

Alcohol Consumption and Cancer Risk: Understanding Possible Causal Mechanisms for Breast and Colorectal Cancers

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. The Centers for Disease Control and Prevention (CDC) requested and funded this report. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by E-mail to **epc@ahrq.gov**.

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Structured Abstract

Objectives: The purpose of this report is to systematically examine the possible causal mechanism(s) that may explain the association between alcohol (ethanol) consumption and the risk of developing breast and colorectal cancers.

Data Sources: We searched 11 external databases, including PubMed and EMBASE, for studies on possible mechanisms. These searches used Medical Subject Headings and free text words to identify relevant evidence.

Review Methods: Two reviewers independently screened search results, selected studies to be included, and reviewed each trial for inclusion. We manually examined the bibliographies of included studies, scanned the content of new issues of selected journals, and reviewed relevant gray literature for potential additional articles.

Results:

Breast Cancer. Five human and 15 animal studies identified in our searches point to a connection between alcohol intake and changes in important metabolic pathways that when altered may increase the risk of developing breast cancer. Alterations in blood hormone levels, especially elevated estrogen-related hormones, have been reported in humans. Several cell line studies suggest that the estrogen receptor pathways may be altered by ethanol. Increased estrogen levels may increase the risk of breast cancer through increases in cell proliferation and alterations in estrogen receptors. Human studies have also suggested a connection with prolactin and with biomarkers of oxidative stress. Of 15 animal studies, six reported increased mammary tumorigenesis (four administered a co-carcinogen and two did not). Other animal studies reported conversion of ethanol to acetaldehyde in mammary tissue as having a significant effect on the progression of tumor development. Fifteen cell line studies suggested the following mechanisms:

- increased hormonal receptor levels
- increased cell proliferation
- a direct stimulatory effect
- DNA adduct formation
- increase cyclic adenosine monophosphate (cAMP)
- change in potassium channels
- modulation of gene expression.

Colorectal Cancer. One human tissue study, 19 animal studies (of which 12 administered a co-carcinogen and seven did not), and 10 cell line studies indicate that ethanol and acetaldehyde may alter metabolic pathways and cell structures that increase the risk of developing colon cancer. Exposure of human colonic biopsies to acetaldehyde suggests that acetaldehyde disrupts epithelial tight junctions.

Among 19 animal studies the mechanisms considered included:

- mucosal damage after ethanol consumption
- increased degradation of folate
- stimulation of rectal carcinogenesis
- increased cell proliferation
- increased effect of carcinogens.

Ten cell line studies suggested:

- folate uptake modulation
- tumor necrosis factor modulation
- inflammation and cell death
- DNA adduct formation
- cell differentiation
- modulation of gene expression.

One study used a combination of animal and cell line and suggested intestinal cell proliferation and disruption of cellular signals as possible mechanisms.

Conclusions: Based on our systematic review of the literature, many potential mechanisms by which alcohol may influence the development of breast or colorectal cancers have been explored but the exact connection or connections remain unclear. The evidence points in several directions but the importance of any one mechanism is not apparent at this time.

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<http://www.ahrq.gov/downloads/pub/evidence/pdf/alccan/alccan.pdf>.

Executive Summary

Alcohol Consumption and Cancer Risk: Understanding Possible Mechanisms for Breast and Colorectal Cancers

The purpose of our assessment of alcohol and cancer induction is to explore the possible underlying causal mechanism(s) of the association between alcohol consumption and breast and colorectal cancers. Therefore, we developed four Key Questions that address the potential mechanism(s) by which alcohol might be involved in the development of breast and colorectal cancers. The primary evidence base to address these questions consisted of experimental studies of humans, animals, and cell lines where alcohol exposure could be controlled. In addition to this evidence base we also considered epidemiology studies where alcohol exposure was not controlled (including those in patients with or without cancer) and hypothesis-generating studies that examined potential metabolic pathways connecting alcohol to cancer risk. These studies were considered in a separate evidence base that did not directly address the Key Questions.

Methods

The following Key Questions will be addressed in this report:

1. *What are the likely causal mechanisms by which alcohol contributes to the development of breast cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in breast cancer development?*
2. *For the most likely mechanisms of action involving alcohol and the development of breast cancer, how might other factors modify the effect of alcohol on breast cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?*
3. *What are the likely causal mechanisms by which alcohol contributes to the development of colorectal cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in colorectal cancer development?*
4. *For the most likely mechanisms of action involving alcohol and the development of colorectal cancer, how might other factors modify the effect of alcohol on colorectal cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?*

To address these Key Questions we searched electronic databases for information on ethanol consumption and the possible risks for breast and colorectal cancers. Thirty-five breast cancer

studies (five in humans, 15 in animals, and 15 in cell lines) and 31 colorectal cancer studies (one in humans, 19 in animals, 10 in cell lines, and one combination [animal and cell lines]) were included in the report. Information on study design and conduct was used to judge individual study internal validity. Data on experimental model, mechanism(s) examined, amount and duration of ethanol exposure, cancer formation, and intermediate outcomes were abstracted and tabled for review and discussion.

Evidence for Alcohol Consumption and Cancer Risk: Understanding Possible Mechanisms for Breast and Colorectal Cancers

Breast Cancer Studies

Human studies. We included five studies to evaluate the possible mechanisms for alcohol consumption and breast cancer risk: the first study examined effects of alcohol on estradiol, estrone, estrone sulfate, testosterone, androstenedione, progesterone, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenediol; the second study examined the effects of alcohol on plasma and urinary hormone concentrations in premenopausal women; a third study examined the effect of alcohol on prolactin levels in menopausal women using estradiol replacement; a fourth study examined the effects of alcohol on estrogen levels in postmenopausal women; and a fifth study examined the relationship of alcohol consumption with antioxidant nutrients and biomarkers of oxidative stress. Although none of these five studies reported direct evidence of cancer, we included them given that alcohol was administered to assess possible hormonal mechanism(s) and biomarkers of oxidative stress.

Animal studies. We included 15 studies using animal models to evaluate the possible mechanisms for alcohol consumption and breast cancer risk. Outcomes measured varied across studies. Of the 15 included studies, 14 reported on the type of mechanism(s) examined and one did not. The type of mechanisms examined in the 14 studies included elevated levels of estrogen and or progesterone, biotransformation to acetaldehyde, formation of deoxyribonucleic acid (DNA) adducts, elevation of serum prolactin, suppression of cellular immunity, enhancement of rate of tumor progression, and effect on DNA synthesis. Administration and duration of ethanol exposure varied across all studies. Studies also varied on whether a carcinogen was administered to induce carcinogenesis. Of the 15 studies, 10 reported the use of a carcinogen to induce cancer:

- dimethylene (a) anthracene [DMBA] (five studies)
- N-methyl-N-nitrosurea [MNU] (two studies)
- N-nitrosodimethylamine [NMDA] and 4-methylnitrosoamino-1-3-pyridyl-1-butanone [NNK] (one study)
- MADB106 [one study]
- bittner virus [one study].

Cell line studies. We included 15 studies using cell lines to evaluate the possible mechanisms for alcohol consumption and breast cancer risk. Twelve studies administered ethanol alone, and two studies administered ethanol combined with acetaldehyde. Cell lines examined in the studies included:

- MCF-7 (six studies)

- MCF-10F (two studies)
- T47D (one study)
- MM46 tumor cells (one study)
- MCF-7 + T47D (one study)
- MCF-7 + T84 (one study)
- MDA-MB-453 (one study)
- MCF-7 + T47D + MDA-MB-231 (one study)
- MCF-7 + ZR75.1 + BT-20 + MDA-MB-231 (one study).

Various mechanisms were reported by these studies: hormonal-related, DNA-adduct formation, inflammation and cell death, cell differentiation, increase cyclic adenosine monophosphate (cAMP), change in potassium channels, and modulation of gene expression.

Colorectal cancer studies.

Human study. We included one study using human tissues to evaluate the possible mechanism for alcohol consumption and colorectal cancer risk. The study exposed colonic mucosa to acetaldehyde vapor. Although the study did not report direct evidence to show causation of cancer, the authors concluded that acetaldehyde may cause an increase in risk of colon cancer via loss of cell-cell adhesion.

Animal studies. We included 19 studies using animal models to evaluate the possible mechanisms for alcohol consumption and colorectal cancer risk. Outcomes varied across all studies. Of the 19 included studies, 17 reported on the type of mechanism(s) examined and two did not. The type of mechanisms examined in the 17 studies included:

- cytochrome system expression
- generation of acetaldehyde
- DNA methylation
- effect of folate metabolism
- cell proliferation
- formation of acetaldehyde by human colonic bacteria
- local mucosal effect
- effect on various phases of carcinogenesis.

Administration and duration of ethanol exposure varied across all animal studies. Studies also varied on whether a carcinogen was administered to induce carcinogenesis. Of the 19 studies, 12 reported the use of a carcinogen to induce cancer:

- 1,1-dimethylhydrazine (DMH) (six studies)
- methylazoxymethanol (MAM) acetate (one study)
- acetoxymethyl-methylnitrosamine (AMMN) (one study)
- AMMN + cyanamide (CY) (one study)
- azoxymethane (AOM) (three studies).

Cell line studies. We included 10 studies using cell lines to evaluate the possible mechanisms for alcohol consumption and colorectal cancer risk. Cell lines examined in the studies included:

- Caco-2 (six studies)
- HT-29 (one study)
- colonic mucosa cells (one study)
- Caco-2 + HT-29 (one study)
- HT-29 + SW-1116 + HCT-15 (one study).

Various mechanisms were reported by these studies:

- folate uptake modulation
- tumor necrosis factor modulation
- inflammation and cell death
- formation of crosslinks with DNA
- cell differentiation
- modulation of gene expression.

Amount and duration of ethanol and/or acetaldehyde varied across all studies. Seven studies administered ethanol alone, while three studies administered ethanol combined with acetaldehyde.

Combination study (animal, cell line). We included one study that used a combination of animal (mice) and cell line (Caco-2) to evaluate the possible mechanisms for alcohol consumption and colorectal cancer risk. Intestinal cell proliferation as a result of phosphatidylethanol accumulation was the examined mechanism. The animal study administered ethanol, and the cell line study administered either ethanol or acetaldehyde. The primary outcome reported was disruption of cellular signals.

Discussion

The relationship between alcohol consumption and the risk of breast and colorectal cancers has been assessed in several systematic reviews and epidemiology studies (cohort and case-control studies). In this report, we looked at the potential mechanism(s) connecting both breast and colorectal cancers with alcohol consumption, under the assumption that there is a causal relationship. Our report did not focus on such a causal relationship reported in epidemiology literature where alcohol consumption was not under experimental control, but rather on potential mechanism(s) in studies that administered either alcohol or acetaldehyde in the absence of cancer. Only the human studies that actually administered ethanol regardless of experimental model were abstracted and included in the primary evidence base to assess possible mechanism(s). In addition, given that acetaldehyde is a metabolite of ethanol, we included animal studies that administered either alcohol and/or acetaldehyde in our evidence base. In humans, acetaldehyde levels in the blood are either very low or undetectable following alcohol consumption. Epidemiology studies that administered survey questionnaires to assess alcohol consumption and cancer risk and hypothesis-generating studies that examined potential pathways connecting alcohol to cancer risk were included as a separate evidence base.

The majority of the animal studies that chemically induced tumors through the administration of both alcohol and a carcinogen reported an increase in the carcinogenic effect; however, these studies can only offer indirect evidence of a connection between alcohol consumption and

increased cancer risk in humans. Most of these studies varied in terms of quantity of ethanol and timing of administration relative to the carcinogen that was used in the study to induce carcinogenesis. Though some of the possible mechanisms identified in this report have been evaluated in a variety of experimental models (i.e., human, animals, cell lines), others have simply been examined as hypothesis generating and as such may call for future research.

Breast cancer. Both human and animal studies included in our primary evidence base point to a connection between alcohol intake and changes in blood hormone levels, especially elevated levels of estrogen and androgens in humans. Several cell line studies also suggest that estrogen receptor pathways may be altered by ethanol. Increased estrogen levels may increase the risk of breast cancer through increases in cell proliferation and alterations in estrogen receptors. Elevation in prolactin levels were also examined in human and animal studies. While not as extensive as the estrogen-related studies, these studies give some indication that alcohol consumption may alter prolactin levels and increase the risk of developing breast cancer. In order to report the role of oxidative stress in breast cancer, one human study measured changes in the levels of serum biomarkers.

The formation of acetaldehyde after ethanol consumption and its involvement in breast cancer has been examined in human epidemiology studies of enzyme polymorphism. Polymorphism in the enzymes that metabolize ethanol may increase an individual's exposure to toxic metabolites such as acetaldehyde and influence cancer risk if acetaldehyde is involved in breast cancer development. In animal studies, conversion of ethanol to acetaldehyde in mammary tissue has been reported to have a significant effect on the progression of tumor development. Events downstream from acetaldehyde are likely being altered by the presence of acetaldehyde and may lead to enhanced tumor development.

Enhancement of cell proliferation and tumor progression related to ethanol consumption and conversion to acetaldehyde were examined in animal and cell line studies. The findings of these studies suggest that alterations in cell proliferation due to alcohol exposure may be a possible mechanism increasing breast cancer risk.

Colorectal cancer. One human study reported that acetaldehyde disrupts epithelial tight junctions and cell adhesion. Several animal studies also looked at the effects of acetaldehyde in the colon and reported the following: mucosal damage after ethanol consumption, increased degradation of folate, stimulation of rectal carcinogenesis, and an increased effect of carcinogens in the presence of acetaldehyde. In cell line studies, acetaldehyde exposure was reported to influence the initial steps of colonic carcinogenesis and later tumor development and decrease the activity of some brush border enzymes. Finally, a study using human tissue, animal tissue, and a cell line found evidence that acetaldehyde stimulates cell proliferation in intestinal crypt cells and therefore acetaldehyde may act as a cocarcinogen in the colon. These studies (human, animal, and cell line) combine to suggest that acetaldehyde production in the colon may provide a potential causal mechanism by which alcohol contributes to the development of colon cancer.

An effect of ethanol consumption on cell proliferation in the colon was investigated in a combination study (animal and cell line). In this study, chronic alcohol exposure resulted in disruption of signals that normally restrict proliferation in highly confluent intestinal cells, thereby facilitating abnormal intestinal proliferation. Several animal studies reported enhanced growth of mucosal tissue after chronic ethanol consumption. Cell studies indicate that exposure to ethanol and acetaldehyde increases cell proliferation and damages DNA which may contribute to cancer development. Together these studies suggest that ethanol and acetaldehyde exposure in

the colorectal mucosa may increase cell proliferation and be a potential mechanism connecting alcohol consumption to colorectal cancer risk.

Conclusions

Based on our systematic review of the literature, many potential mechanisms by which alcohol may influence the development of breast or colorectal cancers have been explored but the exact connection or connections remain unclear. The evidence points in several directions but the importance of any one mechanism is not apparent at this time. Several mechanisms have been proposed and human, animal, and cell line studies have provided evidence in support of several mechanisms, but the findings have been inconsistent. The diversity of experimental protocols among the studies included in this report could have contributed to the lack of consistency. Furthermore, variation across included studies for both the route of administration and amount of ethanol may have influenced results. Based on animal studies alone, researchers may be inclined to infer a causal link between alcohol and the risk of breast or colorectal cancers. In addition, although a majority of the epidemiology studies reported that alcohol increased the risk of both breast and colorectal cancers, we cannot discount uncontrolled confounding by diet and related lifestyles.

Evidence Report

Chapter 1. Introduction

Scope

The purpose of this report is to systematically and objectively synthesize evidence from the basic science literature to clarify the possible causal mechanisms by which alcohol may contribute to cancer risk, focusing on the induction and development of breast cancer and colorectal cancer under the assumption that there is a causal relationship. Therefore, the primary evidence base for this report consists of studies that administer ethanol or acetaldehyde to humans, animals, tissues, or cells and then look for the development of breast or colorectal cancer, or for changes in metabolic pathways and cellular structures that may increase the risk for developing these cancers. Case-control and other epidemiology studies are not included in the primary evidence base for assessment of possible mechanisms. However, such studies may provide insight into the dose/response relationship between alcohol consumption and cancer risk.

Apart from alcohol (i.e., ethanol) and water, the exact composition of most alcoholic beverages (e.g., beer, wine, or distilled spirits) on the market remains confidential proprietary information.¹ Therefore, the scope of this report is limited to ethanol. Other compounds (or contaminants) found in various alcoholic beverages that may play a role in the development of breast and colorectal cancers are outside the scope of this report. These compounds include nitrosamines, aflatoxins, polyphenols, ethyl carbamate (urethane), asbestos, and arsenic compounds.¹⁻⁴

In addition, studies that evaluated tumor progression or metastatic spread of either breast or colorectal cancer during alcohol consumption are outside the scope of this report because they are not examining the mechanisms underlying the association of alcohol and the risk of developing cancer.

Ethanol Metabolism

Orally-ingested ethanol from an alcoholic drink is rapidly and almost completely absorbed by the stomach, small intestines, and colon. The bioavailability of ethanol, the fraction of the ingested dose that reaches the systemic circulation, is about 80%.⁵ Therefore a large portion of ingested ethanol reaches the circulation (i.e., blood alcohol concentration) and is distributed to all body tissues including the breast, colon, and rectum. Blood alcohol concentration, however, may vary depending on the rate of gastric emptying and degree of metabolism during this first pass via the stomach and liver (i.e., first-pass metabolism of ethanol).⁶⁻⁸

Ethanol is metabolized in the body by two pathways (i.e., oxidative and nonoxidative).⁸ However, the nonoxidative pathway is minimal compared to the oxidative pathway.⁸ The liver is the major organ for the oxidative metabolism of ethanol.^{9,10} Ethanol is converted into acetaldehyde by cytosolic alcohol dehydrogenase (ADH).⁹⁻¹¹ Due to variation in gene encoding there are multiple isoenzymes of ADH that vary in their enzyme activity (ADH1A, ADH1B*1, ADH1B*2, ADH1B*3, ADH1C*1, ADH1C*2, ADH4, ADH5, ADH6, and ADH7).^{2,3,9,11-17} The ADH1B*2 is lower in frequency amongst Caucasians and higher among Asians and is about 40 times more active compared to the ADH1B*1 in the conversion of ethanol to acetaldehyde.¹⁸ ADH1C*1 is very common in Asians, and metabolizes ethanol 2.5 times faster compared to ADH1C*2.^{18,19} Among individuals who consume alcohol, ADH1C*1, a fast-acting metabolizer

of ethanol, results in accumulation of acetaldehyde. As a result of increased levels of acetaldehyde, these individuals may experience uncomfortable side effects, and may well have a tendency to consume less alcohol.^{18,19} The genetic polymorphism of ADH leads to differences in individual ethanol metabolism and individual differences in the susceptibility to alcohol-related tissue damage.^{8,18}

Acetaldehyde, a metabolite of ethanol, is further metabolized to acetate primarily by mitochondrial aldehyde dehydrogenase (ALDH2).^{9,11} ALDH2 accounts for the greater part of acetaldehyde breakdown and exists as ALDH2*1 and ALDH2*2. Individuals with ALDH2*2 have blood acetaldehyde levels 20 times higher compared to those with ALDH2*1.¹⁸ Acetaldehyde is a highly toxic metabolite that binds to many cellular proteins and may be responsible for damage in the liver as well as other body tissues.⁸ It binds to deoxyribonucleic acid (DNA), resulting in the formation of a DNA adduct which may influence cancer development.^{3,11} Presence of a DNA adduct is a sign of exposure to specific cancer-causing agent, and is indicative of growing damage to the DNA.^{3,11,13} Acetaldehyde is a cancer-causing agent in animals.¹⁴

During each oxidative process, nicotinamide adenine dinucleotide (NAD⁺) is reduced to NADH. In the liver, ethanol metabolism also involves microsomal cytochromes P450 2E1 (CYP2E1).¹¹ This pathway produces reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals which may increase the risk of tissue damage.^{2,8,11}

Nonoxidative metabolism of alcohol involves two pathways.⁸ One pathway results in the formation of fatty acid ethyl esters and the other the formation of phosphatidyl ethanol.^{8,9}

ADH is present in the human colonic mucosa as well as in the microflora inhabiting the colon, and ethanol is metabolized to acetaldehyde by ADH in both of these locations.^{20,21} ADH activity is significantly higher in the mucosa of the rectum than the colon.²¹ Aldehyde dehydrogenase activity is much greater in the liver than in the colonic mucosa, which favors the accumulation of acetaldehyde in the colon.²⁰ Breast tissue contains ADH and CYP2E1.¹⁰ Breast tissue converts ethanol to acetaldehyde which is then metabolized to acetate by xanthine oxidoreductase.

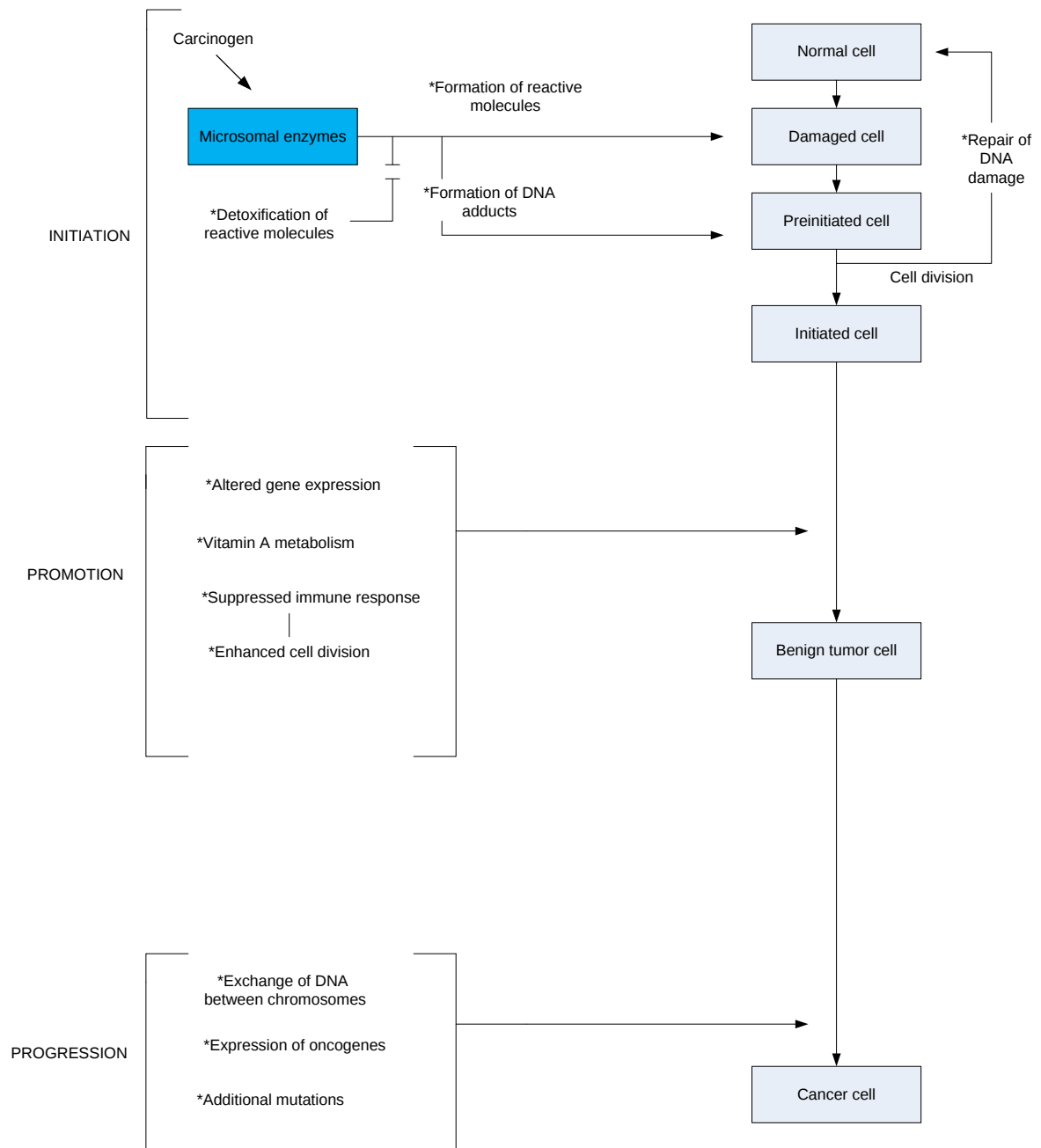
Alcohol and Cancer

Fewer than 10% of cancers can be attributed to an inherited genetic abnormality.²² The majority of cancers are the result of changes in the gene structure due to the loss of control mechanisms that prevent cancer development.²² Control mechanisms that may be altered during cancer development are: 1) tumor suppressor genes that lose their function causing a disruption in cellular adhesion and abnormal cell cycle progression, 2) DNA repair enzymes that become nonfunctional due to distorted methylation, and 3) proto-oncogenes that mutate into oncogenes.²²

The course by which normal cells are transformed into cancer cells is termed carcinogenesis (see Figure 1).^{3,14} When administered in combination with a recognized carcinogen, ethanol or its metabolite (acetaldehyde) produces reactive oxygen species (ROS).¹⁰ ROS may increase the transformation of normal cells into cancerous cells in various organs by inhibition of DNA methylation as well as by interacting with metabolism of retinoids.^{3,10,14,23,24} Alcohol and its metabolites have been implicated in all three stages of cancer formation (see the asterisks in Figure 1):^{3,9,11,13,14,24,25}

- initiation stage by impact on DNA repair
- promotion stage by altered gene expression, enhanced cell division, suppression of immune response, and change in metabolism of vitamin A
- progression stage by expression of oncogenes, exchange of DNA between chromosomes, and additional mutations.

Figure 1. Three stages of carcinogenesis



*Source from <http://www.niaaa.nih.gov/resources/graphicsgallery/immunesystem/lieb.htm>²⁶

Alcohol consumption is highly prevalent in the general U.S. population. The 2008 prevalence and trends data from the Behavioral Risk Factor Surveillance System indicate that about 54% of U.S. adults consumed alcohol within the past 30 days.²⁷ Though moderate alcohol consumption may have some potential health benefits, alcohol consumption has been identified as one of the major worldwide risks for burden of disease.²⁸ In the U.S., a standard drink is 12 fl oz (beer), 8 fl oz of malt liquor, 5 fl oz (wine), and 1.5 fl oz (80% proof distilled spirit).²⁹⁻³² Each is equivalent to 0.6 fluid ounces (12-14 g) of ethanol.²⁹⁻³² Moderate daily alcohol consumption in the U.S. for men is two drinks and for women is one drink.²⁹⁻³² However, variations have been reported worldwide in the definition of what is moderate for men and women.²⁹

Several epidemiology studies have reported moderate to strong associations between the level of alcohol consumption and the incidence of cancers of the mouth, pharynx, larynx, esophagus, and liver.^{2,24,33-35} Although the association between alcohol and breast and colorectal cancer is comparatively less strong than the association with these other cancers, given the high prevalence and incidence of breast and colorectal cancer, reducing the effect of any contributing factor may have a large overall impact on cancer incidence and prevalence.^{3,24,33,34,36-41} Observed associations of alcohol consumption and cancer, however, can be confounded by other risk factors for cancer, such as age, smoking, family history, obesity and physical activity, race or ethnicity, and nutrition.^{14,36,42-44} Because of the high prevalence of alcohol consumption, exploring the potential underlying mechanism(s) of the association between alcohol consumption and breast and colorectal cancers, if any, is essential in developing primary preventive measures. In view of the fact that alcohol consumption is a modifiable behavior,⁴⁵ recommending and promoting changes in behavior and appropriate preventive interventions may help reduce cancer risks in the general population.

Breast Cancer

According to the US National Cancer Institute (NCI), breast cancer is the most common cancer among women.⁴⁶ In 2009, it was anticipated that of the 192,370 women who were diagnosed, 40,170 would die of breast cancer.⁴⁶ Risk factors include family history, age at first birth, obesity in post menopausal women, dietary factors, alcohol consumption, early menarche, hormonal replacement therapy, low-dose irradiation, and lactation.^{18,46} Estrogen-induced breast cancer may result from cell proliferation, activation of cytochrome P450, and DNA damage.¹⁰ Cell proliferation is significant in the maintenance of normal and healthy breast tissue and these risk factors may alter cell proliferation in a direction that favors cancer development. Furthermore, enzyme polymorphism affects alcohol metabolism and could influence the effect of alcohol consumption on hormonal levels, thereby resulting in an increased risk of breast cancer.⁴⁷⁻⁵⁰ Among patients diagnosed with breast cancer, unregulated breast epithelial cell growth has been reported.⁵¹ Alcohol consumption has been investigated as a risk factor in the development of breast cancer. In a 2006 meta-analysis of 98 studies of alcohol and breast cancer, Key et al. reported that each additional 10 g ethanol/day resulted in a 10% increase in the odds ratio (OR) of risk of breast cancer associated with alcohol consumption.⁵²

Colorectal Cancer

Of the estimated 75,590 men and 71,380 women diagnosed with colorectal cancer, 49,920 men and women were expected to die of the disease in 2009.⁵³ Among adults with cancer, colorectal cancer is the second most common cause of death.⁵⁴ Risk factors include:^{13,14,53-58}

- age
- smoking
- low fiber diet
- high red meat/low fish intake
- inadequate intake of folate, B6 and retinoids
- obesity
- lack of physical activity
- low calcium intake
- alcohol (heavy consumption)
- an increase in colonic acetaldehyde level concentration
- chronic ulcerative colitis
- granulomatous colitis
- adenomatous polyps

In addition, following alcohol consumption, intracolonic ethanol is metabolized by colonic mucosal cells and intracolonic microbes. The risks of colorectal cancer development associated with alcohol consumption have been examined in epidemiology studies. In a 2004 meta-analysis of eight studies, Cho et al. reported that daily consumption of more than 45 g of alcohol increased the risk of colorectal cancer by 45%.³⁶ In addition, Homann et al. in a 2009 study reported that individuals with ADH1C1*1 homozygosity and consumption of more than 30 g of alcohol per day have significant increase risk of colorectal cancer.¹⁹

Chapter 2. Methods

Technical Expert Panel

ECRI Institute, in consultation with AHRQ, recruited a technical expert panel (TEP) to give input on key steps including the selection and refinement of the questions to be examined. Broad expertise and perspectives were sought. Divergent and conflicted opinions are common and perceived as healthy scientific discourse that results in a thoughtful, relevant systematic review. Therefore, in the end, study questions, design and/or methodologic approaches do not necessarily represent the views of individual technical and content experts. The expert panel membership is provided in the front matter of this report.

ECRI Institute created a protocol for developing the evidence report. The process consisted of working with AHRQ and the TEP to outline the report's objectives and to finalize Key Questions for the review. These Key Questions are presented in the Scope and Key Questions section of the Introduction. Upon AHRQ approval, the draft protocol was posted on the AHRQ Web site at <http://www.ahrq.gov/clinic/tp/alccantp.htm>.

Peer Review and Public Commentary

A draft of the completed report was sent to the peer reviewers and the representatives of AHRQ. In response to the comments of the peer reviewers, revisions were made to the evidence report, and a summary of the comments and their disposition was submitted to AHRQ. Peer reviewer comments on a preliminary draft of this report were considered by the EPC in preparation of this final report. Synthesis of the scientific literature presented here does not necessarily represent the views of individual reviewers.

Key Questions

The purpose of our assessment of the basic science literature concerning alcohol and cancer induction is not to determine the extent to which alcohol is a risk factor for breast and colorectal cancers, but instead to explore the evidence suggesting possible underlying causal mechanism(s) of the association between alcohol consumption and breast and colorectal cancers (see broken arrows from alcohol to cancer induction in Figure 2 and Figure 3). Therefore, we developed four Key Questions that address the potential mechanism(s) by which alcohol might be involved in the development of breast and colorectal cancers.

Key Question 1. What are the likely causal mechanisms by which alcohol contributes to the development of breast cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in breast cancer development?

Key Question 2. For the most likely mechanisms of action involving alcohol and the development of breast cancer, how might other factors modify the effect of alcohol on breast cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?

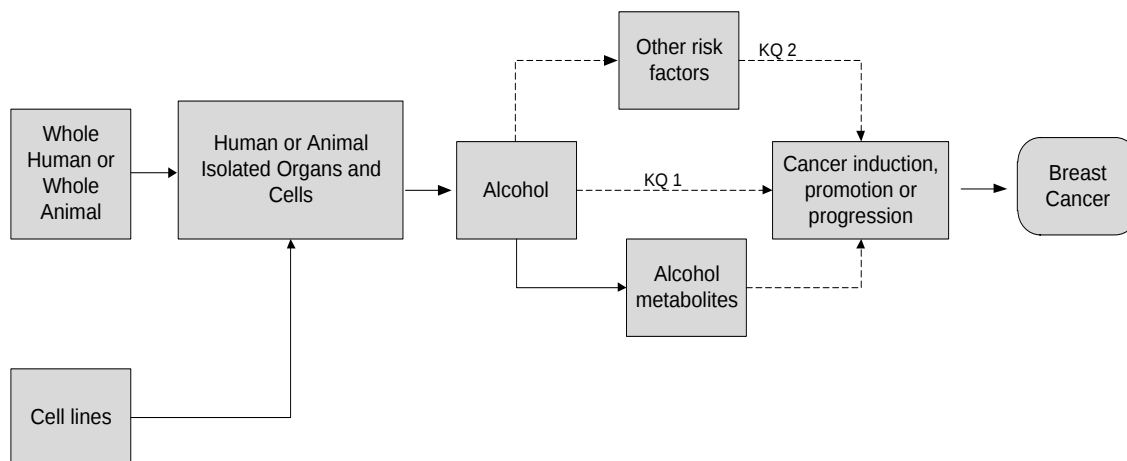
Key Question 3. What are the likely causal mechanisms by which alcohol contributes to the development of colorectal cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in colorectal cancer development?

Key Question 4. For the most likely mechanisms of action involving alcohol and the development of colorectal cancer, how might other factors modify the effect of alcohol on colorectal cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?

Analytical Framework

Figure 2 for breast and Figure 3 colorectal cancer portray analytical framework that visually describe the potential links in a chain of evidence that connect alcohol to breast and colorectal cancers. Contained within the framework are the Key Questions being addressed by this report and the potential areas of study (humans, animals, tissues, cells, ethanol and its metabolites) that can be manipulated to examine the assumed connection between alcohol consumption and an increased risk of developing breast or colorectal cancer.

Figure 2. Analytical framework for breast cancer

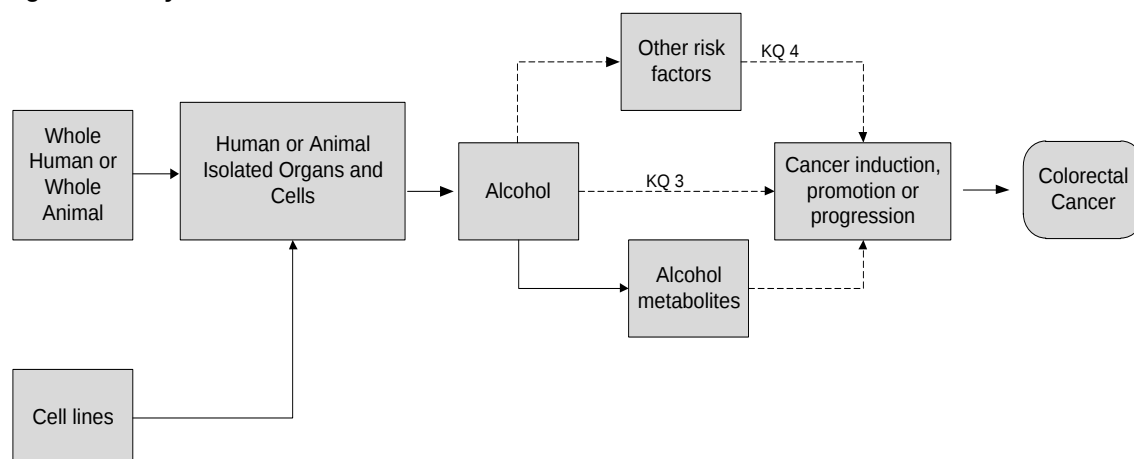


KQ: Key Question

KQ 1: effect of alcohol on stages of carcinogenesis

KQ 2: effect of alcohol and other risk factors on stages of carcinogenesis

Figure 3. Analytical framework for colorectal cancer



KQ: Key Question

KQ 3: effect of alcohol on stages of carcinogenesis

KQ 4: effect of alcohol and other risk factors on stages of carcinogenesis

Identification of Clinical Studies

The studies included in the primary evidence base for this technology assessment were identified using a multi-staged study selection process, and were based on inclusion criteria that were determined *a priori*, after the creation of the Key Questions and before any detailed examination of the literature base. Use of *a priori* inclusion criteria reduces the risk of bias because the decision to include or exclude each study is independent of the results of the study. In the first stage of the selection process, we performed a comprehensive literature search using broad criteria. In the second stage, we retrieved all articles that appeared to meet the *a priori* inclusion criteria, based on their published abstracts. In the final stage of the study selection, we reviewed the full text of each retrieved article, assessed its internal validity, and verified whether or not it met the *a priori* inclusion criteria.

