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Oral Cell DNA Adducts as Potential Biomarkers for Lung Cancer Susceptibility in Cigarette Smokers

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Abstract

This perspective considers the use of oral cell DNA adducts, together with exposure and genetic information, to potentially identify those cigarette smokers at highest risk for lung cancer, so that appropriate preventive measures could be initiated at a relatively young age before too much damage has been done. There are now well established and validated analytical methods for the quantitation of urinary and serum metabolites of tobacco smoke toxicants and carcinogens. These metabolites provide a profile of exposure and in some cases lung cancer risk. But they do not yield information on the critical DNA damage parameter that leads to mutations in cancer growth control genes such as *KRAS* and *TP53*. Studies demonstrate a correlation between changes in the oral cavity and lung in cigarette smokers, due to the field effect of tobacco smoke. Oral cell DNA is readily obtained in contrast to DNA samples from the lung. Studies in which oral cell DNA and salivary DNA have been analyzed for specific DNA adducts are reviewed; some of the adducts identified have also been previously reported in lung DNA from smokers. The multiple challenges of developing a panel of oral cell DNA adducts that could be routinely quantified by mass spectrometry are discussed.

Introduction

The tobacco control community has made great strides in the past several decades to de-normalize the use of tobacco products, especially combusted ones such as cigarettes, cigars, and pipes. Smoking has become, in part, a socioeconomic phenomenon, so most North American and Western European readers of this perspective will never encounter cigarette smokers in indoor environments and probably rarely in the outdoors during their normal daily routine. They may not realize that in spite of this remarkable progress there are still about 40 million adult smokers in the U.S. and an astounding one billion in the world.^{1,2} It is likely that many of these smokers are generally aware of the hazards of cigarette smoking but they cannot break their habit because nicotine, the principal alkaloid of the tobacco plant, is highly addictive.³

Cigarette smoking is the major cause of lung cancer, a disease that is generally detected too late for effective curative therapy. While some remarkable advances in lung cancer therapy have been achieved, the overall 5 year survival rate varies from 4 - 17% depending on stage

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and regional differences, and median survival following diagnosis is typically measured in months.⁴ Cigarette smoking causes 90% of lung cancer in the U.S., where 158,080 deaths were expected in 2016, making it the leading cause of cancer death in both men and women.⁵ Worldwide, there were 1,589,000 deaths from lung cancer in 2012, an average of about 3 per minute.⁶ Cigarette smoking caused 80% of this worldwide death toll in males and 50% in females.⁷ This is an immense public health crisis but it receives relatively little attention because it is so common.

While cigarette smoking is clearly the major cause of lung cancer, only 11% of female and 24% of male lifetime smokers will get lung cancer by age 85 or greater, and this relatively small percentage is not due to competing causes of death from smoking.⁸ The major goal of the research approach discussed in this perspective is to identify individuals who are highly susceptible to the carcinogenic effects of tobacco products. These individuals would be candidates for intensive lung cancer surveillance and screening, increasing the probability of detection of a tumor at an early stage. When lung cancer is detected in its early stages, surgical resection can achieve far higher 5 year survival rates, 50-70%, than when it is detected at later stages. In this perspective, we are not proposing methods for early detection of tumors such as the identification in exhaled breath condensate of specific proteins which are characteristic of lung tumors,⁹ but rather identification of susceptible individuals. While there are already algorithms relating various parameters to lung cancer susceptibility, they are mostly retrospective in nature, with pack-years of cigarette smoking being a major prognostic factor.¹⁰⁻¹⁵ Thus, these algorithms are typically applied to subjects who are older, when the process may be more advanced.¹⁶⁻²⁰ Our ultimate goal is to develop a risk model that is prospective in nature. Overall, there would be a greater probability of success if one identifies high risk individuals early in the carcinogenic process. Even if this were effective in only 10% of tobacco users, the outcome could be prevention of more than 15,000 lung cancer deaths per year in the U.S. alone and massive savings in terms of dollars not spent on therapy.

