demonstrated in the study of transmission of *M. bovis* among wild deer (6).

Although there is abundant literature regarding the caution that immunocompromised patients should exercise around pets, there is scarce discussion about the risks that immunocompromised pets may pose to healthy pet owners. There are many conditions for which household dogs are iatrogenically immunocompromised, with modern treatments for inflammatory conditions in pets including corticosteroids, as well as chemotherapeutic agents and biologic agents such as tumor necrosis factor-α blockers (7). This outbreak shows how an immunocompromised pet can be the conduit for a zoonotic infection in cohabiting humans. It raises the issue that immunocompromised pets may potentially pose a risk to the health of their owners and other close contacts. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Correspondence and requests for reprints should be addressed to Ciara Ottewill, B.A., M.B. B.Ch., B.A.O., M.Sc. (Oxon), Department of Respiratory Medicine, National Tuberculosis Centre, St. James Hospital, James Street, Dublin 8, Ireland D08 NHY1. Email: cottewill@gmail.com.

References

1. Phillips CJC, Foster CRW, Morris PA, Teverson R. The transmission of *Mycobacterium bovis* infection to cattle. *Res Vet Sci* 2003; 74:1–15.
2. O'Reilly LM, Daborn CJ. The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tuber Lung Dis* 1995; 76:1–46.
3. O'Connor CM, Abid M, Walsh AL, Behbod B, Roberts T, Booth LV, *et al.* Cat-to-human transmission of *Mycobacterium bovis*, United Kingdom. *Emerg Infect Dis* 2019;25:2284–2286.
4. Shrikrishna D, de la Rua-Domenech R, Smith NH, Colloff A, Coutts I. Human and canine pulmonary *Mycobacterium* bovis infection in the same household: re-emergence of an old zoonotic threat? *Thorax* 2009; 64:89–91.
5. Satta G, Lipman M, Smith GP, Arnold C, Kon OM, McHugh TD. *Mycobacterium tuberculosis* and whole-genome sequencing: how close are we to unleashing its full potential? *Clin Microbiol Infect* 2018;24: 604–609.
6. Palmer MV, Whipple DL. Survival of *Mycobacterium bovis* on feedstuffs commonly used as supplemental feed for white-tailed deer (*Odocoileus virginianus*). *J Wildl Dis* 2006;42: 853–858.
7. Mauldin EA, Morris DO, Brown DC, Casal ML. Exfoliative cutaneous lupus erythematosus in German shorthaired pointer dogs: disease development, progression and evaluation of three immunomodulatory drugs (ciclosporin, hydroxychloroquine, and adalimumab) in a controlled environment. *Vet Dermatol* 2010;21: 373–382.

Distinct Genomic Landscape of Lung Adenocarcinoma from Household Use of Smoky Coal

Tongwu Zhang[1], Phuc H. Hoang[1], Jason Y. Y. Wong[1], Kaiyun Yang[2], Kexin Chen[3], Maria Pik Wong[4], Roel C. H. Vermeulen[5], Xuanwei study team, Yunchao Huang[2]*, Stephen J. Chanock[1]*, Nathaniel Rothman[1]*, Qing Lan[1]*, and Maria Teresa Landi[1]*

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; [2]The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Yunnan Cancer Center, Kunming, Yunnan, China; [3]Department of Epidemiology and Biostatistics, National Clinical Research Center for Cancer, Key Laboratory of Molecular Cancer Epidemiology of Tianjin, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin, China; and [4]Queen Mary Hospital, The University of Hong Kong, Hong Kong, China; [5]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands

ORCID ID: 0000-0003-2124-2706 (T.Z.).

To the Editor:

Lung adenocarcinoma (LUAD) rates among never-smoking women in Xuanwei, China, are among the highest in the world among never-smokers (1, 2) and are attributed to indoor air pollution from smoky (i.e., bituminous) coal combustion. We have previously reported that never-smoking female smoky coal users had a 100-fold increased risk of lung cancer compared with smokeless (i.e., anthracite) coal users (2), demonstrating that smoky coal in Xuanwei is one of the strongest environmental risk factors for any cancer reported to date (3, 4). We also found that smoky coal used earlier in life was more strongly associated with lung cancer risk (3) and that one or more polycyclic aromatic hydrocarbons (PAHs), such as 5-methylchrysene (5-MC), could be a key component of coal carcinogenesis based on estimated air exposures linked to lung cancer risk in epidemiologic studies (4). However, the underlying mutational processes are unknown.

