Agnostic Cys34-albumin adductomics and DNA methylation: Implication of N-acetylcysteine in lung carcinogenesis years before diagnosis

Sonia Dagnino, Barbara Bodinier, Hasmik Grigoryan, Stephen M. Rappaport, Maryam Karimi, Florence Guida, Silvia Polidoro, William B. Edmands, Alessio Naccarati, Giovanni Fiorito, Carlotta Sacerdote, Vittorio Krogh, Roel Vermeulen, Paolo Vineis and Marc Chadeau-Hyam

Although smoking and oxidative stress are known contributors to lung carcinogenesis, their mechanisms of action remain poorly understood. To shed light into these mechanisms, we applied a novel approach using Cys34-adductomics in a lung cancer nested case-control study (n = 212). Adductomics profiles were integrated with DNA-methylation data at established smoking-related CpG sites measured in the same individuals. Our analysis identified 42 Cys34-albumin adducts, of which 2 were significantly differentially abundant in cases and controls: adduct of N-acetylcysteine (NAC, p = 4.15 × 10⁻⁷) and of cysteinyl-glycine (p = 7.89 × 10⁻³). Blood levels of the former were found associated to the methylation levels at 11 smoking-related CpG sites. We detect, for the first time in prospective blood samples, and irrespective of time to diagnosis, decreased levels of NAC adduct in lung cancer cases. Altogether, our results highlight the potential role of these adducts in the oxidative stress response contributing to lung carcinogenesis years before diagnosis.

Introduction

Lung cancer is the leading cause of cancer mortality worldwide, and is causally linked to smoking. Using high throughput DNA-methylation assays, recent studies have identified epigenetic biomarkers of smoking exposure and smoking history. Some of these markers were also found to be associated with lung cancer risk prospectively, suggesting the involvement of DNA methylation changes in the mediation of the adverse effect of smoking.
Although smoking and oxidative stress are known contributors to lung carcinogenesis, their action mechanisms remain poorly understood. Here, using human serum albumin Cys-34 adductomics to gauge exposure to reactive oxygen species, the authors detected for the first time lower levels of N-acetylcysteine (NAC) adducts in lung cancer cases years before diagnosis. This variation was associated with smoking and hypomethylation at certain smoking-related CpGs. The results indicate a perturbation in the oxidative stress pathways in future cases and call for further investigation into the role of oxidative stress in lung carcinogenesis and the potential use of NAC as a preventive drug.

### Materials and Methods

#### Study population

Plasma samples from study participants were recruited between 1993 and 1998 as part of the Italian branch of the EPIC. Our study population includes 106 prospective lung cancer cases (median follow-up 7 years) and healthy controls, which were matched by gender, age, recruiting center and year and season of blood collection from the centers of Turin and Varese (details are available in Supporting Information). Our study complies with the Declaration of Helsinki and protocol was approved by the Ethics Committees at the Human Genetics Foundation (IIGM, Turin, Italy).

#### Exposure data

Detailed information on cigarette smoking was collected at enrollment from questionnaires in the EPIC study and included smoking status and smoking intensity (in cigarettes/day), smoking duration, time since smoking cessation (in former smokers). As previously proposed, the comprehensive smoking index (CSI) capturing the dynamics of lifelong smoking exposure and possible smoking cessation was calculated from this data.

#### Adductomics measurements

The 212 plasma samples were analyzed in duplicate following a previously described protocol based on the identification of third largest tryptic peptide of HSA (“T3,” sequence ALVLIAFAQ YLQQCC4PFEDHVK, mass 2,432 Da) containing the Cys34 locus and its potential modifications. Plasma samples were processed for digestion and dilution and subsequently analyzed by Nano Liquid Chromatography and HRMS (NanoHPLC-HRMS) with an Orbitrap Elite coupled with an ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) operated in positive ionization mode. Spectral data were preprocessed using an in-house R script (see Supporting Information for details). Peak areas of T3 adducts were normalized with the corresponding peak area of the “housekeeping” HK peptide (LVNEVTEFAK) to account for differential amounts of HSA. Adducts abundance was expressed as PAR (peak area ratio as defined by adduct peak area/HK peptide peak area × 1,000). Signal missing in both duplicates was considered below the limit of quantitation (LOQ) and set to LOQ/√2. If only one measurement was missing, it was imputed in cases and controls separately, using nearest neighbor averaging. From our analysis, a total of five adduct features were excluded because they were missing in more than 60% of the samples. While this cutoff was arbitrary, we ensured that we did not induce any bias in our data or exclude any informative or disease-relevant features, by checking that these five excluded features were (i) not differentially missing in cases and controls, (ii) missing across batches and (iii) had poor quality spectral data. All adducts PAR levels were log-transformed.