Urinary and Serum Metabolites as Biomarkers of Lung Cancer Risk

In our ongoing research to date, we have focused on several biomarkers of tobacco smoke toxicant and carcinogen uptake as quantified by the relevant parent substances and metabolites detected in urine.²¹ Thus, we and others have developed and applied analytically validated methods for urinary substances such as total nicotine equivalents (the sum of nicotine (**1**), cotinine (**2**), 3'-hydroxycotinine (**3**) and their glucuronides, as well as nicotine-*N*-oxide (**4**)); total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL, **5**), a metabolite of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK, **6**); total *N*'-nitrosonornicotine (NNN, **7**), an important tobacco-specific carcinogen; *trans*-1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT, **8**) and 3-hydroxyphenanthrene (**9**), metabolites of the representative polycyclic aromatic hydrocarbon phenanthrene (**10**); *S*-phenylmercapturic acid (SPMA, **11**), a metabolite of the carcinogen benzene; 3-hydroxypropylmercapturic acid (HPMA, **12**), a metabolite of acrolein; and 3-hydroxy-1-methylpropylmercapturic acid (HMPMA, **13**), a metabolite of crotonaldehyde (see **Figure 1** for structures).²²⁻²⁵ Massive amounts of data demonstrate the involvement of

these classes of compounds in cancer induction by tobacco smoke.^{3,8} Some advantages and disadvantages of selected urinary biomarkers are summarized in **Table 1**.

We have collaborated with epidemiologists to evaluate the relationship of these urinary metabolites to cancer, as determined in prospective epidemiology studies. These studies collect and store bio-samples such as blood or urine from large numbers of healthy subjects, then follow the subjects for decades until sufficient numbers of cancer cases occur for statistical analysis. Samples from the cases and matched controls without cancer are recovered from freezers and analyzed for specific biomarkers. This is the most powerful epidemiologic study design because the subjects are recruited and interviewed when healthy, so the eventual occurrence of disease has no bearing on their biomarker levels. The results of these studies have been reviewed.^{26,27} In summary, statistically significant relationships of urinary total cotinine (**2** plus its glucuronide), total NNAL (**5**), and PheT (**8**) with lung cancer risk, and total NNN (**7**) with esophageal cancer risk were observed in prospective epidemiological studies of male smokers in Shanghai. Urinary total cotinine and total NNAL were related to lung cancer risk in a study of male and female smokers in Singapore, and total NNAL in serum was related to lung cancer risk in a study of male and female smokers in the U.S.^{26,27} Levels of urinary SPMA (**11**), HPMA (**12**), and HMPMA (**13**) were not independently related to lung cancer (after correction for cotinine levels) in the Shanghai study. These results support the use of total cotinine, total NNAL, and PheT as possible biomarkers of lung cancer risk, but the relationships uncovered to date, when examined in the context of receiver operating characteristics analysis, are not strong enough to support their independent application as predictive biomarkers.

Specific DNA Adducts as Biomarkers of Lung Cancer Risk

The urinary and serum metabolites discussed above are mainly biomarkers of exposure and do not inform us about the critical parameter - DNA adduct levels. Thus, an individual with high exposure but excellent detoxification or DNA repair capacity will not have relatively high DNA adduct levels and would be misclassified as having high risk based on exposure measurements only. Conversely, an individual with relatively low exposure but high levels of metabolic activation or low DNA repair capacity would be classified as low risk based on exposure biomarkers only. Therefore, the next logical step in the development of a predictive biomarker formula is to quantify DNA adducts. In this perspective, we discuss DNA adducts in human oral cells, quantified by mass spectrometry, as potentially useful biomarkers for lung cancer risk. Our main hypothesis is that these DNA adduct measurements in combination with certain exposure biomarkers as well as selected genetic information will produce a predictive algorithm for susceptibility to lung cancer in smokers.

The oral mucosa is the mucous membrane lining the inside of the mouth. Oral mucosa cells are an excellent source of material for evaluating DNA adducts and molecular changes potentially related to cancer.²⁸⁻³¹ Collection of oral mucosa cells is relatively simple, in contrast to bronchial brushings and sputum, which can be difficult, expensive, and impractical to obtain. Multiple studies have demonstrated similarities in molecular changes between oral mucosa cells and bronchial cells obtained from the same individuals, particularly in smokers, consistent with the field carcinogenesis concept of lung and upper

aerodigestive tract cancer.³²⁻³⁸ In one large study, a strong association was found between promoter methylation patterns of the *p16* and *FHIT* genes in oral tissues and bronchial brush specimens from smokers.³² In another study, similarities were found in gene expression changes in the oral and bronchial mucosa, and smoking altered the expression of numerous genes compared to observations in never smokers.³⁵ A third study demonstrated that smoking-induced gene expression changes in the bronchial airway were reflected both in the nasal and buccal epithelium.³⁴ Collectively, these results support the use of oral mucosa cells as a surrogate for DNA adduct formation in the lung.^{38,39}

