Special Issue: Biomarkers of Substance Abuse
Opinion

Aldehyde-Induced DNA and Protein Adducts as Biomarker Tools for Alcohol Use Disorder

Helen M. Heymann,[1] Adriana M. Gardner,[1] and Eric R. Gross[1,*]

Alcohol use disorder (AUD) screening frequently involves questionnaires complemented by laboratory work to monitor alcohol use and/or evaluate AUD-associated complications. Here we suggest that measuring aldehyde-induced DNA and protein adducts produced during alcohol metabolism may lead to earlier detection of AUD and AUD-associated complications compared with existing biomarkers. Use of aldehyde-induced adducts to monitor AUD may also be important when considering that approximately 540 million people bear a genetic variant of aldehyde dehydrogenase 2 (*ALDH2*) predisposing this population to aldehyde-induced toxicity with alcohol use. We posit that measuring aldehyde-induced adducts may provide a means to improve precision medicine approaches, taking into account lifestyle choices and genetics to evaluate AUD and AUD-associated complications.

Highlights

During alcohol consumption, reactive aldehydes are formed that can damage DNA and proteins. Recent evidence suggests that aldehyde-induced DNA adducts formed following human alcohol consumption are detectable in epithelial cells from the upper digestive tract and from DNA in blood samples.

Biomarker tools to monitor aldehyde-induced adducts are particularly relevant since ~560 million people worldwide cannot efficiently metabolize reactive aldehydes. These individuals are more susceptible to AUD-associated complications although they may consume less alcohol.

Measurement of aldehyde-induced adducts may serve as a promising biomarker to advance precision medicine for AUD by allowing earlier detection and more precise management strategies for AUD and AUD-associated complications.

AUD and Risky Alcohol Use: A Global Problem

An estimated ~16 million individuals in the USA have an AUD [1]. The complications caused by alcohol use also produce an annual economic burden of ~US$250 billion and AUD constitutes the third leading cause of preventable death in the USA [2,3]. Just as concerning is that a recent analysis of a national survey from the USA conducted in 2012–2013, compared with the results from this survey in 2001–2002, highlights that alcohol use, high-risk drinking, and AUD are steadily increasing across all socioeconomic groups [4].

In East Asia alcohol use is also on the rise and the Republic of Korea has the highest annual alcohol consumption *per capita* among all countries in the Asia Pacific region (12.3 L) [5]. This is concerning as nearly 540 million people of East Asian descent are carriers of the ***ALDH2*** (see Glossary) genetic variant ***ALDH2*2*** and cannot metabolize acetaldehyde (the metabolite of alcohol) efficiently. The resultant accumulation of acetaldehyde leads to an increased risk for the development of alcohol-induced complications such as head and neck cancers, including esophageal cancer, relative to individuals with the ***ALDH2*1*** genetic variant [6].

Aldehydes, including acetaldehyde, possess an electrophilic carbon that reacts with nucleophilic groups in DNA or protein resulting in **adduct** formation [7]. Here we propose that measurement of aldehyde-induced adducts may be used to complement existing alcohol biomarkers for the earlier detection of AUD and AUD-related complications. This may allow prompt intervention and could be exploited to achieve a **precision medicine** approach for the diagnosis of AUD at an early stage. In this Opinion article, we discuss the biochemistry of aldehyde-induced adduct formation and delineate recent studies measuring aldehyde-induced adducts to identify AUD and AUD-related complications.

[1]Department of Anesthesiology, Perioperative, and Pain Medicine, School of Medicine, Stanford University, Stanford, CA 94305, USA

*Correspondence: ergross@stanford.edu (E.R. Gross).

 https://doi.org/10.1016/j.molmed.2017.12.003

AUD Screening

AUD is determined by a ***Diagnostic and Statistical Manual of Mental Disorders* (5th edn) (DSM-V)** diagnosis and is on a spectrum of alcohol consumption (that also includes risky use) known as unhealthy alcohol use. To receive a diagnosis of AUD, individuals must meet at least two of 11 outlined DSM-V criteria for AUD during a 12-month period [8].

To complement the AUD diagnosis, acute alcohol concentrations can be detected by breath or blood tests and are commonly used by law enforcement officials to monitor for acute alcohol intoxication. Further, monitoring for urine ethanol biomarkers including ethyl glucuronide (EtG) and ethyl sulfate (EtS) can detect alcohol consumption up to ~80 h after use [9]. Although these biomarkers can detect ethanol use, they do not provide a means to identify AUD or the extent of AUD-associated complications. In turn, biomarkers such as circulating liver enzymes, red blood cell volume, and transferrin can gauge the damage caused by alcohol consumption (Box 1). However, these biomarkers are elevated only after extensive cellular damage occurs and are not a means to formally identify AUD [75].

Since some AUD screening tools are based on an evaluation of the number of **standard drinks** consumed (e.g., **AUDIT**, the shortened **AUDIT-C**, the **single-question screen**) [10–12], assessment of aldehyde-induced adducts, particularly for those with a deficiency in aldehyde metabolism (which can lead to the accumulation of more acetaldehyde for each standard drink consumed), could provide valuable information regarding the carcinogenic effects of acetaldehyde on the individual. This monitoring could potentially be used to fill a void in identifying AUD and AUD-associated complications earlier to provide more timely interventions (Figure 1).

DNA and Protein Adduct Formation during Alcohol Consumption

Alcohol metabolism in humans occurs through oxidation reactions in the liver, primarily by alcohol dehydrogenase (ADH) and ALDH2 (Figure 2). Metabolism of ethanol to acetaldehyde also occurs through an alternative pathway by **cytochrome P450 2E1 (CYP2E1)**, which is activated either when ADH becomes saturated or by chronic alcohol consumption [13,14]. During alcohol metabolism, CYP2E1 induction by ethanol is a predominant source of reactive oxygen species (ROS). The ROS produced cause **lipid peroxidation** of the cell membrane, which forms toxic reactive aldehydes including 4-hydroxynonenal (4-HNE) [15].

Box 1. Current AUD Biomarkers

Acute consumption of alcohol can be detected by a blood or breath test. In addition, urine biomarkers including EtG and EtS can be used to detect whether alcohol was used within the past 80 h. Further, blood work to monitor alcohol use disorder can involve the measurement of circulating liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and γ-glutamyl transferase [GGT]) in addition to red blood cell mean corpuscular volume (MCV) and carbohydrate-deficient transferrin (CDT) [71]. Chronic alcohol abuse is suggested if blood work measures an AST:ALT ratio of at least 2:1, indicative of liver disease, and an increased MCV [72]. Recent moderate alcohol consumption is indicated by increased GGT and CDT. In turn, CDT levels are also used to monitor abstinence from alcohol [72]. Generally, the sensitivity and specificity of these biomarkers are imperfect and frequently lower than required for diagnostic purposes [71]. The expression of these biomarkers varies depending on the amount and pattern of alcohol consumption in addition to gender, age, weight, and the existence of other diseases [73].

