature 4: P = 0.70; **Figure 4**).[9]
  - We observed similar results in another independent data set with whole exome sequencing performed on 110 samples from patients with SCLC (data not shown).[10]

Figure 3. Smoking signature of patients with SCLC from ASTRUM-005

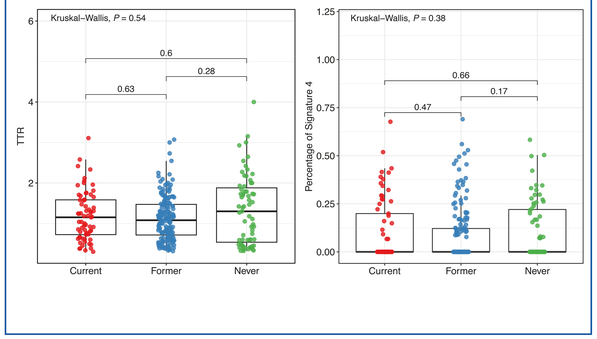
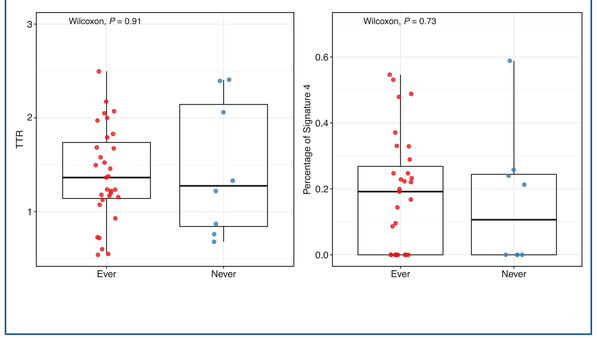


Figure 4. Smoking signature of patients with SCLC from a public database



- Patients were further grouped into high/low TTR groups using the median TTR of 1.12 as the cutoff value.
- The mutation analysis showed that *REL* was more frequently mutated in non-smokers (P < 0.001) (**Figure 5**).
- Higher TTR resulted in shorter median OS (HR [95% CI], 1.65 [1.05-2.62]; P = 0.03) for patients who only received chemotherapy, while both the high and low TTR groups gained similar benefits for patients who received serplulimab plus chemotherapy (HR [95% CI], 0.97 [0.67-1.4]; P = 0.87) (**Figure 6A**).
- Patients in both treatment groups (chemotherapy and serplulimab plus chemotherapy) showed similar benefits in PFS, regardless of the TTR (HR [95% CI], 1.25 [0.83-1.88]; P = 0.29 and 0.95 [0.67-1.34]; P = 0.77, respectively) (**Figure 6B**).

References

1. Govindan R, et al. *Cell*. 2012;150:1121-1134.
2. Wang X, et al. *Cancer Res*. 2021;81:2566-2573.
3. Song K, et al. *Transl Lung Cancer Res*. 2018;7:439-449.
4. Tseng JS, et al. *JAMA Netw Open*. 2022;5:e224830.
5. Mayakonda A, et al. *Genome Res*. 2018;28:1747-1756.
6. Alexandrov LB, et al. *Nature*. 2013;500:415-421.
7. Rosenthal R, et al. *Genome Biol*. 2016;17:31.
8. Rizvi H, et al. *J Clin Oncol*. 2018;36:1645.
9. Zhou H, et al. *Nat Commun*. 2021;12:5431.
10. George J, et al. *Nature*. 2015;524:47-53.