Why not quantify DNA adducts directly in lung tissue? As noted above, bronchial brushings and sputum can be difficult to obtain. However, there have been many studies of DNA adduct levels in lung tissue from smokers, mainly obtained during surgery for lung cancer or other diseases. These studies have been reviewed.⁴⁰⁻⁴³ Multiple investigations using non-specific DNA adduct detection methods such as ³²P-postlabelling and immunoassay demonstrate higher levels of DNA adducts of unknown structure in lung tissue from smokers than from non-smokers. The characterization of the DNA adducts frequently seen in ³²P-postlabelling studies, and often elevated in smokers' lung tissue, typically described as "a diagonal radioactive zone", remains a challenge. This material was originally thought to be derived from polycyclic aromatic hydrocarbons, but studies by Arif et al do not support that assignment.⁴⁴ Relatively few structurally specific DNA adducts have been characterized in lung tissue.⁴⁵ These are summarized in **Figure 2**, the content of which is from a review published in 2012;⁴² there have been no new characterizations since then. Detected adducts include 7- and *O*⁶-alkylguanines such as **14-17**, aromatic-substituted guanines such as **20** and **22**, and a variety of cyclic and etheno-type adducts such as **23-26** and **28-31**, as well as the HPB releasing adducts **32**. Most of the DNA adducts illustrated in **Figure 2** were detected in relatively small studies, and some of them such as the benzo[*a*]pyrene adduct **22** were frequently not detected.⁴⁶ None have been unequivocally related to lung cancer risk. Even the sources and identities of some of these DNA adducts are in many cases unclear. For example, the simple 7-alkylguanine, *O*⁶-alkylguanine, and *O*⁴-ethylthymidine adducts could result from direct acting methylating or ethylating agents in cigarette smoke, which are mainly uncharacterized, or from the very small amounts of dialkylnitrosamines present in smoke.

DNA Adducts in Human Oral Tissue and Saliva

Earlier studies on DNA adducts in oral tissue, detected exclusively by non-specific immunoassay and ³²P-postlabelling assays, have been reviewed.²⁸ More recent studies which used chemically specific assays, mainly but not exclusively based on mass spectrometry, are summarized here.

Borthakur et al quantified 8-oxo-dGuo (**19**, **Figure 2**) in buccal mucosa cells as a marker of oxidative damage. Subjects were non-smokers who reported being healthy and not drinking alcohol excessively. The subjects gave blood and buccal cells at baseline and after 3 and 7 days. The participants rinsed their mouth with distilled water before collection of buccal cells using a soft bristle toothbrush. Levels of 8-oxo-dGuo were quantified by HPLC with electrochemical detection. The results demonstrated that 8-oxo-dGuo/dGuo levels were

relatively stable over a 7 day period and were 3-4 times higher in buccal cell DNA (30-40 adducts per 10^6 dGuo) than in leukocyte DNA.³¹

Bessette *et al*⁴⁷ screened for DNA adducts in buccal cell DNA of cigarette smokers using data-dependent constant neutral loss MS³ in which the neutral loss of the deoxyribose moiety in the MS/MS scan triggers the acquisition of MS³ product ion spectra of the aglycone. The study was initiated by spiking buccal cell DNA samples with several authentic DNA adducts derived from acrolein, 4-hydroxynonenal, 4-aminobiphenyl, and benzo[*a*]pyrene, and from metabolically activated forms of the heterocyclic aromatic amines 2-amino-3,8-dimethyl-3H-imidazo[4,5-*f*]quinoxaline (methyl-IQx), 2-amino- α -carboline, and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), at a level of 5 adducts per 10^8 bases. All of the adducts spiked into the buccal cell DNA were clearly detected. However, in unspiked buccal cell DNA samples, only adducts **23** and **25** (**Figure 2**), derived from acrolein, were unequivocally detected. The MS response for these adducts in the unspiked samples were 10-50 times greater than the response of the other adducts. The levels of the acrolein adducts were estimated to be above 5 adducts per 10^7 DNA bases. These levels were quite similar to those first reported more than 10 years earlier in gingival tissue of cigarette smokers using ³²P-postlabelling.⁴⁸ That study provided convincing evidence based on HPLC analysis for the presence in gingival tissue of smokers of acrolein adduct **23** and both stereoisomers of the crotonaldehyde adduct **24** (**Figure 2**). Levels of all of these adducts were significantly higher in smokers than in non-smokers. Further studies by this group have investigated the use of monoclonal antibodies and immunohistochemistry for analysis of the acrolein-DNA adducts in human oral cells.^{49,50}