The elevated concentrations of these biomarkers are primarily caused by alcohol-induced organ injury [75]. The elevation of AST, ALT, and GGT can be secondary to damage to hepatocytes or, for GGT, biliary tract damage occurring in alcoholic liver disease [75]. Increased MCV with alcohol consumption is caused by direct toxicity to the bone marrow in addition to folic acid deficiency or impaired B12 absorption associated with alcoholism [72]. CDT elevation following alcohol consumption is independent of the severity of liver disease and caused by transient changes in the glycosylation pattern of transferrin [74].

Glossary

Adduct: a complex that forms when a chemical reacts with a cellular macromolecule such as DNA or protein.

Alcohol-induced liver disease: alcohol abuse leads to liver pathology progressing from steatosis to steatohepatitis, fibrosis, and cirrhosis, which leads to end-stage liver disease.

Alcohol Use Disorders Identification Test (AUDIT): a ten-item clinician-administered or self-reported screening tool that utilizes the concept of a standard drink to screen for alcohol consumption, drinking behavior, and alcohol-related problems.

Alcohol Use Disorders Identification Test (AUDIT-C): a modified three-item version of the ten-item AUDIT.

Aldehyde dehydrogenase 2 (ALDH2): a mitochondrial enzyme encoded on chromosome 12q24 that detoxifies and removes acetaldehyde and other reactive aldehydes.

***ALDH2*1*:** the wild-type allele encoding ALDH2, which metabolizes acetaldehyde.

***ALDH2*2*:** the East Asian variant encoding ALDH2; caused by a single point mutation of guanine to adenine that decreases the ability to metabolize reactive aldehydes by 60–90% compared with carriers of the *ALDH2*1* gene.

Cirrhosis: a late stage of progressive liver fibrosis, generally considered irreversible.

Cytochrome P450 2E1 (CYP2E1): enzyme responsible for metabolizing several molecules including ethanol to acetaldehyde when ALDH2 is saturated. CYP2E1 activity generates ROS that cause aldehyde formation through lipid peroxidation.

***Diagnostic and Statistical Manual of Mental Disorders* (5th edn) (DSM-V):** published by the American Psychiatric Association; an outline of the standard criteria for the classification of mental disorders.

Fibrosis: the formation of excess fibrous connective tissue, representing (in this case) the liver's response to injury.

Hepatic stellate cells: liver pericytes involved in regulating the turnover of the extracellular matrix. In

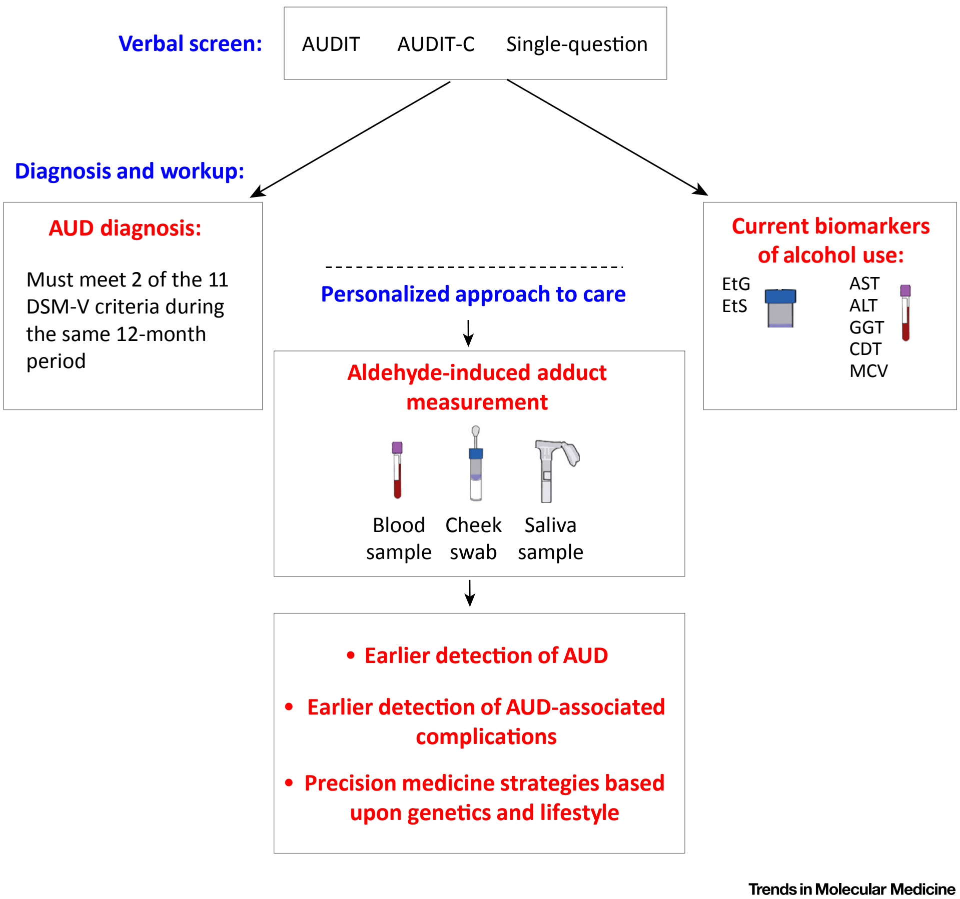
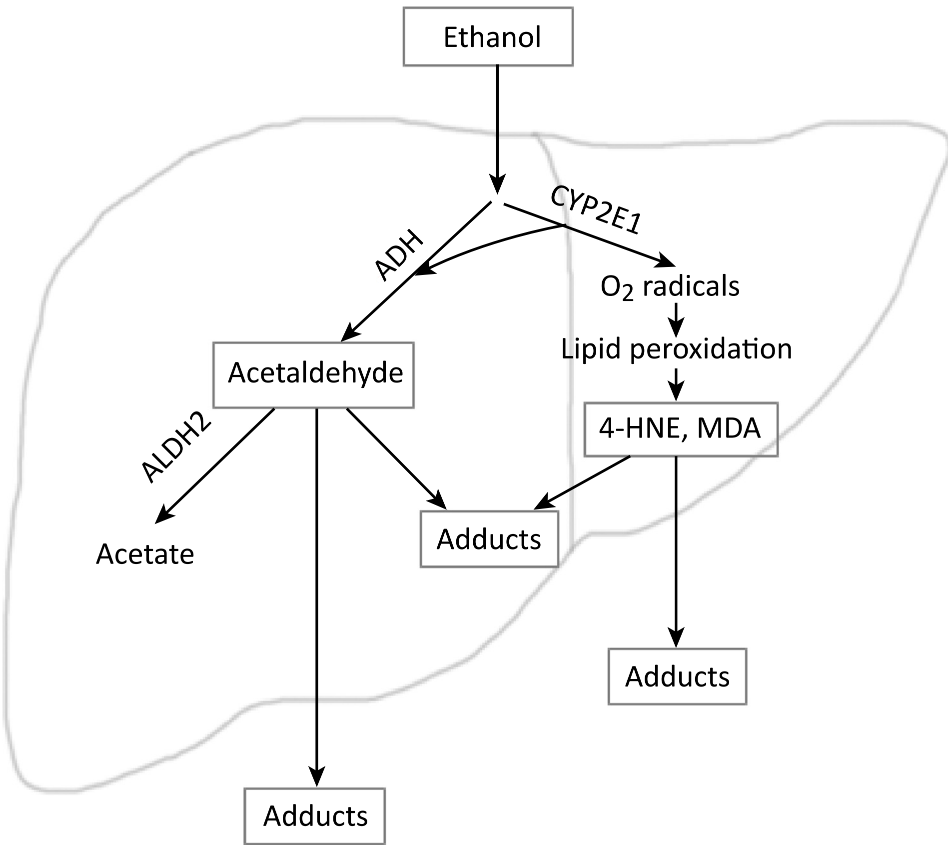