To comprehensively characterize the mutational landscape and signatures in lung cancer associated with exposure to smoky coal, we performed deep (tumor, 109.4×; normal, 27.6×) whole-genome sequencing (WGS) in LUAD from Xuanwei ($n = 18$) and Kunming ($n = 6$), a control area with no Xuanwei smoky coal exposure. We also analyzed all LUAD WGS raw data from the PCAWG (Pan-Cancer Analysis of Whole Genomes) study (5), in which smoking status was defined by the presence of the tobacco smoking–associated single base substitution signature 4 (SBS4) in the tumor genomes. Details

*These authors contributed equally to this work.

A complete list of Xuanwei study team members may be found before the beginning of the References.

Supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute; National Science Foundation of China (No: 82060426, 81702274).

Originally Published in Press as DOI: 10.1164/rccm.202302-0340LE on July 5, 2023

Table 1. Comparison of Demographics, Xuanwei Smoky Coal Exposure and Genomic Features of Lung Adenocarcinoma Tumors in Cases from Xuanwei, Kunming, and the Pan-Cancer Analysis of Whole Genomes Study

	Xuanwei Nonsmokers (*n* = 18)	Kunming Nonsmokers (*n* = 6)	PCAWG Smokers (*n* = 21)	PCAWG Nonsmokers (*n* = 17)
Demographics and coal exposure*				
Age, yr, median (range)	55 (44–64)	61 (52–66)	70 (41–81)	64 (52–79)
Sex, M/F	1/17	0/6	13/8	5/12
Cumulative tons Xuanwei smoky coal burned in home before age 18 yr, median (range)	102 (3–170)	0	0	0
Cumulative tons Xuanwei smoky coal burned in home age ⩾18 yr, median (range)	130 (0–364)	0	0	0
Tumor molecular characteristics				
Tumor mutational burden† (median)	7.55	1.05	13.15	1.63
Percentage of genomic alterations by copy number, median	79%	84%	84%	78%
Structural variants, median	77	92	66	71
Whole-genome doubling, no. of tumors (%)	11 (61.11%)	4 (66.67%)	12 (57.14%)	11 (64.75%)
Kataegis,‡ no. of tumors (%)	10 (55.55%)	3 (50.00%)	14 (66.67%)	8 (47.06%)
Tumor driver mutations, no. of tumors (%)				
EGFR mutation	12 (66.67%)	3 (50.00%)	2 (9.52%)	3 (17.65%)
EGFR (double mutations)	7 (38.89%)	0 (0%)	0 (0%)	1 (5.88%)
EGFR (hotspot)	p.S768I, 8 (44.44%)	p.L858R, 2 (33.33%)	No hotspot	No hotspot
KRAS mutation	1 (5.56%)	0 (0%)	5 (23.81%)	2 (11.76%)
TP53 mutation	9 (50.0%)	1 (16.67%)	11 (52.38%)	4 (23.53%)
SFTPB (noncoding)	2 (11.11%)	0 (0%)	3 (14.29%)	1 (5.88%)
ALK (fusion)	0 (0%)	2 (33.33%)	0 (0%)	0 (0%)
CDKN2A deletion	5 (27.78%)	0 (0%)	3 (14.29%)	3 (17.65%)
Tumor mutational signatures,§ median no. (%) mutations assigned to each signature				
COSMIC Signature SBS4	18,646 (80.3%)	0 (0%)	26,887 (69.9%)	0 (0%)
COSMIC Signature DBS2	106 (47.44%)	—	781 (100%)	—
COSMIC Signature ID3	644 (100%)	—	1,007 (100%)	—

Definition of abbreviations: DBS = doublet base substitution; ID = insertions and deletions; SBS = single base substitution.
*No cases from Xuanwei or Kunming had a history of tuberculosis. Nine of 18 cases from Xuanwei and no cases from Kunming had a family history of lung cancer. One of 18 cases from Xuanwei and no cases from Kunming had a history of previous noncancer lung disease. Seventeen of 18 and 2 of 6 cases from Xuanwei and Kunming, respectively, had a history of exposure to environmental tobacco smoke. Seventeen of 18 cases from Xuanwei had lived in a home where smoky coal combustion was not ventilated to the outside by a chimney. None of these variables were statistically significantly associated with the mutational signatures presented in the table and were not included in analytical models. Tumor stage: Xuanwei (seven IA, three IB, two IIA, four IIIA); Kunming (one IA, three IB, one IIA, one IIB).
†Tumor mutational burden estimated as the number of mutations per megabase.
‡Kataegis, a pattern of localized hypermutations identified in cancer genomes.
§Signature deconvolution analysis performed by SigProfilerExtractor algorithm and using COSMIC mutational signatures version 3 as reference (10); COSMIC: Catalogue of Somatic Mutations in Cancer (https://cancer.sanger.ac.uk/signatures).

regarding WGS and downstream bioinformatic analyses are described in our previous publication (6). To investigate the relationship between patterns of exposure to smoky coal and mutational signatures, we conducted an exposure assessment of smoky coal use as previously described and systematically investigated the genomic alterations induced by different PAH exposures in LUAD (3, 4). Analyses were conducted using the NIH High-Performance Computing Biowulf cluster (https://hpc.nih.gov).