Annotation of assayed adducts was based on the identification of the monoisotopic mass of the calculated added masses to the T3-peptide, MS2 spectra and also used the library of the adducts constructed in previous work.

### What’s new?

Although smoking and oxidative stress are known contributors to lung carcinogenesis, their action mechanisms remain poorly understood. Here, using human serum albumin Cys-34 adductomics to gauge exposure to reactive oxygen species, the authors detected for the first time lower levels of N-acetylcysteine (NAC) adducts in lung cancer cases years before diagnosis. This variation was associated with smoking and hypomethylation at certain smoking-related CpGs. The results indicate a perturbation in the oxidative stress pathways in future cases and call for further investigation into the role of oxidative stress in lung carcinogenesis and the potential use of NAC as a preventive drug.
Methylation data
DNA-methylation data from the Infinium HumanMethylation450 BeadChip assay was also available in our participants from a previous study.10 Sample preparation and data processing were described elsewhere26 and are detailed in Supporting Information. We only considered here the 2,671 CpG sites which were found to be associated to smoking in a recent meta-analysis,9 of which 2,670 were assayed after probes filtering in our data set.

Statistical analyses
Adductomics profiling. The association of the measured levels of a given adduct and (i) exposure to tobacco smoke, and (ii) prospective lung cancer status was evaluated using linear mixed models where the adduct level was modeled as the response, and smoking exposure or disease status as explanatory variable. To model the repeated measurement design, the participant ID was included as a random intercept in the model. Similarly, nuisance variation was modeled including random intercepts for technical confounders27,28 (see Supporting Information).

Statistical significance of the effect of the variable of interest was evaluated using the p-value of likelihood ratio test comparing the model with and without that variable.27,29,30 We corrected for multiple testing using a permutation-based approach estimating the per-test significance level to be applied to control the family wise error rate (FWER).31,32 Adductomics profiling was conducted for three different measures of exposure to tobacco smoke (smoking status, pack-years and CSI), and for lung cancer status. For lung cancer analyses, the model was further adjusted on smoking status (current, former or never smoker), and was stratified on the most represented histological subtypes in our study (Supporting Information Table S1).

For all disease-related adducts, we regressed, in cases only, the adduct level (as predictor) against the time to diagnosis (TtD) as defined by the time elapsed from blood collection to lung cancer diagnosis, adjusting for the same variables.

OMICs integration. We investigated if and how adducts that were found to be differentially abundant between lung cancer cases and healthy controls were also related to smoking, by integrating adducts levels and DNA methylation levels at the smoking-related CpG sites. As detailed in the Supporting Information, we ran a series of linear mixed models for each disease-related adduct and each of the 2,670 smoking-related CpG, setting the methylation level as the variable of interest. These models also accounted for technical variation in the methylation data using a two-step strategy first estimating, and second removing technically-induced shifts in measured methylation levels.22,29,33 Results from these analyses were visualized as a bipartite network where edges were selected based on statistical significance of the pairwise associations, correcting for 2,670 tests.

Receiver operating characteristic (ROC) analyses. In order to quantify the disease-relevant information brought about by the disease-related adducts, we ran series of unconditional logistic models setting the adduct level as predictors. Results were visualized by means of ROC curves and corresponding area under the curve (AUC). To prevent overfitted results, we constructed ROC curves using (N = 5,000) independent subsamples (see Supporting Information). We compared models with (i) only smoking (as measured by pack-years and/or principal components (PCs) from the smoking-related CpG sites), (ii) only the disease-related adducts and (iii) the disease-related adducts and smoking metrics.

Data availability
The data that support the findings of our study are available from the corresponding author upon reasonable request.