Further evidence for the presence of DNA adducts of acrolein and crotonaldehyde in oral samples was provided by Chen and Lin who used a validated nanoflow-LC-nanospray ionization tandem MS method to quantify these adducts in 27 human salivary DNA samples from healthy volunteers.⁵¹ They found mean adduct levels of 104 ± 50 adducts/ 10^8 nucleotides for acrolein adducts (consisting mostly of **23**, **Figure 2**) and 7.5 adducts/ 10^8 nucleotides for **24**. They also used this method to quantify the etheno adducts **28** and **31** (**Figure 2**) as well as 1,*N*²-ethenodGuo (**33**, **Figure 3**), with average levels of 99 ± 50 , 72 ± 49 , and 391 ± 198 adducts/ 10^8 nucleotides, respectively. These three adducts were highly correlated with each other suggesting a common source, most likely lipid peroxidation.

The Chen group also used nanoflow-LC-nanospray ionization tandem MS to quantify several other DNA adducts in human saliva.^{52,53} Neutral thermal hydrolysis of salivary DNA released 3-Et-Ade (**34**, **Figure 3**) and 7-Et-Gua (**15**, **Figure 2**). The mean levels of 3-Et-Ade in 15 smokers and 15 non-smokers were 12.6 ± 7.0 and 9.7 ± 5.3 per 10^8 normal nucleotides, respectively, while those of 7-Et-Gua were 14.1 ± 8.2 and 3.8 ± 2.8 per 10^8 normal nucleotides.⁵² Further studies focused on thymidine adducts. Starting with 50 μ g of DNA isolated from 3.5 ml of saliva, they were able to detect O²-Et-dThd (**35**, **Figure 3**), N³-Et-dThd (**36**, **Figure 3**), and O⁴-Et-dThd (**27**, **Figure 2**) in saliva DNA samples from 20 smokers, with levels ranging from 4-5 adducts per 10^8 normal nucleotides, while these adducts were non-detectable in saliva samples from non-smokers.⁵³

Saliva samples from 37 human volunteers on unrestricted diets were analyzed for DNA adducts of heterocyclic aromatic amines and related compounds by LC-MS/MSⁿ by Bessette et al.⁵⁴ The dGuo-C8 adducts of PhIP (**37**, **Figure 3**), 2-amino- α -carboline (**38**, **Figure 3**), MeIQx (**39**, **Figure 3**) and 4-aminobiphenyl (**20**, **Figure 2**) were characterized and quantified using consecutive reaction monitoring. The PhIP-DNA adducts were detected most frequently, in saliva samples from 13 of 29 ever-smokers, and in 2 of 8 samples from never-smokers. Levels of these adducts ranged from 1 to 9 per 10⁸ DNA bases.

Alcohol consumption is an established risk factor for cancer of the upper aerodigestive tract, including oral cavity cancer. Acetaldehyde, the primary metabolite of alcohol, is considered to be crucial in DNA damage by alcohol and is regarded by the International Agency for Research on Cancer as “carcinogenic to humans” when associated with the consumption of alcoholic beverages.⁵⁵ The major DNA adduct of acetaldehyde is *N*²-ethylidene-dGuo (**21**, **Figure 2**), which can be quantified by LC-MS/MS as *N*²-ethyl-dGuo in DNA that has been treated with NaBH₃CN. Our group quantified levels of this adduct in oral cells collected by mouthwash at various time points after consumption by non-smokers of increasing alcohol doses, administered as vodka to reach target blood alcohol concentrations of 0.03-0.07%.⁵⁶ Levels of *N*²-ethylidene-dGuo in oral cell DNA increased as much as 100-fold from baseline within 4h after each dose for all subjects and in a dose-responsive manner, reaching levels as high as 1 adduct per 10⁵ nucleotides. A time dependent increase and decrease in levels of this adduct in oral cells was clearly observed after all three alcohol doses. Levels of the adduct also increased in lymphocytes and granulocytes of the subjects but there was substantial intra-individual variability which obscured clear elucidation of alcohol's effects.⁵⁷ Acetaldehyde exposure from alcohol consumption is expected to greatly exceed that from smoking, but further studies are needed on the potential interaction of drinking and smoking on oral cell DNA adduct levels.