Demographic, exposure, and genomic feature data are shown in Table 1. The tumor mutational burden for Xuanwei samples was strikingly sevenfold higher than in samples from Kunming (Wilcoxon rank-sum test; $P < 0.001$) and in samples from nonsmokers in the PCAWG study ($P < 0.001$), but it was not significantly different compared with smokers in the PCAWG study ($P = 0.14$) (Table 1). In contrast, frequencies and patterns of copy number and structural alterations did not substantially differ between Xuanwei and Kunming or PCAWG LUAD tumors, suggesting that tumors from smoky coal exposure arise through distinct mutagenic processes. Overall, five of six tumors from Kunming had subclonal mutation clusters, with a total subclonal mutation ratio of 9.82%. In contrast, only 4 of 18 tumors from Xuanwei had subclonal mutation clusters, with a total subclonal mutation ratio of 1.07% (Fisher's exact test; $P = 0.015$), suggesting that smoky coal exposure drives tumor clonal more than subclonal expansion.

In Xuanwei tumors, p.S768I mutations were the most recurrent *EGFR* mutations ($n = 8$; 66.67%) as previously reported (7). Interestingly, seven of these Xuanwei tumors carried double nonsynonymous *EGFR* mutations, and the most frequent partners of the p.S768I *EGFR* mutation were p.G719A ($n = 3$) and p.G719C ($n = 2$). The high frequency of specific *EGFR* double mutations