Results
Study population and adductomics measurements
Of the 212 participants originally included in the study, 15 (5 controls and 10 cases) were excluded from the analysis due to missing data. Main characteristics of the resulting 101 controls and 96 cases are summarized in Table S1, and show, as expected, similar center, gender, age and body mass index

Figure 1. HRMS data acquired for the adduct of N-acetylcysteine. (a) Isotope distribution for the putative N-acetylcysteine adduct in a plasma sample. (b) MS/MS spectrum of the precursor ion at m/z = 865.43 (m+1) acquired in the ion trap. The Δm/z used to identify the adducted mass is calculated from the shift in the y’’ series. [Color figure can be viewed at wileyonlinelibrary.com]
(BMI) distributions in cases and controls, and an excess of current and former smokers in cases. The TID ranged from 0.27 to 13.89 years (median 7.03 years).

Our untargeted adductomics approach located a total of 42 T3-peptide modifications in our samples. Of these 42 adduct features, 25 were annotated as part of this work and 17 remain unknown (see Table S2). Coefficients of variation among duplicate measurements ranged from 0.22 and 0.5 with a median of 0.32 (Table S3). The differential mass of the measured adducts included truncations and additions and ranged from −65.1159 to 462.2047 Da. Annotations were made on the basis of MS and MS² data (and example of MS and MS² data used for annotations is shown for LC34-N-acetylcysteine in Fig. 1). The larger amount of annotated adducts resulted in Cys34 disulfides (cysteine, homocysteine, cysteinyl-glycine [Cys-Gly], glutathione, N-acetylcysteine [NAC], etc.). Other annotations included sulfoxidation products, truncations and addition of cyanide, crotonaldehyde and benzaldehyde. The full description of measured adducts is available in Table S2.

The distribution of all the adducts levels (expressed as PAR) showed right-skewed distributions, and log-transformation attenuated the asymmetric distribution for all adducts (Fig. S1).

**Association studies**

As summarized in Table S4, we found a general lower abundance of the assayed T3-peptide adducts in relation to smoking status (Fig. S2). In particular, for cumulative exposure (pack-years) and CSI, the 42 effect size estimates were negative. We found that the levels of 13 adducts were associated with at least one smoking exposure metric at a nominal significance level of 0.05. Only four of these—LC19, benzaldehyde; LC20, unknown; LC34, NAC; and LC35, Cys-Gly—were associated with more than one smoking exposure metric. However, none of these associations survived correction for multiple testing.

Analyses of the lung cancer status indicated that the levels of six T3-peptide adducts were different at a nominal significance level of 0.05 (Fig. 2) between cases and controls, and for all six adducts, lower levels were observed in cases (Fig. 3 and Table S5). Of these, the adduct of NAC, (LC34, p = 4.15 × 10⁻³⁵, Table S5) survived the correction for multiple testing based on our permutation-based per-test significance level (α') required to control the family wise error rate (FWER) at a 0.1 level for Models 1 and 2, respectively. The plain blue line represents the m/z density assayed in our study along the spectrum. [Color figure can be viewed at wileyonlinelibrary.com]

Figure 2. Manhattan plot summarizing the results of the association study relating each of the 42 T3 adducts level in relation to lung cancer disease status, using a univariate linear mixed model approach. Each adduct is represented by its −log₁₀ p-value testing the null hypothesis of no difference in adduct level in cases and controls (Y axis) for each assayed T3 adduct, in function of their m/z ratio (X axis). Results are presented for the model adjusting for technical confounders, age, gender and BMI (Model 1, bullets), and for the model additionally adjusting for smoking status (Model 2, triangle). Adducts with p-values above 0.05 are colored in gray, and those with p-values below 0.05 in dark red. The blue horizontal dashed line represents the nominal 0.05 significance level and the red and green dashed line represent the permutation-based per-test significance level (α') required to control the family wise error rate (FWER) at a 0.1 level for Models 1 and 2, respectively. The plain blue line represents the m/z density assayed in our study along the spectrum. [Color figure can be viewed at wileyonlinelibrary.com]
differentially abundant adducts for squamous cell carcinomas (results not shown). However, we found two T3-peptide adducts with lower levels in adenocarcinomas \((p < 0.05)\). These included LC19 (adduct of benzaldehyde), which was not identified in the analysis of lung cancer as a whole, and Cys-Gly-(H2O), which remained nominally associated to adenocarcinoma status upon adjustment for smoking status (Table 1). Analyses restricted to LCC identified five of the six lung cancer-related T3-peptide adducts as being significantly less abundant in cases \((p < 0.05)\). All these associations remained nominally significant when adjusting for smoking status. Of the five adducts found to be less abundant in LCC cases, NAC and LC1, despite smaller sample size, exhibited larger differences \((\beta < -1.22)\) and stronger associations \((p < 3.5 \times 10^{-4})\), Table 1) than those observed in relation to lung cancer (i.e., irrespective of histological subtype).