Figure 1. Proposed Scheme to Incorporate the Measurement of Aldehyde-Induced Adducts in Clinical Practice. Following a positive verbal screen, an evaluation might comprise formal diagnosis of alcohol use disorder (AUD) using DSM-V criteria. In addition, measurement of aldehyde-induced adducts might provide an additional dimension to this analysis, potentially providing more specific and personalized recommendations for individuals. This might be used to complement other biomarkers of alcohol use, including monitoring alcohol use by measurement of urine ethyl glucuronide (EtG) or ethyl sulfate (EtS) or by surveying AUD-associated complications via blood tests to measure aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), carbohydrate-deficient transferrin (CDT), and mean corpuscular volume (MCV). AUDIT, Alcohol Use Disorders Identification Test.

The reaction forming an aldehyde-induced adduct occurs by either a Michael addition or a Schiff base. In a Michael addition, the β-carbon of the aldehyde reacts with the nucleophilic group to form a double bond. For a Schiff base, the carbonyl carbon of the aldehyde reacts with a DNA amine group or lysine residue [7]. Several aldehydes, including acetaldehyde and malondialdehyde (MDA), can also react with DNA or protein together to generate **hybrid adducts**, yielding a product such as a MDA-acetaldehyde (MAA) adduct [16].

Aldehydes form DNA adducts preferentially at the deoxyguanosine amino group and less frequently at deoxyadenosine and deoxycytidine amino groups [76]. By causing DNA damage, aldehyde-induced DNA adducts can promote carcinogenesis through transversion. Under cellular stress, DNA can become oxidized and form 8-hydroxydeoxyguanosine (8-OH-dG) adducts, which can occur with alcohol consumption. More specific to alcohol is the aldehyde-induced DNA adduct N^2-ethylidene-deoxyguanosine (N^2-ethylidene-dG). However, since N^2-ethylidene-dG is highly unstable this adduct is frequently measured by quantifying a stabilized

response to cytokines produced in chronic injury, stellate cells differentiate into myofibroblasts.
Hybrid adducts: several aldehydes, in particular acetaldehyde and MDA, can react with DNA or protein to form these mixed compounds.
Kupffer cells: group of resident macrophages accounting for 20% of non-parenchymal cells in the liver; responsible for clearing toxins, microorganisms, and cell debris.
Lipid peroxidation: initiated by ROS, the oxidative degradation of fatty acids in the cell plasma membrane results in a series of autocatalytic reactions. During this process a variety of small molecules are produced including reactive aldehydes.
Myofibroblasts: derived from stellate cells; myofibroblasts have contractive, proinflammatory, and fibrogenic properties and are key mediators of liver fibrosis.
Precision medicine: medical care that takes into account individual variability in genes, environment, and lifestyle.
Single-question screen: a one-question screen for alcohol use that asks how many times in the past year an individual has had ≥*X* drinks in a day (*X* = 4 for women, 5 for men).
Standard drink: constitutes approximately 14 g of alcohol, equivalent to 12 oz of beer, 5 oz of wine, or 1.5 oz of 80-proof spirits.
Steatosis: the abnormal infiltration of liver cells with fat, also known as fatty liver; constitutes the earliest stage of alcoholic liver disease.



Trends in Molecular Medicine

Figure 2. Production of Aldehyde-Induced Adducts Following Alcohol Consumption in Humans. The enzyme alcohol dehydrogenase (ADH) converts ethanol to the highly reactive intermediate acetaldehyde. Acetaldehyde is then converted by aldehyde dehydrogenase 2 (ALDH2) to the nontoxic molecule acetate. Alternatively, cytochrome P450 2E1 (CYP2E1) metabolizes alcohol when ADH is saturated and is induced by chronic alcohol consumption. Acetaldehyde is highly reactive and can form complexes with protein or DNA known as adducts. CYP2E1 also generates acetaldehyde from ethanol and its induction is a major source of oxygen radicals that can react with lipids in the cell, forming additional reactive aldehydes such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). Acetaldehyde, 4-HNE, MDA, and MDA-acetaldehyde (MAA) can lead to adducts on DNA and protein. Aldehydes can also easily diffuse through cell membranes, forming aldehyde-induced adducts in the blood or other tissues.

and reduced form of N^2-ethylidene-deoxyguanosine, N^2-ethyl-2′-deoxyguanosine (N^2-ethyl-dG) [77]. Acetaldehyde can also produce by the condensation of two acetaldehyde molecules a crotonaldehyde-derived propano-deoxyguanosine (Cr-PdG) DNA adduct [78]. Additional adducts, such as MDA-deoxyguanosine and 4-hydroxynonenal-deoxyguanosine, are also formed.

For proteins, aldehydes form adducts primarily at lysine, histidine, and cysteine amino acids and protein function may be altered particularly when aldehydes bind to a protein at a critical location [18,28,79]. One role of aldehyde-induced adducts is to function as an autofeedback mechanism to limit reactive aldehyde formation. This occurs when aldehyde-induced protein adducts form on cytochrome CYP2E1 and function as an autofeedback mechanism to reduce aldehyde accumulation. This limits the CYP2E1-dependent metabolism of alcohol, which can produce reactive aldehydes [17]. Conversely, reactive aldehydes can reduce the enzymatic

Table 1. Key Table (References cited in Table: 84–90)