Electronic Database Searches

We searched 11 external databases, including PubMed and EMBASE, for studies on possible mechanisms of alcohol and breast and colorectal cancer development (i.e., initiation, promotion, and progression) to identify evidence relevant to the Key Questions 1-4 using Medical Subject Headings and free text words. Additionally, we used some of the search terms and sources that were suggested by the Technical Expert Panel members on October 28, 2009. Two reviewers in the investigative team independently screened search results, selected studies to be included and reviewed each trial for inclusion. To supplement the electronic searches, we manually examined the bibliographies of included studies, scanned the content of new issues of selected journals, and reviewed relevant gray literature for potential additional articles. Gray literature includes reports and studies produced by local government agencies, private organizations, educational facilities, and corporations that do not appear in the peer-reviewed literature. Although we examined gray literature sources to identify relevant information, we only consider published, peer-reviewed literature in this report. During the peer review process, any new studies or data recommended

were subjected to the same inclusion and exclusion criteria. A complete list of the databases searched and the search strategy used to identify relevant studies are presented in Appendix A.

Study Selection

Use of explicit inclusion criteria, decided upon before any data have been extracted from studies, is a vital tool in preventing reviewer biases. Some of the *a priori* criteria are based on study design, and other criteria ensure that the evidence is not derived from unusual patients or interventions, and/or outmoded technologies. We developed the same inclusion criteria for each Key Question that this report addresses.

Criteria for Inclusion/Exclusion of Studies in the Review

We used the following formal criteria to determine which studies were included in the primary evidence base that addresses each Key Question. These studies are primarily experimental studies where the exposure to ethanol or acetaldehyde could be controlled and precise biochemical measurements could be made.

1. Any study, regardless of design, that provides data on the possible causal mechanism(s) of any association between alcohol consumption and the development of breast and colorectal cancers in any population setting, including humans, animals, and in vitro experimental studies.
2. In order to assess the outcome measure of carcinogenesis, there must be no breast or colorectal cancer present in human and animal studies prior to the start of the study.
3. Cell lines should be appropriate to the study of breast and colorectal cancers in humans.
4. Studies that report on metastatic lesions or tumor invasion were excluded because they do not discuss the likely causal mechanism(s) of the tumor at the primary site (breast or colorectal).
5. When the same study was published more than once, we used the data from the most recent publication. However, if the older report had provided data that was not provided by the most recent report, we included such data.
6. Studies must have administered ethanol. Studies that administered alcoholic beverages such as beer or malt liquor were excluded given that the exact composition of such drinks remains confidential.

Studies that did not specifically control alcohol exposure were also considered in this report but were not included in the primary evidence base addressing the Key Questions. Hypothesis-generating studies examining metabolic pathways that may connect alcohol to cancer risk and epidemiology studies of alcohol exposure (including those in patients with or without cancer) were incorporated into the report in order to review and discuss this literature for comparison with our primary evidence base from experimental studies.

Literature Review Procedures

The abstracts for all identified documents were downloaded into the Mobius Analytics SRS 4.0 Web-based system for conducting systematic reviews. Using this system, we assessed abstracts in order to either include or exclude identified documents based on our inclusion

criteria. If the abstract was missing or had insufficient information to make a decision on inclusion we ordered the full article. Full articles were then retrieved for review and categorization using Web-based forms. The Web-based system provided a structured framework to build and manage the numerous documents identified by our searches.

The review process underwent four levels:

- **Level 1** – Abstract Review
- **Level 2** – Full Document Review
- **Level 3** – Background Document Review
- **Level 4** – Evidence Base Document Review.

Each level has an electronic form for capturing data about each document identified in our searches (see Appendix B for sample data abstraction forms).

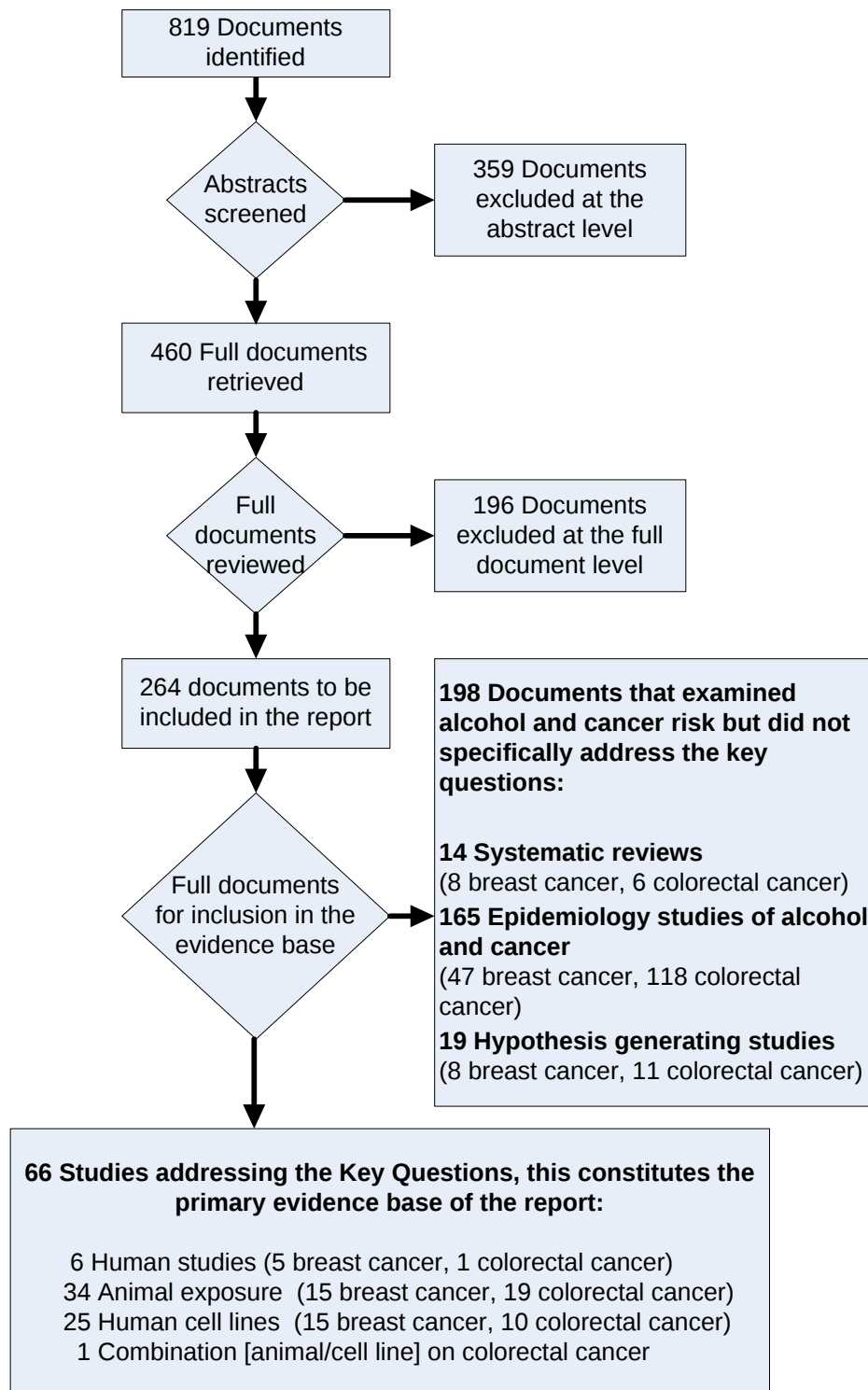
Data Abstraction and Data Management

All documents that were identified as belonging in the evidence base of the report underwent data abstraction using EXCEL spreadsheets. Table B-1 in Appendix B provides a list of the data abstracted from each study and placed in to a separate column in the spreadsheet. Some of the columns were modified depending on whether a study examined humans, animals, or cell lines. The information in the spreadsheets was later used to create the evidence tables in this report.

Disposition of the Documents Identified by Literature Searches

The SRS Web-based system allowed us to track all identified documents along with their complete citation. Literature searches were updated periodically and the new documents were added to the system and reviewed. Using the information contained in the SRS database we were able to create Figure 4 to illustrate an attrition diagram as well as separate tables that show the disposition of the documents identified by our literature searches. A total of 819 documents were identified by our searches. After review of the abstracts and then full documents, we included 264 documents for discussion within the report. Of these 264 documents, 66 met the requirements for the primary evidence base because they addressed one of the Key Questions. An additional 197 documents were included because they addressed issues related to alcohol and breast or colorectal cancer risk.

Figure 4. Disposition of the documents identified by literature searches



Assessing the Evidence for Each Key Question

Assessment of Internal and External Validity

A critical part in the process of creating a systematic review is assessing the validity of the results reported in each included study in the review. The validity of individual study results is determined in the context of the Key Questions these studies address. Internal validity is the extent to which a study's design and conduct are likely to have prevented bias and produced results that describe a true relationship.⁵⁹

The members of the Technical Expert Panel proposed several methods for evaluating the internal validity of studies using animals, tissues, or cells as the primary experimental model.

- Evidence from experimental studies offer the most compelling evidence that a mechanism/pathway is directly involved in increasing cancer risk with alcohol intake.
- Use of alcohol concentration levels in animal studies that far exceed levels that occur in humans are considered of low applicability.
- Cell lines should be appropriate to the study of breast and colorectal cancer in humans.

To ultimately establish the presence of a contributory cause between alcohol consumption and breast or colorectal cancer, the following criteria have to be fulfilled: association, exposure prior to the association, and demonstration that changing the cause alters the effect.^{14,60-63}

Other supportive criteria such as strength of association, consistency of association, biological plausibility, and a dose-response relationship can be used to establish contributory cause.⁶⁰⁻⁶³

For this systematic review, we applied the “direct” vs. “indirect” evidence concept.⁶⁴ Direct assessment measures are those which provide *direct* evidence that alcohol causes either breast or colorectal cancer. Such evidence as shown in Figure 1 may confirm the steps during cancer formation and possible sites of action of alcohol thus demonstrating a contributory cause.

Indirect measures typically focus on predictors that are correlated to carcinogenesis, but do not measure actual causation. Some of the most common indirect assessment measures include.^{3,10,13,14,18,41,55,65-81}

- increased androgen and estrogen concentration
- inactivation of the BRCA1 gene
- formation of new capillaries (angiogenesis)
- depletion of s-adenosylmethionine (SAM)
- low iron levels, low folate and vitamin B₁₂ levels
- induction of epidermal growth factor
- increase in tumor necrosis factor-alpha receptor
- acetaldehyde formation by colonic bacteria
- induction of CYP2E1
- impairment of retinoic acid

- generation of reactive oxygen species and reactive nitrogen species
- immune suppression (effects on peripheral T- and B-lymphocytes)
- increase in cell membrane permeability
- interference with DNA repair (acetaldehyde-DNA adducts)
- increased levels of biomarkers of oxidative stress.

Because of the focus on the *how* and *why* of causation of cancer, indirect measures are critical in our efforts to improve the evidence of direct causation in ongoing and future research of possible causal mechanisms explaining the increased risk of breast and colorectal cancer with alcohol consumption.

For this report, experimental studies that show direct evidence were treated as stronger evidence than studies of association which only showed indirect evidence. The strength of evidence supporting each proposed mechanism relating alcohol intake to the development of breast or colorectal cancer were categorized as either “Sufficient” or “Insufficient.” Three domains were evaluated: the potential risk of bias, or “internal validity” of the evidence base, the size of the evidence base (number of studies examining any one proposed mechanism), and the consistency of the findings (agreement across studies examining the same proposed mechanism).

External validity is the extent to which the findings and conclusions from a study or report can be translated to a specific setting or population (i.e., generalizability).⁵⁹ Generalizability is always strongest when results are collected in the specific setting or population of interest. However, clinical studies often cannot be conducted in such a setting or population, and results are instead collected from a more rigidly defined and less generalizable patient population. Human studies have more external validity than animal or cell line studies.

Data Synthesis

No meta-analyses were planned for this report. Given that this systematic review is hypothesis-summarizing and generating, we present a narrative summary of the findings based on the number of different mechanisms proposed and the studies showing support or lack of support for each mechanism.

Assessment of Internal Validity of Breast and Colorectal Studies

Internal validity, especially in the context of clinical studies, is the extent to which a study’s design and conduct are likely to have prevented bias.^{59,82} However, in the context of this report, which is assessing the results of human, animal and in vitro studies, we defined internal validity as the extent to which a direct relationship can be seen between the result of a given study and an increase in the risk of developing breast or colorectal cancer following ethanol consumption. Although we believe that the included studies are valid in design and outcomes measured for their intended purpose, we needed a measure of internal validity that was relevant to the connection between study results and cancer risk in humans. Therefore we considered human studies that administered alcohol having a higher internal validity than animal or in vitro studies. Animal studies that administered alcohol and did not use any known co-carcinogen were considered as having a higher internal validity (more direct relationship to an increase in cancer risk) than studies that administered a carcinogen. Studies that administered acetaldehyde or

known carcinogens were considered as having lower internal validity and a less direct relationship with an increase in cancer risk in humans who consume alcohol.

Assessment of External Validity of Breast and Colorectal Studies

In our report we did not identify any studies using human subjects that directly assessed the possible mechanism(s) associated with risk of breast cancer following alcohol consumption. However, we did identify one human study that indirectly reported on colorectal cancer risk association with alcohol consumption: exposure of colonic biopsy tissues to acetaldehyde.⁸³ For the animal studies, generalizability may be compromised by administering ethanol concentrations that far exceed levels suitable for human consumption, by administering acetaldehyde, and by co-administering a known carcinogen.^{73,82,84} Therefore, the results of these studies may not be directly applicable to human settings.

Chapter 3. Results

Evidence Base Describing Possible Mechanisms Connecting Alcohol Consumption and Breast Cancer Risk

Human Studies

We included five studies (see Table C-1 in Appendix C) that evaluated the possible mechanisms connecting alcohol consumption and breast cancer risk: the first study examined effects of alcohol on estradiol, estrone, estrone sulfate, testosterone, androstenedione, progesterone, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenediol;⁸⁵ the second study⁸⁶ examined the effects of alcohol on plasma and urinary hormone concentrations in premenopausal women; the third study⁸⁷ examined the effect of alcohol on prolactin levels in menopausal women using estradiol replacement; the fourth study⁸⁸ examined the effects of alcohol on estrogen levels in postmenopausal women; and the fifth study⁷⁶ examined the relationship of alcohol consumption with antioxidant nutrients and a biomarker of oxidative stress. Although none of these five studies reported direct evidence of cancer, we included them because alcohol was administered to examine alterations in hormonal mechanism(s) and biomarkers of oxidative stress that have been suggested to be linked to the development of breast cancer. Four studies⁸⁷⁻⁹⁰ reported increased serum hormonal levels and one study⁷⁶ reported an increase in isoprostane levels, a biomarker of oxidative stress. Table C-1 in Appendix C provides a summary of study design, mechanisms examined, amount and duration of ethanol or acetaldehyde exposure, study results, and authors' conclusions.

In the study by Dorgan et al., 51 healthy postmenopausal women consumed 15 or 30 grams of alcohol per day or an alcohol-free placebo beverage through three 8-week dietary periods. Each dietary period was preceded by a 2- to 5-week washout period when participants did not consume any alcohol. The results showed an increase in serum levels of both estrone sulfate and DHEAS. While this study did not report any direct evidence to show causation of cancer, Dorgan et al. concluded that results suggest a possible mechanism by which consumption of one or two alcoholic drinks per day by postmenopausal women could increase their risk of breast cancer.⁸⁵ In the second study Reichman et al. examined 34 premenopausal women who consumed 30 g of ethanol daily for three menstrual cycles and no alcohol during three other cycles.⁸⁶ The results showed an increase in plasma DHEA sulfate, plasma estrone, plasma estradiol, and urinary estradiol. Reichman et al. concluded that these results suggest a possible mechanism between alcohol consumption and risk of breast cancer again because of changes in hormone levels.⁸⁶ In the third study, Ginsburg et al.⁸⁷ conducted two randomized, crossover studies in postmenopausal women: study 1 administered ethanol (1 mL/kg, 95% ethanol) vs. isocaloric drink; study 2 was similar to study 1 except authors removed transdermal estradiol patches after administration of either ethanol or isocaloric drink. In both crossover studies, Ginsburg et al.⁸⁷ reported an increase in serum prolactin levels. In the fourth study Ginsburg et al.⁸⁸ administered ethanol (pineapple juice and 40% ethanol at a dose of 2.2 mL/kg of body weight [0.7 g/kg of body weight] in a total volume of 300 mL) vs. placebo to 24 postmenopausal women and reported a 3-fold increase in circulating estradiol levels in women on estrogen replacement therapy (ERT). In the fifth study Hartman et al.⁷⁶ administered a controlled diet plus each of three treatments (15 or 30 g alcohol/day or no-alcohol placebo beverage) to 53 postmenopausal women, during three 8-week periods in random order and reported that moderate alcohol consumption increased isoprostane, a biomarker of oxidative stress by 4.9%.

Animal Studies

We included 15 studies using animal models to evaluate possible mechanisms connecting alcohol consumption with breast cancer risk (see Table C-2 in Appendix C). Of the 15 included studies, 14 reported on the mechanism(s) and one⁹¹ did not. The mechanisms examined in the 14 studies were:

- elevated levels of estrogen and or progesterone⁹²⁻⁹⁴
- biotransformation to acetaldehyde⁹⁵
- formation of DNA adducts⁹⁶
- elevation of serum prolactin^{97,98}
- suppression of cellular immunity⁹⁹
- enhancement of rate of tumor progression¹⁰⁰⁻¹⁰³
- effect on DNA synthesis^{104,105}

Administration and duration of ethanol exposure varied across studies. Studies also varied on whether a carcinogen was co-administered to induce carcinogenesis. Of the 15 studies, 10 reported the use of a known carcinogen to induce cancer:

- dimethylene (a) anthracene [DMBA] (five studies)
- N-methyl-N-nitrosurea [MNU] (two studies)
- N-nitrosodimethylamine [NMDA] and 4-methylnitrosoamino-1-3-pyridyl-1-butanone [NNK] (one study)
- MADB106 [one study]
- bittner virus [one study].

Table C-2 in Appendix C provides a summary of mechanisms examined, amount and duration of ethanol or acetaldehyde exposure, carcinogen use, study results, and authors' conclusions.

Outcomes measured varied across studies. Overall, six studies reported increased cancer formation (four studies co-administered a carcinogen^{92,93,103} and two studies did not^{91,97}). The reported results of intermediate outcomes included:

- biotransformation of ethanol to acetaldehyde⁹⁵
- increase in the formation of DNA adducts⁹⁶
- increase in terminal-end bud density and a decrease in alveolar bud structures^{94,104,105}
- a reduction in blood natural killer cytotoxicity⁹⁹

Three studies reported no changes in outcomes and concluded that their findings did not support a link between alcohol consumption and the risk of breast cancer.¹⁰⁰⁻¹⁰²

Cell Line Studies

We included 15 studies using cell lines to evaluate possible mechanisms connecting alcohol consumption with breast cancer risk (see Table C-3 in Appendix C). Cell lines examined in the studies included:

- MCF-7 (six studies)
- MCF-10F (two studies)
- T4TD (one study)
- MM46 tumor cells (one study)
- MDA-MB-453 (one study)
- MCF-7 + T47D (one study)
- MCF-7 + T84 (one study)
- MCF-7 + T47D + MDA-MB-231 (one study)
- MCF-7 + ZR75.1 + BT-20 + MDA-MB-231 (one study).

Various types of mechanism were reported by these studies:

- hormonal-related^{65,67-69,106}
- DNA adduct formation^{107,108}
- effect on cell proliferation^{51,109,110}
- increase cAMP¹¹¹
- change in potassium channels¹¹²
- mammary gland mucin upregulation¹¹³
- smooth muscle up-regulation during transcription¹¹⁴

Amount and duration of ethanol and/or acetaldehyde exposure varied across all studies. Ten studies administered ethanol alone, and two studies administered ethanol combined with acetaldehyde. Table C-3 in Appendix C provides a summary of mechanisms examined, amount and duration of ethanol or acetaldehyde exposure, study results, and authors' conclusions.

Five studies reported an increase in the expression of mRNA,^{68,106,113,115} two studies reported an increase in the formation of DNA adducts,^{107,108} two studies reported an increase in cell proliferation,^{65,69} two studies reported enhancement of ³H-thymidine uptake,^{51,110} one study reported up-regulation of smooth muscle myosin alkali light chain,¹¹⁴ and one study¹⁰⁹ reported reduction in the expression ribosomal protein L7a.

Evidence Base for Describing Possible Mechanisms Connecting Alcohol Consumption and Colorectal Cancer Risk

Human Studies

We included one study (see Table C-4 in Appendix C) using human tissues to evaluate the possible mechanism connecting alcohol consumption with colorectal cancer risk. The study exposed colonic mucosa to acetaldehyde vapor.⁸³ Although no direct evidence to show a connection between acetaldehyde exposure and cancer risk was reported, the authors concluded that acetaldehyde may cause an increase in risk of colon cancer via loss of cell-cell adhesion.⁸³ Table C-4 in Appendix C provides a summary of study design, mechanisms examined, amount and duration of acetaldehyde exposure, study results, and authors' conclusions.

Animal Studies

We included 19 studies using animal models to evaluate the possible mechanisms for alcohol consumption and colorectal cancer risk (see Table C-5 in Appendix C). Of the 19 included studies, 17 reported on the mechanism(s) examined and two^{116,117} did not. The mechanisms examined in the 17 studies included:

- cytochrome system expression^{118,119}
- generation of acetaldehyde^{70,120-123}
- DNA methylation¹²⁴
- cell proliferation¹²⁵⁻¹²⁷
- local mucosal effect^{128,129}
- effect on various phases of carcinogenesis^{73,130,131}

Administration and duration of ethanol exposure varied across all studies. Studies also varied on whether a carcinogen was co-administered. Of the 19 studies, 12 reported the use of a known carcinogen to induce cancer:

- 1,1-dimethylhydrazine (DMH) (six studies)
- methylazoxymethanol (MAM) acetate (one study)
- acetoxymethyl-methylnitrosamine (AMMN) (one study)
- AMMN + cyanamide (CY) (one study)
- azoxymethane (AOM) (three studies).

Table C-5 in Appendix C provides a summary of mechanisms examined, amount and duration of ethanol or acetaldehyde exposure, carcinogen use, study results, and authors' conclusions.

Outcomes measured varied across studies. Among the studies that co-administered a carcinogen, six^{73,123,126,128,129,132} reported increased cancer formation, one¹³¹ reported suppression

of cancer formation, and two reported no effect.^{116,117} Another study that did not co-administer a carcinogen reported an increase in cancer formation.¹²¹ The reported results of intermediate outcomes include:

- increase in the number of aberrant crypt foci^{118,122,125,127}
- increase in microsomal ethanol-oxidizing system activity¹²⁰
- increase in acetaldehyde level resulting in folate degradation⁷⁰
- undermethylation of DNA¹²⁴
- increase in the expression of CYP2E1¹¹⁹
- decrease in the formation of DNA adducts¹³⁰

Cell Line Studies

We included 10 studies using cell lines to evaluate possible mechanisms connecting alcohol consumption with colorectal cancer risk (see Table C-6 in Appendix C). Cell lines examined in the studies included:

- Caco-2 (six studies)
- HT-29 (one study)
- colonic mucosa cells (one study)
- Caco-2 + HT-29 (one study)
- HT-29 + SW-1116 + HCT-15 (one study).

Various mechanisms were reported by these studies:

- folate uptake modulation¹³³
- tumor necrosis factor modulation^{75,133}
- inflammation and cell death¹³⁴
- formation of crosslinks with DNA¹³⁵
- initiation of cancer^{136,137}
- cell differentiation¹³⁸
- modulation of gene expression¹³⁹

Amount and duration of ethanol and/or acetaldehyde varied across all studies (seven studies administered ethanol alone, three studies administered ethanol combined with acetaldehyde). Table C-6 in Appendix C provides a summary of mechanisms examined, amount and duration of ethanol or acetaldehyde exposure, study results, and authors' conclusions.

Outcomes varied across all studies. Reported results included:

- inhibitory effect on both 3H-folic and 3H-methotrexate uptake¹⁴⁰
- increase in tumor necrosis factor-alpha receptor-1⁷⁵
- inflammation resulting in increased phosphatidylserine production¹³⁴
- increase in mRNA expression⁷⁴

- dual effect on cell proliferation (acute acetaldehyde exposure inhibitory and chronic acetaldehyde exposure stimulating)¹³⁶
- increase in sucrase and maltase activity^{137,138}
- increase in alkaline phosphatase and sucrose activities, limited cytotoxicity¹³³
- damage to DNA strands¹³⁵
- lack of effect on the expression of HLA class 1 antigens¹³⁹

Combination Study (Animal, Cell Line)

We included one study¹⁴¹ that used a combination of animal (mice) and cell line (Caco-2) to evaluate the possible mechanisms connecting alcohol consumption with colorectal cancer risk (see Table C-7 in Appendix C). Intestinal cell proliferation as a result of phosphatidylethanol accumulation was the examined mechanism. The animal study administered ethanol and the cell line study administered either ethanol or acetaldehyde. Outcome reported was disruption of cellular signals. Chronic alcohol exposure resulted in an increase of maximal intestinal density.¹⁴¹

Systematic Reviews and Narrative Reviews of Epidemiology Studies

We identified and summarized the reported results and conclusions from 13 systematic reviews of epidemiology studies looking for an association between alcohol intake and cancer risk (seven on breast cancer [see Table 1], six on colorectal cancer [see Table 2]). While these studies were not considered part of our primary evidence base addressing the key questions of this report, they do provide important evidence connecting alcohol intake with breast and colorectal cancer risk in humans and provide a context for discussing the findings of the studies included in our primary evidence base. The tables provide the review objectives, the resources searched, inclusion criteria, a summary of results, and the authors' conclusions. Key areas examined by the systematic reviews of breast cancer included:

- alterations in estrogen-dependent pathways
- polymorphisms in one-carbon metabolism pathways
- interaction with dietary folate intake
- dose-response relationships between alcohol intake and cancer risk.

Key areas examined by the systematic reviews of colorectal cancer include differences in Japanese versus western populations, and amount of alcohol intake and cancer risk.

Table 1. Systematic reviews/meta-analyses for breast cancer epidemiology studies

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Suzuki et al. 2008 ¹⁴²	To quantitatively assess the accumulated evidence on the association between alcohol intake and the risk of estrogen receptor (ER) and progesterone receptor (PR)– defined breast cancer subtypes and to evaluate whether the observed association differs across ER/PR status.	Eligible studies were identified by searching the MEDLINE database from January 1, 1970 through April 20, 2007 for relevant epidemiology studies of alcohol consumption in relation to the risk of breast cancer defined by ER/PR without any language restriction. <u>Evidence base:</u> Nineteen studies (4 prospective cohort studies and 16 case-control studies)	The risk of developing breast cancer was statistically significant comparing the highest vs. lowest consumption categories for developing: ER+ tumors 27% (1.17-1.38), all ER- tumors 14% (1.03-1.26), ER+PR+ tumors 22% (1.11-1.34), ER+PR- tumors 28% (1.07-1.53), but not ER-PR- tumors. An increase in alcohol consumption of 10 g of ethanol per day was associated with statistically significant increased risks for: all ER+ 12% (8%-15%), all ER- 7% (0%-14%), ER+PR+ 11% (7%-14%) and ER+PR- 15% (2%-30%), but not ER-PR-.	Estrogen-dependent pathway alone cannot account for the detected positive associations with alcohol for ER+PR+ and ER-PR+ tumors.
Lissowska et al. 2007 ¹⁴³	To examine the role of genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk.	Epidemiology studies of methylenetetrahydrofolate gene (MTHFR A222V and E429A) polymorphisms and breast cancer risk published through August 2006 were identified through a PubMed search.	There was no significant association of breast cancer risk with nutrients involved in one carbon metabolism (i.e., folate, vitamins B2, B6, B12, methionine) or with alcohol intake.	Study did not support association between polymorphisms in the one-carbon metabolism pathway and the risk of breast cancer.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Lewis et al. 2006 ¹⁴⁴	To summarize the available evidence from observational studies on this issue and a meta-analysis of the association between a common polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene.	MEDLINE and ISI Web of knowledge databases for relevant studies that were published through May 31, 2006. <u>Evidence base:</u> 19 studies (13 case-control studies and 9 cohort studies) of which seven cohort studies and one case-control study examined the interaction between alcohol and folate intakes with respect to risk of breast cancer.	Only two studies used the same cut off points for alcohol intake. Therefore, evidence for interaction between alcohol and folate intakes with respect to risk of breast cancer was inconclusive.	There is no association between a lack of dietary folate intake and breast cancer risk.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Key et al. 2006 ⁵²	To give an up-to-date assessment of the association of alcohol with female breast cancer, addressing methodological issues and shortfalls in previous overviews.	MEDLINE, EMBASE, Pascal (BIDS), Science Citation Index (BIDS), Social Sciences Citation Index (BIDS), Index to Scientific and Technical Proceedings (via BIDS), Biological Abstracts (BIOSIS), Biological Sciences, AIDS and Cancer Research Abstracts, Biology Digest, Conference Papers Index, Cochrane Library, NHS National Research Register (NRR), SIGLE (System for Information on Grey Literature), NTIS (National Technical Information Service), TOXLINE. <u>Evidence base:</u> 98 studies (75,728 drinkers vs. 60,653 non-drinkers)	Excess risk associated with alcohol consumption was 22% (9%-37%). Each additional 10 g ethanol per day increases breast cancer risk by 10% (5%-15%). Estimated population attributable risk in the U.S.A. and U.K. were, 1.6% and 6.0%, respectively.	Association between alcohol and breast cancer may be causal.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Hamajima et al. 2002 ³⁴	A collaborative reanalysis of individual data from 53 epidemiology studies, including 58,515 women with breast cancer and 95,067 women without the disease.	Resources searched were not reported by study authors. <u>Evidence base:</u> 53 studies (51 published, 2 unpublished)	The average consumption of alcohol reported by controls from developed countries was 6.0 g per day, i.e., about half a unit/drink of alcohol per day; greater in ever-smokers than never-smokers, (8.4 g per day and 5.0 g per day, respectively). Compared with women who reported no alcohol, relative risk (RR) of breast cancer was 1.32 (1.19-1.45, $p < 0.00001$) for an intake of 35-44 g per day alcohol, and 1.46 (1.33-1.61, $p < 0.00001$) for ≥ 45 g per day alcohol. For each additional 10 g per day intake of alcohol, the relative risk of breast cancer increased by 7.1% (5.5%-8.7%, $p < 0.00001$).	Caution is needed to interpret the effect of alcohol on risk of breast cancer.
Corrao et al. 1999 ¹⁴⁵	To compare the strength of the evidence provided by the epidemiology literature on the association between alcohol consumption and the risk of six cancers (oral cavity, esophagus, colorectum, liver, larynx, breast).	MEDLINE from 1966 up to and including 1998, articles reported by other bibliographic databases available at the University of Miami (<i>Current Contents</i> from 1996, EMBASE from 1980, CAB abstracts from 1973, and <i>Core Biomedical Collection</i> from 1993). <u>Evidence base:</u> 200 epidemiology studies (29 breast).	RR for dose of alcohol intake for breast cancer in the Mediterranean region* were: 1.6 (1.6-1.7) for 25 g per day, 2.7 (2.4-2.9) for 50 g per day, and 7.1 (5.8-18.6) for 100 g per day. RR for dose of alcohol intake in other areas* were: 1.2 (1.1-1.3) for 25 g per day, 1.5 (1.2-1.8) for 50 g per day, and 2.1 (1.4-3.1) for 100 g per day. *strata by region	Based on weak dose-response relationship, there is need for well-conducted epidemiology studies.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Smith-Warner et al. 1998 ³⁹	To assess the risk of invasive breast cancer associated with total and beverage-specific alcohol consumption and to evaluate whether dietary and nondietary factors modify the association.	Resources searched were not reported by study authors. <u>Evidence base:</u> 6 prospective studies that had at least 200 incident breast cancer cases, assessed long-term intake of food and nutrients, and used a validated diet assessment instrument.	For alcohol intake less than 60 g per day breast cancer risk increased linearly with increasing intake. Pooled multivariate RR for an increment of 10 g per day of alcohol (about 0.75-1 drink) was 1.09 (1.04-1.13). Multivariate-adjusted RR for total alcohol intake of 30 to <60 g per day (about 2-5 drinks) vs. nondrinkers was 1.41 (1.18-1.69). Limited data suggested that alcohol intake of at least 60 g per day were not associated with further increased risk. The specific type of alcoholic beverage did not strongly influence risk estimates. The association between alcohol intake and breast cancer was not modified by other factors.	Alcohol consumption is associated with a linear increase in breast cancer incidence in women over the range of consumption reported by most women. Among women who consume alcohol regularly, reducing alcohol consumption is a potential means to reduce breast cancer risk.
Longnecker 1993 ¹⁴⁶	To evaluate the association between alcohol consumption and risk of breast cancer.	MEDLINE from 1996 through September 1992, all abstracts presented at the society for Epidemiology Research from 1989-1994. <u>Evidence base:</u> 38 epidemiology studies	RR of breast cancer following daily alcohol consumption were 1.11 (1.07-1.16) for one drink, 1.24 (1.15-1.34) for two drinks, and 1.38 (1.23-1.55) for three drinks.	Causal role of alcohol remains uncertain.

Table 2. Systematic reviews/meta-analyses for colorectal cancer epidemiology studies

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Mizoue et al. 2008 ¹⁴⁷	To examine the association between alcohol consumption and colorectal cancer in Japanese.	Population-based cohort studies that were conducted in Japan, started between the mid-1980s and the mid-1990s, included more than 30,000 participants, obtained information on diet, including alcohol intake, using a validated questionnaire or a similar one at baseline, and collected incidence data for colorectal cancer during the follow-up period. <u>Evidence base (5 cohort studies):</u> • The Japan Public Health Center-based Prospective Study (JPHC) • The Japan Collaborative Cohort Study (JACC) • The Miyagi Cohort Study • The Takayama Study ○ According to the authors, the JPHC was treated as two independent studies (JPHC I and JPHC II) because of a difference in the dietary questionnaires used; thus, data from a total of five studies were analyzed.	In men, multivariate-adjusted pooled hazard ratios for alcohol intake of 23-45.9 g per day, 46-68.9 g per day, 69-91.9 g per day, and >92 g per day, compared with nondrinkers, were 1.42 (1.21-1.66), 1.95 (1.53-2.49), 2.15 (1.74-2.64), and 2.96 (2.27-3.86), respectively (p for trend <0.001). The association was evident for both the colon and the rectum. A significant positive association was also observed in women. Twenty-five percent of colorectal cancer cases were attributable to an alcohol consumption of >23 g per day.	When compared to Western populations, alcohol-colorectal cancer association seems to be more evident in Japanese.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Moskal et al. 2007 ¹⁴⁸	To examine if current alcohol intake is associated with risk of colon and rectal cancer by summarizing the results of published prospective cohort studies with meta-analytic techniques.	Prospective cohort studies in MEDLINE published in English between 1990 and June 2005; (iii) referenced in MEDLINE. Since studies on specific types of alcohol (beer, wine, and liquor) were limited, the authors restricted the meta-analyses to total alcohol consumption on colorectal cancer risk. Studies in particular populations (i.e., cohorts of alcoholics or brewery workers) were not included. <u>Evidence base:</u> Sixteen prospective cohort studies	High alcohol intake was significantly associated with increased risk of colon 1.50 (1.25-1.79) and rectal cancer 1.63 (1.35-1.97). This was comparable to a 15% increase of risk of colon or rectal cancer for an increase of 100 g of alcohol intake per week. The association did not change significantly by anatomical site (colon, rectum).	Lifestyle recommendations for prevention of colorectal cancer should consider limiting alcohol intake.
Mizoue et al. 2006 ¹⁴⁹	To review epidemiology findings regarding the association between alcohol drinking and colorectal cancer among the Japanese population.	MEDLINE from 1965 to 2005 <u>Inclusion criteria:</u> Epidemiology studies on the association between alcohol drinking and colorectal cancer incidence or mortality among Japanese. <u>Evidence base:</u> Eighteen studies (5 cohort studies and 13 case-control studies).	A moderate or strong positive association was observed between alcohol drinking and colon cancer risk in all large-scale cohort studies, with some showing a dose-response relationship, and among several case-control studies. A positive association with rectal cancer was also reported, but it was less consistent, and the magnitude of the association was generally weaker compared with colon cancer. The RR of colon or colorectal cancer increased even among moderate drinkers consuming <46 g of alcohol per day, levels at which no material increase in the risk was observed in a pooled analysis of Western studies.	Among the Japanese population, alcohol consumption perhaps may increase the risk of colorectal cancer. Association with colon cancer is probable, and that for rectal cancer is possible.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Cho et al. 2004 ³⁶	To examine the relationship of total alcohol intake and intake from specific beverages to the incidence of colorectal cancer and to evaluate whether other potential risk factors modify the association.	The authors reported a pooled analysis of primary data from 8 cohort studies in 5 countries.	Increased risk for colorectal cancer was limited to persons with an alcohol intake of 30 g/day or greater (approximately >2 drinks per day), a consumption level reported by 4% of women and 13% of men. Compared with nondrinkers, the pooled RR were 1.16 (0.99-1.36) for persons who consumed 30 to <45 g per day and 1.41 (1.16-1.72) for those who consumed ≥45 g per day (<i>p</i> for trend <0.001). Evident for cancers of the proximal colon, distal colon, and rectum. No clear difference in relative risks was found among specific alcoholic beverage.	There was a correlation between a single determination of alcohol consumption and a modest relative elevation in the rate of colorectal cancer, mostly at the highest levels of consumption.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Corrao et al. 1999 ¹⁴⁵	To compare the strength of the evidence provided by the epidemiology literature on the association between alcohol consumption and the risk of six cancers (oral cavity, esophagus, colorectum, liver, larynx, breast).	MEDLINE from 1966 up to and including 1998, articles reported by other bibliographic databases available at the University of Miami (<i>Current Contents</i> from 1996, EMBASE from 1980, CAB abstracts from 1973, and <i>Core Biomedical Collection</i> from 1993). <u>Evidence base:</u> 200 epidemiology studies (16 colon [12 case-control, 4 cohort], 14 rectum [11 case-control, 3 cohort]).	<u>Colon studies</u>** RR for dose of alcohol intake in colon studies (case-control) were 1.0 (1.0-1.1) for 25 g per day, 1.1 (1.0-1.2) for 50 g per day, and 1.1 (1.0-1.3) for 100 g per day. RR for dose alcohol intake in colon studies (cohort studies) were 1.4 (1.1-1.7) for 25 g per day, 1.9 (1.3-2.9) for 50 g per day, and 3.6 (1.6-8.5) for 100 g per day. <i>**Reported results were stratified by study design</i> <u>Rectum studies</u>*** RR for dose of alcohol intake in rectum studies among men were 1.1 (1.0-1.2) for 25 g per day, 1.2 (1.1-1.5) for 50 g per day, and 1.5 (1.2-2.2) for 100 g per day. RR for dose of alcohol in rectum studies among women were 2.3 (1.3-4.0) for 25 g per day, 5.0 (1.6-16.4) for 50 g per day, and 25.7 (2.5-267.6) for 100 g per day. <i>***Reported results were stratified by gender</i>	Based on weak dose-response relationship, there is need for well-conducted epidemiology studies.