Plasma levels of the seven adducts found differentially abundant in lung cancer cases did not support any linear trend in relation to TtD \((p > 0.24)\), Table S6). In particular, the levels of NAC adduct were found particularly stable with TtD for lung cancer as a whole \((\beta < 10^{-3}, p > 0.9)\), and for both histological subtypes separately \((p > 0.23)\). We only identified a significant (and decreasing) trend for adducts plasma levels of Cys-Gly-(H2O), LC10 (unknown) and in a lesser extent, of Cys-Gly (Table S6) in relation to the TtD in prospective LCC cases.

Integration of DNA methylation data
In order to further explore the role of smoking in the adduct-lung cancer associations, we explored the pairwise associations linking adduct levels of the six lung cancer-related T3 adducts to methylation levels at each of the 2,670 assayed CpG sites that were recently established as epigenetics markers of smoking status \(^9\) (Fig. 4). From a preliminary PC analysis, we estimated that 110 effective tests were performed for each adduct. Using a Bonferroni-corrected per-test significance for that number of tests, we identified 34 CpG sites that were associated to the adduct level of at least one of the lung cancer-related adducts. Of these 34 CpG sites, six were associated with smoking status, cumulative smoking exposure and CSI in our study population (Table S7). The levels of LC1, LC35 and NAC adducts were associated to the methylation levels of 17, 15 and 11 smoking-related CpG sites, respectively (including all the CpG sites associated with smoking in our data at a Bonferroni-corrected significance level). Conversely, levels of Cys-Gly-(H2O) were not found to be associated to the methylation levels of any of the smoking-related CpG site \((p < 0.001)\).

ROC analyses
We restricted our ROC analyses to the two adducts found to be most differentially abundant between lung cancer cases and
Table 1. Results from the association study of the log-transformed levels of the (W = 42) T3 adducts in relation to the two most represented histological subtypes of lung cancer in our study. Results are summarized by the effect size estimate (regression coefficient $\beta$) and the corresponding p-value testing the null hypothesis of no association ($\beta = 0$). We report results for the model adjusting for technical confounders, age, gender and BMI (Model 1), and for the model additionally adjusting for smoking status (Model 2). For readability, we only report the adducts whose level were found significantly different in cases and controls at a nominal significance level of 0.05 (shown in bold) for at least one histological subtype.

| Adduct Description | Full sample (96 cases) | Adenocarcinoma (41 cases) | Large cell carcinoma (20 cases) |
|--------------------|------------------------|---------------------------|--------------------------------|
|                    | Model 1 | Model 2 | Model 1 | Model 2 | Model 1 | Model 2 |
|                    | $\beta$ | p-Value | $\beta$ | p-Value | $\beta$ | p-Value |
| LC1, unknown       | -0.461 | 4.10e-02 | -0.355 | 1.44e-01 | -0.076 | 6.81e-01 |
| LC10, unknown      | -0.652 | 2.18e-02 | -0.579 | 6.01e-02 | -0.522 | 1.13e-01 |
| LC19, benzaldehyde | -0.502 | 1.22e-01 | -0.414 | 2.40e-01 | -0.888 | 4.74e-02 |
| LC26, unknown      | -0.368 | 3.19e-02 | -0.329 | 7.58e-02 | -0.37 | 2.16e-01 |
| LC33, S-Cys-Gly-(-H2O) | -0.489 | 7.89e-03 | -0.51 | 1.07e-02 | -0.587 | 2.22e-02 |
| LC34, S-(N-acetyl)Cys | -0.587 | 4.15e-03 | -0.505 | 2.25e-02 | -0.229 | 2.85e-01 |
| LC35, S-Cys-Gly     | -0.315 | 2.56e-02 | -0.302 | 4.85e-02 | -0.069 | 6.81e-01 |