Adducted Protein	Reactive aldehyde used or studied to determine adduct formation	Protein Source	Proposed Pathophysiologic implications
α-tubulin	[^{14}C]Acetaldehyde	Rodent Liver	Impaired microtubule formation [28, 84]
	4-HNE	Bovine brain	
Collagen type I and type III	Acetaldehyde (using an antibody)	Human liver biopsies of alcoholic and non-alcoholic subjects	Inflammation; fibrosis [20, 29]
	[^{14}C] Acetaldehyde	Calf skin	
ALDH2	4-HNE	Human recombinant ALDH2	Possible negative feedback mechanism to facilitate alcohol aversion [26]
AMPK	4-HNE	Recombinant AMPK, HepG2 cells, and liver from rodents fed an alcohol or non-alcohol diet	Regulation of β-oxidation[85]
Calmodulin	[^{14}C] Acetaldehyde	Bovine brain	May impair calcium homeostasis [30]
Cytochrome C oxidase	4-HNE	Liver from rodents fed an alcohol or non-alcohol diet	Modulate electron transport chain [27, 86]
	MDA	Liver from rodents fed an alcohol or non-alcohol diet	
CYP2E1	Acetaldehyde	Liver from rodents fed an alcohol or non-alcohol diet	May limit aldehyde accumulation [17]
Electron transfer flavoprotein α	4-HNE (using an antibody)	Liver from rodents fed an alcohol or non-alcohol diet	Altered metabolism of fatty acyl-CoA [67]
ERK1/2	4-HNE	Rodent primary hepatocyte culture, liver from rodents fed an alcohol or non-alcohol diet	Inhibit prosurvival and proliferation pathways [87]
Glutathione S-transferase	Acetaldehyde	Rodent Liver	Limit capacity to handle cellular stress [31]
GRP78	4-HNE	Liver from rodents fed an alcohol or non-alcohol diet, human recombinant GRP78	Limit capacity to handle cellular stress[88]
Ketosteroid reductase	Acetaldehyde (using an antibody)	Liver from rodents fed an alcohol or non-alcohol diet	Possible abnormal bile synthesis [21]
Surfactant Protein D	MAA (using an antibody)	Rodent Lung	May interfere with surfactant production or function; inflammation [32]
Albumin	Acetaldehyde (using an antibody)	Human serum from subjects with alcohol versus non-alcohol history	Possible immune-mediated pathology [19, 89]
	[^{14}C] Acetaldehyde	Bovine Serum	
Carbonic anhydrase II	Acetaldehyde	Human recombinant protein, Human blood samples	Possible link to osteoporosis or renal tubular acidosis [33]
Coagulation factors I, II, IIa, VII, X, Xa	Acetaldehyde	Reconstituted factors	Increased clotting time [34-36, 90]
Coagulation factor IX	Acetaldehyde	Reconstituted factor	Decreased clotting time [34]
Hemoglobin	Acetaldehyde (using an antibody)	Human blood collected from subjects with alcohol versus non-alcohol history	May decrease oxygen binding; RBC vulnerability to hemolysis [23, 37, 38]
	Acetaldehyde	Human blood	
VLDL and LDL	Acetaldehyde (using an antibody)	Human plasma collected from subjects with alcohol versus non-alcohol history	Possible accelerated clearance of VLDL and LDL, decreased conversion of VLDL to LDL [22]

[a]Described are the types of aldehyde-induced protein adducts that form following alcohol consumption or exposure to ethanol-derived reactive aldehydes, the reactive aldehydes causing an adduct, the source of the studied adduct, and documented associated pathophysiology based on the aldehyde-induced biomarkers. LDL, low-density lipoprotein; RBCs, red blood cells; VLDL, very-low-density lipoprotein.

activity of ALDH2 (e.g., 50 μM of 4-HNE *in vitro*) [18]. Although more detailed studies are needed, this autofeedback mechanism inhibiting ALDH2 – which is likely to increase acetaldehyde levels – may cause behavioral aversion to the consumption of additional alcohol. This seems likely given that the ALDH2 inhibitor disulfiram, an alcohol aversion therapy agent, leads to the avoidance of alcohol by causing nausea and vomiting with alcohol consumption.

Relevant to this discussion, several other aldehyde-induced protein adducts from alcohol consumption have been reported [16,17,19–39] (Table 1, Key Table). We discuss some of these examples in the following sections.

Monitoring Aldehyde-Induced Adducts and Influence of the *ALDH2*2* Genetic Variant

Individuals with the *ALDH2*2* genetic variant tend to limit alcohol consumption due to the unpleasant side effects of acetaldehyde accumulation, including elevated heart rate and facial flushing [40]. Although the *ALDH2*2* genotype is considered to curb alcohol consumption, there are concerning trends that the number of heterozygotes for the *ALDH2*2* genetic variant with AUD is steadily rising in East Asian countries [41,42].

Heterozygotes for the *ALDH2*2* variant who consume alcohol have an increased risk for the development of head- and neck-related cancers, including esophageal cancer, compared with individuals with the *ALDH2*1* variant [41]. To support this association of aldehyde-induced adducts as a possible mechanism for the development of esophageal cancer, N^2-ethylidene-dG concentrations were measured in the esophagus of *Aldh2* knockout mice and were linked to a ~100-fold greater level of DNA damage in the esophagus following 8 weeks of alcohol consumption relative to wild-type controls under the same alcohol regimen [43]. Consistent with these data, in human esophageal keratinocytes a ~15-fold increase in N^2-ethylidene-dG concentrations was observed on siRNA knockdown of *ALDH2* relative to untreated human keratinocytes *in vitro* [43].

To support the feasibility of measuring N^2-ethylidene-dG in humans, after five male and five female participants without AUD had consumed alcohol (to achieve a target blood alcohol level of 0.03%), the DNA adduct N^2-ethylidene-dG was measured in cells collected from the upper digestive tract with a saline wash and from granulocytes and lymphocytes collected from blood [44,45]. In rhesus monkeys, the highest concentration of N^2-ethylidene-dG following alcohol consumption was present in the upper digestive track and concentrations of N^2-ethylidene-dG decreased in samples collected further from the upper digestive track [46]. In addition, heterozygote *ALDH2*2* individuals with AUD could exhibit significant elevations of N^2-ethyl-dG in DNA isolated from blood samples compared with *ALDH2*1* individuals with AUD [47]. Consumption of a standard drink also resulted in higher concentrations of acetaldehyde-induced hemoglobin adduct formation in *ALDH2*2* heterozygotes compared with *ALDH2*1* individuals [48]. Together these data suggest that, in humans, it is possible to survey alcohol-induced DNA damage and specifically monitor individual cell types in the body for aldehyde-induced protein adducts.

With alcohol abstinence, recent evidence would also imply that the DNA damage caused by alcohol consumption might be reversible. Individuals with AUD who abstained from alcohol had reduced amounts of alcohol-induced cellular dysplasia in their esophagus identified by esophagoduodenoscopy [49]. These data favor the development of strategies to measure acetaldehyde-induced DNA adducts at or near the esophagus or in the blood; this may prove to be a valuable approach to detecting potential risks for the development of esophageal cancer and determining how to prevent advanced stages of development, although extensive testing will be needed.

Individuals with an *ALDH2*2* variant might receive lower scores on an AUDIT or AUDIT-C questionnaire since, as discussed, smaller quantities of alcohol produce acetaldehyde-induced intoxicating effects in individuals heterozygotic for *ALDH2*2* compared with *ALDH2*1*. Therefore, surveys based on the number of standard drinks consumed may not accurately screen for AUD in these individuals. We propose that it might be more informative to combine written screening tools with aldehyde-induced adduct quantification to determine a threshold of alcohol consumption that is harmful. Although this remains to be tested, we hypothesize that alcohol consumption levels might be evaluated to assess the genetic predisposition of individuals to metabolize acetaldehyde at a personalized level. This approach might be potentially useful to set the stage for the establishment of a precision medicine platform to diagnose and treat individuals with AUD.

Aldehyde-Induced Adduct Biomarkers to Detect Specific AUD-Related Complications

Several additional examples exist of how aldehyde-induced biomarkers might be useful to detect and monitor specific AUD complications such as alcohol-induced cardiomyopathy, malignancy, and liver disease [50,52,53,80]. The mechanistic role of aldehyde production and aldehyde metabolism in alcoholic cardiomyopathy has been recently reviewed [50]. New mechanistic insights on alcoholic cardiomyopathy may encourage the development of tools that might specifically detect alcohol-induced cardiomyopathy to correlate with disease progression. Below we briefly discuss the implications that such putative biomarkers may have for the detection of **alcohol-induced liver disease** and/or various malignancies.