The tobacco-specific nitrosamines NNK (**6**, **Figure 1**) and NNN (**7**, **Figure 1**) undergo metabolic α -hydroxylation reactions catalyzed by cytochrome P450 enzymes yielding a pyridyloxobutylating intermediate which reacts with cellular DNA to produce a variety of characterized adducts with dGuo, dThd, and dCyd.⁵⁸ Treatment of this DNA with acid causes hydrolysis of several pyridyloxobutyl adducts (see **32**, **Figure 2**) with the release of 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). Thus, HPB-releasing DNA adducts are a measure of pyridyloxobutylation of DNA by NNK, NNN, and possibly other tobacco-specific compounds. Our group quantified HPB-releasing DNA adducts in oral cells collected by buccal brushing and by mouthwash.⁵⁹ In smokers' samples collected by mouthwash, the levels of HPB-releasing DNA adducts averaged 12.0 pmol HPB/mg DNA (4 adducts per 10⁶ nucleotides), and they were detected in 20 of the 28 samples with quantifiable yield of DNA. Samples were also collected by buccal brushing and the levels of adducts correlated with those collected by mouthwash, and averaged 44.7 pmol HPB/mg DNA (15 adducts per 10⁶ nucleotides) in smokers. HPB-releasing DNA adducts in non-smoker samples were mostly undetectable.

Overall, there are no coordinated studies of DNA adducts in human oral tissue and saliva vs. levels of the same adducts in human lung. **Figure 2** demonstrates that there are 10 DNA adducts that have been detected in both human oral tissue or saliva and human lung, but

never from the same humans. **Figure 3** demonstrates that there are 7 adducts that have been detected in human saliva but have not been reported in human lung. It would most likely be impractical to carry out a study in which adducts from lung tissue and oral tissue or saliva from the same human were analyzed. Therefore, it will be necessary in future studies to depend on the assumption, supported by the studies discussed in the previous section, that genetic alterations in oral tissue reflect those in the lung because both are in the field affected by cigarette smoke. The relationship of salivary DNA adducts to adduct levels in the lung may be more complex.

Developing a Research Approach

The development of a panel of oral cell DNA adducts that might predict lung cancer susceptibility is indeed challenging. The first challenge is the selection of DNA adducts to be quantified. Considering what is known about tobacco smoke carcinogenesis, it would be prudent to include adducts derived from a variety of different types of carcinogens and relevant enhancing processes. Tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, and volatiles such as formaldehyde, acetaldehyde, 1,3-butadiene, and acrolein are representative of major classes of carcinogens and toxicants in cigarette smoke. Inflammation and oxidative damage are also likely to play a significant role in lung carcinogenesis by tobacco smoke. Thus, as an initial approach, one could consider a panel of DNA adducts consisting of HPB-releasing DNA adducts (**32, Figure 2**), benzo[*a*]pyrene-DNA adducts (**22, Figure 2**) or their hydrolysis products (such as benzo[*a*]pyrene tetraols), formaldehyde-DNA adducts, 1,3-butadiene-DNA adducts, acrolein-DNA adducts (**23 and 25, Figure 2**), 8-oxo-dGuo (**19, Figure 2**), and etheno-dGuo (**33, Figure 3**). Among these, only the HPB-releasing DNA adducts, acrolein-DNA adducts, and 8-oxo-dGuo have been identified and quantified in human oral tissues. Formaldehyde-DNA adducts have been quantified in human leukocyte DNA,⁶⁰ benzo[*a*]pyrene-DNA adducts have been occasionally quantified in human lung DNA,^{46,61} and etheno-dGuo has been measured in human salivary DNA.⁵¹ Considering that only 1 -10 µg of oral cell DNA would be available from a typical collection, the quantitation of multiple adducts, some of which are typically found at levels of 1 per 10⁸ nucleotides, represents a major analytical chemistry challenge. It is now realistic to consider addressing this challenge because contemporary nanoflow-LC - nanoelectrospray ionization techniques coupled with state of the art mass spectrometers can achieve detection limits in the low amol, or even high zmol range, sufficient for this type of analysis, although it still may require multiple collections per individual.