Study	Objective	Resources Searched and Inclusion Criteria	Results	Conclusions as Reported by Study Authors
Franceschi and La Vecchia 1994 ¹⁵⁰	To evaluate alcohol consumption and the risk of cancers of the stomach and colon-rectum.	<u>Evidence base:</u> 34 studies (15 cohort, 19 case-control).	Among the 15 <i>cohort studies</i>: seven studies were not very informative; and overall evidence from 8 studies showed colon cancer RR estimates varying within a narrow range of 1.0-1.7 [ranging between 1.1-1.3 in most studies], and rectal cancer 1.0-2.5 [ranging between 1.0-1.7 in most studies]. Among the 19 <i>case-control</i> studies: five studies were totally negative, and showed no evidence of association; 3 other studies showed overall significant associations; and remaining 11 studies showed no consistent overall association.	Epidemiology evidence regarding a causal role of alcoholic beverage consumption and colorectal carcinogenesis remains inconclusive.

Reported Mechanisms in the Epidemiology Literature

A search of the literature identified the following mechanisms reported in breast and colorectal cancer epidemiology studies that were not included in our primary evidence base (see Table 3 and Table 4, respectively). These studies investigated the association between alcohol consumption and increased cancer risk primarily by administering questionnaires to study dietary behavior and amount of alcohol consumption and correlated these findings with cancer incidence. Some of these studies looked at different alcoholic beverages, for example wine, beer, and other spirits. However, none of these studies controlled alcohol exposure.

Our searches of the literature identified hypothesis-generating studies that provide indirect evidence of potential mechanisms. These studies examined various metabolic pathways that have been proposed as potential connections between alcohol exposure and increased breast or colorectal cancer risk (see Table 5 and Table 6, respectively).

These hypothesis-generating studies and epidemiology studies were incorporated into this report in order to review and discuss this literature base in comparison with our primary evidence base.

Table 3. Breast cancer epidemiology studies

Proposed Mechanism	References
Changes in circulating hormone levels	37,151-158
DNA-adduct formation	159
Changes in levels of insulin-like growth factor	160
Changes in levels of biomarkers of inflammation	77-81
Cytochrome P450 polymorphism	161,162
Methylenetetrahydrofolate reductase polymorphism/Dietary/Vitamins	38,163-173
Alcohol dehydrogenase/Acetaldehyde dehydrogenase polymorphism	45,47-50,174-178
Other types of polymorphism	179-184

Table 4. Colorectal cancer epidemiology studies

Proposed Mechanism	References
DNA repair polymorphisms	90,166,185-234
Hyperproliferation of rectal mucosa	141,235
Colonic microbial metabolism resulting in the generation of acetaldehyde	55,56
Cytochrome P450 polymorphism	236-240
Alcohol dehydrogenase and acetaldehyde dehydrogenase polymorphism	19,241-252
Changes in levels of insulin-like growth factor	253-256
Impact of C-reactive protein and Inflammation	257,258
Methylenetetrahydrofolate reductase polymorphism/Dietary/Vitamins	189,259-263
Other types of polymorphism and mechanisms	259,264-294

Table 5. Hypothesis-generating breast cancer studies

Study	Reported Mechanism
Marietta et al. 2009 ²⁹⁵	Stimulation of Fanconi anemia–breast cancer associated (FANC–BRCA) DNA damage response network by acetaldehyde
Taibi et al. 2009 ²⁹⁶	Low levels of both xanthine dehydrogenase and cellular retinol binding protein
Jin et al. 2008 ²⁹⁷	Activation of BRCA2 transcription by estrogen receptor-beta
Maciel et al. 2004 ²⁹⁸	Inhibition of bioactivation of ethanol to acetaldehyde by folic acid
Jordao et al. 2004 ²⁹⁹	Increased lipid peroxidation
Stevens et al. 2000 ³⁰⁰	Change in estrogen levels
Colantoni et al. 2000 ³⁰¹	Increased levels of malondialdehyde
Jones et al. 1998 ³⁰²	Response of MCF-7 cells to potential estrogens and non-estrogenic substances

Table 6. Hypothesis-generating colorectal cancer studies

Study	Reported Mechanism
Jelski et al. 2004 ¹⁵	Alcohol dehydrogenase and aldehyde dehydrogenase polymorphisms
Vincon et al. 2003 ³⁰³	Generation of free radicals.
Leuratti et al. 2002 ³⁰⁴	DNA adduct formation
Parlesak et al. 2000 ³⁰⁵	Inhibition of retinol oxidation
Koivisto et al. 1996 ³⁰⁶	Alcohol dehydrogenase polymorphism
Jokelainen et al. 1996 ⁵⁷	Generation of acetaldehyde by human colonic bacteria
Seitz et al. 1996 ²¹	Alcohol dehydrogenase polymorphism
Nosova et al., 1996 ³⁰⁷	Generation of acetaldehyde by human colonic bacteria
Rosenberg et al. 1994 ³⁰⁸	Induction of cytochrome P450
Jokelainen et al. 1994 ³⁰⁹	Generation of acetaldehyde by human colonic bacteria
Shimizu et al. 1990 ³¹⁰	Induction of cytochrome P450

Ongoing Clinical Trials

A search of the clinicaltrials.gov (<http://clinicaltrials.gov/>) Web site did not identify any ongoing trials related alcohol consumption and possible causal mechanisms for breast and colorectal cancers.

Chapter 4. Discussion

Breast Cancer

Key Question 1. What are the likely causal mechanisms by which alcohol contributes to the development of breast cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in breast cancer development?

Alcohol-related Changes in Circulating Hormones

Changes in circulating hormone levels due to chronic alcohol intake have been demonstrated in several epidemiology studies (see Table 3). Our searches identified eight epidemiology studies that looked at this connection.^{37,151-157} Seven studies¹⁵¹⁻¹⁵⁷ made specific reference that moderate alcohol consumption may be responsible for increasing breast cancer risk by influencing hormonal levels and estrogen receptors and one study³⁷ reported light-to-moderate alcohol consumption was not associated with increase breast cancer risk. The findings from these seven studies suggest that alcohol interferes with estrogen pathways, thereby causing changes in hormonal levels and estrogen receptors. This may then have a direct effect on breast tissue and cancer risk. Given this apparent connection between alcohol intake and alterations in circulating hormones seen in the epidemiology literature, we looked for hypothesis-generating studies that examined this connection.

A majority of the human and animal studies identified in our searches and included in our primary evidence base also point to a connection between alcohol intake and changes in blood hormone levels, especially elevated levels of estrogen-related hormones in humans (see Table C-1 in Appendix C) and animals (see Table C-2 in Appendix C). Several cell line studies also suggest that estrogen receptor pathways may be altered by ethanol (see Table C-3 in Appendix C). Increased estrogen levels may increase the risk of breast cancer through increases in cell proliferation and alterations in estrogen receptors. Suzuki et al.¹⁴² looked at the possible connection between estrogen receptor (ER) alterations, alcohol intake, and the risk of breast cancer in a meta-analysis of epidemiology studies (see Table 1.). The highest versus the lowest alcohol consumption categories were analyzed for their association with all ER+ and ER- subtype tumors. Meta-analysis of all studies using relative risk (RR) indicated a statistically significant 27% higher risk of developing ER+ tumors (95% CI: 1.17 to 1.38) and a 14% higher risk for developing ER- tumors (95% CI: 1.03 to 1.26) in the high consumption group. The authors concluded that they had “found support for a positive relationship between alcohol consumption and the development of all ER+ tumors.” The authors also concluded that “The results from these meta-analyses suggest that the biological mechanism for development of breast cancer due to alcohol intake could be explained not only through ER-mediated classical estrogen-dependent pathway but also through other mechanisms” such as DNA damage or increased expression of other signaling pathways leading to cell proliferation. These studies (human, animal, and cell line) combine to suggest that estrogen-related mechanisms may be altered by alcohol consumption and provide a potential causal mechanism by which alcohol affects the estrogen receptors thereby contributing to the increased risk of development of breast cancer.

Elevation in prolactin levels was examined in one human study. Ginsburg et al.⁸⁷ reported that serum prolactin levels increased in menopausal women during acute ethanol ingestion. In animal studies, ethanol-induced hyperprolactinemia in mice was associated with the development of mammary tumors.^{97,98} While not as extensive as the estrogen-related studies, these studies give some indication that alcohol consumption may alter prolactin levels and increase the risk of developing breast cancer.

Cell Proliferation and Tumor Progression

Although we did not identify any epidemiology study that reported on hyperproliferation as a possible mechanism, enhancement of cell proliferation and tumor progression related to ethanol consumption and conversion to acetaldehyde and its connection to breast cancer has been examined in numerous animal (Table C-2 in Appendix C) and cell line studies (Table C-3 in Appendix C). Several of the animal studies used carcinogens such as MNU^{93,94} or DMBA.^{100,101,105} However, the DMBA studies were not as consistent in showing a relationship between ethanol and mammary tumorigenesis as the MNU studies (see Table C-2 in Appendix C). The effect of ethanol on cell proliferation in cell lines was examined in three studies included in this report. Izevbigie et al.⁵¹ reported that ethanol stimulated cell proliferation in the MCF-7 cell line, Zhu et al.¹⁰⁹ reported that ethanol induced changes that could promote cancer development in the T4TD cell line, and Przylipiak et al.¹¹⁰ reported that ethanol had direct growth stimulatory effects on the MCH cell line. Enhancement of cell proliferation and tumor progression as a potential causal mechanism linking ethanol and breast cancer has some support but human subject studies are needed to further explore this connection. According to Dumitrescu and Shields, estrogen-induced breast cancer may be as a result of cell proliferation, activation of CYP2E1, and DNA damage.¹⁰

Polymorphism in Ethanol Metabolism

Our searches identified a number of epidemiology studies proposing that both genetic and enzyme polymorphisms contribute to the promotion of breast cancer development in individuals who consume alcohol (see Table 3). Polymorphisms examined in these studies include cytochrome P450,^{161,162} methylenetetrahydrofolate reductase,^{38,163-173} and alcohol dehydrogenase and acetaldehyde dehydrogenase.^{45,47-50,174-178} The majority of these studies reported enzyme polymorphism as a risk marker for breast cancer following moderate alcohol consumption. Our searches did not identify any experimental studies in humans or animals that examined this issue.

DNA Adduct Formation

DNA adduct formation was examined in an epidemiology study by Rundle et al.¹⁵⁹ The authors investigated the association between alcohol consumption and DNA adduct levels in breast tissue in women diagnosed with ductal carcinoma *in situ* and invasive ductal or lobular cancer (i.e., cases) vs. women with benign conditions without atypia (i.e., controls). In tumor and nontumor tissue from cases, adduct levels were increased among drinkers compared to nondrinkers. However, among controls, no increase in adduct levels were found regardless of drinking status.¹⁵⁹

We identified no experimental human studies that examined this mechanism. We did identify experimental studies using animals that suggest intake of ethanol does increase adduct formation and could contribute to breast cancer risk.⁹⁶ Cell line studies also suggested that the formation of DNA adducts increases after incubation with ethanol.^{107,108}

Other Potential Mechanisms

A single human study by Hartman et al.⁷⁶ reported on increased level of biomarkers of oxidative stress such as α -tocopherol and isoprostane after alcohol consumption (see Table C-1 in Appendix C). Our searches identified five epidemiology studies⁷⁷⁻⁸¹ that also postulated a connection between biomarkers of inflammation, alcohol intake, and risk of breast cancer. Increased levels of biomarkers such as malondialdehyde,^{77,79} isoprostanes,⁸¹ and catalase activity^{78,80} were reported. We did not identify any experimental studies using animal or cell line models that examined other potential mechanisms.

Key Question 2. For the most likely mechanisms of action involving alcohol and the development of breast cancer, how might other factors modify the effect of alcohol on breast cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?

For this Key Question, we looked for studies that evaluated factors that modify the association of alcohol with biomarkers of risk of breast cancer. The human studies of alcohol consumption and hormone changes were performed in pre- and postmenopausal women but an actual age effect was not examined in these studies. The duration of consumption was relatively short; long term effects could not be calculated in these studies. However, we did identify one human study that examined biomarkers of oxidative stress and risk of carcinogenesis. Hartman et al. reported that in postmenopausal women who consumed 30 g alcohol per day, α -tocopherol decreased by 4.6% and isoprostane levels increased by 4.9%.⁷⁶ This study provides a possible link between oxidative stress and risk of breast cancer formation.

Table 7 and Table 8 contain an overview of the breast cancer studies included in this report in terms of study design and reporting issues that determined whether the study provides evidence of a direct or an indirect association between alcohol consumption and breast cancer. Route of administration, rate of absorption and metabolism, formulation and quantity of ethanol, and timing of the intervention, however, may reduce the generalizability of animal studies to a clinical setting. Although we evaluated cell line studies as part of our overall evidence evaluation, we did not include them in this table given that events such as confounding exposure, control for other risk factors, and cancer formation are not applicable to this model.

Table 7. Overall results from human breast cancer studies

Study	*Confounding Exposure	Cancer Formation	Surrogate Outcome Measure	Authors Reported on Causal Mechanism	Number of Links in the Pathway of Carcinogenesis
Hartman et al. 2005 ⁷⁶	N	N	Y	Y	1
Dorgan et al. 2001 ⁸⁵ Same as ³¹¹	N	N	Y	Y	1
Ginsburg et al. 1996 ⁸⁸	N	N	Y	Y	1
Ginsburg et al. 1995 ⁸⁷	N	N	Y	Y	1
Reichman et al. 1993 ⁸⁶	N	N	Y	Y	1

*Confounding exposure: did study administer a carcinogen and /or acetaldehyde?

Y: there was confounding exposure

N: there was no confounding exposure

Table 8. Overall results from animal breast cancer studies

Study	*Confounding Exposure	Cancer Formation	Surrogate Outcome Measure	Authors Reported on Causal Mechanism	Number of Links in the Pathway of Carcinogenesis
Hilakivi-Clarke et al. 2004 ⁹²	Y	Y	N	Y	1
Castro et al. 2003 ⁹⁵	N	N	Y	Y	1
Chhabra et al. 2000 ⁹⁶	Y	N	Y	Y	1
Watabiki et al. 2000 ⁹⁷	N	N	Y	Y	1
Holmberg et al. 1995 ⁹¹	N	Y	N	N	0
Singletary et al. 1995 ⁹³	Y	N	Y	N	0
Singletary and McNary 1994 ⁹⁴	Y	N	Y	Y	1
Taylor et al. 1993 ⁹⁹	Y	N	Y	Y	1
McDermott et al. 1992 ¹⁰¹	Y	Y	N	N	0
Hackney et al. 1992 ¹⁰²	N	Y	N	N	0
Singletary and McNary 1994 ¹⁰⁴	N	N	Y	Y	1
Singletary et al. 1991 ¹⁰⁵	Y	N	Y	Y	1
Rogers and Conner 1990 ¹⁰⁰	Y	Y	N	N	0
Grubbs et al. 1988 ¹⁰³	Y	Y	N	N	1
Schrauzer et al. 1979 ⁹⁸	Y	N	Y	Y	1

*Confounding exposure: did study administer a carcinogen and /or acetaldehyde?

Y: there was confounding exposure

N: there was no confounding exposure

Colorectal Cancer

Key Question 3. What are the likely causal mechanisms by which alcohol contributes to the development of colorectal cancer? Which of the possible mechanisms (e.g., induction of P450 cytochromes and carcinogen metabolism, effects on blood hormone concentrations, effect of acetaldehyde or other alcohol metabolite on apoptosis and DNA repair, interactive effects on other nutritional factors, or others) are likely to be most important in colorectal cancer development?

Acetaldehyde production in the colon. Exposure of colon mucosa to acetaldehyde from microbial metabolism of ethanol has been postulated as a mechanism for increasing the risk of developing colorectal cancer in two epidemiology studies (see Table 4)^{55,56} and three experimental studies (see Table 5).^{57,307,309} According to study authors, individual variations in human colonic flora may contribute to the risk of alcohol-related colorectal cancer,⁵⁵ and increased activity of intracolonic bacterial alcohol dehydrogenase may also play a role in increasing cancer risk.^{56,57,307,309}

Experimental human studies examining this subject are few (see Table C-4 in Appendix C). A study by Basuroy et al.⁸³ suggests that acetaldehyde disrupts epithelial tight junction and cell adhesion and through this mechanism increases the risk of colon cancer. Several animal studies also looked at the effects of acetaldehyde in the colon (see Table C-5 in Appendix C). These studies showed mucosal damage after ethanol consumption,¹²⁰ increased degradation of folate,⁷⁰ stimulation of rectal carcinogenesis,¹²² and an increased effect of carcinogens in the presence of acetaldehyde.¹²³ In cell line studies acetaldehyde exposure was reported to influence the initial steps of colonic carcinogenesis and later tumor development¹³⁶ and decrease the activity of some brush border enzymes.¹³⁷ Finally, a study using animal and cell line tissue found evidence that acetaldehyde stimulates cell proliferation in animal intestinal crypt cells and therefore acetaldehyde may act as a cocarcinogen in the colon.¹⁴¹ These studies suggest that acetaldehyde production in the colon may provide a potential causal mechanism by which alcohol contributes to the development of colon cancer.

Cell proliferation. Hyperproliferation of rectal mucosa after exposure to alcohol was postulated as a mechanism for increasing the risk of developing colorectal cancer in an epidemiology study by Simanowski et al.²³⁵ The authors examined rectal biopsies for proliferation markers such as histone H3 and Ki67 in 44 heavy drinkers and 26 controls. Heavy drinkers showed an increase in cell proliferation markers in the rectal mucosa compared to controls.²³⁵

An effect of ethanol consumption on cell proliferation in the colon was investigated in both animal and cell line studies in our primary evidence base. Several animal studies reported enhanced growth of mucosal tissue after chronic ethanol consumption.¹²⁵⁻¹²⁷ Cell studies indicated that exposure to ethanol and acetaldehyde increases cell proliferation^{74,136} and damages DNA which may contribute to cancer development.¹³⁵ Together these studies suggest that ethanol and acetaldehyde exposure in the colorectal mucosa may increase cell proliferation and be a potential mechanism connecting alcohol consumption to colorectal cancer risk.

DNA repair polymorphism. We identified 52 epidemiology studies that assessed DNA repair polymorphism and alcohol consumption. The majority of these studies suggested that DNA repair polymorphism may influence the risk of colorectal cancer.

Enzyme polymorphism. We identified 19 studies that assessed enzyme polymorphism in epidemiology studies: 13 examined alcohol and acetaldehyde dehydrogenase polymorphism;^{19,241-252} five examined cytochrome P450 polymorphism;²³⁶⁻²⁴⁰ and six examined methylenetetrahydrofolate reductase polymorphism.^{189,259-263} The majority of these studies reported enzyme polymorphism as a risk marker for colorectal cancer following moderate alcohol consumption.

Other potential mechanisms. Ethanol may also influence carcinogenesis in the colon and rectum through an interaction with carcinogens. Animal studies suggest that ethanol exposure in the colon increases the chances of tumor development,¹³² but other studies found no association between ethanol ingestion and colorectal carcinogenesis or instead reported inhibition of

tumorigenesis.^{73,130,131} Other possible mechanisms reported in animal studies include alcohol's inhibition of folate metabolism⁷⁰ and DNA hypomethylation.¹²⁴

Key Question 4. For the most likely mechanisms of action involving alcohol and the development of colorectal cancer, how might other factors modify the effect of alcohol on colorectal cancer (for example, age, latency of effect, intensity, duration, and recency of exposure, presence of co-carcinogens, presence of threshold effect)? Do the causal mechanisms vary by cell type or other tumor characteristics?

For this Key Question, we looked for studies that evaluated factors that modify the association of alcohol with biomarkers of colorectal cancer risk. Few studies are available that examined factors that modify the effects of ethanol consumption on the risk of developing colorectal cancer. The study in human subjects in which biopsy samples were examined for damage after exposure to acetaldehyde did not report the influence of personal factors on the degree of damage generated.⁸³

Table 9 and Table 10 contain an overview of the colorectal cancer studies included in this report in terms of study design and reporting issues that determined whether the study provides evidence of a direct or an indirect association between alcohol consumption and colorectal cancer. Route of administration, rate of absorption and metabolism, formulation and quantity of ethanol, and timing of the intervention however may reduce the generalizability of animal studies to a clinical setting. Although we evaluated cell line studies as part of our overall evidence evaluation, we did not include them in this table given that events such as confounding exposure, control for other risk factors, and cancer formation are not applicable to this model.

Table 9. Overall results from human colorectal cancer study

Study	*Confounding Exposure	Cancer Formation	Surrogate Outcome Measure	Authors Reported on Causal Mechanism	Number of Links in the Pathway of Carcinogenesis
Basuroy et al. 2005 ⁸³	Y	N	Y	Y	1

*Confounding exposure: did study administer a carcinogen and/or acetaldehyde?

Y: there was confounding exposure

N: there was no confounding exposure

Table 10. Overall results from animal colorectal cancer studies

Study	*Confounding Exposure	Cancer Formation	Surrogate Outcome Measure	Authors Reported on Causal Mechanism	Number of Links in the Pathway of Carcinogenesis
Hayashi et al. 2007 ¹¹⁸	Y	N	Y	Y	1
Perez-Holanda et al. 2005 ⁷³	Y	N	N	N	0
Pronko et al. 2002 ¹²⁰	N	N	Y	Y	1
Roy et al. 2002 ¹²¹	N	N	Y	N	1
Homann et al. 2000 ⁷⁰	N	N	Y	N	1
Choi et al. 1999 ¹²⁴	N	N	Y	Y	1
Hakkak et al. 1996 ¹¹⁹	N	N	Y	Y	1
Simanowski et al. 1994 ¹²⁵	N	N	Y	Y	1
Niwa et al. 1991 ¹²⁶	Y	N	Y	Y	1
Seitz et al. 1990 ¹²²	Y	N	Y	Y	1
McGarrity et al. 1988 ¹²⁹	Y	N	Y	Y	1
Hamilton et al. 1988 ¹³⁰	Y	N	Y	Y	1
Garzon et al. 1987 ¹²⁸	Y	Y	N	Y	1
Hamilton et al. 1987 ¹³²	Y	N	Y	Y	1
Hamilton et al. 1987 ¹³¹	Y	N	Y	Y	1
Simanowski et al. 1986 ¹²⁷	N	N	Y	Y	1
Nelson et al. 1985 ¹¹⁶	Y	N	N	N	0
Seitz et al. 1985 ¹²³	Y	N	Y	Y	1
Howarth et al. 1984 ¹¹⁷	Y	N	N	N	0

*Confounding exposure: did study administer a carcinogen and/or acetaldehyde?

Y: there was confounding exposure

N: there was no confounding exposure

Excluded Studies

Because this is a systematic review using specific inclusion and exclusion criteria with the creation of specific Key Questions, the report is directed at evidence that addresses each Key Question. None of the excluded studies (see Table D-1 in Appendix D) were left out for quality, design, conduct, integrity, or inaccuracy but rather because they did not address these Key Questions.

Future Research Goals

Our examination of the epidemiology literature correlating alcohol consumption with cancer risk has suggested many areas in which experimental research may provide insight into the actual mechanisms connecting cancer risk and alcohol consumption. For breast cancer these potential mechanisms are changes in circulating hormone levels and changes in hormone receptors, DNA-adduct formation, and various enzyme polymorphisms related to alcohol metabolism. For colorectal cancer these areas are DNA repair polymorphisms, mucosal cell proliferation, and various enzyme polymorphisms related to alcohol metabolism. Experimental studies in humans, animals, or cell lines have provided basic information on some but not all of these potential mechanisms.

The connection between alcohol intake and changes in estrogen levels and breast cancer risk has been studied in human, animal, and cell line studies. Future research in this area would seem to be warranted to determine the exact level of risk imposed by this pathway. A connection between cell proliferation and tumor progression in breast cancer has been suggested by animal studies but not in human studies and human-based studies in this area would seem to be warranted. Enzyme polymorphism in ethanol metabolism as well as in other metabolic pathways that may be influenced by alcohol may require more human-based studies as opposed to animal studies where polymorphism is not a factor. DNA adduct formation has not been well studied in human or animal studies and research in this area should be expanded. Oxidative stress and inflammation associated with alcohol consumption have been postulated as risk factors in epidemiology studies but not studied to any extent in hypothesis-generating studies. Oxidative stress and inflammation should be examined with better experimentally controlled studies.

Experimental human studies examining the connection between alcohol intake and colorectal cancer are few. Many potential mechanisms related to acetaldehyde production in the colon, cell proliferation due to ethanol or acetaldehyde exposure, alterations in DNA repair mechanisms, and the influence of carcinogens and alcohol in the colon need to be examined in human-based studies. Animal studies are also needed to examine the influence of bacterial flora, the effects of ethanol and acetaldehyde on the colon, especially changes in cell proliferation and DNA, and the interaction between carcinogens and ethanol and acetaldehyde.

Conclusions

Based on our systematic review of the literature, many potential mechanisms by which alcohol may influence the development of breast or colorectal cancers have been explored but the exact connection or connections remain unclear. The evidence points in several directions but the importance of any one mechanism is not apparent at this time.

Table 11 through Table 13 summarizes the mechanisms on alcohol consumption and the risk of breast cancer as presented in studies identified in this report. Six human, five animal and five cell line studies reported on changes in hormonal levels as the potential causal mechanism

by which alcohol consumption may contribute to the development of breast cancer. Our findings are comparable to the most commonly reported mechanisms in most of the breast cancer epidemiology studies summarized in Table 3.

Table 11. Reported mechanisms in human breast cancer studies

Mechanism Reported by Study Authors	Number of Studies	References
Change in levels of estrogen, progesterone, and DHEA	2	85,311
Change in level of estrogen	1	88
Elevation of prolactin	1	87
Elevation of estrogens and DHEA	1	86

Table 12. Reported mechanisms in animal breast cancer studies

Mechanism Reported by Study Authors	Number of Studies	References
Change in level of estrogen	3	92-94
Biotransformation of ethanol to acetaldehyde	1	95
Formation of DNA adducts	1	96
Elevation of prolactin	2	97,98
Effects on DNA synthesis	2	104,105
Suppression of cellular immunity	1	99

Table 13. Reported mechanisms in cell line breast cancer studies

Mechanism Reported by Study Authors	Number of Studies	References
Effect on estrogen receptor- α expression	5	65,67-69,106
Effect on peroxisome proliferator-activated receptor (PPAR)- α and PPAR- β transactivation	1	115
Formation of DNA adducts	2	107,108
Disruption and modulation of cell proliferation	2	51,109
Upregulation of transcription of smooth muscle myosin alkali light chain	1	114
Upregulation of mammary gland mucin	1	113
Direct growth stimulatory effect by enhancement on 3H-thymidine	1	110
Change in potassium channels	1	112
Increase cAMP levels	1	111

Table 14 through Table 17 summarizes the mechanisms of alcohol consumption and the risk of colorectal cancer as presented in studies identified in this report. One human study exposed colonic mucosa biopsies to vapor-phase acetaldehyde and reported an effect of acetaldehyde on cell adhesion as the most likely causal mechanisms by which alcohol consumption may contribute to the development of colorectal cancer. In contrast, nine animal studies reported a local toxic effect of acetaldehyde resulting in mucosal damage as the most likely causal mechanism by which alcohol consumption may contribute to development of colorectal cancer. Other mechanisms identified in this report include:

- increase in cytochrome P4502E1 expression (two animal studies)
- effect on DNA synthesis and methylation (two animal studies, two cell line studies)
- effect on cell proliferation (two cell line studies)
- apoptotic cell death (three cell line studies)
- effect on various stages of carcinogenesis (two animal studies)
- changes in polyamine content (one animal study)
- effect of acetaldehyde on brush border enzymes (one cell line study)
- modulation of gene expression (one cell line study).

Our findings are comparable to some of the most common mechanisms (e.g., colonic microbial production of acetaldehyde, effect on DNA methylation and synthesis) reported by the colorectal cancer epidemiology studies summarized in Table 4.

Table 14. Reported mechanisms in human colorectal cancer study

Mechanism Reported by Study Authors	Number of Studies	References
Effect of acetaldehyde on cell to cell adhesion	1	83

Table 15. Reported mechanisms in animal colorectal cancer studies

Mechanism Reported by Study Authors	Number of Studies	References
Local toxic effect of acetaldehyde resulting in mucosal damage and cell proliferation	9	70,120-123,125-128
Increase cytochrome P4502E1 expression	2	118,119
DNA methylation and synthesis	2	132,139
Effect on various stages of carcinogenesis	2	130,131
Changes in polyamine content	1	129

Table 16. Reported mechanisms in cell line colorectal cancer studies

Mechanism Reported by Study Authors	Number of Studies	References
Effect on DNA methylation and synthesis	2	134,140
Apoptotic cell death	3	75,133,134
Effect on cell proliferation	3	74,136,138
Effect of acetaldehyde on brush border enzyme	1	137
Modulation of gene expression	1	139

Table 17. Reported mechanisms in combination (animal, cell lines) colorectal cancer study

Mechanism Reported by Study Authors	Number of Studies	References
Effect on cell proliferation	1	141

Limitations

The evidence base for the report included 66 studies:

- six human studies (five breast cancer, one colorectal cancer)
- 34 animal studies (15 breast cancer, 19 colorectal cancer)
- 25 cell line studies (15 breast cancer, 10 colorectal cancer)

- one combination study (animal, cell line) on colorectal cancer.

Therefore the evidence in support of any potential mechanism connecting alcohol intake to cancer development is based largely on animal models. Animal models are important tools for understanding disease mechanisms but they have limitations when predicting the actual course of events in humans.⁸² Reviews of animal studies have shown that there is a tendency to publish studies with positive results and not to publish studies that suggest no difference in measured outcomes (i.e., publication bias). Therefore studies that could possibly rule out mechanisms connecting alcohol and cancer may not be published. Positive results in animal studies may not translate to a clinical setting because carcinogens were administered in a controlled setting that is not characteristic of human conditions. Most experimental animals are young and rarely have comorbidities, a situation that may also limit generalizability of animal studies to clinical studies.³¹²

Few human studies met the inclusion criteria for this report and this limited the comparisons that could be made between the findings of animal studies and those in human studies. Exact alcohol exposure can be controlled in animal studies but few human studies have done the same. While the four breast cancer human studies actually administered and quantified the amount of ethanol, the only colorectal cancer study administered acetaldehyde to biopsied colonic mucosa. Because of the limited number of human studies in our evidence base, we did look at potential mechanisms suggested in epidemiology studies and compared them to mechanisms examined in animal and cell line studies.