Alcohol-Induced Liver Disease

Aldehyde-induced adducts contribute to the initial development and later stages of alcohol-induced liver disease [51–53]. For instance, in rats receiving a diet containing ethanol, acetaldehyde, 4-HNE, and MDA adducts colocalize with perivenous lipid deposits, indicative of the earliest lesion observed in alcohol-induced liver disease, known as **steatosis** [51,52]. In humans similar colocalization of acetaldehyde-induced adducts and steatosis has been reported in alcoholic individuals with no clinical signs or laboratory tests suggesting the presence of liver disease [53]. Moreover, in non-alcoholics acetaldehyde-induced adducts were not identified in liver biopsies [53].

In humans with advanced stages of liver disease, aldehyde-induced adducts are localized in **hepatic stellate cells** associated with **fibrosis** and **cirrhosis** as well as in **myofibroblasts** in liver regions bearing fibrotic bridging [54]. Aldehyde hybrid adducts can additionally stimulate the secretion/production of fibronectin *in vitro* by hepatic stellate cells, as well as inflammatory cytokines and adhesion molecules from endothelial cells [55]. These findings are relevant in that hybrid adducts can increase the development of scar tissue and fibrosis compared with individual adducts [56]. In addition, aldehyde-induced adducts can directly activate immune cells, **Kupffer cells**, and endothelial cells to produce profibrogenic mediators [81]. Consequently, the ability to monitor specific aldehyde-induced protein cellular adducts such as these may provide a basis for specific monitoring of the progression of liver disease.

Autoimmunity to aldehyde-induced adducts might also be implicated in the pathogenesis of alcoholic liver disease. To support this notion, IgA, IgM, and IgG antibodies directed against acetaldehyde-induced protein adducts have been documented in the serum of chronic alcoholics compared with non-alcoholics [57]. In patients with alcoholic liver disease, anti-adduct IgA titers significantly correlated with the combined clinical and laboratory index of liver disease severity [57]. IgG antibodies reacting with MDA-, 4HNE-, and MAA-induced adducts were also significantly increased in alcoholic patients with alcohol-induced hepatitis or cirrhosis compared with alcoholics without liver damage, patients with nonalcoholic liver disease, and healthy controls. The titers measured in alcoholic patients with alcohol-induced hepatitis or cirrhosis also correlated with the severity of liver damage [58,59]. Although further studies are warranted, these studies taken together suggest that antibodies against aldehyde-induced protein adducts might have potential as putative biomarkers to stratify liver disease severity in AUD.

Alcohol-Induced Malignancies

AUD has been associated with an increased risk for the development of cancers of the head and neck, gastrointestinal tract, breast, and liver [49,82,83], with aldehyde-induced adducts contributing to DNA damage that can promote carcinogenesis [15,60,61]. For example, alcohol consumption associated with the development of hepatocellular carcinoma is caused by transversion of p53 at codon 249 (by a G-to-T transversion). 4-HNE is known to form DNA adducts on deoxyguanosine, and when 4-HNE is directly applied to wild-type p53 TK-6 lymphoblastoid cells it causes increased transversion of p53 at codon 249 [62,63]. Specific quantification of acetaldehyde-induced DNA adducts, as we describe for esophageal cancer above, may be a powerful method for earlier detection and cancer screening.

Recent Techniques to Detect Aldehyde and Aldehyde-Induced Adducts

Although techniques to measure aldehyde-induced DNA and protein adducts have existed for several decades, new technology for the assessment of aldehyde-induced adducts can now be exploited to improve on existing biomarkers in addition to developing novel candidate biomarkers to detect AUD and AUD-associated complications. Here we discuss these novel methods, including probes to quantify aldehydes using live-cell imaging techniques, the advances made in detecting DNA-induced aldehyde adducts, and the use of mass spectrometry (MS) to further identify proteins modified by aldehyde-induced adducts.

Live-Cell Detection of Aldehydes

One challenge in quantifying reactive aldehydes is that, with the techniques available, the sample requires processing to evaluate aldehyde-induced adducts. However, recently a reporter probe was developed using dark hydrazone fluorescence labeling, which can quantify alkyl aldehydes (e.g., acetaldehyde, 4-HNE) in live cells. This study illustrated the ability to measure dose-dependent changes in alkyl aldehyde levels in HeLa cells by both fluorescence imaging and flow cytometry [64]. Additionally, a hydrazinyl naphthalimide fluorescent probe was recently developed that can monitor aldehyde load in lung epithelial cells exposed to ethanol [65]. Overall, the recent reports of fluorescent dyes for monitoring aldehydes in live cells provides exciting and valuable research tools to potentially study AUD and AUD-associated complications in cellular systems.

DNA Adduct Detection

Recently, an effective method of quantifying N^2-ethyl-dG was developed using liquid chromatography–electrospray ionization tandem MS (LC-ESI-MS/MS) with hydrophobic interaction chromatography (HILIC) to improve the ionization efficiency of detection of N^2-ethyl-dG. Although further corroborating evidence is needed, use of HILIC increased the MS signal intensity 97-fold compared with using reversed-phase chromatography [66]. This is encouraging since a LC-ESI-MS/MS system may allow quantification of N^2-ethyl-dG without requiring additional tools such as a nanoelectrospray interface to detect N^2-ethyl-dG, potentially making N^2-ethyl-dG quantification easier and more feasible [66].

Protein Adduct Detection

Since a number of proteins that form aldehyde-induced protein adducts were identified over a decade ago, detection of new and novel targets with existing technology for AUD and AUD-associated complications may be possible. Potentially, antibodies that detect aldehyde-induced protein adducts, such as MDA and 4-HNE, can be used in combination with MS to understand specifically how aldehyde-induced adducts of the proteome are altered when exposed to alcohol. This approach recently identified, in rat liver mitochondrial fractions, several proteins harboring 4-HNE-induced adducts [67]. In particular, electron transfer flavoprotein alpha was identified to exhibit significant enhancement of 4-HNE-induced adducts in rats fed a Lieber–DeCarli ethanol diet for 5 weeks compared with rats not receiving alcohol [67]. Further application of this and similar methods may uncover a previously unrecognized subset of the proteome and/or novel putative protein targets for modification by aldehyde-induced adducts. Presumably these might also be used as biomarkers for AUD and AUD-associated complications.

Limitations to Quantifying Aldehyde-Induced Adducts

One potential challenge in implementing aldehyde-induced adducts to monitor AUD and AUD-associated complications is that endogenous and exogenous sources of acetaldehyde other than alcohol consumption may exist. Although acetaldehyde is produced by bacteria in the gastrointestinal tract, alcohol-induced acetaldehyde adducts – particularly DNA adducts – are present at sufficient concentrations to be detected above natural biological processes, such as those of bacteria, that may produce aldehydes; this is based on studies measuring DNA adducts in epithelial cells and in blood samples [45,48]. However, if additional biomarkers are developed, the utility of these could potentially be limited if changes in aldehyde-induced adducts caused by alcohol consumption are subtle compared with natural biological processes that can produce aldehydes.