Each method must be fully validated for accuracy, precision, linearity, ruggedness, and freedom from artifacts. Another variable is the site and method of sample collection from the oral cavity. It may also be necessary to explore the contribution of oral microorganisms to DNA adduct formation. When each method has been validated, and shown not to result from the possible contribution of adducted bacterial DNA, it would be tested for inclusion in the panel. The first test would be comparison of DNA adduct levels from smokers versus non-smokers. If the DNA adduct is not elevated in smokers, or if there is not a sufficient range of values in smokers, it would not likely be a valuable addition to the panel. Typically, an initial evaluation of this type requires at least 30 samples from smokers and 30 from non-smokers, which could then be confirmed in larger studies.^{59,60,62} The second test would be

longitudinal or temporal stability of the DNA adduct level. This is important because the adduct levels are proposed to reflect the balance between DNA adduct formation and repair. If this is the case, it would be expected that the DNA adduct level would remain relatively constant over time, if the cigarette smoker maintains his/her habit. Studies of this type have been performed for urinary metabolites, and it would be important to carry out similar studies for the oral cell DNA adducts. For example, we showed that the intra-class correlation coefficient for urinary total NNAL was 76% in 70 smokers sampled every other month for one year indicating relative stability of these measurements in a given individual.⁶³ When these tests of the oral cell DNA adducts would be completed, the panel could be applied in prospective epidemiology studies to test its ability in risk prediction. Prospective epidemiology studies of lung cancer with collected oral cells include the Shanghai Cohort study, the Multi-Ethnic Cohort study, the Wayne State University and Karmanos Cancer Institute study, the University of Toronto study, the Los Angeles population-based case-control study, and the UCSF Northern California Lung Cancer study.⁶⁴ These studies also collect extensive data on factors such as diet and alcohol consumption which would be included in the final statistical model.

While the development of a panel of oral cell DNA adducts is clearly challenging, the same could have been said about the panel of urinary metabolites summarized in **Table 1**. In practice, these biomarkers as well as a number of additional ones are now routinely measured in large studies of tobacco smoke toxicant and carcinogen exposure. The Population Assessment of Tobacco and Health (PATH) study, jointly sponsored by the National Institutes of Health and the Food and Drug Administration, follows 46,000 people, some of whom are tobacco users, some of whom are not, for at least 3 years. The PATH study thus generates many thousands of urine samples which are being analyzed in the laboratories of the Centers for Disease Control and Prevention using mass spectrometry-based robotically driven analytical systems. Our own relatively small laboratory is able to quantify thousands of urinary biomarker samples from smokers using high throughput technology coupled to mass spectrometry, and starting with small amounts of urine, typically 1 – 2 ml.^{65,66} These activities would have been incomprehensible in the early part of this century.

Exposure parameters and oral cell DNA adducts would likely only be part of an ultimate model for susceptibility to lung cancer, and it is recognized that these could be affected by polymorphisms in carcinogen metabolizing genes such as glutathione-S-transferases or UDP-glucuronosyl transferases. The model would also need to include genotyping data for *CYP2A6*, the highly polymorphic major enzyme involved in nicotine metabolism, with low activity forms affecting smoking behavior, nicotine uptake, and lung cancer risk.⁶⁷ It would likely also include genotyping for variation in the $\alpha 5$ nicotinic cholinergic receptor subunit gene (*CHRNA5*) associated with nicotine dependence.⁶⁴ The association with quantity smoked has been reported for rs16969968 and rs1051730, which are correlated genetic variants. These variants are also associated with lung cancer and COPD,⁶⁴ and a haplotype rs588765-rs16969968 is also significantly associated with lung cancer (2-3 fold increased risk).⁶⁸ Some studies have examined the use of these and related SNPs to assess efficacy in smoking cessation.⁶⁹⁻⁷¹ Additional findings could be included as they evolve in genomics

and epigenetics studies. For example, hypomethylation of CpG sites in smoking-related genes such as the *AHRR* gene has been shown to be associated with future lung cancer in some prospective epidemiology studies.⁷²