**Discussion**

In the present work, we used a recently established technique for agnostic Cys34 adductomics, in order to discover adducts potentially involved in lung carcinogenesis. Based on 212 biobanked blood samples, we successfully measured 42 adducts, and multivariate analysis showed a superior performance of the model including adduct levels and smoking exposure (mean AUC = 0.61), and a modest performance improvement while considering both variables in the model (mean AUC = 0.68).

Regarding the association study, we found that the variation in adduct levels of NAC adducts is related to smoking exposure, which contrasts with our previous results from the follow-up of the Lung Cancer Epidemiology study. Our results suggest an inverse association of the Cys-Gly adducts with smoking. Our results suggest an inverse association of the Cys-Gly adducts with smoking.

In conclusion, our results support the hypothesis that smoking exposure is associated with the variation in adduct levels of NAC adducts, and that this association is related to smoking exposure. Further studies are needed to confirm these findings and to better understand the mechanisms underlying this association.
Figure 4. Bipartite network representation of the pairwise associations between each of the six lung cancer related T3 adducts and the methylation levels at the 2,670 CpG sites found associated to smoking in Joehanes et al. and assayed in our study population. Pairwise association was evaluated using a linear mixed model setting the log-transformed adduct levels as outcome and the methylation M-values as predictor. Results are adjusted for technical confounders, age, gender and BMI. In order to correct for multiple testing, we only represent edges if the $p$-value for the regression coefficient linking the adduct level and the DNA methylation M-value is below 0.05/110, where 110 is the number of principal components required to explain more than 90% of the variance of the original (2,670 dimensional) DNA methylation matrix. For clarity, we only represent CpG sites that are found associated to at least one T3 adduct. The CpG sites are colored according to the $p$-value measuring their association with smoking status in our study population, and edges are colored according the direction of the adduct-CpG site association: red for direct associations ($\beta > 0$), and blue for inverse associations ($\beta < 0$). [Color figure can be viewed at wileyonlinelibrary.com]

Our analysis of lung cancer outcome identified six differentially abundant adducts in prospective cases compared to controls ($p < 0.05$). The adduct of NAC showed significantly lower levels according to our permutation-based per-test significance level ensuring a FWER $< 0.1$. The adduct of Cys-Gly-(H$_2$O) was also found to be lower in cases, and only the NAC adduct was also nominally associated with smoking exposure metrics ($p < 0.05$).

The adduct of Cys-Gly-(H$_2$O) was the only lung cancer-related adduct that was differentially abundant in adenocarcinoma cases and controls, whereas NAC showed larger and more marked differences between LCC cases and controls ($n = 20$, $\beta = -1.22, p = 3.41 \times 10^{-3}$) than in all lung cancer cases ($n = 96$, $\beta = -0.59, p = 4.15 \times 10^{-3}$).

Integrative analyses of DNA methylation identified 11 (of the 2,670) smoking-related CpG sites that were associated with the NAC adduct, whereas none of the CpG sites were associated with plasma levels of the adduct of Cys-Gly-(H$_2$O). The smoking-related CpG sites associated with the NAC adduct ($p < 0.05$), were all directly associated with adduct levels (except for cg04095045 which showed an inverse association), suggesting that a reduced adduct level was associated to hypomethylation at these CpG sites. Previous findings have already shown a hypomethylation at these sites in smokers. These associations could indicate a potential similar smoking-induced pathway resulting in or involving hypomethylation and reduced adduct levels at these specific sites.

Our ROC analyses suggest that both adducts have modest predictive abilities for lung cancer as a whole but complement disease-related information from smoking exposure. Analyses restricted to LCC cases showed that plasma levels of the NAC adduct reasonably predicted disease outcome and that predictions were improved by including smoking exposure variables.