Exogenous sources of aldehyde exposure, such as tobacco cigarettes, could also be difficult to differentiate from aldehyde-induced adducts produced by alcohol [32,68]. However, it is important to recognize that acetaldehyde levels measured in the saliva are sevenfold higher when tobacco cigarettes are smoked with alcohol consumption compared with alcohol consumption alone [68]. This interplay between lifestyle choices and genetics is also eloquently supported by a small clinical study reporting the odds ratio of developing esophageal cancer as the highest among people who drink alcohol, smoke tobacco cigarettes, and have the *ALDH2*2* genotype [69]. Overall, although measuring acetaldehyde-induced adducts may not be specific to a process that occurs only with alcohol consumption, these measurements may provide a valuable tool to define a level of risky alcohol use that may be relevant to the development of potential AUD-associated complications when factoring in additional lifestyle choices such as cigarette smoking.

Although tools to measure aldehyde-induced adducts may not be easily applied to all patient communities, the development of such biomarker tools may allow stratification of patient populations based on lifestyle and genetics. This in turn might provide general recommendations for patients who cannot undergo testing to assess aldehyde-induced adducts. Therefore, although biomarkers of aldehyde-induced adducts are not intended to replace existing verbal screening tools and urine EtG and EtS quantification, they might potentially provide an added dimension to the evaluation of AUD and AUD-associated complications.

Box 2. Clinician's Corner

- Alcohol consumption, especially 12-month alcohol use, high-risk drinking, and AUD, are on the rise in the USA compared with a decade ago.
- Following alcohol consumption, reactive aldehydes are produced that modify DNA and protein. These modifications are called aldehyde-induced adducts and can lead to cellular damage that can potentially result in alcohol-induced complications such as cancer or cardiomyopathy [50,60,61].
- People of East Asian descent carry a genetic variant in the enzyme ALDH2 known as ALDH2*2. The ALDH2*2 variant severely limits aldehyde metabolism after alcohol consumption and results in facial flushing and tachycardia [40]. Frequent alcohol consumption by people heterozygous for the *ALDH2*2* variant is associated with an increased risk for development of head and neck cancers, including esophageal cancer. Monitoring of aldehyde-induced adducts may be a means to develop more precise care for this particular patient population.
- In the future, monitoring the presence of aldehyde-induced adducts in patient biospecimens may complement questionnaires and biomarkers for AUD and AUD-associated complications. This could ultimately improve management strategies, stratify risk for alcohol-related diseases, and allow timely interventions for AUD and AUD-associated complications.

Concluding Remarks

We posit that aldehyde-induced adducts may prove to be promising biomarkers for AUD and AUD-associated complications. Additional research is still required to validate these findings and to fully develop these candidate biomarkers for possible clinical use (see Outstanding Questions and Box 2). Since adduct quantification can correlate with the amount of alcohol consumed both acutely and chronically, aldehyde-induced adducts might be exploited to better characterize the degree of unhealthy alcohol use (risky use or AUD) and, presumably, the severity of AUD. Additionally, the concept of DNA and protein adduct detection might be extended as a possible useful tool to monitor other substance abuse disorders, such as cocaine [70].

In the era of precision medicine, aldehyde-induced adducts combined with written questionnaires might provide more detailed tools to help evaluate AUD and AUD-associated complications. In the future it will be exciting to see whether quantification of aldehyde-induced adducts from a blood test, saliva sample, or cheek swab might allow earlier detection and possible intervention for AUD and AUD-associated complications.

Outstanding Questions

How does the aldehyde-induced DNA adduct N^2-ethylidene-2′-dG specifically contribute to human diseases caused by alcohol consumption?

Following alcohol consumption, what is the time course of adduct formation in biospecimens for different proteins? How does that compare with the time course of DNA adducts isolated from different cell lines?

How will blood test, saliva sample, or cheek swab results differ in the quantification of aldehyde-induced adducts? Is one test best suited for detection of the concentration of alcohol that can lead to AUD or the risk of developing specific alcohol-related diseases?

Acknowledgments

This work was supported by NIH GM119522 (E.R.G.), a California TRDRP High Impact Pilot Research Award (E.R.G.), and a FAER Medical Student Anesthesia Research Fellowship (H.M.H.).

References

1. Substance Abuse and Mental Health Services Administration (2017) Key Substance Use and Mental Health Indicators in the United States: Results from the 2016 National Survey on Drug Use and Health (HHS Publication No. SMA 17-5044, NSDUH Series H-52), Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. https://www. samhsa.gov/data/.
2. Centers for Disease Control and Prevention (CDC) Alcohol and Public Health: Alcohol-Related Disease Impact (ARDI). Average for United States 2006–2010 – Alcohol-Attributable Deaths Due to Excessive Alcohol Use, CDC.
3. Sacks, J.J. *et al.* (2015) 2010 national and state costs of excessive alcohol consumption. *Am. J. Prev. Med.* 49, e73–e79
4. Grant, B.F. *et al.* (2017) Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001–2002 to 2012–2013: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA Psychiatry* 74, 911–923
5. Monzavi, S.M. *et al.* (2015) Alcohol related disorders in Asia Pacific region: prevalence, health consequences and impacts on the nations. *Asia Pac. Med. Toxicol.* 4, 1–8
6. Brooks, P.J. *et al.* (2009) The alcohol flushing response: an unrecognized risk factor for esophageal cancer from alcohol consumption. *PLoS Med.* 6, e50
7. Esterbauer, H. *et al.* (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic. Biol. Med.* 11, 81–128
8. APA (2013) *Diagnostic and Statistical Manual of Mental Disorders.* (5th edn), American Psychiatric Association
9. Helander, A. *et al.* (2009) Detection times for urinary ethyl glucuronide and ethyl sulfate in heavy drinkers during alcohol detoxification. *Alcohol Alcohol.* 44, 55–61
10. Babor, T.F. *et al.* (2001) *The Alcohol Use Disorders Identification Test: Guidelines for Use in Primary Care,* World Health Organization