References and Included Studies

1. US Department of Health and Human Services, Public Health Service, National Toxicology Program. Alcoholic beverage consumption. Known to be a human carcinogen. Rockville (MD): U.S. Department of Health and Human Services; 2000. 2 p.
2. Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Coglian V, WHO International Agency for Research on Cancer Monograph Working Group. Carcinogenicity of alcoholic beverages. *Lancet Oncol* 2007 Apr;8(4):292-3. PMID: 17431955
3. Seitz HK, Simanowski UA. Alcohol and carcinogenesis. *Annu Rev Nutr* 1988;8:99-119. PMID: 3060182
4. Monteiro R, Calhau C, Silva AO, Pinheiro-Silva S, Guerreiro S, Gartner F, Azevedo I, Soares R. Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J Cell Biochem* 2008 Aug 1;104(5):1699-707. PMID: 18348194
5. Holford NH. Clinical pharmacokinetics of ethanol. *Clin Pharmacokinet* 1987 Nov;13(5):273-92. PMID: 3319346
6. Levitt DG. PKQuest: measurement of intestinal absorption and first pass metabolism - application to human ethanol pharmacokinetics. *BMC Clin Pharmacol* 2002 Aug 15;2:4. PMID: 12182761
7. Oneta CM, Simanowski UA, Martinez M, Allali-Hassani A, Pares X, Homann N, Conradt C, Waldherr R, Fiehn W, Coutelle C, Seitz HK. First pass metabolism of ethanol is strikingly influenced by the speed of gastric emptying. *Gut* 1998 Nov;43(5):612-9. PMID: 9824340
8. Zakhari S. Overview: how is alcohol metabolized by the body? *Alcohol Res Health* 2006;29(4):245-54. PMID: 17718403
9. Nagy LE. Molecular aspects of alcohol metabolism: transcription factors involved in early ethanol-induced liver injury. *Annu Rev Nutr* 2004;24:55-78. PMID: 15189113
10. Dumitrescu RG, Shields PG. The etiology of alcohol-induced breast cancer. *Alcohol* 2005 Apr;35(3):213-25. PMID: 16054983
11. Alcohol metabolism, tobacco and cancer. In: Cho C. *Alcohol, Tobacco, and Cancer*. Switzerland: S. Karger AG; 2006 Jul. p. 29-47.
12. Jelski W, Szmikowski M. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the cancer diseases. *Clin Chim Acta* 2008 Sep;395(1-2):1-5. PMID: 18505683
13. Alcohol and cancer of the large intestine. In: Cho C. *Alcohol, Tobacco, and Cancer*. Switzerland: S. Karger AG; 2006 Jul. p. 63-77.
14. Seitz HK, Stickel F. Molecular mechanisms of alcohol-mediated carcinogenesis. *Nat Rev Cancer* 2007 Aug;7(8):599-612. PMID: 17646865
15. Jelski W, Zalewski B, Chrostek L, Szmikowski M. The activity of class I, II, III, and IV alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in colorectal cancer. *Dig Dis Sci* 2004 Jun;49(6):977-81. PMID: 15309886
16. Jelski W, Orywal K, Kedra B, Szmikowski M. [Alcohol dehydrogenase and aldehyde dehydrogenase as tumour markers and factors intensifying carcinogenesis in colorectal cancer]. *Pol Merkur Lekarski* 2008 Jun;24(144):506-10. (Pol). PMID: 18702331
17. Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Coglian V, WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens--Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol* 2009 Nov;10(11):1033-4. PMID: 19891056
18. Alcohol and breast cancer risk. In: Cho C. *Alcohol, Tobacco, and Cancer*. Switzerland: S. Karger AG; 2006 Jul. p. 119-39.
19. Homann N, Konig IR, Marks M, Benesova M, Stickel F, Millonig G, Mueller S, Seitz HK. Alcohol and colorectal cancer: the role of alcohol dehydrogenase 1C polymorphism. *Alcohol Clin Exp Res* 2009 Mar;33(3):551-6. PMID: 19120062
20. Salaspuro M. Bacteriocolon pathway for ethanol oxidation: characteristics and implications. *Ann Med* 1996 Jun;28(3):195-200. PMID: 8811162
21. Seitz HK, Egerer G, Oneta C, Kramer S, Sieg A, Klee F, Simanowski UA. Alcohol dehydrogenase in the human colon and rectum. *Digestion* 1996 Mar;57(2):105-8. PMID: 8785998
22. General mechanisms of cancer. In: Cho C. *Alcohol, Tobacco, and Cancer*. Switzerland: S. Karger AG; 2006 Jul. p. 1-12.
23. Ketcham AS, Wexler H, Mantel N. Effects of alcohol in mouse neoplasia. *Cancer Res* 1963 Jun;23:667-70. PMID: 14032175
24. Poschl G, Seitz HK. Alcohol and cancer. *Alcohol Alcohol* 2004 May-Jun;39(3):155-65. PMID: 15082451
25. Wright RM, McManaman JL, Repine JE. Alcohol-induced breast cancer: a proposed mechanism. *Free Radic Biol Med* 1999 Feb;26(3-4):348-54. PMID: 9895226

26. National Institute on Alcohol Abuse and Alcoholism (NIAAA). Three stages of carcinogenesis. Bethesda (MD): National Institutes of Health (NIH); 2001 Feb. 1 p. Also available: <http://www.niaaa.nih.gov/NR/rdonlyres/9E893CA5-39E0-47D8-B924-E4B883006642/0/lieb.pdf>.
27. Prevalence and trends data. Nationwide (states, DC, and territories) [internet]. Atlanta (GA): Centers for Disease Control and Prevention (CDC); 2008. [accessed 2009 Aug 21]. 2008 alcohol consumption: adults who have had at least one drink of alcohol within the past 30 days. [2 p]. Available: <http://apps.nccd.cdc.gov/brfss/index.asp>.
28. WHO. Global status report on alcohol 2004. Geneva: World Health Organization (WHO); 2004. 94 p.
29. Dufour MC. What is moderate drinking? Defining “drinks” and drinking levels. *Alcohol Res Health* 1999;23(1):5-14. PMID: 10890793
30. U.S. Department of Health and Human Services, U.S. Department of Agriculture. Dietary guidelines for Americans, 2005. Washington (DC): U.S. Department of Health and Human Services, U.S. Department of Agriculture; 2005. 84 p. Also available: www.healthierus.gov/dietaryguidelines.
31. Harvard School of Public Health (HSPH). The nutrition source. Alcohol: balancing risks and benefits. [internet]. Boston (MA): Harvard School of Public Health (HSPH); 2009 [9 p]. Available: <http://www.hsph.harvard.edu/nutritionsource/what-should-you-eat/alcohol-full-story/>.
32. CDC. Alcohol. [internet]. Atlanta (GA): Centers for Disease Control and Prevention (CDC); 2009 [6 p]. Available: <http://www.cdc.gov/alcohol/faqs.htm>.
33. Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. *J Nutr* 2004 Sep;134(9):2475S-81S. PMID: 15333745
34. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW Jr, Coates RJ, Liff JM, Talamini R, Chantarakul N, Koetsawang S, Rachawat D, Morabia A, Schuman L, Stewart W, Szklo M, Bain C, Schofield F, Siskind V, Band P, Coldman AJ, Gallagher RP, Hislop TG, Yang P, Kolonel LM, Nomura AM, Hu J, Johnson. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer* 2002 Nov 18;87(11):1234-45. PMID: 12439712
35. O'Hanlon LH. Studies seek molecular clues on alcohol's role in cancer. *J Natl Cancer Inst* 2005 Nov 2;97(21):1563-4. PMID: 16264173
36. Cho E, Smith-Warner SA, Ritz J, van den Brandt PA, Colditz GA, Folsom AR, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Holmberg L, Kim DH, Malila N, Miller AB, Pietinen P, Rohan TE, Sellers TA, Speizer FE, Willett WC, Wolk A, Hunter DJ. Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. *Ann Intern Med* 2004 Apr 20;140(8):603-13. PMID: 15096331
37. Zhang Y, Kreger BE, Dorgan JF, Splansky GL, Cupples LA, Ellison RC. Alcohol consumption and risk of breast cancer: the Framingham Study revisited. *Am J Epidemiol* 1999 Jan 15;149(2):93-101. PMID: 9921953
38. Feigelson HS, Jonas CR, Robertson AS, McCullough ML, Thun MJ, Calle EE. Alcohol, folate, methionine, and risk of incident breast cancer in the American Cancer Society Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev* 2003 Feb;12(2):161-4. PMID: 12582027
39. Smith-Warner SA, Spiegelman D, Yaun SS, van den Brandt PA, Folsom AR, Goldbohm RA, Graham S, Holmberg L, Howe GR, Marshall JR, Miller AB, Potter JD, Speizer FE, Willett WC, Wolk A, Hunter DJ. Alcohol and breast cancer in women: a pooled analysis of cohort studies. *JAMA* 1998 Feb 18;279(7):535-40. PMID: 9480365
40. Purohit V, Khalsa J, Serrano J. Mechanisms of alcohol-associated cancers: introduction and summary of the symposium. *Alcohol* 2005 Apr;35(3):155-60. PMID: 16054976
41. Singletary K. Ethanol and experimental breast cancer: a review. *Alcohol Clin Exp Res* 1997 Apr;21(2):334-9. PMID: 9113272
42. Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. Diet, nutrition and the prevention of cancer. *Public Health Nutr* 2004 Feb;7(1A):187-200. PMID: 14972060
43. Schatzkin A, Longnecker MP. Alcohol and breast cancer. Where are we now and where do we go from here? *Cancer* 1994 Aug 1;74(3 Suppl):1101-10. PMID: 8039145
44. Epidemiology of alcohol-associated cancers. In: Cho C. Alcohol, Tobacco, and Cancer. Switzerland: S. Karger AG; 2006 Jul. p. 13-28.
45. Lilla C, Koehler T, Kropp S, Wang-Gohrke S, Chang-Claude J. Alcohol dehydrogenase 1B (ADH1B) genotype, alcohol consumption and breast cancer risk by age 50 years in a German case-control study. *Br J Cancer* 2005 Jun 6;92(11):2039-41. PMID: 15886702
46. Surveillance Epidemiology and End Results. SEER stat fact sheets - cancer of the breast. [internet]. Bethesda (MD): National Cancer Institute (NCI); [accessed 2007 Feb 5]. [3 p]. Available: <http://seer.cancer.gov/statfacts/html/breast.html>.

47. Hines LM, Hankinson SE, Smith-Warner SA, Spiegelman D, Kelsey KT, Colditz GA, Willett WC, Hunter DJ. A prospective study of the effect of alcohol consumption and ADH3 genotype on plasma steroid hormone levels and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000 Oct;9(10):1099-105. PMID: 11045794
48. Coutelle C, Hohn B, Benesova M, Oneta CM, Quattrocchi P, Roth HJ, Schmidt-Gayk H, Schneeweiss A, Bastert G, Seitz HK. Risk factors in alcohol associated breast cancer: alcohol dehydrogenase polymorphism and estrogens. *Int J Oncol* 2004 Oct;25(4):1127-32. PMID: 15375565
49. Freudenheim JL, Ambrosone CB, Moysich KB, Vena JE, Graham S, Marshall JR, Muti P, Laughlin R, Nemoto T, Harty LC, Crits GA, Chan AW, Shields PG. Alcohol dehydrogenase 3 genotype modification of the association of alcohol consumption with breast cancer risk. *Cancer Causes Control* 1999 Oct;10(5):369-77. PMID: 10530606
50. Terry MB, Knight JA, Zablotzka L, Wang Q, John EM, Andrulis IL, Senie RT, Daly M, Ozcelik H, Briollais L, Santella RM. Alcohol metabolism, alcohol intake, and breast cancer risk: a sister-set analysis using the Breast Cancer Family Registry. *Breast Cancer Res Treat* 2007 Dec;106(2):281-8. PMID: 17268812
51. Izevbogie EB, Ekwunsi SI, Jordan J, Howard CB. Ethanol modulates the growth of human breast cancer cells in vitro. *Exp Biol Med (Maywood)* 2002 Apr;227(4):260-5. PMID: 11910048
52. Key J, Hodgson S, Omar RZ, Jensen TK, Thompson SG, Boobis AR, Davies DS, Elliott P. Meta-analysis of studies of alcohol and breast cancer with consideration of the methodological issues. *Cancer Causes Control* 2006 Aug;17(6):759-70. PMID: 16783604
53. National Cancer Institute (NCI). SEER stat fact sheets: cancer, colon and rectum. [internet]. Bethesda (MD): National Cancer Institute (NCI); [accessed 2008 Oct 10]. [4 p]. Available: <http://seer.cancer.gov/statfacts/html/colorect.html>.
54. Markowitz SD, Bertagnolli MM. Molecular basis of colorectal cancer. *N Engl J Med* 2009 Dec 17;361(25):2449-60. Also available: <http://content.nejm.org/cgi/reprint/361/25/2449.pdf>. PMID: 20018966
55. Nosova T, Jousimies-Somer H, Kaihovaara P, Jokelainen K, Heine R, Salaspuro M. Characteristics of alcohol dehydrogenases of certain aerobic bacteria representing human colonic flora. *Alcohol Clin Exp Res* 1997 May;21(3):489-94. PMID: 9161610
56. Jokelainen K, Siitonen A, Jousimies-Somer H, Nosova T, Heine R, Salaspuro M. In vitro alcohol dehydrogenase-mediated acetaldehyde production by aerobic bacteria representing the normal colonic flora in man. *Alcohol Clin Exp Res* 1996 Sep;20(6):967-72. PMID: 8892513
57. Jokelainen K, Matysiak-Budnik T, Makisalo H, Hockerstedt K, Salaspuro M. High intracolonic acetaldehyde values produced by a bacteriocolon pathway for ethanol oxidation in piglets. *Gut* 1996 Jul;39(1):100-4. PMID: 8881818
58. Slattery ML, Wolff RK, Herrick J, Caan BJ, Samowitz W. Tumor markers and rectal cancer: support for an inflammation-related pathway. *Int J Cancer* 2009 Oct 1;125(7):1698-704. PMID: 19452524
59. Higgins JP, Green S. Cochrane handbook for systematic reviews of interventions 5.0.2. In: Cochrane Library [book online]. The Cochrane Collaboration; 2008 [updated 2009 Sep 1]. [accessed 2009 Oct 7]. Available: <http://www.cochrane-handbook.org/>.
60. Riegelman R. Studying a study & testing a test: how to read the medical evidence. 5th ed. Philadelphia (PA): Lippincott Williams & Wilkins; 1975 Nov. Chapter 7: Interpretation. p. 50-7.
61. Doll R, Hill AB. Smoking and carcinoma of the lung; preliminary report. *Br Med J* 1950 Sep 30;2(4682):739-48. PMID: 14772469
62. Doll R, Hill AB. A study of the aetiology of carcinoma of the lung. *Br Med J* 1952 Dec 13;2(4797):1271-86. PMID: 12997741
63. Hussain SP, Harris CC. Molecular epidemiology of human cancer. *Toxicol Lett* 1998 Dec 28;102-103:219-25. PMID: 10022257
64. Owens D, Lohr K, Atkins D, Treadwell J, Reston J, Bass E, Chang S, Helfand M. Grading the strength of a body of evidence when comparing medical interventions. In: *Methods guide for comparative effectiveness reviews*. Rockville (MD): Agency for Healthcare Research and Quality (AHRQ); 2009 Jul. p. 19. Also available: http://effectivehealthcare.ahrq.gov/ehc/products/122/328/2009_0805_grading.pdf.
65. Etique N, Chardard D, Chesnel A, Merlin JL, Flament S, Grillier-Vuissoz I. Ethanol stimulates proliferation, ERalpha and aromatase expression in MCF-7 human breast cancer cells. *Int J Mol Med* 2004 Jan;13(1):149-55. PMID: 14654987
66. Acetaldehyde-DNA adducts: implications for the molecular mechanism of alcohol-related carcinogenesis. In: Cho C. Alcohol, tobacco, and cancer. Switzerland: S. Karger AG; 2006 Jul. p. 78-94.
67. Fan S, Meng Q, Gao B, Grossman J, Yadegari M, Goldberg ID, Rosen EM. Alcohol stimulates estrogen receptor signaling in human breast cancer cell lines. *Cancer Res* 2000 Oct 15;60(20):5635-9. PMID: 11059753
68. Etique N, Flament S, Lecomte J, Grillier-Vuissoz I. Ethanol-induced ligand-independent activation of ERalpha mediated by cyclic AMP/PKA signaling pathway: an in vitro study on MCF-7 breast cancer cells. *Int J Oncol* 2007 Dec;31(6):1509-18. PMID: 17982678

69. Singletary KW, Frey RS, Yan W. Effect of ethanol on proliferation and estrogen receptor- α expression in human breast cancer cells. *Cancer Lett* 2001 Apr 26;165(2):131-7. PMID: 11275361
70. Homann N, Tillonen J, Salaspuro M. Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. *Int J Cancer* 2000 Apr 15;86(2):169-73. PMID: 10738242
71. Salaspuro M. Microbial metabolism of ethanol and acetaldehyde and clinical consequences. *Addict Biol* 1997;2(1):35-46.
72. Vitale JJ, Gottlieb. Alcohol and alcohol-related deficiencies as carcinogens. *Cancer Res* 1975 Nov;35(11 Pt. 2):3336-8. PMID: 1192405
73. Perez-Holanda S, Rodrigo L, Vinas-Salas J, Pinol-Felis C. Effect of ethanol consumption on colon cancer in an experimental model. *Rev Esp Enferm Dig* 2005 Feb;97(2):87-96. PMID: 15801884
74. Tong WM, Manhardt T, Lassnig H, Cross HS. Induction of epidermal growth factor receptor expression and mitogenesis by alcohol in human colon adenocarcinoma-derived Caco-2 cells. *Anticancer Res* 1999 Jul-Aug;19(4B):3321-5. PMID: 10652628
75. Rodriguez DA, Moncada C, Nunez MT, Lavandero S, Ponnappa BC, Israel Y. Ethanol increases tumor necrosis factor- α receptor-1 (TNF-R1) levels in hepatic, intestinal, and cardiac cells. *Alcohol* 2004 May;33(1):9-15. PMID: 15353169
76. Hartman TJ, Baer DJ, Graham LB, Stone WL, Gunter EW, Parker CE, Albert PS, Dorgan JF, Clevidence BA, Campbell WS, Tomer KB, Judd JT, Taylor PR. Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. *Eur J Clin Nutr* 2005 Feb;59(2):161-8. PMID: 15367922
77. Ray G, Batra S, Shukla NK, Deo S, Raina V, Ashok S, Husain SA. Lipid peroxidation, free radical production and antioxidant status in breast cancer. *Breast Cancer Res Treat* 2000 Jan;59(2):163-70. PMID: 10817351
78. Afrasyap L, Guvenen G, Turkmen S. Plasma and erythrocyte total antioxidant status in patients with benign and malign breast disease. *Cancer Biochem Biophys* 1998 Jun;16(1-2):129-38. PMID: 9923972
79. Huang YL, Sheu JY, Lin TH. Association between oxidative stress and changes of trace elements in patients with breast cancer. *Clin Biochem* 1999 Mar;32(2):131-6. PMID: 10211630
80. Kumar K, Thangaraju M, Sachdanandam P. Changes observed in antioxidant system in the blood of postmenopausal women with breast cancer. *Biochem Int* 1991 Sep;25(2):371-80. PMID: 1789800
81. Meagher EA, Barry OP, Burke A, Lucey MR, Lawson JA, Rokach J, FitzGerald GA. Alcohol-induced generation of lipid peroxidation products in humans. *J Clin Invest* 1999 Sep;104(6):805-13. PMID: 10491416
82. van der Worp HB, Howells DW, Sena ES, Porritt MJ, Rewell S, O'Collins V, Macleod MR. Can animal models of disease reliably inform human studies? *PLoS Med* 2010;7(3):e1000245. PMID: 20361020
83. Basuroy S, Sheth P, Mansbach CM, Rao RK. Acetaldehyde disrupts tight junctions and adherens junctions in human colonic mucosa: protection by EGF and L-glutamine. *Am J Physiol Gastrointest Liver Physiol* 2005 Aug;289(2):G367-75. PMID: 15718285
84. Perse M, Cerar A. Dimethylhydrazine model is not appropriate for evaluating effect of ethanol on colorectal cancer. *Rev Esp Enferm Dig* 2007 Aug;99(8):463-6. PMID: 18020863
85. Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK, Campbell WS, Hartman TJ, Tejpar AA, Clevidence BA, Giffen CA, Chandler DW, Stanczyk FZ, Taylor PR. Serum hormones and the alcohol-breast cancer association in postmenopausal women. *J Natl Cancer Inst* 2001 May 2;93(9):710-5. PMID: 11333294
86. Reichman ME, Judd JT, Longcope C, Schatzkin A, Clevidence BA, Nair PP, Campbell WS, Taylor PR. Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. *J Natl Cancer Inst* 1993;85(9):722-7. PMID: 8478958
87. Ginsburg ES, Walsh BW, Shea BF, Gao X, Gleason RE, Feltmate C, Barbieri RL. Effect of acute ethanol ingestion on prolactin in menopausal women using estradiol replacement. *Gynecol Obstet Invest* 1995;39(1):47-9. PMID: 7890253
88. Ginsburg ES, Mello NK, Mendelson JH, Barbieri RL, Teoh SK, Rothman M, Gao X, Sholar JW. Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA* 1996 Dec 4;276(21):1747-51. PMID: 8940324
89. Saitoh M, Ramirez E, Babaian RJ. Ultrasonic volume monitoring in patient with prostate cancer treated by external beam radiation therapy. *Urology* 1994 Mar;43(3):342-8. PMID: 7510913
90. Marugame T, Tsuji E, Inoue H, Shinomiya S, Kiyohara C, Onuma K, Hamada H, Koga H, Handa K, Hayabuchi H, Kono S. Methylene-tetrahydrofolate reductase polymorphism and risk of colorectal adenomas. *Cancer Lett* 2000 Apr 14;151(2):181-6. PMID: 10738112
91. Holmberg B, Ekstrom T. The effects of long-term oral administration of ethanol on Sprague-Dawley rats--a condensed report. *Toxicology* 1995 Feb 6;96(2):133-45. PMID: 7886684

92. Hilakivi-Clarke L, Cabanes A, de Assis S, Wang M, Khan G, Shoemaker WJ, Stevens RG. In utero alcohol exposure increases mammary tumorigenesis in rats. *Br J Cancer* 2004 Jun 1;90(11):2225-31. PMID: 15150620
93. Singletary K, Nelshoppen J, Wallig M. Enhancement by chronic ethanol intake of N-methyl-N-nitrosourea-induced rat mammary tumorigenesis. *Carcinogenesis* 1995 Apr;16(4):959-64. PMID: 7728981
94. Singletary KW, McNary MQ. Influence of ethanol intake on mammary gland morphology and cell proliferation in normal and carcinogen-treated rats. *Alcohol Clin Exp Res* 1994 Oct;18(5):1261-6. PMID: 7847617
95. Castro GD, Delgado de Layno AM, Costantini MH, Castro JA. Rat breast microsomal biotransformation of ethanol to acetaldehyde but not to free radicals: Its potential role in the association between alcohol drinking and breast tumor promotion. *Teratog Carcinog Mutagen* 2003;(Suppl 1):61-70. PMID: 12616597
96. Chhabra SK, Anderson LM, Perella C, Desai D, Amin S, Kyrtopoulos SA, Souliotis VL. Coexposure to ethanol with N-nitrosodimethylamine or 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone during lactation of rats: marked increase in O(6)-methylguanine-DNA adducts in maternal mammary gland and in suckling lung and kidney. *Toxicol Appl Pharmacol* 2000 Dec 1;169(2):191-200. PMID: 11097872
97. Watabiki T, Okii Y, Tokiyasu T, Yoshimura S, Yoshida M, Akane A, Shikata N, Tsubura A. Long-term ethanol consumption in ICR mice causes mammary tumor in females and liver fibrosis in males. *Alcohol Clin Exp Res* 2000 Apr;24(4 Suppl):117S-22S. PMID: 10803793
98. Schrauzer GN, McGinness JE, Ishmael D, Bell LJ. Alcoholism and cancer. I. Effects of long-term exposure to alcohol on spontaneous mammary adenocarcinoma and prolactin levels in C3H/St mice. *J Stud Alcohol* 1979 Mar;40(3):240-6. PMID: 571941
99. Taylor AN, Ben-Eliyahu S, Yirmiya R, Chang MP, Norman DC, Chiappelli F. Actions of alcohol on immunity and neoplasia in fetal alcohol exposed and adult rats. *Alcohol Alcohol Suppl* 1993;2:69-74. PMID: 7748350
100. Rogers AE, Conner BH. Dimethylbenzanthracene-induced mammary tumorigenesis in ethanol-fed rats. *Nutr Res* 1990;10(8):915-28.
101. McDermott EW, O'Dwyer PJ, O'Higgins NJ. Dietary alcohol intake does not increase the incidence of experimentally induced mammary carcinoma. *Eur J Surg Oncol* 1992 Jun;18(3):251-4. PMID: 1607037
102. Hackney JF, Engelman RW, Good RA. Ethanol calories do not enhance breast cancer in isocalorically fed C3H/Ou mice. *Nutr Cancer* 1992;18(3):245-53. PMID: 1296198
103. Grubbs C, Juliana M, Whitaker L. Effect of ethanol on initiation of methylnitrosourea (MNU)- and dimethylbenzanthracene (DMBA)-induced mammary cancers. *Proc Am Assoc Cancer Res* 1988;29:148.
104. Singletary KW, McNary MQ. Effect of moderate ethanol consumption on mammary gland structural development and DNA synthesis in the female rat. *Alcohol* 1992 Mar-Apr;9(2):95-101. PMID: 1599632
105. Singletary KW, McNary MQ, Odoms AM, Nelshoppen J, Wallig MA. Ethanol consumption and DMBA-induced mammary carcinogenesis in rats. *Nutr Cancer* 1991;16(1):13-23. PMID: 1923906
106. Etique N, Grillier-Vuissoz I, Lecomte J, Flament S. Crosstalk between adenosine receptor (A2A isoform) and ERalpha mediates ethanol action in MCF-7 breast cancer cells. *Oncol Rep (Athens)* 2009 Apr;21(4):977-81. PMID: 19287996
107. Singletary KW, Barnes SL, Van Breemen RB. Ethanol inhibits benzo[a]pyrene-DNA adduct removal and increases 8-oxo-deoxyguanosine formation in human mammary epithelial cells. *Cancer Lett* 2004 Jan;203(2):139-44. PMID: 14732221
108. Barnes SL, Singletary KW, Frey R. Ethanol and acetaldehyde enhance benzo[a]pyrene-DNA adduct formation in human mammary epithelial cells. *Carcinogenesis* 2000;21(11):2123-8. PMID: 11062178
109. Zhu Y, Lin H, Li Z, Wang M, Luo J. Modulation of expression of ribosomal protein L7a (rpL7a) by ethanol in human breast cancer cells. *Breast Cancer Res Treat* 2001;69(1):29-38. PMID: 11759826
110. Przylipek A, Rabe T, Hafner J, Przylipek M, Runnebaum R. Influence of ethanol on in vitro growth of human mammary carcinoma cell line MCF-7. *Arch Gynecol Obstet* 1996;258(3):137-40. PMID: 8781701
111. Cyong JC, Okada H, Ishihara K, Hasegawa F. Effect of ethanol on cyclic nucleotide levels and the electron microscopic morphology of murine mammary tumor cells. *Kitasato Arch Exp Med* 1978 Dec;51(3-4):73-80. PMID: 232731
112. Dhar MS, Plummer HK III. Protein expression of G-protein inwardly rectifying potassium channels (GIRK) in breast cancer cells. *BMC Physiol* 2006 Aug 31;6:8. PMID: 16945134
113. Verma M, Davidson EA. MUC1 upregulation by ethanol. *Cancer Biochem Biophys* 1999 Jul;17(1-2):1-11. PMID: 10738897
114. Zhu Y, Lin H, Wang M, Li Z, Wiggins RC, Luo J. Up-regulation of transcription of smooth muscle myosin alkali light chain by ethanol in human breast cancer cells. *Int J Oncol* 2001 Jun;18(6):1299-305. PMID: 11351266

115. Venkata NG, Aung CS, Cabot PJ, Monteith GR, Roberts-Thomson SJ. PPARalpha and PPARbeta Are differentially affected by ethanol and the ethanol metabolite acetaldehyde in the MCF-7 breast cancer cell line. *Toxicol Sci* 2008 Mar;102(1):120-8. PMID: 18003597
116. Nelson RL, Samelson SL. Neither dietary ethanol nor beer augments experimental colon carcinogenesis in rats. *Dis Colon Rectum* 1985 Jun;28(6):460-2. PMID: 4006641
117. Howarth AE, Pihl E. High-fat diet promotes and causes distal shift of experimental rat colonic cancer--beer and alcohol do not. *Nutr Cancer* 1984;6(4):229-35. PMID: 6545579
118. Hayashi N, Tsutsumi M, Fukura M, Yano H, Tsuchishima M, Takase S. Effect of chronic dietary ethanol consumption on colonic cancer in rats induced by 1,1-dimethylhydrazine. *Alcohol Clin Exp Res* 2007 Jan;31(1 Suppl):S72-6. PMID: 17331170
119. Hakkak R, Korourian S, Ronis MJ, Ingelman-Sundberg M, Badger TM. Effects of diet and ethanol on the expression and localization of cytochromes P450 2E1 and P450 2C7 in the colon of male rats. *Biochem Pharmacol* 1996 Jan 12;51(1):61-9. PMID: 8534269
120. Pronko P, Bardina L, Satanovskaya V, Kuzmich A, Zimatkin S. Effect of chronic alcohol consumption on the ethanol- and acetaldehyde-metabolizing systems in the rat gastrointestinal tract. *Alcohol Alcohol* 2002;37(3):229-35. PMID: 12003909
121. Roy HK, Gulizia JM, Karolski WJ, Ratashak A, Sorrell MF, Tuma D. Ethanol promotes intestinal tumorigenesis in the MIN mouse. Multiple intestinal neoplasia. *Cancer Epidemiol Biomarkers Prev* 2002 Nov;11(11):1499-502. PMID: 12433735
122. Seitz HK, Simanowski UA, Garzon FT, Rideout JM, Peters TJ, Koch A, Berger MR, Einecke H, Maiwald M. Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. *Gastroenterology* 1990 Feb;98(2):406-13. PMID: 2295396
123. Seitz HK, Czygan P, Simanowski U, Waldherr R, Veith S, Raedsch R, Kommerell B. Stimulation of chemically induced rectal carcinogenesis by chronic ethanol ingestion. *Alcohol Alcohol* 1985;20(4):427-33. PMID: 3910061
124. Choi SW, Stickel F, Baik HW, Kim YI, Seitz HK, Mason JB. Chronic alcohol consumption induces genomic but not p53-specific DNA hypomethylation in rat colon. *J Nutr* 1999 Nov;129(11):1945-50. PMID: 10539767
125. Simanowski UA, Suter P, Russell RM, Heller M, Waldherr R, Ward R, Peters TJ, Smith D, Seitz HK. Enhancement of ethanol induced rectal mucosal hyper regeneration with age in F344 rats. *Gut* 1994 Aug;35(8):1102-6. PMID: 7926914
126. Niwa K, Tanaka T, Sugie S, Shinoda T, Kato K, Tamaya T, Mori H. Enhancing effect of ethanol or sake on methylazoxymethanol acetate-initiated large bowel carcinogenesis in ACI/N rats. *Nutr Cancer* 1991;15(3-4):229-37. PMID: 1866316
127. Simanowski UA, Seitz HK, Baier B, Kommerell B, Schmidt-Gayk H, Wright NA. Chronic ethanol consumption selectively stimulates rectal cell proliferation in the rat. *Gut* 1986 Mar;27(3):278-82. PMID: 3699547
128. Garzon FT, Simanowski UA, Berger MR, Schmahl D, Kommerell B, Seitz HK. Acetoxymethyl-methylnitrosamine (AMMN) induced colorectal carcinogenesis is stimulated by chronic alcohol consumption. *Alcohol Alcohol Suppl* 1987;1:501-2. PMID: 3426722
129. McGarrity TJ, Peiffer LP, Colony PC, Pegg AE. The effects of chronic ethanol administration on polyamine content during dimethylhydrazine-induced colorectal carcinogenesis in the rat. *Carcinogenesis* 1988 Nov;9(11):2093-8. PMID: 3180344
130. Hamilton SR, Sohn OS, Fiala ES. Inhibition by dietary ethanol of experimental colonic carcinogenesis induced by high-dose azoxymethane in F344 rats. *Cancer Res* 1988 Jun 15;48(12):3313-8. PMID: 3370634
131. Hamilton SR, Sohn OS, Fiala ES. Effects of timing and quantity of chronic dietary ethanol consumption on azoxymethane-induced colonic carcinogenesis and azoxymethane metabolism in Fischer 344 rats. *Cancer Res* 1987 Aug 15;47(16):4305-11. PMID: 3111683
132. Hamilton SR, Hyland J, McAvinchey D, Chaudhry Y, Hartka L, Kim HT, Cichon P, Floyd J, Turjman N, Kessie G, et al. Effects of chronic dietary beer and ethanol consumption on experimental colonic carcinogenesis by azoxymethane in rats. *Cancer Res* 1987 Mar 15;47(6):1551-9. PMID: 3815356
133. Vaculova A, Hofmanova J, Soucek K, Andera L, Kozubik A. Ethanol acts as a potent agent sensitizing colon cancer cells to the TRAIL-induced apoptosis. *FEBS Lett* 2004 Nov 5;577(1-2):309-13. PMID: 15527805
134. Asai K, Buurman WA, Reutelingsperger CP, Schutte B, Kaminishi M. Low concentrations of ethanol induce apoptosis in human intestinal cells. *Scand J Gastroenterol* 2003 Nov;38(11):1154-61. PMID: 14686719
135. Blasiak J, Trzeciak A, Malecka-Panas E, Drzewoski J, Wojewodzka M. In vitro genotoxicity of ethanol and acetaldehyde in human lymphocytes and the gastrointestinal tract mucosa cells. *Toxicol In Vitro* 2000 Aug;14(4):287-95. PMID: 10906435
136. Koivisto T, Salaspuro M. Acetaldehyde alters proliferation, differentiation and adhesion properties of human colon adenocarcinoma cell line Caco-2. *Carcinogenesis* 1998 Nov;19(11):2031-6. PMID: 9855020

137. Koivisto T, Salaspuro M. Effects of acetaldehyde on brush border enzyme activities in human colon adenocarcinoma cell line Caco-2. *Alcohol Clin Exp Res* 1997 Dec;21(9):1599-605. PMID: 9438518
138. Malagolini N, Dall'Olio F, Turrini I, Cessi C, Serafini-Cessi F. Effect of ethanol on human colon carcinoma CaCo-2 and HT-29 cell lines during the maturation process. *Alcohol Clin Exp Res* 1994 Dec;18(6):1386-91. PMID: 7695034
139. Papavassiliou ED, Arvind P, Tsioulas GJ, Qiao L, Goldin E, Staiano-Coico L, Rigas B. The effect of ethanol on the expression of HLA class I genes in human colon adenocarcinoma cell lines. *Cancer Lett* 1994 Jun 15;81(1):33-8. PMID: 8019985
140. Lemos C, Peters GJ, Jansen G, Martel F, Calhau C. Modulation of folate uptake in cultured human colon adenocarcinoma Caco-2 cells by dietary compounds. *Eur J Nutr* 2007 Sep;46(6):329-36. PMID: 17712586
141. Pannequin J, Delaunay N, Darido C, Maurice T, Crespy P, Frohman MA, Balda MS, Matter K, Joubert D, Bourgaux JF, Bali JP, Hollande F. Phosphatidylethanol accumulation promotes intestinal hyperplasia by inducing ZONAB-mediated cell density increase in response to chronic ethanol exposure. *Mol Cancer Res* 2007 Nov;5(11):1147-57. PMID: 18025260
142. Suzuki R, Orsini N, Mignone L, Saji S, Wolk A. Alcohol intake and risk of breast cancer defined by estrogen and progesterone receptor status--a meta-analysis of epidemiological studies. [erratum appears in *Int J Cancer*. 2008 Aug 15;123(4):981]. *Int J Cancer* 2008 Apr 15;122(8):1832-41. PMID: 18067133
143. Lissowska J, Gaudet MM, Brinton LA, Chanock SJ, Peplonska B, Welch R, Zatonski W, Szeszenia-Dabrowska N, Park S, Sherman M, Garcia-Closas M. Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: A population-based case-control study and meta-analyses. *Int J Cancer* 2007 Jun 15;120(12):2696-703. PMID: 17311260
144. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. *J Natl Cancer Inst* 2006 Nov 15;98(22):1607-22. PMID: 17105984
145. Corrao G, Bagnardi V, Zambon A, Arico S. Exploring the dose-response relationship between alcohol consumption and the risk of several alcohol-related conditions: a meta-analysis. *Addiction* 1999 Oct;94(10):1551-73. PMID: 10790907
146. Longnecker MP. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. *Cancer Causes Control* 1994 Jan;5(1):73-82. PMID: 8123780
147. Mizoue T, Inoue M, Wakai K, Nagata C, Shimazu T, Tsuji I, Otani T, Tanaka K, Matsuo K, Tamakoshi A, Sasazuki S, Tsugane S, Research Group for Development and Evaluation of Cancer Prevention Strategies in . Alcohol drinking and colorectal cancer in Japanese: a pooled analysis of results from five cohort studies. *Am J Epidemiol* 2008 Jun 15;167(12):1397-406. PMID: 18420544
148. Moskal A, Norat T, Ferrari P, Riboli E. Alcohol intake and colorectal cancer risk: a dose-response meta-analysis of published cohort studies. *Int J Cancer* 2007 Feb 1;120(3):664-71. PMID: 17096321
149. Mizoue T, Tanaka K, Tsuji I, Wakai K, Nagata C, Otani T, Inoue M, Tsugane S, Research Group for the Development and Evaluation of Cancer Prevention Strategies. Alcohol drinking and colorectal cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol* 2006 Sep;36(9):582-97. PMID: 16870695
150. Franceschi S, La Vecchia C. Alcohol and the risk of cancers of the stomach and colon-rectum. *Dig Dis* 1994 Sep-Oct;12(5):276-89. PMID: 7882548
151. Zhang SM, Lee IM, Manson JE, Cook NR, Willett WC, Buring JE. Alcohol consumption and breast cancer risk in the Women's Health Study. *Am J Epidemiol* 2007 Mar 15;165(6):667-76. PMID: 17204515
152. Li Y, Baer D, Friedman GD, Udaltsova N, Shim V, Klatsky AL. Wine, liquor, beer and risk of breast cancer in a large population. *Eur J Cancer* 2009 Mar;45(5):843-50. PMID: 19095438
153. Li CI, Malone KE, Porter PL, Weiss NS, Tang MT, Daling JR. The Relationship between Alcohol Use and Risk of Breast Cancer by Histology and Hormone Receptor Status among Women 65-79 Years of Age. *Cancer Epidemiol Biomarkers Prev* 2003 Oct;12(10):1061-6. PMID: 14578143
154. Lew JQ, Freedman ND, Leitzmann MF, Brinton LA, Hoover RN, Hollenbeck AR, Schatzkin A, Park Y. Alcohol and risk of breast cancer by histologic type and hormone receptor status in postmenopausal women: the NIH-AARP Diet and Health Study. *Am J Epidemiol* 2009 Aug 1;170(3):308-17. PMID: 19541857
155. Wayne S, Neuhauser ML, Ulrich CM, Koprowski C, Wiggins C, Baumgartner KB, Bernstein L, Baumgartner RN, Gilliland F, McTiernan A, Ballard-Barbash R. Association between alcohol intake and serum sex hormones and peptides differs by tamoxifen use in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev* 2008 Nov;17(11):3224-32. PMID: 18957523