The main limitation of our study is the small sample size, which hampers our ability to perform refined sensitivity and/or stratified analyses. The technical challenges and costs of the method have not yet permitted the analysis of a validation set. However, our data arise from two different populations (from Torino and Varese recruiting centers), and as an internal validation approach, we analyzed both data sets separately. Despite resulting lower sample sizes, our conclusions remained unchanged in both subpopulations (results not shown). Nevertheless, our study is the largest (in terms of the number of samples analyzed) performed to date using HRMS-based untargeted adductomics.

NAC is a known antioxidant capable of reducing OS through its ability to scavenge ROS. It is a precursor of L-Cysteine, an essential amino acid involved in the synthesis of reduced glutathione. The lower abundance of the NAC adduct in cases may reflect the lower bioavailability of NAC in serum suggestive of (potentially smoking-induced) dysregulation of redox control. Several studies have indicated that elevated levels of ROS and perturbation of redox homeostasis are hallmarks of carcinogenesis. Therefore, maintaining ROS balance appears important to ensure normal cell growth and development. Studies in mice have indicated the ability of NAC supplementation to hinder the carcinogenicity of cigarette smoke after in utero exposure. Supplementation with prenatal NAC was shown to inhibit genomic and postgenomic alterations in the lung due to cigarette smoke...
exposure as a consequence of OS, hence implying the potential utility of such a supplementation in the at-risk population. In a multicenter intervention study, NAC supplementation had no effect on former or current smoker patients affected with the lung, head or neck cancer. In another study on healthy smoking volunteers, a supplementation of NAC was associated with a lower level of DNA adducts, micronuclei in mouth floor and urinary excretion of mutagens. Our findings are in line with these

Figure 5. Receiver operating characteristic (ROC) curves from logistic models regressing the disease risk against the adduct levels of NAC (LC34) (left, a and c), and Cys-Gly-(H2O) (LC33, right, b and d). Models were fitted using either lung cancer status, irrespective of the histological subtype (top panel, a and b), or restricting the cases to LCC for NAC adduct (c) and to adenocarcinomas for Cys-Gly-(H2O) adduct (d). In each case, we ran three different models: one including smoking exposure as measured by pack-years (in red), one including the level of the adduct of interest (in green), one including both the adduct level and the smoking exposure (in light blue). For the analyses of lung cancer (all subtypes), we also investigated a model including the 76 principal components explaining >90% of the variance of the 2,670 smoking-related CpG sites (orange), and the model including both these components and the adduct level (dark blue). We used a subsampling procedure (repeated independently 5,000 times) of 80% of the study population as training set and report the ROC curves and corresponding AUC in the validation set as defined by remaining 20% of the population. The plain ROC curve (and the point estimate of the AUC) corresponds to the average performance yielded across the 5,000 subsamples, and the colored areas (and AUC ranges) reflect the extreme performances yielded across the subsamples. [Color figure can be viewed at wileyonlinelibrary.com]
results and are the first to report, using prospective samples from an observational study, in vivo decreased NAC adduct levels in future lung cancer cases, years before onset.

The peptide Cys-Gly is known to be involved, as a precursor, in the glutathione (GSH) pathway. The γ-glutamyl transferase catalyzes conversion of GSH to Cys-Gly, subsequently to glycine and cysteine, which is further used to resynthesize glutathione. The lower level of the Cys-Gly-(H₂O) adduct in cases, therefore, also points to a perturbation in redox biology, with possible depletion of GSH. Interestingly, our results suggest that these modifications in the Cys-Gly-(H₂O) adduct are not directly related to smoking exposure or its downstream consequences.

Both our findings are consistent with a perturbation in redox pathways and consequently a potential imbalance in ROS levels and elimination in future lung cancer cases. The perturbation created in the NAC pathway seems to be related to smoking, whereas the effects expressed by the modifications of Cys-Gly do not seem directly related to smoking.

Altogether our approach provides interesting new insights into lung carcinogenesis that is consistent with results from previous trials establishing NAC as a potential preventive drug for OS in smokers and triggers grounds for further investigation of these preventive treatments. Our results highlight the ability of Cys34 adductomics to explore the biological processes by which OS and redox balance dysregulation may contribute, years before clinical manifestations, to carcinogenesis and to investigate the role of smoking in these processes.