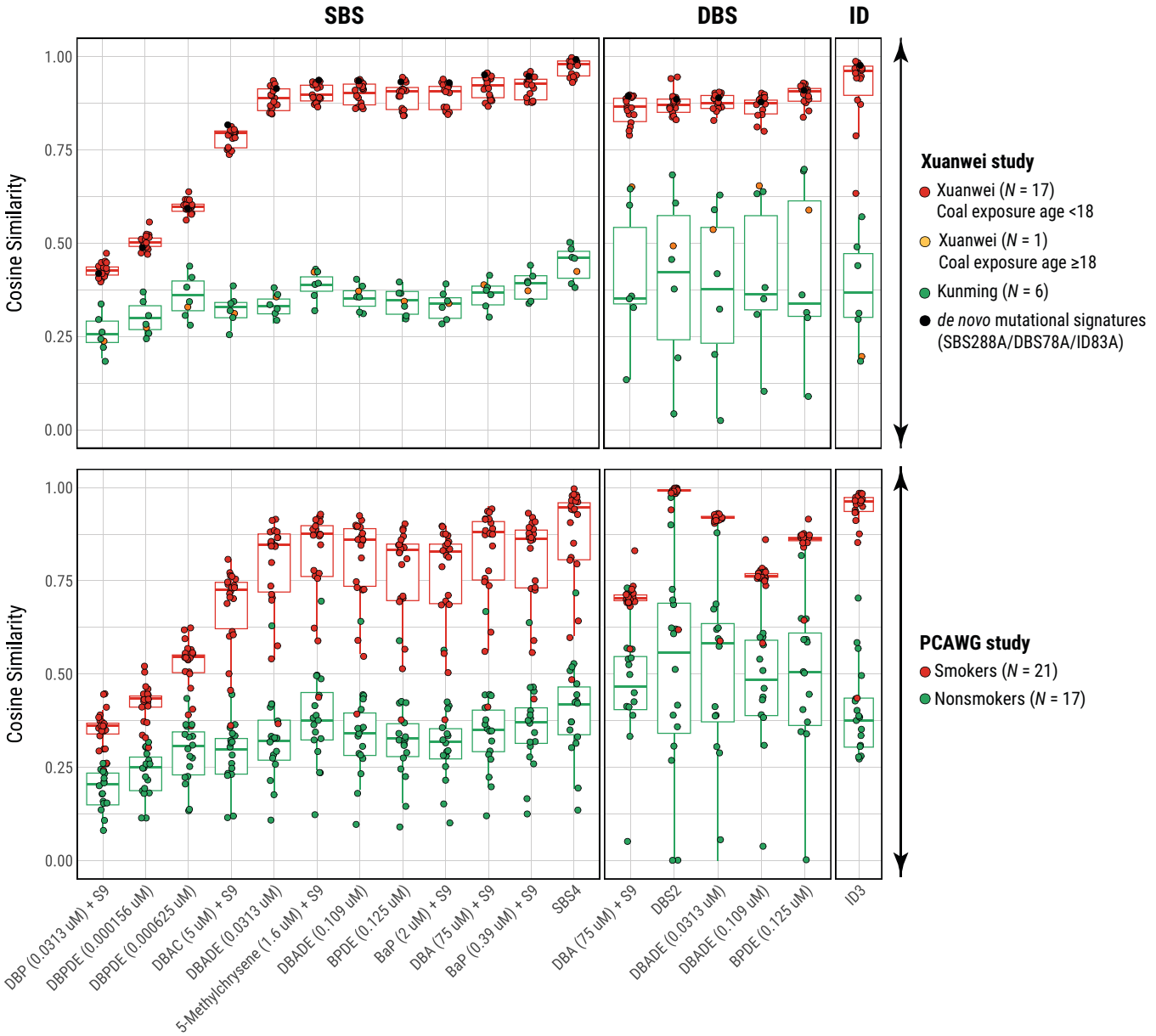

Figure 1. Cosine similarity distributions between the mutational profiles of each sample or *de novo* signatures (SBS288A/DBS78A/ID83A in Xuanwei study only) and known polycyclic aromatic hydrocarbon–related signatures for SBS (left), DBS (middle) and ID (right) profiles. For the Xuanwei study, the tumor sample NALC-0015-T01, which had almost no exposure to smoky coal before age 18, is colored orange. A cosine similarity was used as a measure of closeness between two mutational patterns (profiles or signatures). This ranges between 0 and 1, where a similarity of 1 represents identical mutational patterns and a similarity of 0 represents completely different mutational patterns. 5-MC = 5-methylchrysene; BaP = benzo[a]pyrene; BPDE = benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide; DBA = dibenz[a,h]anthracene; DBAC = dibenz(a,j)acridine; DBADE = dibenz[a,h]anthracene-3,4-diol-1,2-epoxide; DBP = dibenzo[a,l]pyrene; DBPDE = dibenzo[a,l]pyrene-11,12-dihydrodiol-13,14-epoxide; DBS = doublet base substitution; ID = insertions and deletions; SBS = single base substitution.

only in Xuanwei tumors suggests a distinct mutagenic process and selection pressure for *EGFR* mutations during tumorigenesis caused by the smoky coal exposure. No additional significant differences in molecular and clinical features were identified between Xuanwei and smokers or nonsmokers in PCAWG samples (Table 1).

To explore the mutational processes of Xuanwei tumors and compare them with those from specific PAHs and their mutagenic metabolites in experimental systems (8), as well as from smoking-associated signatures in human studies (9), we performed *de novo* mutational signature extractions for different types of somatic mutations and identified three major signatures that showed high cosine similarities to the tobacco smoking signatures: single base substitution SBS288A (cosine similarity, 0.994 to SBS4), doublet base substitution DBS78A (cosine similarity, 0.884 to DBS2), and small

insertion and deletion signature ID83A (cosine similarity, 0.986 to ID3). These signatures were significantly enriched in Xuanwei tumors compared with Kunming (SBS288A, 94.4% vs. 16.7%; DBS78A, 88.9% vs. 0%; ID83A, 94.4% vs. 0%; all Fisher's exact test; $P < 0.001$).

SBS288A was identified in 17 of 18 of the tumors from Xuanwei, but in only 1 tumor from Kunming, which accounted for 88.16% and 0.82% of the total mutations in these two groups, respectively. Although the tumor mutational burden in Xuanwei was lower than in smokers from the PCAWG study, the proportion of mutations assigned to SBS4 was higher in Xuanwei than in PCAWG smokers (80.31% vs. 69.9%; $P = 0.0088$) (Table 1), which suggests that the smoky coal exposure provides a higher mutagenic contribution than tobacco smoking.

Compared with tumors from Kunming, the mutational spectra and *de novo* signatures from almost all tumors in Xuanwei exhibited very strong and almost identical cosine similarities to several experimental PAH signatures (8), including 5-MC and benzo[a]pyrene (Figure 1). These compounds have been identified in coal combustion emissions (4) as well as other combustion sources, including tobacco (8), and 5-MC has previously been linked to lung cancer risk in Xuanwei (4).