156. Deandrea S, Talamini R, Foschi R, Montella M, Dal Maso L, Falcini F, La Vecchia C, Franceschi S, Negri E. Alcohol and breast cancer risk defined by estrogen and progesterone receptor status: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2008 Aug;17(8):2025-8. PMID: 18708394
157. Garcia-Closas M, Herbstman J, Schiffman M, Glass A, Dorgan JF. Relationship between serum hormone concentrations, reproductive history, alcohol consumption and genetic polymorphisms in pre-menopausal women. *Int J Cancer* 2002 Nov 10;102(2):172-8. PMID: 12385014
158. Li CI, Chlebowski RT, Freiberg M, Johnson KC, Kuller L, Lane D, Lessin L, O'Sullivan MJ, Wactawski-Wende J, Yasmeeen S, Prentice R. Alcohol consumption and risk of postmenopausal breast cancer by subtype: the Women's Health Initiative Observational Study. *J Natl Cancer Inst* 2010 Aug 23;Epub ahead of print. PMID: 20733117
159. Rundle A, Tang D, Mooney L, Grumet S, Perera F. The interaction between alcohol consumption and GSTM1 genotype on polycyclic aromatic hydrocarbon-DNA adduct levels in breast tissue. *Cancer Epidemiol Biomarkers Prev* 2003 Sep 1;12(9):911-4. PMID: 14504203
160. Lavigne JA, Baer DJ, Wimbrow HH, Albert PS, Brown ED, Judd JT, Campbell WS, Giffen CA, Dorgan JF, Hartman TJ, Barrett JC, Hursting SD, Taylor PR. Effects of alcohol on insulin-like growth factor I and insulin-like growth factor binding protein 3 in postmenopausal women. *Am J Clin Nutr* 2005 Feb;81(2):503-7. PMID: 15699241
161. Choi JY, Abel J, Neuhaus T, Ko Y, Harth V, Hamajima N, Tajima K, Yoo KY, Park SK, Noh DY, Han W, Choe KJ, Ahn SH, Kim SU, Hirvonen A, Kang D. Role of alcohol and genetic polymorphisms of CYP2E1 and ALDH2 in breast cancer development. *Pharmacogenetics* 2003 Feb;13(2):67-72. PMID: 12563175
162. Lee KM, Abel J, Ko Y, Harth V, Park WY, Seo JS, Yoo KY, Choi JY, Shin A, Ahn SH, Noh DY, Hirvonen A, Kang D. Genetic polymorphisms of cytochrome P450 19 and 1B1, alcohol use, and breast cancer risk in Korean women. *Br J Cancer* 2003 Mar 10;88(5):675-8. PMID: 12618873
163. Kabat GC, Miller AB, Jain M, Rohan TE. Dietary intake of selected B vitamins in relation to risk of major cancers in women. *Br J Cancer* 2008 Sep 2;99(5):816-21. PMID: 18665162
164. Hussien MM, McNulty H, Armstrong N, Johnston PG, Spence RA, Barnett Y. Investigation of systemic folate status, impact of alcohol intake and levels of DNA damage in mononuclear cells of breast cancer patients. *Br J Cancer* 2005 Apr 25;92(8):1524-30. PMID: 15812544
165. Zhu K, Davidson NE, Hunter S, Yang X, Payne-Wilks K, Roland CL, Phillips D, Bentley C, Dai M, Williams SM. Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. *Cancer Causes Control* 2003 Nov;14(9):827-36. PMID: 14682440
166. Eichholzer M, Luthy J, Moser U, Fowler B. Folate and the risk of colorectal, breast and cervix cancer: the epidemiological evidence. *Swiss Med Wkly* 2001 Sep 22;131(37-38):539-49. PMID: 11759174
167. Tjonneland A, Christensen J, Olsen A, Stripp C, Nissen SB, Overvad K, Thomsen BL. Folate intake, alcohol and risk of breast cancer among postmenopausal women in Denmark. *Eur J Clin Nutr* 2006 Feb;60(2):280-6. PMID: 16234832
168. Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, Colditz GA, Hankinson SE. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst* 2003 Mar 5;95(5):373-80. PMID: 12618502
169. Semenza JC, Delfino RJ, Ziogas A, Anton-Culver H. Breast cancer risk and methylenetetrahydrofolate reductase polymorphism. *Breast Cancer Res Treat* 2003 Feb;77(3):217-23. PMID: 12602921
170. Maruti SS, Ulrich CM, White E. Folate and one-carbon metabolism nutrients from supplements and diet in relation to breast cancer risk. *Am J Clin Nutr* 2009 Feb;89(2):624-33. PMID: 19116331
171. Le Marchand L, Haiman CA, Wilkens LR, Kolonel LN, Henderson BE. MTHFR polymorphisms, diet, HRT, and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2004 Dec;13(12):2071-7. PMID: 15598763
172. Freudenheim JL, Bonner M, Krishnan S, Ambrosone CB, Graham S, McCann SE, Moysich KB, Bowman E, Nemoto T, Shields PG. Diet and alcohol consumption in relation to p53 mutations in breast tumors. *Carcinogenesis* 2004 Jun;25(6):931-9. PMID: 14742318
173. Beilby J, Ingram D, Hahnel R, Rossi E. Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. *Eur J Cancer* 2004 May;40(8):1250-4. PMID: 15110890
174. Jelski W, Chrostek L, Szmitkowski M, Markiewicz W. The activity of class I, II, III and IV alcohol dehydrogenase isoenzymes and aldehyde dehydrogenase in breast cancer. *Clin Exp Med* 2006 Jun;6(2):89-93. PMID: 16820997

175. Jelski W, Chrostek L, Markiewicz W, Szmikowski M. Activity of alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) in the sera of patients with breast cancer. *J Clin Lab Anal* 2006;20(3):105-8. PMID: 16721836
176. Triano EA, Slusher LB, Atkins TA, Beneski JT, Gestl SA, Zolfaghari R, Polavarapu R, Fraenhoffer E, Weisz J. Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. *Cancer Res* 2003 Jun 15;63(12):3092-100. PMID: 12810634
177. Visvanathan K, Crum RM, Strickland PT, You X, Ruczinski I, Berndt SI, Alberg AJ, Hoffman SC, Comstock GW, Bell DA, Helzlsouer KJ. Alcohol dehydrogenase genetic polymorphisms, low-to-moderate alcohol consumption, and risk of breast cancer. *Alcohol Clin Exp Res* 2007 Mar;31(3):467-76. PMID: 17295732
178. Sturmer T, Wang-Gohrke S, Arndt V, Boeing H, Kong X, Kreienberg R, Brenner H. Interaction between alcohol dehydrogenase II gene, alcohol consumption, and risk for breast cancer. *Br J Cancer* 2002 Aug 27;87(5):519-23. PMID: 12189549
179. Pezzotti A, Kraft P, Hankinson SE, Hunter DJ, Buring J, Cox DG. The mitochondrial A10398G polymorphism, interaction with alcohol consumption, and breast cancer risk. *PLoS ONE* 2009 Apr 24;4(4):e5356. PMID: 19390621
180. Jin MJ, Chen K, Zhang SS, Zhang YJ, Ren YJ, Xu H, Yao KY, Li QL, Ma XY. [Association of single nucleotide polymorphisms and haplotypes in DNA repair gene XRCC1 with susceptibility of breast cancer]. *Zhejiang Da Xue Xue Bao Yi Xue Ban* 2006 Jul;35(4):370-6. (Chi). PMID: 16924699
181. Zheng T, Holford TR, Zahm SH, Owens PH, Boyle P, Zhang Y, Zhang B, Wise JP Sr, Stephenson LP, Ali-Osman F. Glutathione S-transferase M1 and T1 genetic polymorphisms, alcohol consumption and breast cancer risk. *Br J Cancer* 2003 Jan 13;88(1):58-62. PMID: 12556960
182. Park SK, Yoo KY, Lee SJ, Kim SU, Ahn SH, Noh DY, Choe KJ, Strickland PT, Hirvonen A, Kang D. Alcohol consumption, glutathione S-transferase M1 and T1 genetic polymorphisms and breast cancer risk. *Pharmacogenetics* 2000 Jun;10(4):301-9. PMID: 10862521
183. Vogel U, Christensen J, Nexø BA, Wallin H, Friis S, Tjønneland A. Peroxisome proliferator-activated receptor-gamma2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes [erratum appears in *Carcinogenesis* 2007 Sep;28(9):2062]. *Carcinogenesis* 2007 Feb;28(2):427-34. PMID: 16959787
184. Vogel U, Christensen J, Nexø BA, Wallin H, Friis S, Tjønneland A. Erratum: Peroxisome Proliferator-Activated Receptor-gamma2 Pro12Ala, interaction with alcohol intake and NSAID use, in relation to risk of breast cancer in a prospective study of Danes (*Carcinogenesis* (2007) vol. 2 (427-434)). *Carcinogenesis* 2007 Sep;28(9):2062.
185. Stern MC, Conti DV, Siegmund KD, Corral R, Yuan JM, Koh WP, Yu MC. DNA repair single-nucleotide polymorphisms in colorectal cancer and their role as modifiers of the effect of cigarette smoking and alcohol in the Singapore Chinese Health Study. *Cancer Epidemiol Biomarkers Prev* 2007 Nov;16(11):2363-72. PMID: 18006925
186. Tranah GJ, Bugni J, Giovannucci E, Ma J, Fuchs C, Hines L, Samson L, Hunter DJ. O6-methylguanine-DNA methyltransferase Leu84Phe and Ile143Val polymorphisms and risk of colorectal cancer in the Nurses' Health Study and Physicians' Health Study (United States). *Cancer Causes Control* 2006 Jun;17(5):721-31. PMID: 16633920
187. Jin MJ, Chen K, Song L, Fan CH, Chen Q, Zhu YM, Ma XY, Yao KY. The association of the DNA repair gene XRCC3 Thr241Met polymorphism with susceptibility to colorectal cancer in a Chinese population. *Cancer Genet Cytogenet* 2005 Nov;163(1):38-43. PMID: 16271954
188. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, Willett WC, Selhub J, Hennekens CH, Rozen R. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res* 1997 Mar 15;57(6):1098-102. PMID: 9067278
189. Chen J, Giovannucci EL, Hunter DJ. MTHFR polymorphism, methyl-replete diets and the risk of colorectal carcinoma and adenoma among U.S. men and women: an example of gene-environment interactions in colorectal tumorigenesis. *J Nutr* 1999 Feb;129(2S Suppl):560S-4S. PMID: 10064332
190. Yamaji T, Iwasaki M, Sasazuki S, Sakamoto H, Yoshida T, Tsugane S. Methionine synthase A2756G polymorphism interacts with alcohol and folate intake to influence the risk of colorectal adenoma. *Cancer Epidemiol Biomarkers Prev* 2009 Jan;18(1):267-74. PMID: 19124508
191. Goode EL, Potter JD, Bigler J, Ulrich CM. Methionine synthase D919G polymorphism, folate metabolism, and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev* 2004 Jan;13(1):157-62. PMID: 14744749

192. Weinstein SJ, Albanes D, Selhub J, Graubard B, Lim U, Taylor PR, Virtamo J, Stolzenberg-Solomon R. One-carbon metabolism biomarkers and risk of colon and rectal cancers. *Cancer Epidemiol Biomarkers Prev* 2008 Nov;17(11):3233-40. PMID: 18990766
193. de Vogel S, Bongaerts BW, Wouters KA, Kester AD, Schouten LJ, de Goeij AF, de Bruine AP, Goldbohm RA, van den Brandt PA, van Engeland M, Weijenberg MP. Associations of dietary methyl donor intake with MLH1 promoter hypermethylation and related molecular phenotypes in sporadic colorectal cancer. *Carcinogenesis* 2008 Sep;29(9):1765-73. PMID: 18339680
194. Sharp L, Little J, Brockton NT, Cotton SC, Masson LF, Haites NE, Cassidy J. Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. *Br J Nutr* 2008 Feb;99(2):379-89. PMID: 18053312
195. Otani T, Iwasaki M, Hanaoka T, Kobayashi M, Ishihara J, Natsukawa S, Shaura K, Koizumi Y, Kasuga Y, Yoshimura K, Yoshida T, Tsugane S. Folate, vitamin B6, vitamin B12, and vitamin B2 intake, genetic polymorphisms of related enzymes, and risk of colorectal cancer in a hospital-based case-control study in Japan. *Nutr Cancer* 2005;53(1):42-50. PMID: 16351505
196. Pufulete M, Al-Ghnanien R, Rennie JA, Appleby P, Harris N, Gout S, Emery PW, Sanders TA. Influence of folate status on genomic DNA methylation in colonic mucosa of subjects without colorectal adenoma or cancer. *Br J Cancer* 2005 Mar 14;92(5):838-42. PMID: 15726099
197. Matsuo K, Ito H, Wakai K, Hirose K, Saito T, Suzuki T, Kato T, Hirai T, Kanemitsu Y, Hamajima H, Tajima K. One-carbon metabolism related gene polymorphisms interact with alcohol drinking to influence the risk of colorectal cancer in Japan. *Carcinogenesis* 2005 Dec;26(12):2164-71. PMID: 16051637
198. Hirose M, Kono S, Tabata S, Ogawa S, Yamaguchi K, Mineshita M, Hagiwara T, Yin G, Lee KY, Tsuji A, Ikeda N. Genetic polymorphisms of methylenetetrahydrofolate reductase and aldehyde dehydrogenase 2, alcohol use and risk of colorectal adenomas: Self-Defense Forces Health Study. *Cancer Sci* 2005 Aug;96(8):513-8. PMID: 16108833
199. Larsson SC, Giovannucci E, Wolk A. Vitamin B6 intake, alcohol consumption, and colorectal cancer: a longitudinal population-based cohort of women. *Gastroenterology* 2005 Jun;128(7):1830-7. PMID: 15940618
200. van Engeland M, Weijenberg MP, Roemen GM, Brink M, de Bruine AP, Goldbohm RA, van den Brandt PA, Baylin SB, de Goeij AF, Herman JG. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. *Cancer Res* 2003 Jun 15;63(12):3133-7. PMID: 12810640
201. Chen J, Giovannucci E, Hankinson SE, Ma J, Willett WC, Spiegelman D, Kelsey KT, Hunter DJ. A prospective study of methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms, and risk of colorectal adenoma. *Carcinogenesis* 1998 Dec;19(12):2129-32. PMID: 9886567
202. Curtin K, Slattery ML, Ulrich CM, Bigler J, Levin TR, Wolff RK, Albertsen H, Potter JD, Samowitz WS. Genetic polymorphisms in one-carbon metabolism: Associations with CpG island methylator phenotype (CIMP) in colon cancer and the modifying effects of diet. *Carcinogenesis* 2007 Aug;28(8):1672-9. PMID: 17449906
203. Heijmans BT, Boer JM, Suchiman HE, Cornelisse CJ, Westendorp RG, Kromhout D, Feskens EJ, Slagboom PE. A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. *Cancer Res* 2003 Mar 15;63(6):1249-53. PMID: 12649184
204. Harnack L, Jacobs DR Jr, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer* 2002;43(2):152-8. PMID: 12588695
205. Kim YI, Fawaz K, Knox T, Lee YM, Norton R, Arora S, Paiva L, Mason JB. Colonic mucosal concentrations of folate correlate well with blood measurements of folate status in persons with colorectal polyps. *Am J Clin Nutr* 1998 Oct;68(4):866-72. PMID: 9771864
206. Chen J, Giovannucci E, Kelsey K, Rimm EB, Stampfer MJ, Colditz GA, Spiegelman D, Willett WC, Hunter DJ. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res* 1996 Nov 1;56(21):4862-4. PMID: 8895734
207. Boutron-Ruault MC, Senesse P, Faivre J, Couillaud C, Belghiti C. Folate and alcohol intakes: Related or independent roles in the adenoma-carcinoma sequence? *Nutr Cancer* 1996;26(3):337-46. PMID: 8910915
208. Jiang QT, Chen K, Ma XY, Miao XP, Yao KY, Yu WP, Li LY, Zhu YM, Zhou HG. [A case-control study on the polymorphisms of methylenetetrahydrofolate reductases, drinking interaction and susceptibility in colorectal cancer]. *Chung Hua Liu Hsing Ping Hsueh Tsa Chih* 2004 Jul;25(7):612-6. (Chi). PMID: 15308044

209. Eklof V, Van Guelpen B, Hultdin J, Johansson I, Hallmans G, Palmqvist R. The reduced folate carrier (RFC1) 80G > A and folate hydrolase 1 (FOLH1) 1561C > T polymorphisms and the risk of colorectal cancer: a nested case-referent study. *Scand J Clin Lab Invest* 2008;68(5):393-401. PMID: 19172696
210. Cao HX, Gao CM, Takezaki T, Wu JZ, Ding JH, Liu YT, Li SP, Su P, Cao J, Hamajima N, Tajima K. Genetic polymorphisms of methylenetetrahydrofolate reductase and susceptibility to colorectal cancer. *Asian Pac J Cancer Prev* 2008 Apr-Jun;9(2):203-8. PMID: 18712959
211. Schernhammer ES, Giovannucci E, Fuchs CS, Ogino S. A prospective study of dietary folate and vitamin B and colon cancer according to microsatellite instability and KRAS mutational status. *Cancer Epidemiol Biomarkers Prev* 2008 Oct;17(10):2895-8. PMID: 18843035
212. Lightfoot TJ, Barrett JH, Bishop T, Northwood EL, Smith G, Wilkie MJ, Steele RJ, Carey FA, Key TJ, Wolf R, Forman D. Methylenetetrahydrofolate reductase genotype modifies the chemopreventive effect of folate in colorectal adenoma, but not colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2008 Sep;17(9):2421-30. PMID: 18703816
213. Dahlin AM, Van Guelpen B, Hultdin J, Johansson I, Hallmans G, Palmqvist R. Plasma vitamin B12 concentrations and the risk of colorectal cancer: a nested case-referent study. *Int J Cancer* 2008 May 1;122(9):2057-61. PMID: 18092327
214. Hazra A, Wu K, Kraft P, Fuchs CS, Giovannucci EL, Hunter DJ. Twenty-four non-synonymous polymorphisms in the one-carbon metabolic pathway and risk of colorectal adenoma in the Nurses' Health Study. *Carcinogenesis* 2007 Jul;28(7):1510-9. PMID: 17389618
215. Ashktorab H, Begum R, Akhgar A, Smoot DT, Elbedawi M, Daremipouran M, Zhao A, Momen B, Giardiello FM. Folate status and risk of colorectal polyps in African Americans. *Dig Dis Sci* 2007 Jun;52(6):1462-70. PMID: 17372834
216. Slattery ML, Curtin K, Sweeney C, Levin TR, Potter J, Wolff RK, Albertsen H, Samowitz WS. Diet and lifestyle factor associations with CpG island methylator phenotype and BRAF mutations in colon cancer. *Int J Cancer* 2007 Feb 1;120(3):656-63. PMID: 17096326
217. Wang J, Gajalakshmi V, Jiang J, Kuriki K, Suzuki S, Nagaya T, Nakamura S, Akasaka S, Ishikawa H, Tokudome S. Associations between 5,10-methylenetetrahydrofolate reductase codon 677 and 1298 genetic polymorphisms and environmental factors with reference to susceptibility to colorectal cancer: a case-control study in an Indian population. *Int J Cancer* 2006 Feb 15;118(4):991-7. PMID: 16152599
218. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE. The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2005 May;14(5):1198-203. PMID: 15894672
219. Jiang Q, Chen K, Ma X, Li Q, Yu W, Shu G, Yao K. Diets, polymorphisms of methylenetetrahydrofolate reductase, and the susceptibility of colon cancer and rectal cancer. *Cancer Detect Prev* 2005;29(2):146-54. PMID: 15829374
220. Yin G, Kono S, Toyomura K, Hagiwara T, Nagano J, Mizoue T, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Yasunami Y, Maekawa T, Takenaka K, Ichimiya H, Imaizumi N. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Sci* 2004 Nov;95(11):908-13. PMID: 15546509
221. Kim DH, Ahn YO, Lee BH, Tsuji E, Kiyohara C, Kono S. Methylenetetrahydrofolate reductase polymorphism, alcohol intake, and risks of colon and rectal cancers in Korea. *Cancer Lett* 2004 Dec 28;216(2):199-205. PMID: 15533596
222. Boyapati SM, Bostick RM, McGlynn KA, Fina MF, Roufail WM, Geisinger KR, Hebert JR, Coker A, Wargovich M. Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States). *Cancer Causes Control* 2004 Jun;15(5):493-501. PMID: 15286469
223. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. *Am J Epidemiol* 2004 Mar 1;159(5):423-43. PMID: 14977639
224. Giovannucci E, Chen J, Smith-Warner SA, Rimm EB, Fuchs CS, Palomeque C, Willett WC, Hunter DJ. Methylenetetrahydrofolate reductase, alcohol dehydrogenase, diet, and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2003 Oct;12(10):970-9. PMID: 14578131
225. Marugame T, Tsuji E, Kiyohara C, Eguchi H, Oda T, Shinchi K, Kono S. Relation of plasma folate and methylenetetrahydrofolate reductase C677T polymorphism to colorectal adenomas. *Int J Epidemiol* 2003 Feb;32(1):64-6. PMID: 12690011
226. Keku T, Millikan R, Worley K, Winkel S, Eaton A, Biscocho L, Martin C, Sandler R. 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. *Cancer Epidemiol Biomarkers Prev* 2002 Dec;11(12):1611-21. PMID: 12496052

227. Le Marchand L, White KK, Nomura AM, Wilkens LR, Selhub JS, Tiirikainen M, Goodman MT, Murphy SP, Henderson BE, Kolonel LN. Plasma levels of B vitamins and colorectal cancer risk: The multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2009 Aug;18(8):2195-201. PMID: 19661077
228. Steck SE, Keku T, Butler LM, Galanko J, Massa B, Millikan RC, Sandler RS. Polymorphisms in methionine synthase, methionine synthase reductase and serine hydroxymethyltransferase, folate and alcohol intake, and colon cancer risk. *J Nutrigenet Nutrigenomics* 2008 Jun;1(4):196-204. PMID: 19776626
229. Giovannucci E, Rimm EB, Ascherio A, Stampfer MJ, Colditz GA, Willett WC. Alcohol, low-methionine-low-folate diets, and risk of colon cancer in men. *J Natl Cancer Inst* 1995;87(4):265-73. PMID: 7707417
230. Levine AJ, Siegmund KD, Ervin CM, Diep A, Lee ER, Frankl HD, Haile RW. The methylenetetrahydrofolate reductase 677C-->T polymorphism and distal colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev* 2000 Jul;9(7):657-63. PMID: 10919734
231. Ma J, Stampfer MJ, Christensen B, Giovannucci E, Hunter DJ, Chen J, Willett WC, Selhub J, Hennekens CH, Gravel R, Rozen R. A polymorphism of the methionine synthase gene: association with plasma folate, vitamin B12, homocyst(e)ine, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 1999 Sep;8(9):825-9. PMID: 10498402
232. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev* 1999 Jun;8(6):513-8. PMID: 10385141
233. Ulrich CM, Kampman E, Bigler J, Schwartz SM, Chen C, Bostick R, Fosdick L, Beresford SA, Yasui Y, Potter JD. Colorectal adenomas and the C677T MTHFR polymorphism: evidence for gene-environment interaction? *Cancer Epidemiol Biomarkers Prev* 1999 Aug;8(8):659-68. PMID: 10744125
234. Kono S, Toyomura K, Yin G, Nagano J, Mizoue T. A case-control study of colorectal cancer in relation to lifestyle factors and genetic polymorphisms: design and conduct of the Fukuoka colorectal cancer study. *Asian Pac J Cancer Prev* 2004 Oct-Dec;5(4):393-400. PMID: 15546244
235. Simanowski UA, Homann N, Knuhl M, Arce L, Waldherr R, Conradt C, Bosch FX, Seitz HK. Increased rectal cell proliferation following alcohol abuse. *Gut* 2001 Sep;49(3):418-22. PMID: 11511565
236. Morita M, Tabata S, Tajima O, Yin G, Abe H, Kono S. Genetic polymorphisms of CYP2E1 and risk of colorectal adenomas in the Self Defense Forces Health Study. *Cancer Epidemiol Biomarkers Prev* 2008 Jul;17(7):1800-7. PMID: 18628434
237. Morita M, Le Marchand L, Kono S, Yin G, Toyomura K, Nagano J, Mizoue T, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunami Y, Takenaka K, Ichimiya H, Imaizumi N. Genetic polymorphisms of CYP2E1 and risk of colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Epidemiol Biomarkers Prev* 2009 Jan;18(1):235-41. PMID: 19124503
238. Pereira Serafim PV, Cotrim Guerreiro da Silva ID, Manoukias Forones N. Relationship between genetic polymorphism of CYP1A1 at codon 462 (Ile462Val) in colorectal cancer. *Int J Biol Markers* 2008 Jan-Mar;23(1):18-23. PMID: 18409146
239. Gao CM, Takezaki T, Wu JZ, Chen MB, Liu YT, Ding JH, Sugimura H, Cao J, Hamajima N, Tajima K. CYP2E1 Rsa I polymorphism impacts on risk of colorectal cancer association with smoking and alcohol drinking. *World J Gastroenterol* 2007 Nov 21;13(43):5725-30. PMID: 17963298
240. Ishibe N, Stampfer M, Hunter DJ, Hennekens C, Kelsey KT. A prospective study of cytochrome P450 1A1 polymorphisms and colorectal cancer risk in men. *Cancer Epidemiol Biomarkers Prev* 2000 Aug;9(8):855-6. PMID: 10952105
241. Jung AY, Poole EM, Bigler J, Whitton J, Potter JD, Ulrich CM. DNA methyltransferase and alcohol dehydrogenase: gene-nutrient interactions in relation to risk of colorectal polyps. *Cancer Epidemiol Biomarkers Prev* 2008 Feb;17(2):330-8. PMID: 18268116
242. Yokoyama A, Muramatsu T, Ohmori T, Yokoyama T, Okuyama K, Takahashi H, Hasegawa Y, Higuchi S, Maruyama K, Shirakura K, Ishii H. Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis* 1998 Aug;19(8):1383-7. PMID: 9744533
243. Gao CM, Takezaki T, Wu JZ, Zhang XM, Cao HX, Ding JH, Liu YT, Li SP, Cao J, Matsuo K, Hamajima N, Tajima K. Polymorphisms of alcohol dehydrogenase 2 and aldehyde dehydrogenase 2 and colorectal cancer risk in Chinese males. *World J Gastroenterol* 2008 Aug 28;14(32):5078-83. PMID: 18763293
244. Jelski W, Zalewski B, Chrostek L, Szmitkowski M. Alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) activity in the sera of patients with colorectal cancer. *Clin Exp Med* 2007 Dec;7(4):154-7. PMID: 18188528

245. Yin G, Kono S, Toyomura K, Moore MA, Nagano J, Mizoue T, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Yasunami Y, Maekawa T, Takenaka K, Ichimiya H, Imaizumi N. Alcohol dehydrogenase and aldehyde dehydrogenase polymorphisms and colorectal cancer: the Fukuoka Colorectal Cancer Study. *Cancer Sci* 2007 Aug;98(8):1248-53. PMID: 17517051
246. Kuriki K, Hamajima N, Chiba H, Kanemitsu Y, Hirai T, Kato T, Saito T, Matsuo K, Koike K, Tokudome S, Tajima K. Relation of the CD36 gene A52C polymorphism to the risk of colorectal cancer among Japanese, with reference to with the aldehyde dehydrogenase 2 gene Glu487Lys polymorphism and drinking habit. *Asian Pac J Cancer Prev* 2005 Jan-Mar;6(1):62-8. PMID: 15780035
247. Tiemersma EW, Wark PA, Ocke MC, Bunschoten A, Otten MH, Kok FJ, Kampman E. Alcohol consumption, alcohol dehydrogenase 3 polymorphism, and colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2003 May;12(5):419-25. PMID: 12750236
248. Matsuo K, Hamajima N, Hirai T, Kato T, Koike K, Inoue M, Takezaki T, Tajima K. Aldehyde dehydrogenase 2 (ALDH2) genotype affects rectal cancer susceptibility due to alcohol consumption. *J Epidemiol* 2002 Mar;12(2):70-6. PMID: 12033531
249. Takeshita T, Morimoto K, Yamaguchi N, Watanabe S, Todoroki I, Honjo S, Nakagawa K, Kono S. Relationships between cigarette smoking, alcohol drinking, the ALDH2 genotype and adenomatous types of colorectal polyps in male self-defense force officials. *J Epidemiol* 2000 Nov;10(6):366-71. PMID: 11210104
250. Murata M, Tagawa M, Watanabe S, Kimura H, Takeshita T, Morimoto K. Genotype difference of aldehyde dehydrogenase 2 gene in alcohol drinkers influences the incidence of Japanese colorectal cancer patients. *Jpn J Cancer Res* 1999 Jul;90(7):711-9. PMID: 10470282
251. Yin SJ, Liao CS, Lee YC, Wu CW, Jao SW. Genetic polymorphism and activities of human colon alcohol and aldehyde dehydrogenases: no gender and age differences. *Alcohol Clin Exp Res* 1994 Oct;18(5):1256-60. PMID: 7847616
252. Yang H, Zhou Y, Zhou Z, Liu J, Yuan X, Matsuo K, Takezaki T, Tajima K, Cao J. A novel polymorphism rs1329149 of CYP2E1 and a known polymorphism rs671 of ALDH2 of alcohol metabolizing enzymes are associated with colorectal cancer in a southwestern Chinese population. *Cancer Epidemiol Biomarkers Prev* 2009 Sep;18(9):2522-7. PMID: 19706845
253. Diorio C, Brisson J, Berube S, Pollak M. Intact and total insulin-like growth factor-binding protein-3 (IGFBP-3) levels in relation to breast cancer risk factors: a cross-sectional study. *Breast Cancer Res* 2008;10(3):R42. PMID: 18471292
254. Cleveland RJ, Gammon MD, Edmiston SN, Teitelbaum SL, Britton JA, Terry MB, Eng SM, Neugut AI, Santella RM, Conway K. IGF1 CA repeat polymorphisms, lifestyle factors and breast cancer risk in the long island breast cancer study project. *Carcinogenesis* 2006 Apr;27(4):758-65. PMID: 16332723
255. Cruz-Correa M, Cui H, Giardiello FM, Powe NR, Hyland L, Robinson A, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Feinberg AP. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. *Gastroenterology* 2004 Apr;126(4):964-70. PMID: 15057734
256. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst* 1999 Apr 7;91(7):620-5. PMID: 10203281
257. Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S, Japan Public Health Center-Based Prospective Study Group. Plasma C-reactive protein and risk of colorectal cancer in a nested case-control study: Japan Public Health Center-based prospective study. *Cancer Epidemiol Biomarkers Prev* 2006 Apr;15(4):690-5. PMID: 16614110
258. Otake T, Uezono K, Takahashi R, Fukumoto J, Tabata S, Abe H, Tajima O, Mizoue T, Ohnaka K, Kono S. C-reactive protein and colorectal adenomas: Self Defense Forces Health Study. *Cancer Sci* 2009 Apr;100(4):709-14. PMID: 19469014
259. Diergaarde B, Braam H, van Muijen GN, Ligtenberg MJ, Kok FJ, Kampman E. Dietary factors and microsatellite instability in sporadic colon carcinomas. *Cancer Epidemiol Biomarkers Prev* 2003 Nov;12(11 Pt 1):1130-6. PMID: 14652271
260. Diergaarde B, van Geloof WL, van Muijen GN, Kok FJ, Kampman E. Dietary factors and the occurrence of truncating APC mutations in sporadic colon carcinomas: a Dutch population-based study. *Carcinogenesis* 2003 Feb;24(2):283-90. PMID: 12584179
261. La Vecchia C, Negri E, Pelucchi C, Franceschi S. Dietary folate and colorectal cancer. *Int J Cancer* 2002 Dec 10;102(5):545-7. PMID: 12432561
262. Faivre J, Boutron MC, Senesse P, Couillaud C, Belghiti C, Meny B. Environmental and familial risk factors in relation to the colorectal adenoma-carcinoma sequence: results of a case-control study in Burgundy (France). *Eur J Cancer Prev* 1997 Apr;6(2):127-31. PMID: 9237060

263. Slattery ML, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? *Nutr Cancer* 1997;28(1):52-62. PMID: 9200151
264. Tsong WH, Koh WP, Yuan JM, Wang R, Sun CL, Yu MC. Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. *Br J Cancer* 2007 Mar 12;96(5):821-7. PMID: 17311023
265. Mizoue T, Yamaji T, Tabata S, Yamaguchi K, Shimizu E, Mineshita M, Ogawa S, Kono S. Dietary patterns and colorectal adenomas in Japanese men: the Self-Defense Forces Health Study. *Am J Epidemiol* 2005 Feb 15;161(4):338-45. PMID: 15692077
266. Yamada K, Araki S, Tamura M, Sakai I, Takahashi Y, Kashihara H, Kono S. Case-control study of colorectal carcinoma in situ and cancer in relation to cigarette smoking and alcohol use (Japan). *Cancer Causes Control* 1997 Sep;8(5):780-5. PMID: 9328201
267. Glynn SA, Albanes D, Pietinen P, Brown CC, Rautalahti M, Tangrea JA, Taylor PR, Virtamo J. Alcohol consumption and risk of colorectal cancer in a cohort of Finnish men. *Cancer Causes Control* 1996 Mar;7(2):214-23. PMID: 8740734
268. Boutron MC, Faivre J, Dop MC, Quipourt V, Senesse P. Tobacco, alcohol, and colorectal tumors: a multistep process. *Am J Epidemiol* 1995 Jun 1;141(11):1038-46. PMID: 7771440
269. Martinez ME, McPherson RS, Annegers JF, Levin B. Cigarette smoking and alcohol consumption as risk factors for colorectal adenomatous polyps. *J Natl Cancer Inst* 1995 Feb 15;87(4):274-9. PMID: 7707418
270. Honjo S, Kono S, Shinchi K, Imanishi K, Hirohata T. Cigarette smoking, alcohol use and adenomatous polyps of the sigmoid colon. *Jpn J Cancer Res* 1992 Aug;83(8):806-11. PMID: 1399817
271. Su LJ, Arab L. Alcohol consumption and risk of colon cancer: Evidence from the national health and nutrition examination survey I epidemiologic follow-up study. *Nutr Cancer* 2004;50(2):111-9. PMID: 15623458
272. Ho JW, Lam TH, Tse CW, Chiu LK, Lam HS, Leung PF, Ng KC, Ho SY, Woo J, Leung SS, Yuen ST. Smoking, drinking and colorectal cancer in Hong Kong Chinese: A case-control study. *Int J Cancer* 2004 Apr 20;109(4):587-97. PMID: 14991582
273. Pedersen A, Johansen C, Gronbaek M. Relations between amount and type of alcohol and colon and rectal cancer in a Danish population based cohort study. *Gut* 2003 Jun;52(6):861-7. PMID: 12740343
274. Ye W, Romelsjo A, Augustsson K, Adami HO, Nyren O. No excess risk of colorectal cancer among alcoholics followed for up to 25 years. *Br J Cancer* 2003 Apr 7;88(7):1044-6. PMID: 12671702
275. Sharpe CR, Siemiatycki J, Rachet B. Effects of alcohol consumption on the risk of colorectal cancer among men by anatomical subsite (Canada). *Cancer Causes Control* 2002;13(5):483-91. PMID: 12146853
276. Jedrychowski W, Steindorf K, Popiela T, Wahrendorf J, Tobiasz-Adamczyk B, Kulig J, Penar A, Hartleb M. Alcohol consumption and the risk of colorectal cancer at low levels of micronutrient intake. *Med Sci Monit* 2002;8(5):CR357-63. PMID: 12011778
277. Kune S, Kune GA, Watson LF. Case-control study of alcoholic beverages as etiologic factors: The Melbourne Colorectal Cancer Study. *Nutr Cancer* 1987;9(1):43-56. PMID: 3808969
278. Bongaerts BW, de Goeij AF, van den Brandt PA, Weijenberg MP. Alcohol and the risk of colon and rectal cancer with mutations in the K-ras gene. *Alcohol* 2006 Apr;38(3):147-54. PMID: 16905440
279. Terry MB, Neugut AI, Mansukhani M, Wayne J, Harpaz N, Hibshoosh H. Tobacco, alcohol, and p53 overexpression in early colorectal neoplasia. *BMC Cancer* 2003 Nov 6;3:29. PMID: 14604438
280. Bongaerts BW, de Goeij AF, de Vogel S, van den Brandt PA, Goldbohm RA, Weijenberg MP. Alcohol consumption and distinct molecular pathways to colorectal cancer. *Br J Nutr* 2007 Mar;97(3):430-4. PMID: 17313702
281. Mitrou PN, Watson MA, Loktionov AS, Cardwell C, Gunter MJ, Atkin WS, Macklin CP, Cecil T, Bishop TD, Primrose J, Bingham SA. MTHFR (C677T and A1298C) polymorphisms and risk of sporadic distal colorectal adenoma in the UK Flexible Sigmoidoscopy Screening Trial (United Kingdom). *Cancer Causes Control* 2006 Aug;17(6):793-801. PMID: 16783607
282. Wei Y, Wang L, Lan P, Zhao H, Pan Z, Huang J, Lu J, Wang J. The association between -1304T>G polymorphism in the promoter of MKK4 gene and the risk of sporadic colorectal cancer in southern Chinese population. *Int J Cancer* 2009 Oct 15;125(8):1876-83. PMID: 19610067
283. Andersen V, Agerstjerne L, Jensen D, Ostergaard M, Saebo M, Hamfjord J, Kure E, Vogel U. The multidrug resistance 1 (MDR1) gene polymorphism G-rs3789243-A is not associated with disease susceptibility in Norwegian patients with colorectal adenoma and colorectal cancer; a case control study. *BMC Med Genet* 2009;10:18. PMID: 19250544
284. Hansen RD, Sorensen M, Tjonneland A, Overvad K, Wallin H, Raaschou-Nielsen O, Vogel U. XPA A23G, XPC Lys939Gln, XPD Lys751Gln and XPD Asp312Asn polymorphisms, interactions with smoking, alcohol and dietary factors, and risk of colorectal cancer. *Mutat Res* 2007 Jun 1;619(1-2):68-80. PMID: 17363013