Acknowledgements
We are thankful to Prof. David Phillips and Dr. George Preston (and members of their team) for their collaborations. This work was supported by Horizon 2020 Marie Skłodowska-Curie fellowship EXACT Identifying biomarkers of EXPosure leading to Lung Cancer with AdduCTomics (grant # 708392 to S.D.). S.D. acknowledges support from a pumppriming award from the MRC-PHE Centre for Environment and Health (MR/L019744/1). M.C.-H., B.B., R.V. and P.V. acknowledge support from Cancer Research UK Population Research Committee “Mechanomics” project grant (grant #22184 to M.C.-H.). P.V., M.C.-H., R.V., S.R. and S.D. also acknowledge support from the European Commission FP7 framework “Exposomics” project grant (EU FP7 grant #308610 to P.V.). S.R. acknowledges support from grant R33CA191159 from the National Cancer Institute of the U.S. National Institutes of Health.

Disclaimer
Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

References
1. IARC. Globocan 2018: Lung cancer fact sheet, 2018.
2. WHO. The 10 leading causes of death in the world, 2000 and 2012, vol. 18/01/2015, 2015.
3. Fasanelli F, Baglietto L, Ponzi E, et al. Hypomethylation of smoking-related genes is associated with future lung cancer in four prospective cohorts. Nat Commun 2015;6:10192.
4. Guida F, Sandanger TM, Castagne R, et al. Dynamics of smoking-induced genome-wide methylation changes with time since smoking cessation. Hum Mol Genet 2015;24:2349–59.
5. Vineis P, Alavanja M, Buffa M, et al. Tobacco and cancer: recent epidemiological evidence. J Natl Cancer Inst 2004;96:99–106.
6. O’Keeffe LM, Taylor G, Huxley RR, et al. Smoking as a risk factor for lung cancer in women and men: a systematic review and meta-analysis. BMJ Open 2018;8:e016111.
7. Besingi W, Johansson Å. Smoke-related DNA methylation changes in the etiology of human disease. Hum Mol Genet 2013;22:2290–7.
8. Harlil S, Xu Z, Pandurvi V, et al. CpG sites associated with cigarette smoking: analysis of epigenome-wide data from the sister study. Environ Health Perspect 2014;122:673–8.
9. Johanes R, Just AC, Marioni RE, et al. Epigenetic signatures of cigarette smoking. Circ Cardiovasc Genet 2016;9:436–47.
10. Baglietto L, Ponzi E, Haycock P, et al. DNA methylation changes measured in pre-diagnostic peripheral blood samples are associated with smoking and lung cancer risk. Int J Cancer 2017; 140:50–61.
11. Zhang Y, Breitling LP, Balavarcha Y, et al. Comparison and combination of blood DNA methylation at smoking-associated genes and at lung cancer-related genes in prediction of lung cancer mortality. Int J Cancer 2016;139: 2482–92.
12. Niedzwiecki MM, Walker DJ, Vermuelen R, et al. The exosome: molecules to populations. Annu Rev Pharmacol Toxicol 2018;59:107–27.
13. De Flora S, Ganchev G, Iltcheva M, et al. Pharmacological modulation of lung carcinogenesis in smokers: preclinical and clinical evidence. Trends Pharmacol Sci 2016;37:120–42.
14. Kruk J, Aboul-Enein HY. Reactive oxygen and nitrogen species in carcinogenesis: implications of oxidative stress on the progression and development of several cancer types. Mini Rev Med Chem 2017;17:904–18.
15. Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. Cancer Lett 2017;387: 95–105.
16. Laurent A, Nicco C, Chereau C, et al. Controlling tumor growth by modulating endogenous production of reactive oxygen species. Cancer Res 2005; 65:948–56.
17. Wang H, Gao Z, Liu X, et al. Targeted production of reactive oxygen species in mitochondria to overcome cancer drug resistance. Nat Commun 2018;9:562.
18. Grigoryan H, Edmands W, Lu SS, et al. Adductomics pipeline for untargeted analysis of modifications to Cys34 of human serum albumin. Anal Chem 2016;88:10504–12.
19. Rappaport SM, Li H, Grigoryan H, et al. Adductomics: characterizing exposures to reactive electrophiles. Toxicol Lett 2012;213:83–90.
20. Palli D, Berrino F, Vineis P, et al. A molecular epidemiology project on diet and cancer: the EPIC-Italy prospective study. Design and baseline characteristics of participants. Tumori 2003;89: 586–93.
21. Riboli E, Kaaks R. The EPIC project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26(Suppl 1):S8–14.
22. Sandanger TM, Nost TH, Guida F, et al. DNA methylation and associated gene expression in blood prior to lung cancer diagnosis in the Norwegian Women and Cancer cohort. Sci Rep 2018;8:16714.
23. Lefondre K, Abrahamowicz M, Xiao Y, et al. Modelling smoking history using a comprehensive smoking index: application to lung cancer. Stat Med 2006;25:4132–46.
24. Liu S, Grigoryan H, Edmands WMB, et al. Cys34 adductomes differ between patients with chronic lung or heart disease and healthy controls in central London. Environ Sci Technol 2018;52:2307–13.
25. Lu SS, Grigoryan H, Edmands WM, et al. Profiling the serum albumin Cys34 adductome of solid fuel users in Xuanwei and Fujian, China. Environ Sci Technol 2017;51:46–57.
26. Campanella G, Gunter MJ, Poldoros S, et al. Epigenome-wide association study of adiposity and future risk of obesity-related diseases. Int J Obes (Lond) 2018;42:2022–35.
27. Chadeau-Hyam M, Vermeulen RC, Hebels DG, et al. Prediagnostic transcriptomic markers of chronic...
lymphocytic leukemia reveal perturbations 10 years before diagnosis. *Ann Oncol* 2014;25:1065–72.