We also investigated the relationship between patterns of exposure to smoky coal and mutational signatures. We calculated cumulative tons of smoky coal used in homes at <18 and ≥ 18 years of age. We found that the number of mutations assigned to PAH-related signatures (e.g., SBS288A) was more strongly associated with cumulative tons of smoky coal exposure before 18 years of age (beta = 0.09; 95% confidence interval [CI], 0.06–0.12; $P < 0.001$, by linear regression) than later in life (beta = 0.04; 95% CI, 0.02–0.06; $P = 0.002$). Furthermore, including both age-related exposure variables in a multivariable model to mutually adjust their effects, the regression coefficient for the former association (<18 yr of age) was minimally changed and remained statistically significant (beta = 0.1; 95% CI, 0.04–0.16; $P = 0.0015$), whereas the regression coefficient for the latter association (age ≥ 18 yr) became null and nonsignificant (beta = -0.008; 95% CI, -0.04 to 0.02; $P = 0.62$). Interestingly, we found that the one Xuanwei case (NALC-0015-T01) with almost no exposure to smoky coal before age 18 had very low genomic alterations and no SBS288A and ID83A detected, comparable to unexposed cases from Kunming (Figure 1).

In summary, high mutational burden, *EGFR* double hotspot mutations, and distinct PAH mutational processes associated with early-life smoky coal exposure were identified in samples from Xuanwei and likely contribute to the high lung cancer risk from indoor coal exposure in Xuanwei. Given the ubiquity of coal used for household heating and cooking and for electric power generation worldwide, our results warrant further investigation (e.g., larger sample sizes and more diverse populations with different types and levels of coal [10] and PAH exposure) into the distinct carcinogenic processes of PAH in air pollution to identify possible preventive and therapeutic interventions. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

Correspondence and requests for reprints should be addressed to Qing Lan, M.D., Ph.D., 9609 Medical Center Drive, Rockville, MD 20850. Email: qingl@nih.gov.

Xuanwei study team members: Wei Hu, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; Ying Chen, The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Yunnan Cancer Center, Kunming, Yunnan, China; Jihua Li, Qujing Center for Disease Control and Prevention, Qujing, Yunnan, China; Yongchun Zhou, The Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Yunnan Cancer Center, Kunming, Yunnan, China; George S. Downward, Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands, and Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; Hongji Dai, Department of Epidemiology and Biostatistics, National Clinical Research Center for Cancer, Key Laboratory of Molecular Cancer Epidemiology of Tianjin, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin, China; Lützen Portengen, Division of Environmental Epidemiology, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands; Jianxin Shi, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; Jun Xu, School of Public Health, The University of Hong Kong, Hong Kong, China.

References

1. Lan Q, Chapman RS, Schreinemachers DM, Tian L, He X. Household stove improvement and risk of lung cancer in Xuanwei, China. *J Natl Cancer Inst* 2002;94:826–835.
2. Barone-Adesi F, Chapman RS, Silverman DT, He X, Hu W, Vermeulen R, *et al.* Risk of lung cancer associated with domestic use of coal in Xuanwei, China: retrospective cohort study. *BMJ* 2012;345:e5414.
3. Wong JYY, Downward GS, Hu W, Portengen L, Seow WJ, Silverman DT, *et al.* Lung cancer risk by geologic coal deposits: a case-control study of female never-smokers from Xuanwei and Fuyuan, China. *Int J Cancer* 2019;144:2918–2927.
4. Vermeulen R, Downward GS, Zhang J, Hu W, Portengen L, Bassig BA, *et al.* Constituents of household air pollution and risk of lung cancer among never-smoking women in Xuanwei and Fuyuan, China. *Environ Health Perspect* 2019;127:97001.
5. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. *Nature* 2020;578:82–93.
6. Zhang T, Joubert P, Ansari-Pour N, Zhao W, Hoang PH, Lokanga R, *et al.* Genomic and evolutionary classification of lung cancer in never smokers. *Nat Genet* 2021;53:1348–1359.
7. Guo G, Li G, Liu Y, Li H, Guo Q, Liu J, *et al.* Next-generation sequencing reveals high uncommon EGFR mutations and tumour mutation burden in a subgroup of lung cancer patients. *Front Oncol* 2021;11:621422.
8. Kucab JE, Zou X, Morganella S, Joel M, Nanda AS, Nagy E, *et al.* A compendium of mutational signatures of environmental agents. *Cell* 2019;177:821–836.e16.
9. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, *et al.*; PCAWG Mutational Signatures Working Group; PCAWG Consortium. The repertoire of mutational signatures in human cancer. *Nature* 2020;578:94–101.
10. Cheng ES, Chan KH, Weber M, Steinberg J, Young J, Canfell K, *et al.* Solid fuel, secondhand smoke, and lung cancer mortality: a prospective cohort of 323,794 Chinese never-smokers. *Am J Respir Crit Care Med* 2022;206:1153–1162.