11. Bush, K. *et al.* (1998) The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. *Arch. Intern. Med.* 158, 1789–1795
12. Smith, P.C. *et al.* (2009) Primary care validation of a single-question alcohol screening test. *J. Gen. Intern. Med.* 24, 783–788
13. Lieber, C.S. and DeCarli, L.M. (1968) Ethanol oxidation by hepatic microsomes: adaptive increase after ethanol feeding. *Science* 162, 917–918
14. Lieber, C.S. and DeCarli, L.M. (1970) Hepatic microsomal ethanol-oxidizing system. *In vitro* characteristics and adaptive properties *in vivo*. *J. Biol. Chem.* 245, 2505–2512
15. Wang, Y. *et al.* (2009) Ethanol-induced cytochrome P4502E1 causes carcinogenic etheno-DNA lesions in alcoholic liver disease. *Hepatology* 50, 453–461
16. Tuma, D.J. *et al.* (1996) Acetaldehyde and malondialdehyde react together to generate distinct protein adducts in the liver during long-term ethanol administration. *Hepatology* 23, 872–880
17. Behrens, U.J. *et al.* (1988) Formation of acetaldehyde adducts with ethanol-inducible P450IIE1 *in vivo*. *Biochem. Biophys. Res. Commun.* 154, 584–590
18. Doorn, J.A. and Petersen, D.R. (2003) Covalent adduction of nucleophilic amino acids by 4-hydroxynonenal and 4-oxononenal. *Chem. Biol. Interact.* 143–144, 93–100
19. Romanazzi, V. *et al.* (2013) Immune response to acetaldehyde-human serum albumin adduct among healthy subjects related to alcohol intake. *Environ. Toxicol. Pharmacol.* 36, 378–383
20. Svegliati-Baroni, G. *et al.* (1994) Collagen-acetaldehyde adducts in alcoholic and nonalcoholic liver diseases. *Hepatology* 20, 111–118
21. Zhu, Y. *et al.* (1996) Identification of the 37-kd rat liver protein that forms an acetaldehyde adduct *in vivo* as delta 4-3-ketosteroid 5 beta-reductase. *Hepatology* 23, 115–122
22. Wehr, H. *et al.* (1993) Acetaldehyde adducts and autoantibodies against VLDL and LDL in alcoholics. *J. Lipid Res.* 34, 1237–1244
23. Koivisto, H. *et al.* (2006) Long-term ethanol consumption and macrocytosis: diagnostic and pathogenic implications. *J. Lab. Clin. Med.* 147, 191–196
24. Yoon, Y. *et al.* (1998) Ethanol-induced alterations of the microtubule cytoskeleton in hepatocytes. *Am. J. Physiol.* 274, G757–G766
25. Garro, A.J. *et al.* (1986) The effects of chronic ethanol consumption on carcinogen metabolism and on O^6-methylguanine transferase-mediated repair of alkylated DNA. *Alcohol. Clin. Exp. Res.* 10, 73S–77S
26. Doorn, J.A. *et al.* (2006) Inhibition of human mitochondrial aldehyde dehydrogenase by 4-hydroxynon-2-enal and 4-oxonon-2-enal. *Chem. Res. Toxicol.* 19, 102–110
27. Chen, J. *et al.* (2000) Formation of malondialdehyde adducts in livers of rats exposed to ethanol: role in ethanol-mediated inhibition of cytochrome c oxidase. *Alcohol. Clin. Exp. Res.* 24, 544–552
28. Stewart, B.J. *et al.* (2007) Residue-specific adduction of tubulin by 4-hydroxynonenal and 4-oxononenal causes cross-linking and inhibits polymerization. *Chem. Res. Toxicol.* 20, 1111–1119
29. Jukkola, A. and Niemela, O. (1989) Covalent binding of acetaldehyde to type III collagen. *Biochem. Biophys. Res. Commun.* 159, 163–169
30. Jennett, R.B. *et al.* (1989) Increased covalent binding of acetaldehyde to calmodulin in the presence of calcium. *Life Sci.* 45, 1461–1466
31. Sultana, R. *et al.* (2005) Formation of acetaldehyde adducts of glutathione *S*-transferase A3 in the liver of rats administered alcohol chronically. *Alcohol* 35, 57–66
32. McCaskill, M.L. *et al.* (2011) Hybrid malondialdehyde and acetaldehyde protein adducts form in the lungs of mice exposed to alcohol and cigarette smoke. *Alcohol. Clin. Exp. Res.* 35, 1106–1113
33. Bootorabi, F. *et al.* (2008) Modification of carbonic anhydrase II with acetaldehyde, the first metabolite of ethanol, leads to decreased enzyme activity. *BMC Biochem.* 9, 32
34. Sabol, D.A. *et al.* (1999) Coagulation protein function VII: diametric effects of acetaldehyde on factor VII and factor IX function. *Dig. Dis. Sci.* 44, 2564–2567
35. Basista, M.H. *et al.* (1994) Acetaldehyde alters coagulation protein function. *Dig. Dis. Sci.* 39, 2421–2425
36. Brecher, A.S. *et al.* (1996) Coagulation protein function. IV. Effect of acetaldehyde upon factor X and factor Xa, the proteins at the gateway to the common coagulation pathway. *Alcohol* 13, 539–545
37. De Benedetto, G.E. and Fanigliulo, M. (2009) A new CE-ESI-MS method for the detection of stable hemoglobin acetaldehyde adducts, potential biomarkers of alcohol abuse. *Electrophoresis* 30, 1798–1807
38. Tsuboi, K.K. *et al.* (1981) Acetaldehyde-dependent changes in hemoglobin and oxygen affinity of human erythrocytes. *Hemoglobin* 5, 241–250
39. Hill, G.E. *et al.* (1998) Association of malondialdehyde-acetaldehyde (MAA) adducted proteins with atherosclerotic-induced vascular inflammatory injury. *Atherosclerosis* 141, 107–116
40. Gross, E.R. *et al.* (2015) A personalized medicine approach for Asian Americans with the aldehyde dehydrogenase 2*2 variant. *Annu. Rev. Pharmacol. Toxicol.* 55, 107–127
41. Yokoyama, A. *et al.* (1998) Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis* 19, 1383–1387
42. Chang, J.S. *et al.* (2017) ALDH2 polymorphism and alcohol-related cancers in Asians: a public health perspective. *J. Biomed. Sci.* 24, 19
43. Amanuma, Y. *et al.* (2015) Protective role of ALDH2 against acetaldehyde-derived DNA damage in oesophageal squamous epithelium. *Sci. Rep.* 5, 14142
44. Balbo, S. *et al.* (2012) Time course of DNA adduct formation in peripheral blood granulocytes and lymphocytes after drinking alcohol. *Mutagenesis* 27, 485–490
45. Balbo, S. *et al.* (2012) Kinetics of DNA adduct formation in the oral cavity after drinking alcohol. *Cancer Epidemiol. Biomarkers Prev.* 21, 601–608
46. Balbo, S. *et al.* (2016) Increased levels of the acetaldehyde-derived DNA adduct N^2-ethyldeoxyguanosine in oral mucosa DNA from rhesus monkeys exposed to alcohol. *Mutagenesis* 31, 553–558
47. Matsuda, T. *et al.* (2006) Increased DNA damage in ALDH2-deficient alcoholics. *Chem. Res. Toxicol.* 19, 1374–1378
48. Takeshita, T. and Morimoto, K. (2000) Accumulation of hemoglobin-associated acetaldehyde with habitual alcohol drinking in the atypical ALDH2 genotype. *Alcohol. Clin. Exp. Res.* 24, 1–7
49. Katada, C. *et al.* (2016) Alcohol consumption and multiple dysplastic lesions increase risk of squamous cell carcinoma in the esophagus, head, and neck. *Gastroenterology* 151, 860–869 e7
50. Zhang, Y. and Ren, J. (2011) ALDH2 in alcoholic heart diseases: molecular mechanism and clinical implications. *Pharmacol. Ther.* 132, 86–95
51. Sampey, B.P. *et al.* (2003) Immunohistochemical characterization of hepatic malondialdehyde and 4-hydroxynonenal modified proteins during early stages of ethanol-induced liver injury. *Alcohol. Clin. Exp. Res.* 27, 1015–1022
52. Li, C.J. *et al.* (1997) Acetaldehyde-modified and 4-hydroxynonenal-modified proteins in the livers of rats with alcoholic liver disease. *Hepatology* 26, 650–657
53. Holstege, A. *et al.* (1994) Acetaldehyde-modified epitopes in liver biopsy specimens of alcoholic and nonalcoholic patients: localization and association with progression of liver fibrosis. *Hepatology* 19, 367–374
54. Paradis, V. *et al.* (1996) Cellular and subcellular localization of acetaldehyde-protein adducts in liver biopsies from alcoholic patients. *J. Histochem. Cytochem.* 44, 1051–1057