Summary

This perspective highlights the potential use of oral cell DNA adducts to enhance the predictive power of a proposed model to identify, at a relatively young age, those individuals highly susceptible to lung cancer. Oral cell DNA is easily obtained and is part of the physiologic field affected by tobacco smoke, which includes the lung and upper aerodigestive tract, all of which are highly susceptible to tobacco induced cancer. Oral cell DNA adduct levels would complement the existing panel of urinary tobacco carcinogen and toxicant metabolites which provide excellent exposure data, but only limited information on metabolic activation. Oral cell DNA adducts could potentially identify those individuals who have relatively high DNA adduct loads and consequently are more likely to harbor mutated *KRAS* and *TP53* genes, the most commonly mutated genes in smoking associated lung cancer.

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Abbreviations

NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	<i>N</i> '-nitrosonornicotine
PheT	<i>trans</i> -1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene
SPMA	<i>S</i> -phenylmercapturic acid
HPMA	3-hydroxypropylmercapturic acid
HMPMA	3-hydroxy-1-methylpropylmercapturic acid
methyl-IQx	2-amino-3,8-dimethyl-3H-imidazo[4,5- <i>f</i>]quinoxaline
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
HPB	4-hydroxy-1-(3-pyridyl)-1-butanone
CHRNA5	α 5 nicotinic cholinergic receptor subunit gene

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Figure 1.
Structures of compounds discussed in the text with respect to quantitation of parent compounds and metabolites in the urine of people who use tobacco products.

Figure 2. Structures of DNA adducts reported in human lung tissue, using structure-specific methods (adapted from ⁴²). Mono-substituted dGuo adducts are to the left of the vertical line, and all others to the right. The following DNA adducts have also been detected in DNA from human oral tissue or saliva: **15, 19, 20, 21, 23, 24, 27, 28, 31, and 32.**

Figure 3.
Structures of DNA adducts reported in human saliva, but not in human lung.

Table 1

A panel of tobacco smoke carcinogen and toxicant metabolites quantified in urine.

Urinary Metabolite or Parent Compound	Biological Effect(s) Represented	Advantages	Risk Biomarker in Smokers?	Disadvantages	References
Total nicotine equivalents	Toxicity/addiction	Measures nearly 90% of the nicotine dose; relatively specific to tobacco use	Yes (Shanghai and Singapore cohorts)	Not a direct measure of DNA damage	73
Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)	Lung cancer	Good measure of lung carcinogen dose (NNK); specific to tobacco use	Yes (Shanghai and Singapore cohorts; PLCO – serum)	Not a direct measure of DNA damage	65,74-76
Phenanthrene tetraol (PheT)	Cancer	Good measure of polycyclic aromatic hydrocarbon (PAH) dose plus metabolic activation	Yes (Shanghai cohort)	Not a direct measure of DNA damage	65,75
3-Hydroxyphenanthrene (3-OH-Phe)	Cancer	Good measure of PAH exposure	Not known	Not a direct measure of DNA damage or metabolic activation	77
S-Phenylmercapturic acid (SPMA)	Cancer	Good measure of benzene exposure	No	Confounded by <i>GST-T1</i> status	78,79
3-Hydroxypropylmercapturic acid (3-HPMA)	Toxicity, cancer(?)	Good measure of acrolein exposure	No	Not a direct measure of DNA damage	66
3-Hydroxy-1-methylpropylmercapturic acid (HMPMA)	Toxicity, cancer(?)	Good measure of crotonaldehyde exposure	No	Not a direct measure of DNA damage	66
Monohydroxybutylmercapturic acid (MHBMA)	Cancer	Good measure of 1,3-butadiene exposure	Not known	Confounded by <i>GST-T1</i> status	78,80
F ₂ -Isoprostanes (8- <i>iso</i> -PGF _{2α})	Oxidative damage	Accepted measure of oxidative damage	Not known	Not a direct measure of DNA damage	81
Prostaglandin E2 metabolite (PGEM)	Inflammation	Biomarker of inflammation	Not known	Not a direct measure of DNA damage	82