285. Mitrou PN, Watson MA, Loktionov AS, Cardwell C, Gunter MJ, Atkin WS, Macklin CP, Cecil T, Bishop DT, Primrose J, Bingham SA. Role of NQO1C609T and EPHX1 gene polymorphisms in the association of smoking and alcohol with sporadic distal colorectal adenomas: results from the UKFSS Study. *Carcinogenesis* 2007 Apr;28(4):875-82. PMID: 17082176
286. Chen K, Jin M, Zhu Y, Jiang Q, Yu W, Ma X, Yao K. Genetic polymorphisms of the uridine diphosphate glucuronosyltransferase 1A7 and colorectal cancer risk in relation to cigarette smoking and alcohol drinking in a Chinese population. *J Gastroenterol Hepatol* 2006 Jun;21(6):1036-41. PMID: 16724991
287. Skjelbred CF, Saebo M, Wallin H, Nexø BA, Hagen PC, Lothe IM, Aase S, Johnson E, Hansteen IL, Vogel U, Kure EH. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. *BMC Cancer* 2006;6:67. PMID: 16542436
288. Chen J, Kyte C, Chan W, Wetmur JG, Fuchs CS, Giovannucci E. Polymorphism in the thymidylate synthase promoter enhancer region and risk of colorectal adenomas. *Cancer Epidemiol Biomarkers Prev* 2004 Dec;13(12):2247-50. PMID: 15598787
289. Zhu ZZ, Wang AZ, Jia HR, Jin XX, He XL, Hou LF, Zhu G. Association of the TP53 codon 72 polymorphism with colorectal cancer in a Chinese population. *Jpn J Clin Oncol* 2007 May;37(5):385-90. PMID: 17599946
290. Hansen RD, Sorensen M, Tjonneland A, Overvad K, Wallin H, Raaschou-Nielsen O, Vogel U. A haplotype of polymorphisms in ASE-1, RAI and ERCC1 and the effects of tobacco smoking and alcohol consumption on risk of colorectal cancer: a Danish prospective case-cohort study. *BMC Cancer* 2008;8:54. PMID: 18289367
291. Vogel U, Christensen J, Dybdahl M, Friis S, Hansen RD, Wallin H, Nexø BA, Raaschou-Nielsen O, Andersen PS, Overvad K, Tjonneland A. Prospective study of interaction between alcohol, NSAID use and polymorphisms in genes involved in the inflammatory response in relation to risk of colorectal cancer. *Mutat Res* 2007 Nov 1;624(1-2):88-100. PMID: 17544013
292. Hong YC, Lee KH, Kim WC, Choi SK, Woo ZH, Shin SK, Kim H. Polymorphisms of XRCC1 gene, alcohol consumption and colorectal cancer. *Int J Cancer* 2005 Sep 1;116(3):428-32. PMID: 15800946
293. Tranah GJ, Giovannucci E, Ma J, Fuchs C, Hunter DJ. APC Asp1822Val and Gly2502Ser polymorphisms and risk of colorectal cancer and adenoma. *Cancer Epidemiol Biomarkers Prev* 2005 Apr;14(4):863-70. PMID: 15824157
294. Hansen RD, Krath BN, Frederiksen K, Tjonneland A, Overvad K, Roswall N, Loft S, Dragsted LO, Vogel U, Raaschou-Nielsen O. GPX1 Pro198Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking, and risk of colorectal cancer. *Mutat Res* 2009 May 12;664(1-2):13-9. PMID: 19428376
295. Marietta C, Thompson LH, Lamerdin JE, Brooks PJ. Acetaldehyde stimulates FANCD2 monoubiquitination, H2AX phosphorylation, and BRCA1 phosphorylation in human cells in vitro: implications for alcohol-related carcinogenesis. *Mutat Res* 2009 May 12;664(1-2):77-83. PMID: 19428384
296. Taibi G, Carruba G, Coccidiferro L, Granata OM, Nicotra CM. Low levels of both xanthine dehydrogenase and cellular retinol binding protein are responsible for retinoic acid deficiency in malignant human mammary epithelial cells. *Ann N Y Acad Sci* 2009 Feb;1155:268-72. PMID: 19250215
297. Jin W, Chen Y, Di GH, Miron P, Hou YF, Gao H, Shao ZM. Estrogen receptor (ER) beta or p53 attenuates ERalpha-mediated transcriptional activation on the BRCA2 promoter. *J Biol Chem* 2008 Oct 31;283(44):29671-80. PMID: 18765668
298. Maciel ME, Castro GD, Castro JA. Inhibition of the rat breast cytosolic bioactivation of ethanol to acetaldehyde by some plant polyphenols and folic acid. *Nutr Cancer* 2004;49(1):94-9. PMID: 15456641
299. Jordao AA Jr, Chiarello PG, Arantes MR, Meirelles MS, Vannucchi H. Effect of an acute dose of ethanol on lipid peroxidation in rats: action of vitamin E. *Food Chem Toxicol* 2004 Mar;42(3):459-64. PMID: 14871588
300. Stevens RG, Davis S, Mirick DK, Kheifets L, Kaune W. Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. *Epidemiology* 2000;11(6):660-5. PMID: 11055626
301. Colantoni A, La Paglia N, De Maria N, Emanuele MA, Emanuele NV, Idilman R, Harig J, Van Thiel DH. Influence of sex hormonal status on alcohol-induced oxidative injury in male and female rat liver. *Alcohol Clin Exp Res* 2000 Sep;24(9):1467-73. PMID: 11003215
302. Jones PA, Baker VA, Irwin AJ, Earl LK. Interpretation of the in vitro proliferation response of MCF-7 cells to potential oestrogens and non-oestrogenic substances. *Toxicol In Vitro* 1998 Aug;12(4):373-82. PMID: 20654419
303. Vincon P, Wunderer J, Simanowski UA, Koll M, Preedy VR, Peters TJ, Werner J, Waldherr R, Seitz HK. Inhibition of alcohol-associated colonic hyperregeneration by alpha-tocopherol in the rat. *Alcohol Clin Exp Res* 2003 Jan;27(1):100-6. PMID: 12544013

304. Leuratti C, Watson MA, Deag EJ, Welch A, Singh R, Gottschalg E, Marnett LJ, Atkin W, Day NE, Shuker DE, Bingham SA. Detection of malondialdehyde DNA adducts in human colorectal mucosa: relationship with diet and the presence of adenomas. *Cancer Epidemiol Biomarkers Prev* 2002 Mar;11(3):267-73. PMID: 11895876
305. Parlesak A, Menzl I, Feuchter A, Bode JC, Bode C. Inhibition of retinol oxidation by ethanol in the rat liver and colon. *Gut* 2000 Dec;47(6):825-31. PMID: 11076882
306. Koivisto T, Salaspuro M. Aldehyde dehydrogenases of the rat colon: Comparison with other tissues of the alimentary tract and the liver. *Alcohol Clin Exp Res* 1996;20(3):551-5. PMID: 8727253
307. Nosova T, Jokelainen K, Kaihovaara P, Jousimies-Somer H, Siitonen A, Heine R, Salaspuro M. Aldehyde dehydrogenase activity and acetate production by aerobic bacteria representing the normal flora of human large intestine. *Alcohol* 1996 Nov;31(6):555-64. PMID: 9010546
308. Rosenberg DW, Mankowski DC. Induction of cyp2e-1 protein in mouse colon. *Carcinogenesis* 1994 Jan;15(1):73-8. PMID: 8293551
309. Jokelainen K, Roine RP, Vaananen H, Farkkila M, Salaspuro M. In vitro acetaldehyde formation by human colonic bacteria. *Gut* 1994 Sep;35(9):1271-4. PMID: 7959236
310. Shimizu M, Lasker JM, Tsutsumi M, Lieber CS. Immunohistochemical localization of ethanol-inducible P450IIE1 in the rat alimentary tract. *Gastroenterology* 1990 Oct;99(4):1044-53. PMID: 2203661
311. Laufer EM, Hartman TJ, Baer DJ, Gunter EW, Dorgan JF, Campbell WS, Clevidence BA, Brown ED, Albanes D, Judd JT, Taylor PR. Effects of moderate alcohol consumption on folate and vitamin B12 status in postmenopausal women. *Eur J Clin Nutr* 2004 Nov;58(11):1518-24. PMID: 15138463
312. Perel P, Roberts I, Sena E, Wheble P, Briscoe C, Sandercock P, Macleod M, Mignini LE, Jayaram P, Khan KS. Comparison of treatment effects between animal experiments and clinical trials: systematic review. *BMJ* 2007 Jan 27;334(7586):197-200. PMID: 17175568
313. Yi SW, Sull JW, Linton JA, Nam CM, Ohrr H. Alcohol consumption and digestive cancer mortality in Koreans: The Kangwha Cohort Study. *J Epidemiol* 2010 May 5;20(3):204-11. Epub 2010 Mar 16. PMID: 20234107
314. Scheppach W, Bingham S, Boutron-Ruault MC, De Verdier MG, Moreno V, Nagengast FM, Reifen R, Riboli E, Seitz HK, Wahrendorf J. WHO consensus statement on the role of nutrition in colorectal cancer. *Eur J Cancer Prev* 1999;8(1):57-62. PMID: 10091044
315. Seitz HK, Simanowski UA, Osswald BR. Epidemiology and pathophysiology of ethanol-associated gastrointestinal cancer. *Pharmacogenetics* 1992 Dec;2(6):278-87. PMID: 1306128
316. Weisburger JH. Proceedings: Chemical carcinogenesis in the gastrointestinal tract. *Proc Natl Cancer Conf* 1972;7:465-73. PMID: 4587656
317. Kleinjans JC, Moonen EJ, Dallinga JW, Albering HJ, Van den Bogaard AE, Van Schooten FJ. Polycyclic aromatic hydrocarbons in whiskies. *Lancet* 1996;348(9043):1731. PMID: 8973440
318. Potter JD, McMichael AJ, Hartshorne JM. Alcohol and beer consumption in relation to cancers of bowel and lung: An extended correlation analysis. *J Chronic Dis* 1982;35(11):833-42. PMID: 7142362
319. Siegmund S, Haas S, Schneider A, Singer MV. Animal models in gastrointestinal alcohol research - A short appraisal of the different models and their results. *Best Pract Res Clin Gastroenterol* 2003 Aug;17(4):519-42. PMID: 12828953
320. Aye MM, Ma C, Lin H, Bower KA, Wiggins RC, Luo J. Ethanol-induced in vitro invasion of breast cancer cells: The contribution of MMP-2 by fibroblasts. *Int J Cancer* 2004 Dec 10;112(5):738-46. PMID: 15386367
321. Luo J. Role of matrix metalloproteinase-2 in ethanol-induced invasion by breast cancer cells. *J Gastroenterol Hepatol* 2006;21(Suppl 3):S65-8.
322. Luo J, Miller MW. Ethanol enhances erbB-mediated migration of human breast cancer cells in culture. *Breast Cancer Res Treat* 2000 Sep;63(1):61-9. PMID: 11079160
323. Ma C, Lin H, Leonard SS, Shi X, Ye J, Luo J. Overexpression of ErbB2 enhances ethanol-stimulated intracellular signaling and invasion of human mammary epithelial and breast cancer cells in vitro. *Oncogene* 2003 Aug 14;22(34):5281-90. PMID: 12917629
324. Meng Q, Gao B, Goldberg ID, Rosen EM, Fan S. Stimulation of cell invasion and migration by alcohol in breast cancer cells. *Biochem Biophys Res Commun* 2000 Jul 5;273(2):448-53. PMID: 10873626
325. McGarrity TJ, Nelson RL. Dimethylhydrazine and ethanol. *Dis Colon Rectum* 1986;29(4):289-90. PMID: 3948624
326. Larsen NS. Study suggests mechanism for alcohol-breast cancer link. *J Natl Cancer Inst* 1993;85(9):700-1. PMID: 8478954
327. Colombo LL, Lopez MC, Chen G, Watson RR. In vitro response of v-Ha-ras transgenic mouse lymphocytes after in vivo treatment with alcohol. *Immunopharmacol Immunotoxicol* 2001 Nov;23(4):597-606. PMID: 11792018

328. Fiala ES, Sohn OS, Hamilton SR. Effects of chronic dietary ethanol on in vivo and in vitro metabolism of methylazoxymethanol and on methylazoxymethanol-induced DNA methylation in rat colon and liver. *Cancer Res* 1987;47(22):5939-43. PMID: 3664495
329. Zedeck MS. Colon carcinogenesis and the role of dehydrogenase enzyme activity: Inhibition of tumorigenesis by pyrazole. *Prev Med* 1980;9(3):346-51. PMID: 7208443
330. Weisburger JH, Wynder EL. The role of genotoxic carcinogens and of promoters in carcinogenesis and in human cancer causation. *Acta Pharmacol Toxicol (Copenh)* 1984;55 Suppl 2:53-68. PMID: 6385622
331. Agrawal A, Murphy RF, Agrawal DK. DNA methylation in breast and colorectal cancers. *Mod Pathol* 2007 Jul;20(7):711-21. PMID: 17464311
332. Alberts DS. Reducing the risk of colorectal cancer by intervening in the process of carcinogenesis: a status report. *Cancer J* 2002 May-Jun;8(3):208-21. PMID: 12074318
333. Ambrosone CB. Oxidants and antioxidants in breast cancer. *Antioxid Redox Signal* 2000;2(4):903-17. PMID: 11213491
334. Arasaradnam RP, Commane DM, Bradburn D, Mathers JC. A review of dietary factors and its influence on DNA methylation in colorectal carcinogenesis. *Epigenetics* 2008 Jul-Aug;3(4):193-8. PMID: 18682688
335. Folate, alcohol, methionine, and colon cancer risk: Is there a unifying theme? *Nutr Rev* 1994;52(1):18-20.
336. Bailey LB. Folate, methyl-related nutrients, alcohol, and the MTHFR 677C-->T polymorphism affect cancer risk: intake recommendations. *J Nutr* 2003 Nov;133(11 Suppl 1):3748S-53S. PMID: 14608109
337. Blot WJ. Alcohol and cancer. *Cancer Res* 1992;52(7 Suppl):2119s-23s. PMID: 1544150
338. Boffetta P, Hashibe M. Alcohol and cancer. *Lancet Oncol* 2006 Feb;7(2):149-56. PMID: 16455479
339. Bosetti C, Altieri A, La Vecchia C. Diet and environmental carcinogenesis in breast/gynaecological cancers. *Curr Opin Obstet Gynecol* 2002 Feb;14(1):13-8. PMID: 11801871
340. Brown LM. Epidemiology of alcohol-associated cancers. *Alcohol* 2005 Apr;35(3):161-8. PMID: 16054977
341. Campos FG, Logullo Waitzberg AG, Kiss DR, Waitzberg DL, Habr-Gama A, Gama-Rodrigues J. Diet and colorectal cancer: current evidence for etiology and prevention. *Nutr Hosp* 2005 Jan-Feb;20(1):18-25. PMID: 15762416
342. Chhabra SK, Souliotis VL, Kyrtopoulos SA, Anderson LM. Nitrosamines, alcohol, and gastrointestinal tract cancer: Recent epidemiology and experimentation. *In Vivo* 1996;10(3):265-84. PMID: 8797028
343. Correa Lima MP, Gomes-da-Silva MH. Colorectal cancer: lifestyle and dietary factors. *Nutr Hosp* 2005 Jul-Aug;20(4):235-41. PMID: 16045124
344. Dossus L, Kaaks R. Nutrition, metabolic factors and cancer risk. *Baillieres Best Pract Res Clin Endocrinol Metab* 2008 Aug;22(4):551-71. PMID: 18971118
345. Dumitrescu RG, Cotarla I. Understanding breast cancer risk -- where do we stand in 2005? *J Cell Mol Med* 2005 Jan-Mar;9(1):208-21. PMID: 15784178
346. Ferguson LR, Bronzetti G, De Flora S. Mechanistic approaches to chemoprevention of mutation and cancer. *Mutat Res* 2005 Dec 11;591(1-2):3-7. PMID: 16095634
347. Filion K. Rectal proliferation and alcohol abuse. *Gut* 2002 Oct;51(4):611; author reply 611-2. PMID: 12235092
348. Forman MR, Hursting SD, Umar A, Barrett JC. Nutrition and cancer prevention: a multidisciplinary perspective on human trials. *Annu Rev Nutr* 2004;24:223-54. PMID: 15189120
349. Fraumeni JF Jr. Epidemiological opportunities in alcohol-related cancer. *Cancer Res* 1979 Jul;39(7 Pt 2):2851-2. PMID: 445491
350. Washington State Department of Labor and Industries. Work-related carpal tunnel syndrome diagnosis and treatment guideline. Olympia (WA): Washington State Department of Labor and Industries; 2009 Apr. 16 p.
351. Giovannucci E. Epidemiologic studies of folate and colorectal neoplasia: a review. *J Nutr* 2002 Aug;132(8 Suppl):2350S-5S. PMID: 12163691
352. Goodwin PJ. Host-related factors in breast cancer: An underappreciated piece of the puzzle? *J Clin Oncol* 2008 Jul 10;26(20):3299-300. PMID: 18612145
353. Hamid A, Wani NA, Kaur J. New perspectives on folate transport in relation to alcoholism-induced folate malabsorption--association with epigenome stability and cancer development. *FEBS J* 2009 Apr;276(8):2175-91. PMID: 19292860
354. Heavey PM, McKenna D, Rowland IR. Colorectal cancer and the relationship between genes and the environment. *Nutr Cancer* 2004;48(2):124-41. PMID: 15231447
355. Homann N, Seitz HK, Wang XD, Yokoyama A, Singletary KW, Ishii H. Mechanisms in alcohol-associated carcinogenesis. *Alcohol Clin Exp Res* 2005 Jul;29(7):1317-20. PMID: 16088994
356. Huxley R, Asia Pacific Cohort Studies Collaboration. The role of lifestyle risk factors on mortality from colorectal cancer in populations of the Asia-Pacific region. *Asian Pac J Cancer Prev* 2007 Apr-Jun;8(2):191-8. PMID: 17696730
357. Key TJ, Verkasalo PK. Endogenous hormones and the aetiology of breast cancer. *Breast Cancer Res* 1999;1(1):18-21. PMID: 11250677

358. Kim DH. The interactive effect of methyl-group diet and polymorphism of methylenetetrahydrofolate reductase on the risk of colorectal cancer. *Mutat Res* 2007 Sep 1;622(1-2):14-8. PMID: 17602711
359. Klatsky AL. Diet, alcohol, and health: A story of connections, confounders, and cofactors. *Am J Clin Nutr* 2001;74(3):279-80. PMID: 11522547
360. La Vecchia C. Nutritional factors and cancers of the breast, endometrium and ovary. *Eur J Cancer Clin Oncol* 1989 Dec;25(12):1945-51. PMID: 2698810
361. Lands WE. A review of alcohol clearance in humans. *Alcohol* 1998 Feb;15(2):147-60. PMID: 9476961
362. Ledermann S. [Cancer, tobacco, wine and alcohol; analysis of their influence, if such should be the case.]. *Concours Med* 1955 Mar 19;77(12):1218-25. PMID: 14365150
363. Li FY, Lai MD. Colorectal cancer, one entity or three. *J Zhejiang Univ Sci B* 2009 Mar;10(3):219-29. PMID: 19283877
364. Lieber CS. ALCOHOL: its metabolism and interaction with nutrients. *Annu Rev Nutr* 2000;20:395-430. PMID: 10940340
365. Lindahl R. Aldehyde dehydrogenases and their role in carcinogenesis. *Crit Rev Biochem Mol Biol* 1992;27(4-5):283-335. PMID: 1521460
366. Longnecker MP. Alcohol consumption and risk of cancer in humans: An overview. *Alcohol* 1995;12(2):87-96. PMID: 7772271
367. Longnecker MP. Do hormones link alcohol with breast cancer? *J Natl Cancer Inst* 1993;85(9):692-3. PMID: 8478949
368. Lowenfels AB. Alcohol and breast cancer. *Lancet* 1990;335(8699):1216. PMID: 1971051
369. Mason JB, Choi SW. Effects of alcohol on folate metabolism: implications for carcinogenesis. *Alcohol* 2005 Apr;35(3):235-41. PMID: 16054985
370. Nanri A, Moore MA, Kono S. Impact of C-reactive protein on disease risk and its relation to dietary factors. *Asian Pac J Cancer Prev* 2007 Apr-Jun;8(2):167-77. PMID: 17696726
371. Payne JE. Colorectal carcinogenesis. *Aust N Z J Surg* 1990 Jan;60(1):11-8. PMID: 2183744
372. Potter JD. Colon cancer--do the nutritional epidemiology, the gut physiology and the molecular biology tell the same story? *J Nutr* 1993 Feb;123(2 Suppl):418-23. PMID: 8429397
373. Potter JD. Risk factors for colon neoplasia--epidemiology and biology. *Eur J Cancer* 1995 Jul-Aug;31A(7-8):1033-8. PMID: 7576987
374. Poschl G, Stickel F, Wang XD, Seitz HK. Alcohol and cancer: genetic and nutritional aspects. *Proc Nutr Soc* 2004 Feb;63(1):65-71. PMID: 15070439
375. Pufulete M, Emery PW, Sanders TA. Folate, DNA methylation and colo-rectal cancer. *Proc Nutr Soc* 2003 May;62(2):437-45. PMID: 14506892
376. Purohit V. Can alcohol promote aromatization of androgens to estrogens? A review. *Alcohol* 2000 Nov;22(3):123-7. PMID: 11163119
377. Rampersaud GC, Bailey LB, Kauwell GP. Relationship of folate to colorectal and cervical cancer: review and recommendations for practitioners. *J Am Diet Assoc* 2002 Sep;102(9):1273-82. PMID: 12792626
378. Rogers AE, Conner MW. Alcohol and cancer. *Adv Exp Med Biol* 1986;206:473-95. PMID: 3035901
379. Rogers AE, Zeisel SH, Groopman J. Diet and carcinogenesis. *Carcinogenesis* 1993;14(11):2205-17. PMID: 8242845
380. Rothman KJ. Research and prevention priorities for alcohol carcinogenesis. *Environ Health Perspect* 1995 Nov;103 Suppl 8:161-3. PMID: 8741777
381. Sarkar DK, Liehr JG, Singletary KW. Role of estrogen in alcohol promotion of breast cancer and prolactinomas. *Alcohol Clin Exp Res* 2001 May;25(5 Suppl ISBRA):230S-6S. PMID: 11391076
382. Salaspuro MP, Mezey E. Acetaldehyde, microbes, and cancer of the digestive tract. *Crit Rev Clin Lab Sci* 2003;40(2):183-208. PMID: 12755455
383. Seitz HK, Becker P. Alcohol metabolism and cancer risk. *Alcohol Res Health* 2007;30(1):38-41, 44-7. PMID: 17718399
384. Seitz HK, Homann N. The role of acetaldehyde in alcohol-associated cancer of the gastrointestinal tract. *Novartis Found Symp* 2007;285:110-9; discussion 119. PMID: 17590990
385. Seitz HK, Maurer B. The relationship between alcohol metabolism, estrogen levels, and breast cancer risk. *Alcohol Res Health* 2007;30(1):42-3. PMID: 17718400
386. Seitz H, Poschl G. Alcohol and gastrointestinal cancer: Pathogenic mechanisms. *Addict Biol* 1997;2(1):19-33.
387. Seitz HK, Gartner U, Egerer G, Simanowski UA. Ethanol metabolism in the gastrointestinal tract and its possible consequences. *Alcohol Alcohol Suppl* 1994;2:157-62. PMID: 8974330
388. Seitz HK, Simanowski UA, Homann N, Waldherr R. Cell proliferation and its evaluation in the colorectal mucosa: effect of ethanol. *Z Gastroenterol* 1998 Aug;36(8):645-55. PMID: 9773483
389. Siegmund SV, Haas S, Singer MV. Animal models and their results in gastrointestinal alcohol research. *Dig Dis* 2006 Feb;23(3-4):181-94. PMID: 16508282

390. Simanowski UA, Stickel F, Maier H, Gartner U, Seitz HK. Effect of alcohol on gastrointestinal cell regeneration as a possible mechanism in alcohol-associated carcinogenesis. *Alcohol* 1995 Mar-Apr;12(2):111-5. PMID: 7772260
391. Stoll BA. Alcohol intake and late-stage promotion of breast cancer. *Eur J Cancer* 1999 Nov;35(12):1653-8. PMID: 10674009
392. Tan DJ, Barber JS, Shields PG. Alcohol drinking and breast cancer. *Breast Cancer Online* 2006 Apr;9(4):e15.
393. Taylor B, Rehm J. Moderate alcohol consumption and diseases of the gastrointestinal system: A review of pathophysiological processes. *Dig Dis* 2006 Feb;23(3-4):177-80. PMID: 16508281
394. Thies E, Siegers CP. Metabolic activation and tumourigenesis. *Prog Pharmacol Clin Pharmacol* 1989;7(2):199-214.
395. Tsigris C, Konstantakaki M, Xiromeritis C, Nikiteas N, Yannopouloa A. Animal models of carcinogenesis in the digestive system. *In Vivo* 2007 Sep;21(5):803-12. PMID: 18019415
396. Ulrich CM. Folate and cancer prevention: A closer look at a complex picture. *Am J Clin Nutr* 2007 Aug 1;86(2):271-3. PMID: 17684194
397. Walker AR, Burkitt DP. Colonic cancer--hypotheses of causation, dietary prophylaxis, and future research. *Am J Dig Dis* 1976 Oct;21(10):910-7. PMID: 1015500
398. Wang XD. Retinoids and alcohol-related carcinogenesis. *J Nutr* 2003 Jan;133(1):287S-90S. PMID: 12514311
399. Wang XD. Alcohol, vitamin A, and cancer. *Alcohol* 2005 Apr;35(3):251-8. PMID: 16054987
400. Weisburger JH, Spingarn NE, Wang YY, Vuolo LL. Assessment of the role of mutagens and endogenous factors in large bowel cancer. *Cancer Bull Univ Tex* 1981;33(4):124-9.
401. Weisburger JH. Worldwide prevention of cancer and other chronic diseases based on knowledge of mechanisms. *Mutat Res* 1998 Jun 18;402(1-2):331-7. PMID: 9675332
402. Welsch CW. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. *Cancer Res* 1985 Aug;45(8):3415-43. PMID: 3926298
403. Williams RR. Breast and thyroid cancer and malignant melanoma promoted by alcohol-induced pituitary secretion of prolactin, T.S.H. and M.S.H. *Lancet* 1976 May 8;1(7967):996-9. PMID: 57445
404. Winawer SJ, Shike M. Dietary factors in colorectal cancer and their possible effects on earlier stages of hyperproliferation and adenoma formation. *J Natl Cancer Inst* 1992;84(2):74-5. PMID: 1735881
405. Wynder EL. Nutritional carcinogenesis. *Ann N Y Acad Sci* 1977 Nov 30;300:360-78. PMID: 279278
406. Wynder EL. Environmental carcinogenesis. *Clin Bull* 1978;8(1):3-9. PMID: 352574
407. Nozawa H, Nakao W, Takata J, Arimoto-Kobayashi S, Kondo K. Inhibition of PhIP-induced mammary carcinogenesis in female rats by ingestion of freeze-dried beer. *Cancer Lett* 2006 Apr 8;235(1):121-9. PMID: 15946793
408. Martin AR, Villegas I, La Casa C, de la Lastra CA. Resveratrol, a polyphenol found in grapes, suppresses oxidative damage and stimulates apoptosis during early colonic inflammation in rats. *Biochem Pharmacol* 2004 Apr 1;67(7):1399-410. PMID: 15013856
409. Gierer A. On the temperature dependence and mechanism of action of alcohol dehydrogenase. *Biochim Biophys Acta* 1955 May;17(1):111-21. PMID: 13239634
410. Briviba K, Pan L, Rechkemmer G. Red wine polyphenols inhibit the growth of colon carcinoma cells and modulate the activation pattern of mitogen-activated protein kinases. *J Nutr* 2002 Sep;132(9):2814-8. PMID: 12221251
411. Caderni G, De Filippo C, Luceri C, Salvadori M, Giannini A, Biggeri A, Remy S, Cheynier V, Dolara P. Effects of black tea, green tea and wine extracts on intestinal carcinogenesis induced by azoxymethane in F344 rats. *Carcinogenesis* 2000 Nov;21(11):1965-9. PMID: 11062155
412. Cerda SR, Wilkinson J 4th, Thorgeirsdottir S, Broitman SA. R-(+)-perillyl alcohol-induced cell cycle changes, altered actin cytoskeleton, and decreased ras and p34(cdc2) expression in colonic adenocarcinoma SW480 cells. *J Nutr Biochem* 1999 Jan;10(1):19-30. PMID: 15539246
413. Depeint F, Bruce WR, Shangari N, Mehta R, O'Brien PJ. Mitochondrial function and toxicity: Role of B vitamins on the one-carbon transfer pathways. *Chem Biol Interact* 2006 Oct 27;163(1-2):113-32. PMID: 16814759
414. Farah IO. Assessment of cellular responses to oxidative stress using MCF-7 breast cancer cells, black seed (*N. Sativa L.*) extracts and H2O2. *Int J Environ Res Public Health* 2005 Dec;2(3-4):411-9. PMID: 16819096
415. Femia AP, Caderni G, Vignali F, Salvadori M, Giannini A, Biggeri A, Gee J, Przybylska K, Cheynier V, Dolara P. Effect of polyphenolic extracts from red wine and 4-OH-coumaric acid on 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Eur J Nutr* 2005 Mar;44(2):79-84. PMID: 15309424
416. Gonthier MP, Cheynier V, Donovan JL, Manach C, Morand C, Mila I, Lapierre C, Remesy C, Scalbert A. Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *J Nutr* 2003 Feb 1;133(2):461-7. PMID: 12566484

417. Hall CN, Badawi AF, O'Connor PJ, Saffhill R. The detection of alkylation damage in the DNA of human gastrointestinal tissues. *Br J Cancer* 1991 Jul;64(1):59-63. PMID: 1854628
418. Kabat GC, Rohan TE. Does excess iron play a role in breast carcinogenesis? An unresolved hypothesis. *Cancer Causes Control* 2007 Dec;18(10):1047-53. PMID: 17823849
419. Kabat GC, Miller AB, Jain M, Rohan TE. Dietary iron and heme iron intake and risk of breast cancer: a prospective cohort study. [erratum appears in *Cancer Epidemiol Biomarkers Prev* 2007 Nov;16(11):2519]. *Cancer Epidemiol Biomarkers Prev* 2007 Jun;16(6):1306-8. PMID: 17548704
420. Lagiou P, Samoli E, Lagiou A, Georgila C, Zourna P, Barbouni A, Gkiokas G, Vassilarou D, Tsikkinis A, Sfikas C, Sekeris CE, Hsieh CC, Adami HO, Trichopoulos D. Diet and expression of estrogen alpha and progesterone receptors in the normal mammary gland. *Cancer Causes Control* 2009 Jul;20(5):601-7. PMID: 19037733
421. Etique N, Chardard D, Chesnel A, Flament S, Grillier-Vuissoz I. Analysis of the effects of different alcohols on MCF-7 human breast cancer cells. *Ann N Y Acad Sci* 2004 Dec;1030:78-85. PMID: 15659783
422. Linz AL, Xiao R, Parker JG, Simpson PM, Badger TM, Simmen FA. Feeding of soy protein isolate to rats during pregnancy and lactation suppresses formation of aberrant crypt foci in their progeny's colons: Interaction of diet with fetal alcohol exposure. *J Carcinog* 2004 Oct 15;3:14. PMID: 15488141
423. Luceri C, Caderni G, Sanna A, Dolara P. Red wine and black tea polyphenols modulate the expression of cyclooxygenase-2, inducible nitric oxide synthase and glutathione-related enzymes in azoxymethane-induced F344 rat colon tumors. *J Nutr* 2002 Jun;132(6):1376-9. PMID: 12042461
424. Moon YJ, Wang X, Morris ME. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. *Toxicol In Vitro* 2006 Mar;20(2):187-210. PMID: 16289744
425. Morris JJ, Seifter E. The role of aromatic hydrocarbons in the genesis of breast cancer. *Med Hypotheses* 1992 Jul;38(3):177-84. PMID: 1513270
426. Nozawa H, Yoshida A, Tajima O, Katayama M, Sonobe H, Wakabayashi K, Kondo K. Intake of beer inhibits azoxymethane-induced colonic carcinogenesis in male Fischer 344 rats. *Int J Cancer* 2004 Jan 20;108(3):404-11. PMID: 14648707
427. Nozawa H, Tazumi K, Sato K, Yoshida A, Takata J, Arimoto-Kobayashi S, Kondo K. Inhibitory effects of beer on heterocyclic amine-induced mutagenesis and PhIP-induced aberrant crypt foci in rat colon. *Mutat Res Genet Toxicol Environ Mutagen* 2004 Apr 11;559(1-2):177-87. PMID: 15066585
428. Nozawa H, Nakao W, Zhao F, Kondo K. Dietary supplement of isohumulones inhibits the formation of aberrant crypt foci with a concomitant decrease in prostaglandin E2 level in rat colon. *Mol Nutr Food Res* 2005 Aug;49(8):772-8. PMID: 15968705
429. Peluso M, Airolidi L, Munnia A, Colombi A, Veglia F, Autrup H, Dunning A, Garte S, Gormally E, Malaveille C, Matullo G, Overvad K, Raaschou-Nielsen O, Clavel-Chapelon F, Linseisen J, Boeing H, Trichopoulou A, Palli D, Krogh V, Tumino R, Panico S, Bueno-De-Mesquita BH, Peeters PH, Kumle M, Agudo A, Ma. Bulky DNA adducts, 4-aminobiphenyl-haemoglobin adducts and diet in the European Prospective Investigation into Cancer and Nutrition (EPIC) prospective study. *Br J Nutr* 2008;100(3):489-95. PMID: 18275627
430. Reddy BS, Wang CX, Samaha H, Lubet R, Steele VE, Kelloff GJ, Rao CV. Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. *Cancer Res* 1997 Feb 1;57(3):420-5. PMID: 9012468
431. Robson EJ, Khaled WT, Abell K, Watson CJ. Epithelial-to-mesenchymal transition confers resistance to apoptosis in three murine mammary epithelial cell lines. *Differentiation* 2006 Jun;74(5):254-64. PMID: 16759291
432. Schrauzer GN, Hamm D, Kuehn K, Nakonecny G. Effects of long term exposure to beer on the genesis and development of spontaneous mammary adenocarcinoma and prolactin levels in female virgin C3H/St mice. *J Am Coll Nutr* 1982;1(3):285-91. PMID: 6309941
433. Takechi R, Hiramatsu N, Mamo JC, Pal S. Red wine polyphenolics suppress the secretion and the synthesis of Apo B48 from human intestinal CaCo-2 cells. *Biofactors* 2004;22(1-4):181-3. PMID: 15630279
434. Wulf G, Garg P, Liou YC, Iglehart D, Lu KP. Modeling breast cancer in vivo and ex vivo reveals an essential role of Pin1 in tumorigenesis. *EMBO J* 2004 Aug 18;23(16):3397-407. PMID: 15257284
435. Yamagishi M, Natsume M, Osakabe N, Nakamura H, Furukawa F, Imazawa T, Nishikawa A, Hirose M. Effects of cacao liquor proanthocyanidins on PhIP-induced mutagenesis in vitro, and in vivo mammary and pancreatic tumorigenesis in female Sprague-Dawley rats. *Cancer Lett* 2002 Nov 28;185(2):123-30. PMID: 12169385
436. Gago-Dominguez M, Castela JE, Pike MC, Sevanian A, Haile RW. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005 Dec;14(12):2829-39. PMID: 16364997

437. Gaudet MM, Gammon MD, Santella RM, Britton JA, Teitelbaum SL, Eng SM, Terry MB, Bensen JT, Schroeder J, Olshan AF, Neugut AI, Ambrosone CB. MnSOD Val-9Ala genotype, pro- and anti-oxidant environmental modifiers, and breast cancer among women on Long Island, New York. *Cancer Causes Control* 2005 Dec;16(10):1225-34. PMID: 16215873
438. Giacosa A, Frascio F, Munizzi F. Epidemiology of colorectal polyps. *Tech Coloproctol* 2004 Dec;8 Suppl 2:s243-7. PMID: 15666099
439. Lewis RC, Bostick RM, Xie D, Deng Z, Wargovich MJ, Fina MF, Roufail WM, Geisinger KR. Polymorphism of the cyclin D1 gene, CCND1, and risk for incident sporadic colorectal adenomas. *Cancer Res* 2003 Dec 1;63(23):8549-53. PMID: 14679024
440. Orita S, Hirose M, Takahashi S, Imaida K, Ito N, Shudo K, Ohigashi H, Murakami A, Shirai T. Modifying effects of 1'-acetoxychavicol acetate (ACA) and the novel synthetic retinoids Re-80, Am-580 and Am-55P in a two-stage carcinogenesis model in female rats. *Toxicol Pathol* 2004 Mar-Apr;32(2):250-7. PMID: 15200164
441. Schatzkin A, Freedman L, Schiffman M. An epidemiologic perspective on biomarkers. *J Intern Med* 1993;233(1):75-9. PMID: 8429292
442. Visapaa JP, Jokelainen K, Nosova T, Salaspuro M. Inhibition of intracolonic acetaldehyde production and alcoholic fermentation in rats by ciprofloxacin. *Alcohol Clin Exp Res* 1998 Aug;22(5):1161-4. PMID: 9726290

List of Acronyms/Abbreviations

ADH:	alcohol dehydrogenase
ALDH:	aldehyde dehydrogenase
AJ:	adherens junctions
AMMN:	acetoxymethyl-methylnitrosamine
AOM:	azoxymethane
BPDE:	benzo[a]pyrene diolepoxide
BRCA1:	breast cancer type 1
cAMP:	cyclic adenosine monophosphate
CM:	colonic mucosa
CY:	cyanamide
CYP2E1:	cytochromes P450 2E1
DHEA:	dehydroepiandrosterone
DHEAS:	DHEA sulfate
DMBA:	dimethylene (a) anthracene
DMH:	1,1-dimethylhydrazine
DNA:	deoxyribonucleic acid
EGFR:	epidermal growth factor receptor
ER:	estrogen receptor
ERT:	estrogen replacement therapy
FCS:	fetal calf serum
HLA:	human leukocyte antigen
H₂O₂:	hydrogen peroxide
H₂O:	water
JACC:	Japan Collaborative Cohort Study
JPHC:	Japan Public Health Center-based Prospective Study
MAA:	mutagenic malondialdehyde-acetaldehyde
MAM:	methylazoxymethanol
MEOS:	microsomal ethanol-oxidizing system
MLC:	myosin alkali light chain
MNU:	N-methyl-N-nitrosurea
NAD:	nicotinamide adenine dinucleotide
NADH:	reduced nicotinamide adenine dinucleotide
NMDA:	N-nitrosodimethylamine
NNK:	4-methylnitrosoamino-1-3-pyridyl-1-butanone
PK:	protein kinase
PPAR:	peroxisome proliferator-activated receptor
PR:	progesterone receptor
ROS:	reactive oxygen species
rp:	ribosomal protein
SAM:	s-adenosylmethionine
TNF:	tumor necrosis factor
TJ:	tight junctions