55. Thiele, G.M. *et al.* (2005) Rat sinusoidal liver endothelial cells (SECs) produce pro-fibrotic factors in response to adducts formed from the metabolites of ethanol. *Biochem. Pharmacol.* 70, 1593–1600
56. Tuma, D.J. (2002) Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic. Biol. Med.* 32, 303–308
57. Viitala, K. *et al.* (1997) Serum IgA, IgG, and IgM antibodies directed against acetaldehyde-derived epitopes: relationship to liver disease severity and alcohol consumption. *Hepatology* 25, 1418–1424
58. Rolla, R. *et al.* (2000) Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. *Hepatology* 31, 878–884
59. Mottaran, E. *et al.* (2002) Lipid peroxidation contributes to immune reactions associated with alcoholic liver disease. *Free Radic. Biol. Med.* 32, 38–45
60. Brooks, P.J. and Theruvathu, J.A. (2005) DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. *Alcohol* 35, 187–193
61. Linhart, K. *et al.* (2014) The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts. *Redox Biol.* 3, 56–62
62. Balbo, S. and Brooks, P.J. (2015) Implications of acetaldehyde-derived DNA adducts for understanding alcohol-related carcinogenesis. *Adv. Exp. Med. Biol.* 815, 71–88
63. Hussain, S.P. *et al.* (2000) Increased p53 mutation load in non-tumorous human liver of Wilson disease and hemochromatosis: oxyradical overload diseases. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12770–12775
64. Yuen, L.H. *et al.* (2016) Dark hydrazone fluorescence labeling agents enable imaging of cellular aldehydic load. *ACS Chem. Biol.* 11, 2312–2319
65. Reeves, A.G. *et al.* (2017) Imaging acetaldehyde formation during ethanol metabolism in living cells using a hydrazinyl naphthalimide fluorescent probe. *Anal. Methods* 9, 3418–3421
66. Murakami, H. *et al.* (2018) Progress in a selective method for the determination of the acetaldehyde-derived DNA adducts by using HILIC-ESI-MS/MS. *Talanta* 177, 12–17
67. Andringa, K.K. *et al.* (2014) Proteomic analysis of 4-hydroxynonenal (4-HNE) modified proteins in liver mitochondria from chronic ethanol-fed rats. *Redox Biol.* 2, 1038–1047
68. Salaspuro, V. and Salaspuro, M. (2004) Synergistic effect of alcohol drinking and smoking on *in vivo* acetaldehyde concentration in saliva. *Int. J. Cancer* 111, 480–483
69. Cui, R. *et al.* (2009) Functional variants in ADH1B and ALDH2 coupled with alcohol and smoking synergistically enhance esophageal cancer risk. *Gastroenterology* 137, 1768–1775
70. Ndikum-Moffor, F.M. and Roberts, S.M. (2003) Cocaine-protein targets in mouse liver. *Biochem. Pharmacol.* 66, 105–113
71. Litten, R.Z. *et al.* (2010) Alcohol biomarkers in applied settings: recent advances and future research opportunities. *Alcohol. Clin. Exp. Res.* 34, 955–967
72. Conigrave, K.M. *et al.* (2003) Traditional markers of excessive alcohol use. *Addiction* 98 (Suppl. 2), 31–43
73. Niemela, O. (2016) Biomarker-based approaches for assessing alcohol use disorders. *Int. J. Environ. Res. Public Health* 13, 166
74. Ramskogler, K. *et al.* (2004) CDT values are not influenced by epithelial cell apoptosis in chronic alcoholic patients – preliminary results. *Alcohol. Clin. Exp. Res.* 28, 1396–1398
75. Tavakoli, H.R. *et al.* (2011) Review of current clinical biomarkers for the detection of alcohol dependence. *Innov. Clin. Neurosci.* 8, 26–33
76. Vaca, C.E. *et al.* (1995) Studies of the reaction of acetaldehyde with deoxynucleosides. *Chem. Biol. Interact.* 98, 51–67
77. Wang, M. *et al.* (2006) Identification of an acetaldehyde adduct in human liver DNA and quantitation as N2-ethyldeoxyguanosine. *Chem. Res. Toxicol.* 19, 319–324
78. Garcia, C.C. *et al.* (2011) [^{13}C2]-Acetaldehyde promotes unequivocal formation of 1,N2-propano-2′-deoxyguanosine in human cells. *J. Am. Chem. Soc.* 133, 9140–9143
79. Mauch, T.J. *et al.* (1986) Covalent binding of acetaldehyde selectively inhibits the catalytic activity of lysine-dependent enzymes. *Hepatology* 6, 263–269
80. Munnia, A. *et al.* (2004) Exocyclic malondialdehyde and aromatic DNA adducts in larynx tissues. *Free Radic. Biol. Med.* 37, 850–858
81. Setshedi, M. *et al.* (2010) Acetaldehyde adducts in alcoholic liver disease. *Oxid. Med. Cell. Longev.* 3, 178–185
82. Morgan, T.R. *et al.* (2004) Alcohol and hepatocellular carcinoma. *Gastroenterology* 127, S87–S96
83. McDonald, J.A. *et al.* (2013) Alcohol intake and breast cancer risk: weighing the overall evidence. *Curr. Breast Cancer Rep.* 5 http://dx.doi.org/10.1007/s12609-013-0114-z
84. Jennett, R.B. *et al.* (1989) Preferential covalent binding of acetaldehyde to the alpha-chain of purified rat liver tubulin. *Hepatology* 9, 57–62
85. Shearn, C.T. *et al.* (2014) Identification of 5′ AMP-activated kinase as a target of reactive aldehydes during chronic ingestion of high concentrations of ethanol. *J. Biol. Chem.* 289, 15449–15462
86. Chen, J. *et al.* (1999) Formation of 4-hydroxynonenal adducts with cytochrome c oxidase in rats following short-term ethanol intake. *Hepatology* 29, 1792–1798
87. Sampey, B.P. *et al.* (2007) Ethanol-induced modulation of hepatocellular extracellular signal-regulated kinase-1/2 activity via 4-hydroxynonenal. *J. Biol. Chem.* 282, 1925–1937
88. Galligan, J.J. *et al.* (2014) Oxidative stress-mediated aldehyde adduction of GRP78 in a mouse model of alcoholic liver disease: functional independence of ATPase activity and chaperone function. *Free Radic. Biol. Med.* 73, 411–420
89. Donohue, T.M., Jr *et al.* (1983) Acetaldehyde adducts with proteins: binding of [^{14}C]acetaldehyde to serum albumin. *Arch. Biochem. Biophys.* 220, 239–246
90. Brecher, A. *et al.* (1996) Coagulation protein function. III. Effect of acetaldehyde upon the activation of prothrombin. *Alcohol* 13, 423–429