28. McHale CM, Zhang LP, Lan Q, et al. Global gene expression profiling of a population exposed to a range of benzene levels. *Environ Health Perspect* 2011;119:628–34.

29. Castagne R, Kelly-Irving M, Campanella G, et al. Biological marks of early-life socioeconomic experience is detected in the adult inflammatory transcriptome. *Sci Rep* 2016;6:38705.

30. Castagne R, Delpierre C, Kelly-Irving M, et al. A life course approach to explore the biological embedding of socioeconomic position and social mobility through circulating inflammatory markers. *Sci Rep* 2016;6:25170.

31. Chadeau-Hyam M, Ebbels TM, Brown IJ, et al. Metabolic profiling and the metabolome-wide association study: significance level for biomarker identification. *J Proteome Res* 2010;9: 4620–7.

32. Castagne R, Boulange CL, Karaman I, et al. Improving visualization and interpretation of metabolome-wide association studies: an application in a population-based cohort using untargeted 1H NMR metabolic profiling. *J Proteome Res* 2017;16:3623–33.

33. Vermeulen R, Saberi Hosnijeh F, Bodinier B, et al. Pre-diagnostic blood immune markers, incidence and progression of B-cell lymphoma and multiple myeloma: univariate and functionally informed multivariate analyses. *Int J Cancer* 2018;143:1335–47.

34. Lee PN, Forey BA, Coombs KJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. *BMC Cancer* 2012;12:385.

35. Kawashita S, Hiraku Y, Pinlaor S, et al. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biol Chem* 2006;387:365–72.

36. Toyokuni S, Okamoto K, Yodoi J, et al. Persistent oxidative stress in cancer. *FEBS Lett* 1995;358:1–3.

37. Balansky R, Ganchev G, Ilcheva M, et al. Transplacental antioxidants inhibit lung tumors in mice exposed to cigarette smoke after birth: a novel preventative strategy? *Curr Cancer Drug Targets* 2012;12:164–9.

38. van Zandwijk N, Dalesio O, Pastorino U, et al. EUROSCAN, a randomized trial of vitamin A and N-acetylcysteine in patients with head and neck cancer or lung cancer. For the European Organization for Research and Treatment of Cancer Head and Neck and Lung Cancer Cooperative Groups. *J Natl Cancer Inst* 2000;92: 977–86.

39. van Schooten FJ, Besaratinia A, De Flora S, et al. Effects of oral administration of N-acetyl-L-cysteine: a multi-biomarker study in smokers. *Cancer Epidemiol Biomarkers Prev* 2002; 11:167–75.