Appendix A: Exact Search Strings

Electronic Database Searches

The following databases have been searched for relevant information:

Name	Date Limits	Platform/Provider
Cancerlit	1935 - September 18, 2009	www.pubmed.gov
ClinicalTrials.gov	Searched February 1, 2009	www.clinicaltrials.gov
The Cochrane Central Register of Controlled Trials (CENTRAL)	Through 2010, Issue 1	www.thecochranelibrary.com
The Cochrane Database of Methodology Reviews (Methodology Reviews)	Through 2010, Issue 1	www.thecochranelibrary.com
The Cochrane Database of Systematic Reviews (Cochrane Reviews)	Through 2010, Issue 1	www.thecochranelibrary.com
Database of Abstracts of Reviews of Effects (DARE)	Through 2010, Issue 1	www.thecochranelibrary.com
EMBASE (Excerpta Medica)	1980 through May 3, 2010	OVID
Health Technology Assessment Database (HTA)	Through 2010, Issue 1	www.thecochranelibrary.com
MEDLINE	1965 through May 3, 2010	OVID
U.K. National Health Service Economic Evaluation Database (NHS EED)	Through 2010, Issue 1	www.thecochranelibrary.com

Medical Subject Headings (MeSH), Emtree, PsycINFO and Keywords

Conventions:

OVID

- \$ = truncation character (wildcard)
- exp = “explodes” controlled vocabulary term (e.g., expands search to all more specific related terms in the vocabulary’s hierarchy)
- / = limit controlled vocabulary heading
- .fs. = floating subheading
- .hw. = limit to heading word
- .md. = type of methodology (PsycINFO)
- .mp. = combined search fields (default if no fields are specified)
- .pt. = publication type
- .ti. = limit to title
- .tw. = limit to title and abstract fields

PubMed

- [mh] = MeSH heading
- [majr] = MeSH heading designated as major topic
- [pt] = publication type
- [sb] = subset of PubMed database (PreMEDLINE, Systematic, OldMEDLINE)
- [sh] = MeSH subheading (qualifiers used in conjunction with MeSH headings)
- [tiab] = keyword in title or abstract

Topic-specific search terms – alphabetical listing

Concept	Controlled Vocabulary	Keywords
Adrenal	Adrenal.hw. exp Adrenal gland/ exp Adrenal glands/ exp Endocrine system/	Adrenal Aldosterone Endocrine gland\$ Primary hyperaldosteroneism
Alcohol	Alcohol Alcohol abstinence Alcohol drinking exp Alcohol-related disorders exp Alcoholic beverage exp Alcoholic beverages Alcohol metabolism Drinking behavior Ethanol Feeding behavior Food habits Temperance	Abstinence Alcohol\$ Beer Brandy Cocktail\$ EtOH Gin Liqueur\$ Liquor\$ Mixed drink\$ Schnapps Spirits Vodka
Biochemical Processes	Exp biochemical processes/	
Breast cancer	exp breast cancer Breast carcinoma exp Breast diseases exp Breast neoplasms	Breast\$ Cancer\$ Carcinoma\$ Lesion\$ Lump\$ Mammar\$ Tumo?r\$
Colorectal cancer	Adenomatous polyp Colorectal cancer Colorectal carcinoma Exp colorectal neoplasms	Cancer\$ Carcinoma\$ Colon\$ Colorectal Lesion\$ Polyp\$ Rectal Rectum Tumo?r\$
Experimental neoplasms	Experimental neoplasm/ exp Neoplasms, experimental/	

Concept	Controlled Vocabulary	Keywords
Hypothalamic-hypophyseal system	Hypothalamo-hypophyseal system Hypothalamus hypophysis system Pituitary gland	Hypothalamus hypophysis gonad system
Microbes	Achlorhydria/ Bacteria, aerobic Candida albicans Colon flora Intestine flora Microbial growth Microbiology.fs. microorganism	Bacteria Bacteriocolonial Flora Microb\$ Microflora Yeast\$
Oncogenesis	Breast carcinogenesis Chemical carcinogenesis Colorectal carcinogenesis Malignant neoplastic disease exp neoplastic processes exp Oncogenesis and malignant transformation	Carcinogenesis Oncogenesis Tumorigenesis Tumorigenic effect
Potential mechanisms	5,10 methylenetetrahydrofolate reductase (FADH2) Acetaldehyde Alcohol dehydrogenase Aldehyde dehydrogenase ALDH2 Apoptosis Calcium signaling Cell cycle Cell cycle regulation Cell division Cell membrane permeability Cell nucleus Cell proliferation Cyclin dependent kinase 2 cyp2E1 Cytochrome p-450 enzyme system cytochrome p450 17 cytochrome p450 1A1 cytochrome p450 1A2	Acetaldehyde\$ MAPK MAPKs NFkappaB Proto-oncogene Reactive oxygen

Concept	Controlled Vocabulary	Keywords
	cytochrome P450 1B1 cytochrome P-450 CYP1A1 cytochrome P-450 CYP1A2 cytochrome P-450 CYP2B1 cytochrome P-450 CYP2D6 cytochrome P-450 CYP2E1 cytochrome P-450 CYP3A Deamination DNA adducts exp DNA-binding proteins DNA damage Down regulation Estrogen activity Estrogen metabolism Estrogen receptor, alpha Estrogen receptor beta Fas antigen Folate metabolism exp Folic acid Folic acid Folic acid deficiency Gene control exp Gene expression regulation Gene function Gene mutation Genetic code Genetic polymorphism Genetic variability Growth regulation modulation hydroxylation MAP kinase signaling system Metabolism.fs. Methionine synthase Mitochondria exp Mitogen-activated kinases Mitogen activated protein kinase NF-kappa B Oxidative phosphorylation Oxidative stress	

Concept	Controlled Vocabulary	Keywords
	p16 protein human.sn. Polymorphism, genetic Protein expression Protein p16 Proto oncogene Exp reactive nitrogen species Reactive oxygen metabolite Exp Reactive oxygen species Receptor cross talk Receptor upregulation exp Receptors, estrogen exp Receptors, retinoic acid Retinoid Retinoic acid receptor beta exp Signal transduction exp Transferases Tretinoin	

EMBASE/MEDLINE

English language, remove overlap

Set Number	Concept	Search Statement
1	Alcohol	Alcohol drinking/ or exp alcohol-related disorders/ or alcohol metabolism/
2		exp alcoholic beverage/ or exp alcoholic beverages/ or alcohol/ or ethanol/ or beer or wine or alcohol\$ or brandy\$ or gin or vodka or schnapps or EtOH or liquor\$ or liqueur\$ or spirits or mixed drink\$ or cocktail\$
3		Drinking behavior/ or food habits/ or feeding behavior/ or temperance/ or alcohol abstinence/ or abstinence
4	Combine sets	or/1-3
	Oncogenesis	
5	Carcinogenesis	Exp neoplastic processes/ or exp oncogenesis and malignant transformation/ or malignant neoplastic disease/ or breast carcinogenesis/ or colorectal carcinogenesis/ or chemical carcinogenesis/ or exp neoplasms, experimental/ or experimental neoplasms
6		Carcinogenesis or oncogenesis or tumorigenesis or tumorigenic effect
7	Combine sets	or/5-6
	Potential mechanisms	
8		Proto oncogene/ or proto-oncogene or exp DNA-binding proteins/
9		Metabolism.fs. or deamination/
10	Signaling	Receptor cross-talk/ or exp signal transduction/ or calcium signaling/ or exp gene expression regulation/ or down regulation/ or protein expression/ or receptor upregulation/ or growth regulation modulation/
11	Estrogen	Estrogen receptor, alpha/ or exp receptors, estrogen/ or estrogen activity/ or estrogen metabolism/ or estrogen receptor beta/
12	MAPK	Exp mitogen-activated kinases/ or MAP kinase signaling system/ or mitogen activated protein kinase/ or MAPK or MAPKs
13	Cytochrome P-450	Cytochrome p-450 enzyme system/ or cyp2E1/ or cytochrome P450 17/ or cytochrome P450 1A1/ or cytochrome P450 1A2/ or cytochrome P450 1B1/ or cytochrome P-450 CYP1A1/ or cytochrome P-450 CYP1A2/ or cytochrome P-450 CYP2B1/ or cytochrome P-450 CYP2D6/ or cytochrome P-450 CYP2E1/ or cytochrome P-450 CYP3A/
14	Dehydrogenases	Alcohol dehydrogenase/ or aldehyde dehydrogenase/ or ALDH2/ or acetaldehyde\$
15	Methylation	exp Folic acid/ or Folic acid/ or folic acid deficiency/ or folate metabolism/
16		DNA methylation/ or RNA methylation/ or DNA hypermethylation/ or DNA hypomethylation/ or methylation
17		Methionine synthase/

Set Number	Concept	Search Statement
18		Cyclin dependent kinase 2/ or Fas antigen/ or 5,10 methylenetetrahydrofolate reductase FADH2/ or Protein p16/ or p16 protein human.nm.
19	Cells	Apoptosis/ or cell division/ or cell proliferation/ or cell cycle/ or cell cycle arrest/ or cell cycle regulation/ or cell membrane permeability/ or cell nucleus/
20	Misc. genetic concepts	Gene control/ or gene function/ or gene mutation/ or genetic code/ or genetic polymorphism/ or genetic variability/ or polymorphism, genetic/
21	DNA	DNA adducts/ or DNA damage/ or mitochondria/ or exp DNA-binding proteins/
22	Oxidation	Reactive oxygen metabolite/ or oxidative stress/ or hydroxylation/ or exp reactive oxygen species/ or oxidative phosphorylation/ or reactive oxygen or exp reactive nitrogen species/
23	Retinoic acid	Retinoid/ or retinoic acid receptor beta/ or exp receptors, retinoic acid/ or tretinoin/
24		NF-kappa B/ or NFkappaB
25		Exp transferases/
26	Acetaldehyde	Exp acetaldehyde/ or acetaldehyde\$
27	Biochemical processes (includes DNA repair)	Exp biochemical processes/
28	Adrenal	Exp Adrenal gland/ or exp adrenal glands/ or adrenal.hw. or adrenal.tw. or aldosterone or primary hyperaldosteroneism or exp endocrine system/ or endocrine gland\$
29	Hypothalamic	Hypothalamo-hypophyseal system/ or hypothalamus hypophysis system/ or Hypothalamus hypophysis gonad system or pituitary gland/
30	Microbial	Microflora or microbiology.fs. or microb\$.ti. or achlorhydria/ or bacteria, aerobic/ or candida albicans/ or colon flora/ or intestine flora/ or microbial growth/ or microorganism/ or bacteria or bacteriocolonial or flora or Microflora or yeast\$
31	Combine sets (mechanisms)	or/8-30
32	Breast cancer	exp Breast neoplasms/ or exp breast diseases/ or exp breast cancer/ or breast carcinoma/
33		(breast or mammar\$) and (tumo?r\$ or lesion\$ or cancer\$ or carcinoma\$ or lump\$)
34	Combine sets (breast cancer)	or/32 -33

Set Number	Concept	Search Statement
35	Colorectal cancer	Exp colorectal neoplasms/ or adenomatous polyp/ or colorectal cancer/ or colorectal carcinoma/
36		(colon\$ or rectal or rectum or colorectal) and (tumo?r\$ or lesion\$ or cancer\$ or carcinoma\$ or polyp\$)
37	Combine sets (colorectal cancer)	or/35-36
38	Combine sets Alcohol, oncogenesis & breast cancer	4 and 7 and 34
39	Combine sets Alcohol, mechanisms & breast cancer	4 and 31 and 34
40	Combine sets Alcohol, oncogenesis & colorectal cancer	4 and 7 and 37
41	Combine sets Alcohol, mechanisms & colorectal cancer	4 and 31 and 37
42	Combine sets	38 or 39 or 40 or 41
43	Limit to English	42 and English
44	Non-English	42 not 43
45	Eliminate overlap	Remove duplicates from 43

Appendix B: Sample Data Abstraction Forms

Level 1 – Abstract Review: At this review level abstracts were examined to determine if a document should be retrieved for further review. Checking the inclusion boxes in the form automatically led to retrieval of the full article. All documents selected for inclusion at this level fell to the next level for evaluation.

Keywords:	Submit Data
Abstract:	1. Include or Exclude document ☐ Include ☐ Exclude ☐ Include: Non-English Language Clear Selection Submit Data

Level 2 – Full Document Review: At this level we made the final determination as to whether the document was to be excluded or included in the report. The reason for exclusion was noted in a separate box on the form. All documents selected for inclusion at this level fell to the next level for evaluation.

Keywords:	Submit Data
Abstract:	1. Is this document included in the Report (includes Background and Evidence)? ☐ Include in Report ☐ Exclude Clear Selection 2. Reason for Exclusion Enlarge Shrink Submit Data

Level 3 – Background Document Review: At this level we determined if the document will appear in the Background section of the report or in the Evidence section of the report. If the document was to be used as background material this form was used to indicate which area in the Background section the document belonged. All documents selected for inclusion in the evidence report at this level fell to the next level for evaluation.

Submit Data
1. Is this document included in the Background only or the Evidence Report? ☒ Include in Evidence Report ☐ Include in Background Only Clear Selection
2. If Included for Background only, which of the following apply? ☐ Basic cancer mechanisms ☐ Breast cancer mechanisms ☐ Colorectal cancer mechanisms ☐ Alcohol metabolism ☐ Epidemiology of alcohol and cancer ☐ Other components of alcoholic drinks

Level 4 – Evidence Base Document Review: Only documents that were used in the evidence report appeared at this level. Information recorded at this level was used to organize the documents into specific areas of study depending on study design.

Submit Data
1. Which of the following apply to this document?
☐ Human studies - breast
☐ Animal models - breast
☐ Animal tissues - breast
☐ Cell lines - breast
☐ Human studies - colorectal
☐ Animal models - colorectal
☐ Animal tissues - colorectal
☐ Cell lines - colorectal
Submit Data

Table B-1. Data abstraction and data management

Document ID#: internal ECRI Institute ID number
Article Citation
Country where study was completed
Year in which the study was performed
Experimental Model: type of animal model or cell line
Primary Mechanism examined: mechanism being tested for relationship between alcohol intake and cancer risk
Secondary Mechanism examined: for studies that explore multiple mechanisms
Amount of Alcohol Exposure: levels of alcohol exposure depending on the design of the experiment and the experimental model
Mode of Administration: mode of administration of alcohol depending on the design of the experiment and the experimental model
Duration of Alcohol Exposure: duration of alcohol exposure depending on the design of the experiment and the experimental model
Use of a Carcinogen: the carcinogenic agent, if any, being examined in the study along with alcohol
Use of other non-carcinogen agents: nutritional or other interventional agents utilized to show the relationship between alcohol intake and cancer risk
Description of subject characteristics in human studies: age, male/female ratio, smoking, comorbidities, race/ethnicity, alcoholism
Study design: explains the type of study design
Duration of the study
Direct or Indirect Association: explains evidence of carcinogenesis
Results for Intermediate Outcomes: usually molecular, biochemical, or histological outcomes which may be indicative of a direct or indirect relationship between alcohol intake and cancer risk
Results for Clinical Outcomes: typically broader organ measurements that correlate to a direct or indirect relationship between alcohol intake and cancer risk
Results for Patient Oriented Outcomes: entries in this column are only for human studies
Conclusions: did the study present evidence for or against the proposed mechanism

Appendix C: Evidence Tables

Evidence Base for Breast Cancer

Table C-1. Summary of results from human studies on breast cancer

Study	Model	Study Design	Mechanism Examined	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Hartman et al. 2005 ⁷⁶	53 postmenopausal women	Case control	Increase levels of biomarkers of oxidative stress	Controlled diet plus each of three treatments (15 or 30 g alcohol per day or no-alcohol placebo beverage), during three 8-week periods in random order	After adjusting for body mass index (all models) and total serum cholesterol (tocopherol and isoprostane models), there was a 4.6% decrease ($p = 0.02$) in α -tocopherol and a 4.9% increase ($p = 0.07$) in isoprostane levels when women consumed 30 g alcohol/day ($p = 0.06$ and 0.05 for overall effect of alcohol on α -tocopherol and isoprostanes, respectively).	Moderate alcohol consumption increases some biomarkers of oxidative stress in postmenopausal women.

Study	Model	Study Design	Mechanism Examined	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Dorgan et al. 2001 ⁸⁵ Same as ³¹¹	51 healthy postmenopausal women not using hormone replacement therapy	Three-period crossover design	Elevated serum levels of estradiol, estrone, estrone sulfate, testosterone, androstenedione, progesterone, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS), and androstenediol	15 g or 30 g of alcohol per day or an alcohol-free placebo beverage through a three 8-week dietary period. Alcohol was supplied as 95% ethanol in 12 oz orange juice. Each dietary period was preceded by a 2- to 5-week washout period when participants did not consume any alcohol.	15 g of alcohol/day resulted in an increase of 7.5% (95% confidence interval [CI]: -0.3 to 15.9%; $p = 0.06$) of estrone sulfate. 30 g of alcohol/day resulted in an increase of 10.7% (95% CI: 2.7 to 19.3%; $p = 0.009$) estrone sulfate 15 g of alcohol/day resulted in an increase of 5.1% (95% CI: 1.4 to 9.0%; $p = 0.008$) DHEAS. 30 g of alcohol/day resulted in an increase of 7.5% (95% CI: 3.7 to 11.5%; $p < 0.001$) DHEAS	Results suggest a possible mechanism by which consumption of one or two alcoholic drinks per day by postmenopausal women could increase their risk of breast cancer.

Study	Model	Study Design	Mechanism Examined	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Ginsburg et al. 1996 ⁸⁸	12 postmenopausal women receiving oral estrogen (estradiol, 1 mg/day) and progestin (medroxy-progesterone acetate) replacement therapy were compared with 12 postmenopausal women who were not using estrogen replacement therapy (ERT).	Randomized, double-blind, placebo-controlled crossover study	Effects of alcohol ingestion on estrogens in postmenopausal women	Pineapple juice and 40% ethanol at a dose of 2.2 mL/kg of body weight (0.7 g/kg of body weight) in a total volume of 300 mL over 15 minutes	Within 50 minutes of alcohol ingestion in postmenopausal women on ERT, there was a 3-fold increase in estradiol levels from 297 to 973 pmol/L ($p < 0.001$) No changes in estradiol following alcohol ingestion in postmenopausal women not on ERT	Acute alcohol ingestion may lead to significant and sustained elevations in circulating estradiol.
Ginsburg et al. 1995 ⁸⁷	14 menopausal women using transdermal estradiol	Two randomized crossover studies	Effect of acute ethanol ingestion on prolactin in menopausal women using estradiol replacement	Ethanol (1 mL/kg, 95% ethanol) over 20 minutes	Alcohol when compared to isocaloric carbohydrate drink ingestion resulted in increased serum prolactin levels in both study 1 ($p < 0.03$) and study 2 ($p < 0.001$)	There was an increase in serum prolactin levels.

Study	Model	Study Design	Mechanism Examined	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Reichman et al. 1993 ⁸⁶	34 premenopausal women with a history of regular menstrual cycle	Randomized, diet-controlled crossover intervention	The effects of alcohol consumption on plasma and urinary hormone (DHEA, estrogens) concentrations in premenopausal women	30 g of ethanol daily for three menstrual cycles and no alcohol for the other three	Plasma DHEAS levels increased by 7.0% ($p = 0.05$) in the follicular phase Plasma estrone levels increased by 21.2% ($p = 0.01$) in the peri-ovulatory phase Plasma estradiol increased by 27.5% ($p = 0.01$), urinary estradiol increased by 31.9% ($p = 0.009$) in the peri-ovulatory phase At the luteal phase, urinary estrone increased by 15.2% ($p = 0.05$), estradiol levels increased by 21.6% ($p = 0.02$), and estriol levels increased by 29.1% ($p = 0.03$)	Results suggest a possible mechanism between alcohol consumption and risk of breast cancer.

Table C-2. Summary of results from animal studies on breast cancer

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Hilakivi-Clarke et al. 2004 ⁹²	Elevated levels of estrogen receptors	Rats	<u>Amount in study groups:</u> 0 g/kg ethanol vs. 16 g/kg ethanol vs. 25 g/kg ethanol <u>Duration of Exposure:</u> 12 days	Dimethylene(a)anthracene (DMBA)	Latency to the appearance of first tumor [weeks, mean ([s.e.m))] was 9.7 (0.6) in the control, 8.4 (0.5) in the 16 g alcohol group, and 8.6 (0.5) in the 25 g alcohol group. Tumor incidence and tumor multiplicity were higher in the alcohol groups compared to control. Tumor growth rate was similar in all three groups.	Maternal alcohol intake increased offspring's mammary tumorigenesis.
Castro et al. 2003 ⁹⁵	Biotransformation of ethanol to acetaldehyde	Rats	<u>Amount in study group:</u> 0.21M ethanol <u>Duration of exposure:</u> 1 hour	None	Biotransformation of ethanol to acetaldehyde occurred in mammary tissue microsomes.	Result could have a significant effect in some stages of the process of breast tumor promotion by ethanol.
Chhabra et al. 2000 ⁹⁶	Formation of DNA adducts	Rats	<u>Amount in study group:</u> 1.6 g/kg ethanol <u>Duration of exposure:</u> 14 days	N-nitrosodimethylamine (NDMA), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	There was a 10-fold increase in O ⁶ -methylene adducts from NDMA in mammary gland following cotreatment with ethanol.	Nitrosamines and ethanol are contributors to mammary cancer risk and perinatal carcinogenesis.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Watabiki et al. 2000 ⁹⁷	Ethanol-induced hyperprolactinemia and/or mammary tumor virus increased by the hyperprolactinemia	Rats	<u>Amount in study groups:</u> 10% ethanol vs. 15% ethanol vs. tap water <u>Duration of exposure:</u> 25 months	None	In the ethanol-treated group, tumor occurrence was reported in 9 (45%) of the 20 rats at 8 to 24 months. There no occurrence of tumor in the control.	The murine model may be useful to study the role of ethanol in mammary tumorigenesis.
Holmberg et al. 1995 ⁹¹	None reported	Rats	<u>Amount in study groups:</u> 1 ethanol vs. 3% ethanol <u>Duration of exposure:</u> 2 years	None	Following the administration of low amounts of ethanol, there was an increase in mammary gland fibroma, fibroadenoma or adenoma.	The finding seems not to be consistent in terms of a dose-response relationship or in their interrelation and may thus be regarded as an unspecific phenomenon.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Singletery et al. 1995 ⁹³	Influence on initiation and promotion stages of carcinogenesis through change in blood estrogen and progesterone	Rats	<u>Amount in study groups:</u> Diet containing ethanol at 0% calories vs. 15% calories vs. 20% calories, vs. 30% calories <u>Duration of exposure:</u> 22 days	N-methyl-N-nitrosourea (MNU)	Ethanol consumption at 15% caloric intake resulted in an increase during either the initiation or promotion stages. Ethanol consumption at 20% caloric intake resulted in increase during the promotion stage There was no effect on either stage in the group that received ethanol at 30% caloric intake.	Ethanol at specific intakes can enhance the initiation and promotion stages of MNU-induced mammary tumorigenesis. However, there was not a consistent and proportionate increase in mammary tumor development with increasing intakes of ethanol.
Singletery and McNary 1994 ⁹⁴	Effect on serum estradiol and progesterone	Rats	<u>Amount in study groups:</u> Diet containing ethanol at 0% calories vs. 20% calories, vs. 30% calories <u>Duration of exposure:</u> 35-39 days	MNU	Ethanol consumption at 20% caloric intake resulted in a 19% increase in rat mammary gland terminal-end bud (TEB) density and 49% decrease in alveolar bud (AVB) structures. Ethanol consumption at 30% caloric intake resulted in a 45% increase in rat mammary gland TEB density and 44% decrease in AVB structures.	Ethanol consumption can lead to an increase in the quantity of and the rate of cell proliferation of mammary gland terminal-end bud (TEB) structures.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Taylor et al. 1993 ⁹⁹	Suppression of cellular immunity (T cell activation and proliferation, and on natural killer [NK] cytotoxicity)	Rats	Acute ethanol exposure of 2.5-3.5 g/kg body weight 1 hour before tumor inoculation vs. chronic consumption of liquid diet containing ethanol for 2 weeks before and for 3 weeks after tumor inoculation.	Study authors only reported "Tumor inoculation" by MADB106	Blood NK cytotoxic activity was reduced in ethanol treated rats. Number of blood large granular lymphocytes (LGL) per NK cells at 2 hours post ethanol administration dropped to 86% of control group and at 5 hours post ethanol administration: dropped to 74% of control group.	Alcohol exposure during fetal or adult life has profound immunopathological effects.
McDermott et al. 1992 ¹⁰¹	The aim of the study was to test the hypothesis that dietary alcohol intake increases the incidence of experimental mammary carcinoma	Rats	<u>Amount in study groups:</u> 4.4 g/kg/day ethanol vs. tap water <u>Duration of exposure:</u> 10 days	DMBA	Mean time (days, [SD]) to first tumor appearance in the alcohol group was 63 (16.3) and 67.3 (19) in the control. Mean number of tumors/animal in the alcohol group was 3.2 (2.2) and 2.9 (2.7) in the control. Tumor rate growth (mm ³ /day) in the alcohol group was 30.7 (17.7) and 25.5 (11.8) in the control.	This study failed to support the hypothesis that dietary alcohol intake increases the incidence of mammary carcinoma.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Hackney et al. 1992 ¹⁰²	Enhancement of the rate of mammary tumor development	Rats	<u>Amount in study groups:</u> 4 g/kg/day ethanol vs. 15 g/kg/day ethanol vs. 20 g/kg/day ethanol <u>Duration of exposure:</u> 65 weeks	None	There was no difference among study groups ($p = 0.10$) in development of mammary tumors.	Findings do not support the hypothesis that ethanol augments the risk of breast cancer.
Singletery and McNary 1994 ¹⁰⁴	Effect on mammary gland structural development, DNA synthesis, and decrease in serum progesterone	Rats	<u>Amount in study groups:</u> Diet containing ethanol at 0% vs. 15% vs. 20% vs. 25% of calories <u>Duration of exposure:</u> Experiment 1: 32 days Experiment 2: 28 days Experiment 3: 33 days	None	In experiment 1, TEB increased for rats fed ethanol at 20% and 30% caloric intake and density of AVB decreased at all ethanol concentrations. In experiment 2, TEB density of ethanol-fed rats increased 64%. In experiment 3, there was no change in serum estradiol. However, serum progesterone: decreased by 56% and 51% compared to pair-fed control and ad lib-fed control rats, respectively.	Cancer risk in humans may be proportional to both cell number and rate of cell division within a target tissue.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Singleton et al. 1991 ¹⁰⁵	Effect on mammary gland structural development and DNA synthesis	Rats	<u>Amount in study groups:</u> Diet containing ethanol at 0% vs. 15% vs. 20% vs. 30% of calories <u>Duration of exposure:</u> 29 days	DMBA	Rats that consumed ethanol at 10% and 20% of calories exhibited a significant increase in TEB density and a significant decrease in AVB density. Rats that consumed ethanol at 20% of total calories prior to DMBA administration exhibited a significant 54-74% increase in tumor incidence compared with rats fed the control diet. 78%, 82%, and 91% of tumor-bearing rats possessed adenocarcinomas for rats fed the diets containing 0%, 10%, and 20% of calories as ethanol, respectively. For rats fed ethanol at 30% of calories, tumor incidence was identical to that for rats fed the control diet until 12 weeks following DMBA dosing.	Specific quantities of ethanol can enhance the initiation and the promotion stages of DMBA-induced mammary tumorigenesis.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Rogers and Conner 1990 ¹⁰⁰	Enhancement of carcinogenesis	Rats	<u>Amount in study groups:</u> Diet providing 10% vs. 20% vs. 50% of calories as ethanol <u>Duration of exposure:</u> Exp 1: 10% of ethanol for 1 week, 20% of alcohol for 3 weeks. Exp 2: 10% of ethanol for 4 days, 20% for the remainder of the experiment. Exp 3: 10% of ethanol for 4 weeks, at the beginning of the 4th week the rats were given a single dose of 50% ethanol.	DMBA	In all experiments, there was no detectable effect on mammary tumor latency, incidence, number, weight or histology.	There was no effect of ethanol on mammary gland tumorigenesis induced by DMBA.
Grubbs et al. 1988 ¹⁰³	Enhancement of mammary cancer initiation	Rats	<u>Amount in study groups:</u> 7.0 g/kg ethanol vs. 3.5 g/kg ethanol <u>Duration of exposure:</u> DMBA group: 3 weeks MNU group: 8 weeks	DMBA MNU	Mammary cancer initiation by DMBA was increased by both dose levels of ethanol. Mammary cancer initiation by MNU was increased by high dose of ethanol.	Ethanol enhances mammary cancer initiation.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Schrauzer et al. 1979 ⁹⁸	Change in prolactin levels	Mice	<u>Amount in study groups:</u> 12% ethanol vs. table wine with an alcohol content of 11.5% <u>Duration of exposure:</u> 6 weeks	Bittner virus	Mean serum prolactin levels (ng/ml [SD]) in the alcohol group was 23 (9) and 52 (23) in the control. Tumor incidence, growth rates and latency in the alcohol group occurred in 8 animals that developed adenocarcinoma, the first at the age of 6 months (after 5 months of exposure), the last at 11 months (median: 8). The tumor incidence was 73%. Among the control, animals developed mammary tumors between 12 and 16 months of age (median: 14.2). Tumor incidence was 82% * difference in latency times was significant ($p < 0.001$)	Long-term exposure to ethanol significantly reduced the latency period in the genesis of spontaneous mammary adenocarcinoma.

Table C-3. Summary of results from cell line studies on breast cancer

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Etique et al. 2009 ¹⁰⁶	Cross-talk between A2A Adenosine receptor (A2A AR) and the estrogen receptor-alpha	MCF-7	<u>Amount in study groups:</u> 0.3% ethanol vs. 0.1% ethanol <u>Duration of exposure:</u> 24 hours	There was an increase in the level of progesterone receptor mRNA following 24 hours of treatment with 1uM CGS21680 (a selective agonist). Antagonist (MSX-3) induced a dose-dependent inhibition of an ethanol-induced increase in progesterone receptor expression.	Although results demonstrate cross-talk between A2A AR and estrogen receptor-alpha in the ethanol action on MCF-7 cells, the link between ethanol and A2A AR remains to be determined.
Venkata et al. 2008 ¹¹⁵	Relationship between ethanol and its metabolite acetaldehyde on peroxisome proliferator-activated receptor (PPAR)alpha and PPAR(beta) transactivation	MCF-7	<u>Amount in study groups:</u> 0 mM ethanol vs. 10 mM ethanol vs. 30 mM ethanol vs. 100 mM ethanol vs. 300 mM ethanol <u>Duration of exposure:</u> 24 hours	Over a range of ethanol concentrations up to 300mM, ethanol was able to dose dependently and significantly increase the expression of PPAR(alpha) mRNA in MCF-7 cells. Ethanol also modestly increased the mRNA for PPAR(beta) with a significant increase seen at 30 and 300mM, although not at 100mM. The increased expression for PPAR(beta) mRNA was only in the order of two-fold in contrast to the approximately sevenfold increase seen for PPAR(alpha) compared with the absence of ethanol.	There is likely to be a complex interplay in the way ethanol and/or acetaldehyde acts via the PPARs and other proteins to influence tumorigenic relevant pathways such as proliferation, resistance to apoptosis, and invasiveness.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Etique et al. 2007 ⁶⁸	Activation of the estrogen signaling pathway (cyclic AMP [cAMP]/protein kinase A [PKA]).	MCF-7	<u>Amount in study groups:</u> 0.1% ethanol vs. 0.3% ethanol vs. 0.5% ethanol vs. 0.7% ethanol <u>Duration of exposure:</u> 24 hours	There was a significant 1.6-fold increase in progesterone receptor mRNA level for either 0.1 or 0.3% ethanol and a 1.3-fold increase in pS2 expression for a dose of 0.3%.	Ethanol treatment of MCF-7 breast cells stimulates the cAMP/PKA pathway which triggers two important events: an increase in the expression of genes with cAMP response element (CRE) in their promoter, like aromatase as well as a ligand-independent activation of estrogen receptor-alpha and transcription of target genes.
Etique et al. 2004 ⁶⁵	Stimulation of cell proliferation, estrogen receptor-alpha, and aromatase expression	MCF-7	<u>Amount in study groups:</u> 0.0% ethanol vs. 0.1% ethanol vs. 0.3% ethanol <u>Duration of exposure:</u> Up to 6 days	Ethanol enhanced cell proliferation and clonal growth of MCF-7 cells. In the presence of 0.1% ethanol, there was a significant increase in cell proliferation (11.5%) at day 4 and it peaked at 28% at day 6. In the presence of 0.3% ethanol, there was a significant increase (11%) at day 4, and no significant change at day 6.	Study supports data suggesting that ethanol is an increased risk factor for breast cancer.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Izevbigie et al. 2002 ⁵¹	Disruption and modulation of cell proliferation	MCF-7	Ethanol (0.1%-10%) with or without an inhibitor of mitogen activated protein kinase 1 vs. 0.3%, 3%, and 10% ethanol for 5-, 10-, 20-, and 40-min time course experiments.	Exposure of to 65 mM (0.3% ethanol) increased incorporation of [3-H] thymidiene into MCF-7 cells by approximately two-fold over control. In contrast to the growth stimulatory effect of 0.3% ethanol, both 3% and 10% ethanol significantly inhibited cell growth.	Ethanol stimulates p44/42 mitogen-activated protein kinase's activity and subsequent MCF-7 cell proliferation.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Przylipiak et al. 1996 ¹¹⁰	Direct growth-stimulatory effect on cancer cells by enhancement of 3H-thymidine	MCF-7	<u>Amount in study groups:</u> 0.00001% ethanol vs. 0.0001% ethanol vs. 0.001% ethanol vs. 0.01% ethanol vs. 0.1% ethanol vs. 1% ethanol vs. 10% ethanol <u>Duration of exposure:</u> 5 hours	Ethanol enhanced 3H-thymidine uptake in cultured human mammary carcinoma cell line MCF-7. The most effective concentration was 0.01% which evoked a 202% enhancement of 3H-thymidine uptake, when compared to controls. Concentrations of ethanol between 0.0001% and 10% also significantly enhanced 3H-thymidine uptake. A concentration of 0.00001% ethanol did not affect thymidine incorporation.	Ethanol appears to play a role in tumor promotion in vivo as a result of direct growth-stimulatory effect on human mammary cancer cells in vitro.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Singletery et al. 2004 ¹⁰⁷	Decreased capacity to remove benzo[a]pyrene diolepoxide-DNA (BPDE-DNA) adducts	MCF-10F	<u>Amount in study groups:</u> 0 mM ethanol vs. 15 mM ethanol vs. 25 mM ethanol <u>Duration of exposure:</u> 48 hours	Incubation of cells with ethanol was associated with a significant increase in prevalence of BPDE-DNA adducts compared to controls.	Ethanol- and oxidative stress-associated inhibition of carcinogen-DNA adduct removal in non-neoplastic human mammary cells may be another biological mechanism to explain the increased risk for breast cancer among women consuming alcohol.
Barnes et al. 2000 ¹⁰⁸	DNA adduct formation and enhancement carcinogen-induced DNA damage in target cell DNA	MCF-10F	<u>Amount in study groups:</u> Ethanol: 0, 5, 15, or 25 mM vs. Aldehyde: 0, 0.5, 2.5, or 5.0 µM <u>Duration of exposure:</u> 6 days	Exposure of cells to physiologically relevant concentrations of either ethanol or aldehyde prior to dosing with B[a]P increased adducts formation.	A possible mechanism by which alcohol intake may be enhancing breast cancer risk in humans may be through an ethanol- and aldehyde-associated increase in carcinogen-DNA adducts in the target mammary epithelial cells.
Zhu et al. 2001 ¹⁰⁹	Modulation of expression of ribosomal protein L7a (rpL7a)	T4TD	<u>Amount in study groups:</u> 100-400 mg/dl ethanol <u>Duration of exposure:</u> 16 days	Long-term exposure to ethanol (2 weeks) significantly reduced the transcript of rpL7a by more than 60%.	Ethanol-induced alteration of rpL7a expression may mediate the promoting effects of ethanol on breast cancer development.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Dhar and Plummer 2006 ¹¹²	Protein expression of G-protein inwardly rectifying potassium channels (GIRK)	MDA-MB-453	<u>Amount in study group:</u> 0.12% ethanol <u>Duration of exposure:</u> 16 hours	Transfection of GIRK1 or GIRK4 plasmids decreased gene expression in MDA-MB-453 breast cancer cells.	Functional GIRK channel exists in breast cancer cells that are involved in cellular signaling.
Singletary et al. 2001 ⁶⁹	Proliferation and intracellular content of cAMP in estrogen receptor (ER)-alpha expression	MCF-7, ZR75.1, BT-20, MDA-MB-231	<u>Amount in study groups:</u> 0-100 mM ethanol <u>Duration of exposure:</u> Up to 10 days	Exposure of ER+ cell lines to increasing concentrations of ethanol was associated with an increase in cell proliferation. For example, ethanol added to cultures of cells at concentrations of 20-50 mM significantly stimulated proliferation of MCF-7 and ZR75.1 cells by 53-91% following 7 and 10 days of treatment, compared to controls.	Treatment with ethanol is associated with increased proliferation of two estrogen receptor-positive human breast cancer cell lines.
Zhu et al. 2001 ¹¹⁴	Up-regulation of transcription of smooth muscle myosin alkali light chain (MLC 1sm)	MCF-7, T47D, MDA-MB-231	<u>Amount in study groups:</u> 50-400 mg/dl ethanol <u>Duration of exposure:</u> 16 days	At 400 mg/dl, an ethanol-mediated increase was evident at 6 hours (55% increase), peaked at 24 hours (2.7 fold increase) following exposure. At pharmacologically relevant concentrations (e.g., 100 mg/dl), ethanol produced a significant increase of MLC 1sm expression, and progressively higher ethanol concentrations resulted in more up-regulation.	Alcohol consumption may promote the progression of breast cancer in women.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Fan et al. 2000 ⁶⁷	Stimulation of the estrogen receptor signaling	MCF-7, T47D	<u>Amount in study groups:</u> 60 mM-100 mM ethanol <u>Duration of exposure:</u> 24 hours	Alcohol partially reverses the BRCA1-mediated inhibition of estrogen receptor-alpha transcriptional activity. Alcohol down-regulates BRCA1 and up-regulates estrogen receptor-alpha expression in MCF-7 cells.	Inactivation of BRCA1 and increased estrogen-responsiveness might contribute to alcohol-induced breast cancer.
Verna and Davidson 1999 ¹¹³	Mammary gland mucin (MUC1) upregulation	MCF-7, T84	<u>Amount in study groups:</u> 0 mM ethanol vs. 50 mM ethanol vs. 100 mM ethanol vs. 150 mM ethanol vs. 200 mM ethanol vs. 250 mM ethanol vs. 500 mM ethanol <u>Duration of exposure:</u> Acute exposure: 24 hours Chronic exposure: up to 4 weeks	Ethanol enhanced the expression of MUC1 mRNA in a dose- and time-dependent manner in MCF-7 cells.	Ethanol regulates expression of the MUC1 gene at the transcription level which strongly suggests the existence of ethanol responsive elements in the promoter of the mucin gene.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Cyong et al. 1978 ¹¹¹	Increase cAMP levels	MM46 tumor cells	<u>Amount in study groups:</u> 0% vs. 0.1% vs. 0.5% vs. 1.0% vs. 2.5% vs. 5.0% ethanol <u>Duration of exposure:</u> 30 minutes	Dose-related increases in cAMP were observed at ethanol concentrations from 0.1% to 5.0%.	Results suggest that either tumor cell membrane, or its membrane-associated defense mechanism for detergents, may be incomplete.