Figure 5. Mutation differences by high/low TTR

Top 20 genes in different groups

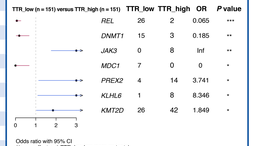
High (n = 151, 50%)

Gene name	Mutated samples	Mutation rate (%)
TP53	136	90.07
RB1	98	64.9
LRP1B	43	28.48
KMT2D	42	27.81
SPTA1	31	20.53
FAT1	24	15.89
CREBBP	24	15.89
TP73	24	15.89
KMT2C	24	15.89
DMD	23	15.23
NOTCH1	21	13.91
PTEN	19	12.58
NOTCH2	17	11.26
PTPRD	16	10.6
NOTCH3	16	10.6
PREX2	16	10.6
COL5A1	15	9.93
PRKDC	15	9.93
ERBB4	13	8.61
PIK3CG	13	8.61

Low (n = 151, 50%)

Gene name	Mutated samples	Mutation rate (%)
TP53	136	90.07
RB1	108	71.52
LRP1B	48	31.79
REL	28	18.54
KMT2D	26	17.22
DMD	24	15.89
NOTCH1	23	15.23
FAT1	22	14.57
KMT2C	22	14.57
SPTA1	22	14.57
AR	18	11.92
CREBBP	17	11.26
GRIN2A	17	11.26
ROS1	17	11.26
TP73	17	11.26
DNMT1	15	9.93
MST1	13	8.61
HLA-B	12	7.95
PRKDC	12	7.95
PTPRD	12	7.95

Differential mutated genes of high/low



OR, odds ratio. *P < 0.05; **P < 0.01; ***P < 0.001.
OR < 1 means more mutants are observed in the low TTR group.
OR > 1 means more mutants are observed in the high TTR group.

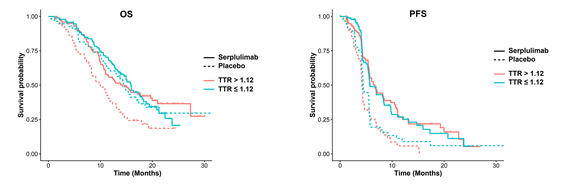
Figure 6. Clinical outcome of ASTRUM-005 by high/low TTR

A. OS

	High (n = 151)	Low (n = 151)	HR	*P* value
Serplulimab (n = 193)	14.16 (95% CI, 10.97-20.99)	15.9 (95% CI, 13.54-18.83)	0.97 (95% CI, 0.67-1.4)	0.87
Placebo (n = 109)	9.99 (95% CI, 8.28-13.11)	14.42 (95% CI, 11.93-21.29)	1.65 (95% CI, 1.05-2.62)	0.03
HR	0.56 (95% CI, 0.38-0.84)	0.93 (95% CI, 0.61-1.43)	-	-
P value	0.007	0.753	-	-

B. PFS

	High (n = 151)	Low (n = 151)	HR	*P* value
Serplulimab (n = 193)	6.67 (95% CI, 5.59-9.76)	5.75 (95% CI, 5.42-8.38)	0.95 (95% CI, 0.67-1.34)	0.77
Placebo (n = 109)	4.21 (95% CI, 3.98-4.67)	4.40 (95% CI, 4.21-5.55)	1.25 (95% CI, 0.83-1.88)	0.29
HR	0.42 (95% CI, 0.29-0.62)	0.58 (95% CI, 0.40-0.85)	-	-
P value	< 0.001	0.006	-	-



Conclusions

- In our ASTRUM-005 study, 67 (22%) patients with SCLC had never smoked.
- Unlike what was observed with NSCLC, patients with SCLC from our study showed similar tobacco-smoking–related genomic mutation patterns, regardless of their smoking status.
- Patients with SCLC who have never smoked may develop transversion mutations from other sources unrelated to direct tobacco exposure.
- Patients with high TTR might gain less benefit from chemotherapy, suggesting mutations in SCLC might be predictive biomarkers for certain therapies.

Acknowledgment and Disclosures

- The authors would like to acknowledge participants in this study and their families, other investigators and staff at all clinical sites, and the members of the Independent Data Monitoring Committee.
- This study was funded by Shanghai Henlius Biotech, Inc. Editorial support was provided by Parexel, and funded by Shanghai Henlius Biotech, Inc.
- Ying Cheng declares no conflict of interest.

Corresponding author: Prof. Ying Cheng. E-mail: jl.cheng@163.com