Evidence Base for Colorectal Cancer

Table C-4. Summary of results from human studies on colorectal cancer

Study	Study Design	Mechanism Examined	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Basuroy et al. 2005 ⁸³	Case series. Mucosal biopsies from the left colon (4 forceps biopsies from visibly normal area of mucosa in each subject) were collected from subjects admitted for colonoscopy for the purpose of cancer surveillance. Authors did not report patients' characteristics and previous alcohol exposure.	The effect of acetaldehyde on tyrosine phosphorylation, immunofluorescence localization, and detergent-insoluble fractions of the tight junctions (TJ) and adherens junctions (AJ).	Biopsies were exposed to vapor-phase acetaldehyde, to achieve acetaldehyde concentration of 100-600 uM in the buffer bathing the tissue. Briefly, biopsies in 24-well culture plates were treated with vapor-phase acetaldehyde by placing stock acetaldehyde solution (0.1%-0.6%) in the reservoir wells and sealing the lid to the plate with tapes. 5 hours	Acetaldehyde resulted in epithelial TJ disruption by inducing tyrosine phosphorylation and dissociation from the cytoskeleton of TJ and AJ proteins.	These may have significant implications for the loss of cell-cell adhesion and increased risk for colon cancer.

Table C-5. Summary of results from animal studies on colorectal cancer

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Hayashi et al. 2007 ¹¹⁸	Increased expression of cytochrome P4502E1 (CYP2E1)	Rats	<u>Amount in study group:</u> Ethanol-containing liquid diet (36% of total calories, 5% ethanol v/v) <u>Duration of exposure:</u> 36 weeks	1,1-dimethylhydrazine (DMH)	The number of aberrant crypt foci (ACF) in colons obtained from ethanol-fed rats with DMH was 24, which was significantly more than that of the other treated rats.	The increased expression of CYP2E1 induced by chronic ethanol consumption promotes the development of DMH-induced colon cancer.
Perez-Holanda et al. 2005 ⁷³	Effect of ethanol consumption on experimental colon carcinogenesis using a dynamic model with concomitant administration of alcohol and dimethylhydrazine (DMH).	Rats	<u>Amount in study group:</u> Ethanol at a dose of 1.23 g/kg of body weight <u>Duration of exposure:</u> 24 weeks	DMH	Tumors developed only in DMH treated groups: 25 rats (89%) in the DMH group and 16 rats (100%) in the DMH + ethanol group. However, when excluding tumor-free animals, no differences were observed in the mean number of tumors per rat (1.67 in the DMH group compared to 1.60 in the DMH + ethanol group).	Addition of an ethanol supplement does not modify colorectal carcinogenesis using a dynamic model of tumor induction with DMH.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Pronko et al. 2002 ¹²⁰	Activities of alcohol dehydrogenase (ADH), catalase, microsomal ethanol-oxidizing system (MEOS), and aldehyde dehydrogenase (ALDH)	Rats	<u>Amount in study group:</u> Ethanol as 25% of calories <u>Duration of exposure:</u> 35 days	None	MEOS activity in the alcohol group was 149% higher compared to control group (increase not statistically significant). Effect of acute alcohol intoxication in rats consuming ethanol chronically (control vs. ethanol diet) as measured by ethanol concentrations in the colon was 9.1 (0.98) vs. 11.1 (1.52) and in the rectum was 13.6 (2.57) vs. 17.9 (2.90). Effect of acute alcohol intoxication in rats consuming ethanol chronically (control vs. ethanol diet) as measured by acetaldehyde concentrations in the colon was 7.93 (1.22) vs. 18.5 (3.94)* and in the rectum: 18.1 (3.95) vs. 30.5 (7.13). *$p < 0.05$	This mechanism can account for the local toxicity of ethanol after its chronic consumption, and relates the development of mucosal damage and compensatory hyper-regenerative processes, and possibly carcinogenesis, in the colonic and rectal mucosa of alcoholics to the effects of acetaldehyde.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Homann et al. 2000 ⁷⁰	Folate deficiency via microbial acetaldehyde production	Rats	<u>Amount in study group:</u> 3 g/kg of ethanol <u>Duration of exposure:</u> 2 weeks	None	Alcohol treatment led to very high intracolonic acetaldehyde levels (387 [185] mM). Erythrocyte, serum and small intestinal folate levels were unaffected by alcohol treatment. Alcohol administration decreased significantly colonic mucosal folate levels by 48%.	Alcohol administration leads to local folate deficiency of colonic mucosa in rats, most probably via the degradation of folate by the high levels of acetaldehyde microbially produced from ethanol.
Choi et al. 1999 ¹²⁴	DNA methylation and methylation of p53 tumor suppressor gene	Rats	<u>Amount in study group:</u> Diet containing 36% of total energy as ethanol <u>Duration of exposure:</u> 4 weeks	None	Titrated methyl uptake by colonic DNA from alcohol-fed rats was 57% less than that in control DNA ($p < 0.05$)	Genomic undermethylation of colonic DNA was observed in the alcohol-fed rats compared to control rats.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Hakkak et al. 1996 ¹¹⁹	The effects on expression of CYP2E1 and CYP2C7	Rats	<u>Amount in study groups:</u> 8-13 g/kg/day ethanol. <u>Duration of exposure:</u> Not reported by authors.	None	CYP2E1 was found to be present in the colon and induced by ethanol. Chronic ethanol treatment increased expression of both hepatic ($p < 0.01$) and colonic ($p < 0.05$) CYP2E1 by three-fold.	CYP2E1 and CYP2C7 are present in the colonic tissue and are inducible by ethanol.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Simanowski et al. 1994 ¹²⁵	Effect on rectal cell proliferation (hyperregeneration)	Rats	<u>Amount in study group:</u> 36% of total calories as ethanol, with an additional acute intraperitoneal dose of 2.5 g/kg body weight <u>Duration of exposure:</u> 4 weeks	None	While age by itself did not affect colorectal cell renewal, chronic ethanol consumption stimulated rectal, but not colonic, crypt cell production rate in an age dependent manner. While no significant effect of ethanol was noted in young animals, cell proliferation was significantly enhanced in middle aged animals by 81% (95% CI: 4.1 (2.7-5.5) v 7.4 (6.0-8.7) cells/crypt/hour, $p < 0.001$) and in old animals by 138% (95% CI: 4.5 (3.3-5.6) v 10.7 (8.9-12.4) cells/crypt/hour, $p < 0.001$), after ethanol ingestion. There was a significant positive correlation between crypt cell production rate and acetaldehyde concentrations measured in the distal and proximal colon after an acute dose of ethanol.	Hyperregeneration of the rectal mucosa after alcohol drinking could by itself favor carcinogenesis, which is especially relevant in old age.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Niwa et al. 1991 ¹²⁶	Hyperproliferation of rectosigmoidal colon	Rats	<u>Amount in study groups:</u> 7.5% ethanol vs. 10% ethanol vs. 15% ethanol <u>Duration of exposure:</u> 414 days	Methylazoxymethanol (MAM) acetate	Incidence of colonic cancer (11/17, 85%) was higher in the group that received 10% ethanol compared to control distilled water, $p = 0.04$.	A relatively short-term administration of ethanol induced significant hyperproliferation of the colonic, especially rectosigmoidal colonic, mucosa.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Seitz et al. 1990 ¹²²	Acetaldehyde generation	Rats	<u>Carcinogenesis Study 1 with a duration of exposure of 15 weeks:</u> Liquid diet containing 36% of total calories as ethanol vs. isocaloric glucose <u>Carcinogenesis Study II with a duration of exposure of 3 hrs:</u> 2.5 ml 0.15 NaCl vs. 2.5 ml 0.15 NaCl + cyanamide (CY) vs. 2.5 ml ethanol vs. 2.5 ml ethanol + CY <u>Acetaldehyde Determination in Blood and Tissues:</u> Acute dose of ethanol (2.5 g/kg body wt)	Acetoxymethyl-methylnitrosamine (AMMN) CY	Using metaphase-arrest technique, administration of alcohol induced rectal (99.1 [2.0] vs. 9.1 [1.8] cells/crypt/hour, $p < 0.01$), but not caecal (18.9 [1.3] vs. 22.2 [3.3]) cells/crypt/hour, $p < 0.05$. <u>Mucosal concentration of acetaldehyde (nmol/g/colon)* in the rectum was 198 (23) and 120 (23) in the caecum.</u> These values were not affected by chronic alcohol feeding. *$p < 0.05$	Chronic ethanol consumption can stimulate under certain experimental conditions chemically induced rectal carcinogenesis by direct mechanisms in the rectal mucosa, possibly mediated by acetaldehyde.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
McGarrity et al. 1988 ¹²⁹	Changes in polyamine content	Rats	<u>Amount in study group:</u> Ethanol as 36% of total calories <u>Duration of exposure:</u> 16 weeks	DMH	DMH and DMH + ethanol groups developed 20 adenocarcinomas: with tumors located in the proximal colon (8 vs. 3), distal colon (11 vs. 114) and rectum (1 vs. 3) for the DMH and DMH + ethanol groups, respectively. No tumors developed in the control or ethanol treated groups.	Chronic ethanol consumption did not alter overall tumor formation, however consumption was reported to increase putrescine content in all 3 regions (proximal, distal colon and rectum) compared to the control liquid diet group. Increase in tissue putrescine levels may possibly reflect increased ornithine decarboxylase activity which has been shown to be increased in human colon adenocarcinomas and premalignant adenomas.
Hamilton et al. 1988 ¹³⁰	Effect on the initiation phase of carcinogenesis	Rats	<u>Amount in study group:</u> Ethanol as 33% of total calories <u>Duration of exposure:</u> 13 weeks	Azoxymethane (AOM)	After 24 hours of AOM administration, levels of DNA adducts O ⁶ -methylguanine and 7-methylguanine were reduced in the clonic mucosa of the ethanol-fed rats to 14 ±7% and 61 ±11% of controls.	Dietary ethanol during the preinduction and induction phase of the AOM model dramatically inhibits tumorigenesis.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Garzon et al. 1987 ¹²⁸	Local effect of ethanol on the colorectal mucosa	Rats	<u>Amount in study group:</u> Liquid diets containing 36% of total calories as ethanol <u>Duration of exposure:</u> 10 weeks	AMMN	Significant difference in occurrence of colorectal tumors following chronic ethanol feeding at weeks 15 (42.1 vs. 15.8, $p < 0.05$). No significant difference was reported at weeks 18 and 21.	Chronic ethanol feeding combined with the direct acting carcinogen AMMN resulted in an earlier occurrence of colorectal tumors.
Hamilton et al. 1987 ¹³²	Effect on fecal bacterial flora, and colonic epithelial DNA synthesis	Rats	<u>Amount in study groups:</u> Liquid diet containing 0% ethanol vs. 9% ethanol vs. 18% ethanol as calories <u>Duration of exposure:</u> 25 weeks	AOM	Low ethanol group demonstrated a trend for higher incidence of left-sided colonic tumors compared to controls (35% vs. 15% controls, $p = 0.06$). The total number of tumors in the high-ethanol group compared to controls was 46% vs. 81%, $p = 0.002$, respectively.	Modulation of experimental colonic tumorigenesis by ethanol consumption was due to alcohol rather than other beverage constituents.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Hamilton et al. 1987 ¹³¹	Effect on preinduction, induction, and postinduction phases of carcinogenesis	Rats	<u>Amount in study groups:</u> Liquid diet containing 11% ethanol vs. 22% ethanol vs. 33% ethanol as calories <u>Duration of exposure:</u> 13 weeks	AOM	Suppression of colonic tumorigenesis occurred in the groups with high levels of chronic dietary ethanol consumption during acclimatization and AOM administration: in the 33% and 22% diet groups, the prevalence of colonic tumors was 3% and 20% as compared with 50% in control ($p < 0.001$ and $p < 0.02$, respectively).	Chronic dietary ethanol effects on experimental colonic tumorigenesis with AOM are: (a) due to mechanisms affecting the preinduction and/or induction phase, including carcinogen metabolism; (b) unrelated to postinduction events such as tumor promotion and progression; and (c) dependent on ethanol dose with a threshold for inhibition of tumorigenesis which is mediated by ethanol inhibition of carcinogen metabolism.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Simanowski et al. 1986 ¹²⁷	Promotion of cell proliferation	Rats	<u>Amount in study group:</u> Liquid diet containing 36% of total calories as ethanol (6.6% v/v) <u>Duration of exposure:</u> 4 weeks	None	Cell proliferation rate was 19.1 (2.0) in the ethanol fed group vs. 9.1 (1.8) cell/crypt/hour in the carbohydrate fed group, $p < 0.005$. Serum gastrin also was elevated in the ethanol fed group 172 (51) vs. 106 (27) pmol/l, $p < 0.01$.	The ethanol dependent proliferative changes in the rectal mucosa are predictive of higher susceptibility of this site to carcinogenesis, supporting experimental and epidemiology data. Increased gastrin concentrations may partly explain the observed rectal hyperproliferation. Other possible causes cannot, however, be excluded.
Nelson et al. 1985 ¹¹⁶	None reported by study authors	Rats	<u>Amount in study groups:</u> 95% laboratory grade ethanol diluted vs. tap water <u>Duration of exposure:</u> 19 weeks	DMH	Number of colonic tumors* in the DMH group was 77 and 88 in the DMH + ethanol group. $*p = 0.764$	No augmentation of colonic tumor induction in rats supplemented by dietary ethanol was seen.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Seitz et al. 1985 ¹²³	Generation of acetaldehyde	Rats	<u>Amount in study groups:</u> Ethanol given as 36% of total calories; ethanol concentration of alcohol diet was 6.6% (v/v) vs. isocaloric carbohydrates <u>Duration of exposure:</u> 4 weeks	DMH	There was a 2.8 fold increase in rectal tumors on the ethanol fed rats compared to controls ($p < 0.02$). All large intestinal tumors were located in the rectum in 47% of ethanol fed rats vs. 27% in controls.	The observed increase of ADH activity in the distal colorectum after chronic ethanol feeding may be of relevance with respect to the cocarcinogenic effect of ethanol in the rectum.
Howarth et al. 1984 ¹¹⁷	None reported by study authors	Rats	<u>Amount in study groups:</u> High-fat diet vs. Beer vs. Alcohol (4.8% v/v) <u>Duration of exposure:</u> 20 weeks	DMH	Alcohol did not affect the incidence of intestinal cancers. The shift of mean tumor distance toward the anus was similar in ethanol drinkers (0.61 [0.33] to 0.33 [0.23], $p < 0.05$).	Alcohol had no effect in our syngeneic model of DMH-induced colorectal cancer, while a high-fat diet had a potent cocarcinogenic effect.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Roy et al. 2002 ¹²¹	Effect on cell proliferation, apoptosis, and formation of mutagenic malondialdehyde-acetaldehyde (MAA)	Mice	<u>Amount in study group:</u> Ethanol supplementation in the drinking water (15% alternating with 20% on a daily basis) <u>Duration of exposure:</u> 10 weeks	None	Ethanol supplementation resulted in a significant increase in tumor number ($135 \pm 35\%$, $p = 0.027$ vs. control). The induction of tumorigenesis by ethanol was most dramatic in the distal small bowel ($167 \pm 56\%$, $p = 0.001$).	Ethanol consumption is a risk factor for colorectal cancer.

Table C-6. Summary of results from cell line studies on colorectal cancer

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Lemos et al. 2007 ¹⁴⁰	Modulation of folate uptake	Caco-2	<u>Amount in study group:</u> 12% alcohol <u>Duration of exposure:</u> Not reported	Ethanol had an acute inhibitory effect on both 3H-folic acid and 3H-methotrexate uptake.	Alcohol inhibited 3H-folic acid uptake in Caco-2 cells.
Rodriguez et al. 2004 ⁷⁵	Increase in tumor necrosis factor-alpha receptor-1 (TNF-R1) levels	Caco-2	<u>Amount in study groups:</u> 25 mM ethanol vs. 50 mM ethanol vs. 100 mM ethanol <u>Duration of exposure:</u> 48 hours	Caco-2 cells showed a significant 80% increase in TNF-R1 levels at 200 mM ethanol ($p < 0.05$).	Exposure of intestinal cells to pharmacologic concentrations of ethanol increases TNF-R1 levels and may augment TNF-alpha-mediated cell injury.
Asai et al. 2003 ¹³⁴	Intestinal epithelial cell death induced by acute, low concentrations of ethanol	Caco-2	<u>Amount in study groups:</u> 0% ethanol vs. 5% ethanol vs. 10% ethanol <u>Duration of exposure:</u> 3 hours	Treatment with 5% and 10% ethanol for 3 hours led to a gradual increase in phosphatidylserine (PS) externalization. Caspase-mediated CK18 was significantly enhanced as early as 1 hour after 10% ethanol incubation, while DNA fragmentation was detected from 2 hours onwards.	Apoptotic cell death in confluent Caco-2 cells was induced by acute and low concentrations of ethanol. These results suggest that clinically achievable doses of ethanol impair intestinal barrier function by induction of apoptosis in intestinal epithelial cells.
Tong et al. 1999 ⁷⁴	Induction of epidermal growth factor receptor (EGFR) expression and mitogenesis	Caco-2	<u>Amount in study group:</u> 0.22 mM of ethanol <u>Duration of exposure:</u> 24 hours	Alcohol affects proliferation of Caco-2 cells, elevates EGFR expression and raises cyclin D1 mRNA and protein expression.	Low blood levels of alcohol may stimulate in vivo proliferation of colonocytes by elevating transcription of a growth factor receptor as well as modifying expression of a cell cycle regulator.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Koivisto and Salaspuro 1998 ¹³⁶	Effect of acetaldehyde alone or in combination with ethanol on cell proliferation rate	Caco-2	<u>Study 1:</u> Acute exposure: cells were exposed to acetaldehyde and/or ethanol for 72 hours. <u>Study 2:</u> Chronic exposure: cells were grown in the presence of acetaldehyde and/or ethanol for five passages with daily change of media.	No significant differences were observed between the four groups in the cytotoxic studies (control vs. 100 mM ethanol vs. 500 uM acetaldehyde vs. 1,000 uM acetaldehyde) suggesting that a 72 hour treatment with 500 or 1,000 uM acetaldehyde, or 100 mM ethanol does not have cytotoxic effects on these cells. In the proliferation studies, the acute effect of acetaldehyde on the proliferation rate of Caco-2 cells was strongly inhibitory. The duplication time of Caco-2 cells was also significantly increased by acute exposure to 100 mM ethanol. Concomitant presence of ethanol did not, however, significantly alter the proliferation rate of acetaldehyde-treated cells. 5-week treatment with 500 uM acetaldehyde, both alone and in combination with 100 mM ethanol, significantly decreased cell duplication time as compared with control. A 5-week treatment with 100 mM alone did not have any significant effect on cell proliferation rate. Acetaldehyde decreased the adhesion of Caco-2 cells to both collagens 1 & IV in the cell adhesion studies.	Ethanol-driven or even endogenous acetaldehyde contributes to the initial steps of colonic carcinogenesis and has an effect on later tumor development.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Koivisto and Salaspuro 1997 ¹³⁷	Effect of acetaldehyde on brush border enzyme activities	Caco-2	<u>Amount in study groups:</u> 500 uM acetaldehyde vs. 500 uM acetaldehyde + 100 mM ethanol vs. 1,000 uM acetaldehyde + 100 mM ethanol vs. 100 mM ethanol <u>Duration of exposure:</u> 13 days	Ethanol alone significantly increased the specific activities of sucrase and maltase, but no significant effect on lactase activity. Only ethanol increased alkaline phosphatase activity. Control cells, as well as cells grown in the presence of 100 mM ethanol alone or 500 uM acetaldehyde, showed a typical pattern of dome formation, with a sharp increase in the number of domes a few days after the confluency, followed by a rapid decrease and plateau. Cells grown in presence of both 100 mM ethanol and 1,000 uM acetaldehyde showed significantly fewer domes 4 and 7 days after the confluency than control cells. The acetaldehyde dehydrogenase (ALDH) activity of Caco-2 cells, measured using 200 uM acetaldehyde as substrate was quite similar to that of normal colonic mucosa.	Acetaldehyde decreases the activities of some, but not all, brush border enzymes in Caco-2 cells.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Vaculova et al. 2004 ¹³³	Modulation of the tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis	HT-29	<u>Experiment 1:</u> 4% ethanol alone or in combination for 4 or 24 h in the medium with 5% of fetal calf serum (FCS). <u>Experiment 2:</u> Using ethanol (0.1–6%) alone, the cells were treated for 48 hours.	There was only a limited cytotoxicity of TRAIL (100 ng/ml) in HT-29 cells. After 24-hour-treatment, the cell viability was 82%. However, when TRAIL was combined with ethanol, only 40% of cells remained viable. There was no significant changes in ethanol-treated cells and about two-fold enhancement of the number of cells with decreased MMP after TRAIL treatment (4 hours) compared to control were detected.	Ethanol acts as a potent agent, sensitizing colon cancer cells to TRAIL-induced apoptosis.
Blasiak et al. 2000 ¹³⁵	Formation of crosslinks with DNA	Colonic mucosa (CM) cells	<u>Single exposure study:</u> CM cells were exposed to ethanol at 10 mM vs. acetaldehyde at 100 mM for 1 hour. <u>Combined exposure study:</u> In combined exposure, the cells were subsequently exposed to ethanol and acetaldehyde at all combinations of the concentrations of the agents for 1 hour	Ethanol caused DNA strand breaks. The CM cells exposed to ethanol at 100 mM were able to remove DNA damage within time period shorter than 2 hours.	Alcohol consumption may lead to the damage to DNA of gastrointestinal tract, which in turn can directly or indirectly contribute to the appearance and development of cancers of this organ.

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Results	Conclusions as Reported by Study Authors
Papavassiliou et al. 1994 ¹³⁹	Modulation of human leukocyte antigen (HLA) class I gene expression	HT-29, SW-1116, HCT-15	<u>Amount in study group:</u> 100% ethanol <u>Duration of exposure:</u> 48 hours	Ethanol had no effect on the expression of HLA class 1 antigens in human colon adenocarcinoma cell lines. Ethanol (1.7×10^{-10} M to 1.7×10^{-1} M), had no effect on the expression of HLA class 1 antigens on these colonocytes, corresponding mRNA levels, or the expression of HLA constructs.	These findings do not support the hypothesis that ethanol may modulate the expression of HLA class 1 genes in human colon cancer cells.
Malagolini et al. 1994 ¹³⁸	Differentiation of intestinal cells	Caco-2, HT-29	<u>Amount in study groups:</u> 0 mM ethanol vs. 50 mM ethanol vs. 100 mM ethanol vs. 200 mM ethanol <u>Duration of exposure:</u> 7 days	The addition of ethanol in the culture medium resulted in a significant increment of sucrase and alpha 2, 6-sialyltransferase activities in all cell lines, as well as the beta 1, 4-N-acetylgalactosaminyl-transferase activity in the Caco-2 cells and alkaline phosphatase activity in HT-29 cells.	Ethanol in vitro affects the differentiation of intestinal cells along the enterocytic lineage.

Table C-7. Summary of results from combination study (animal, cell line) on colorectal cancer

Study	Mechanism Examined	Experimental Model	Amount and Duration of Ethanol and/or Acetaldehyde Exposure	Use of Carcinogen	Results	Conclusions as Reported by Study Authors
Pannequin et al. 2007 ¹⁴¹	Accumulation of phosphatidylethanol resulting in a signal change in intestinal cell proliferation	Mice, Caco-2	<u>Animal study:</u> 2 mol/L (10%) ethanol for 4 months. <u>Cell line study:</u> 10 mmol/L of ethanol or 0.5 mmol/L of acetaldehyde once daily for 48 hours.	None	Chronic exposure to low doses of ethanol (10 mmol/L) induces an increase of maximal intestinal cell density.	The disruption of cellular signals might facilitate the stimulatory role of ethanol metabolites such as acetaldehyde on the proliferation of cells within intestinal crypts, thereby participating in the well-established cocarcinogenic role of alcohol consumption in the colon.

Appendix D: List of Excluded Studies

Table D-1. Excluded full articles

Study	Reason(s) for Exclusion
Yi et al. 2010 ³¹³	Cancer mortality study.
Author(s) not listed 1988 ¹⁰³	Clinical meeting article.
Purohit et al. 2005 ⁴⁰	Clinical meeting article.
Scheppach et al. 1999 ³¹⁴	Clinical meeting article.
Seitz et al. 1992 ³¹⁵	Clinical meeting article.
Weisburger 1992 ³¹⁶	Clinical meeting article.
Kleinjans et al. 1996 ³¹⁷	Contents of alcoholic beverage.
Potter et al. 1982 ³¹⁸	Correlation analysis study.
Siegmund et al. 2003 ³¹⁹	Description of animal models in gastrointestinal alcohol research.
Aye et al. 2004 ³²⁰	Invasion of breast cancer cells.
Luo 2006 ³²¹	Invasion of breast cancer cells.
Luo and Miller 2000 ³²²	Invasion of breast cancer cells.
Ma et al. 2003 ³²³	Invasion of breast cancer cells.
Meng et al. 2000 ³²⁴	Invasion of breast cancer cells.
McGarrity and Nelson 1986 ³²⁵	Letter to editor.
Larsen 1993 ³²⁶	News report publication.
Colombo et al. 2001 ³²⁷	No outcome of interest.
Fiala et al. 1987 ³²⁸	No outcome of interest.
Zedeck 1980 ³²⁹	No outcome of interest.
Holford 1987 ⁵	Pharmacokinetic study.
Weisburger and Wynder 1984 ³³⁰	Review article.
Agrawal et al. 2007 ³³¹	Review article.
Alberts 2002 ³³²	Review article.
Ambrosone 2000 ³³³	Review article.
Arasaradnam et al. 2008 ³³⁴	Review article.
Author(s) not listed 2000 ¹	Review article.
Author(s) not listed 2008 ²⁷	Review article.
Author(s) not listed 1994 ³³⁵	Review article.
Baan et al. 2007 ²	Review article.

Study	Reason(s) for Exclusion
Bailey 2003 ³³⁶	Review article.
Blot 1992 ³³⁷	Review article.
Boffetta and Hashibe 2006 ³³⁸	Review article.
Bosetti et al. 2002 ³³⁹	Review article.
Brown 2005 ³⁴⁰	Review article.
Campos et al. 2005 ³⁴¹	Review article.
Chhabra et al. 1996 ³⁴²	Review article.
Correa Lima and Gomes-da-Silva 2005 ³⁴³	Review article.
Dossus and Kaaks 2008, ³⁴⁴	Review article.
Dumitrescu and Cotaria 2005 ³⁴⁵	Review article.
Ferguson et al. 2005 ³⁴⁶	Review article.
Filion 2002 ³⁴⁷	Review article.
Forman et al. 2004 ³⁴⁸	Review article.
Fraumeni 1979 ³⁴⁹	Review article.
Gago-Dominguez et al. 2007 ³⁵⁰	Review article.
Giovannucci 2002 ³⁵¹	Review article.
Goodwin 2008 ³⁵²	Review article.
Hamid et al. 2009 ³⁵³	Review article.
Heavey et al. 2004 ³⁵⁴	Review article.
Homann et al. 2005 ³⁵⁵	Review article.
Huxley et al. 2007 ³⁵⁶	Review article.
Key and Verkasalo 1999 ³⁵⁷	Review article.
Key et al. 2004 ⁴²	Review article.
Kim et al. 2007 ³⁵⁸	Review article.
Klatsky 2001 ³⁵⁹	Review article.
La Vecchia 1989 ³⁶⁰	Review article.
Lands 1998 ³⁶¹	Review article.
Ledermann 1955 ³⁶²	Review article.
Li and Lai 2009 ³⁶³	Review article.
Lieber 2000 ³⁶⁴	Review article.
Lindhal 1992 ³⁶⁵	Review article.
Longnecker 1995 ³⁶⁶	Review article.

Study	Reason(s) for Exclusion
Longnecker 1995 ³⁶⁷	Review article.
Lowenfels 1990 ³⁶⁸	Review article.
Mason and Choi 2005 ³⁶⁹	Review article.
Nagy 2004 ⁹	Review article.
Nanri et al. 2007 ³⁷⁰	Review article.
O'Hanlon 2005 ³⁵	Review article.
Payne 1990 ³⁷¹	Review article.
Perse and Cerar 2007 ⁸⁴	Review article.
Porter 1993 ³⁷²	Review article.
Porter 1995 ³⁷³	Review article.
Poschl and Seitz 2004 ²⁴	Review article.
Poschl et al. 2004 ³⁷⁴	Review article.
Pufulete et al. 2003 ³⁷⁵	Review article.
Purohit 2000 ³⁷⁶	Review article.
Rampersaud et al. 2002 ³⁷⁷	Review article.
Rogers and Conner 1986 ³⁷⁸	Review article.
Rogers et al. 1993 ³⁷⁹	Review article.
Rothman et al. 1995 ³⁸⁰	Review article.
Sakar et al. 2001 ³⁸¹	Review article.
Salaspuro 1996 ²⁰	Review article.
Salaspuro and Mezey 2003 ³⁸²	Review article.
Schatzkin and Longnecker 1994 ⁴³	Review article.
Secretan et al. 2009 ¹⁷	Review article.
Seitz and Becker 2007 ³⁸³	Review article.
Seitz and Homann 2007 ³⁸⁴	Review article.
Seitz and Maurer 2007 ³⁸⁵	Review article.
Seitz and Poschl 1997 ³⁸⁶	Review article.
Seitz et al. 1994 ³⁸⁷	Review article.
Seitz et al. 2005 ³⁶⁹	Review article.
Seitz et al. 1998 ³⁸⁸	Review article.
Siegmund et al. 2006 ³⁸⁹	Review article.
Simanowski et al. 1995 ³⁹⁰	Review article.

Study	Reason(s) for Exclusion
Stoll 1999 ³⁹¹	Review article.
Tan et al. 2006 ³⁹²	Review article.
Taylor and Rehm 2006 ³⁹³	Review article.
Thies and Siegers 1989 ³⁹⁴	Review article.
Tsigris et al. 2007 ³⁹⁵	Review article.
Ulrich 2007 ³⁹⁶	Review article.
Walker and Burkitt 1976 ³⁹⁷	Review article.
Wang 2003 ³⁹⁸	Review article.
Wang 2005 ³⁹⁹	Review article.
Weisburger et al. 1981 ⁴⁰⁰	Review article.
Weisburger 1998 ⁴⁰¹	Review article.
Welsch 1985 ⁴⁰²	Review article.
Williams 1976 ⁴⁰³	Review article.
Winawer and Shike 1992 ⁴⁰⁴	Review article.
Wright et al. 1999 ²⁵	Review article.
Wynder 1977 ⁴⁰⁵	Review article.
Wynder 1978 ⁴⁰⁶	Review article
Nozawa et al. 2006 ⁴⁰⁷	Study administered freeze-dried beer.
Martin et al. 2004 ⁴⁰⁸	Study administered Resveratrol, a polyphenol found in grapes.
Gierer 1955 ⁴⁰⁹	Study did not look at cancer causation.
Briviba et al. 2002 ⁴¹⁰	Study did not report on consumption/administration of ethanol.
Caderni et al. 2000 ⁴¹¹	Study did not report on consumption/administration of ethanol.
Cerda et al. 1999 ⁴¹²	Study did not report on consumption/administration of ethanol.
Depeint et al. 2006 ⁴¹³	Study did not report on consumption/administration of ethanol.
Diergaarde et al. 2003 ²⁵⁹	Study did not report on consumption/administration of ethanol.
Dolara et al. 2005 ³⁵⁸	Study did not report on consumption/administration of ethanol.
Farah 2005 ⁴¹⁴	Study did not report on consumption/administration of ethanol.

Study	Reason(s) for Exclusion
Femia et al. 2005 ⁴¹⁵	Study did not report on consumption/administration of ethanol.
Gonthier et al. 2003 ⁴¹⁶	Study did not report on consumption/administration of ethanol.
Hall et al. 1991 ⁴¹⁷	Study did not report on consumption/administration of ethanol.
Kabat and Rohan 2007 ⁴¹⁸	Study did not report on consumption/administration of ethanol.
Kabat et al. 2007 ⁴¹⁹	Study did not report on consumption/administration of ethanol.
Lagiou et al. 2009 ⁴²⁰	Study did not report on consumption/administration of ethanol.
Etique et al. 2004 ⁴²¹	Study did not report on consumption/administration of ethanol.
Linz et al. 2004 ⁴²²	Study did not report on consumption/administration of ethanol.
Luceri et al. 2002 ⁴²³	Study did not report on consumption/administration of ethanol.
Maciel et al. 2004 ²⁹⁸	Study did not report on consumption/administration of ethanol.
Moon et al. 2006 ⁴²⁴	Study did not report on consumption/administration of ethanol.
Morris and Seifter 1992 ⁴²⁵	Study did not report on consumption/administration of ethanol.
Nozawa et al. 2004 ⁴²⁶	Study did not report on consumption/administration of ethanol.
Nozawa et al. 2004 ⁴²⁷	Study did not report on consumption/administration of ethanol.
Nozawa et al. 2005 ⁴²⁸	Study did not report on consumption/administration of ethanol.
Peluso et al. 2008 ⁴²⁹	Study did not report on consumption/administration of ethanol.
Reddy et al. 1997 ⁴³⁰	Study did not report on consumption/administration of ethanol.
Robson et al. 2006 ⁴³¹	Study did not report on consumption/administration of ethanol.
Schrauzer et al. 1982 ⁴³²	Study did not report on consumption/administration of ethanol.
Takechi et al. 2004 ⁴³³	Study did not report on consumption/administration of ethanol.

Study	Reason(s) for Exclusion
Wulf et al. 2004 ⁴³⁴	Study did not report on consumption/administration of ethanol.
Yamagishi et al. 2002 ⁴³⁵	Study did not report on consumption/administration of ethanol.
Slattery et al. 2009 ⁵⁸	Study looked at tumor markers.
Gago-Dominguez et al. 2005 ⁴³⁶	Unrelated epidemiology study.
Gaudet et al. 2005 ⁴³⁷	Unrelated epidemiology study.
Giacosa et al. 2004 ⁴³⁸	Unrelated epidemiology study.
Lewis et al. 2003 ⁴³⁹	Unrelated epidemiology study.
Orita et al. 2004 ⁴⁴⁰	Unrelated epidemiology study.
Schatzkin et al. 1993 ⁴⁴¹	Unrelated epidemiology study.
Visapaa et al. 1998 ⁴⁴²	Inhibition of intracolonic acetaldehyde production by ciprofloxacin.
Vogel et al. 2007 ¹⁸⁴	Title correction.

Appendix E: Peer Reviewers

List of peer reviewers to be provided by AHRQ.