# 3 Antigenotoxic and Cancer Preventive Mechanisms of *N*-Acetyl-*l*-Cysteine

*Silvio De Flora, MD, Alberto Izzotti, Adriana Albini, PhD, Francesco D'Agostini, Maria Bagnasco, and Roumen Balansky*

*CONTENTS*



## 1. INTRODUCTION

The aminothiol *N*-acetyl-*l*-cysteine (NAC) is an analog and precursor of reduced glutathione (GSH). During the last four decades, it has been extensively used as a mucolytic agent. In addition, because of its multiple protective mechanisms, NAC has been proposed for a broad array of applications, both preventive and therapeutic. The scientific community has a continuously growing interest in this molecule, which is being used with increasing frequency in both clinical investigations and experimental studies. As of February 1, 2002, a total of 5153 scientific papers were available in MEDLINE under the query term "acetylcysteine," with an impressive growth during the last 10 years. In one month alone (January 2002), 134 new papers were added to this database.

Several review articles that examine the cancer chemopreventive properties of NAC are available in the literature *(1–9)*. The objective of this chapter is to update the state of the art on this subject. First, we delineate some biochemical and pharmacological features of this drug, also including original data regarding the safety of NAC as evaluated by multigene expression technology (Subheading 2). We then review studies related to the efficacy of NAC as a modulator of mutagenesis and carcinogenesis, as evaluated in in vitro test systems, in animal models that evaluate intermediate biomarkers and tumors at various sites, and in chemoprevention clinical trials (Subheading 3). Finally, we follow a mechanistic approach by discussing the multiple points at which NAC intervenes in the mutagenesis and carcinogenesis processes (Subheading 4).

## 2. BIOCHEMICAL AND PHARMACOLOGICAL ISSUES

### *2.1. NAC and the Glutathione System*

GSH (γ-glutamyl-*l*-cysteinyl glycine) is known to play a central physiological role in maintaining body homeostasis and in protecting cells against oxidants, toxicants, DNA-damaging agents, and carcinogens of either exogenous or endogenous source *(10,11)*. The intracellular ratio of GSH to oxidized glutathione (GSSG) is approx 100:1. This ratio is maintained by the GSH redox cycle, which involves the activities of GSH peroxidase and NADPH-dependant GSSG reductase. The main supply of NADPH is provided by glucose 6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD) in erythrocytes, and additionally by isocitric dehydrogenase and malic dehydrogenase in other tissues *(12)*. Most of the biological functions of GSH depend on the reactivity of

*From: Cancer Chemoprevention, Volume 1: Promising Cancer Chemoprevention Agents*
Edited by: G. J. Kelloff, E. T. Hawk, and C. C. Sigman © Humana Press Inc., Totowa, NJ

the thiol group of its cysteinyl residue *(13)*. The reaction rate of GSH with electrophiles is greatly enhanced by GSH *S*-transferases (GST), which catalyze conjugation processes resulting in detoxification and excretion of water-soluble conjugates *(13–15)*. Genetic polymorphisms have been detected within this broad family of phase 2 isoenzymes. Two of the GST-encoding genes, identified as *GSTM1* (μ) and *GSTT1* (θ), have a null genotype in humans caused by deletion of both paternal and maternal alleles, resulting in a lack of active proteins *(16)*. The absence of these genes has been associated with cancer in various organs *(17)* as well as with disease occurrence and oxidative DNA damage in glaucoma (*18*), and with DNA adduct levels in atherosclerotic lesions *(19)*. In addition, we hypothesised that *GSTM1* and *N*-acetyltransferase *(NAT2)* polymorphisms may affect responsiveness to treatment with NAC, as a first tentative approach to the pharmacogenomics of chemopreventive agents (*see* Subheading 3.3.2.).

Unfortunately, the large GSH molecule is not transported efficiently into cells, and *l*-cysteine, which is the rate-limiting amino acid in the intracellular synthesis of this tripeptide, is toxic to humans *(11)*. The nontoxic molecule of NAC is readily deacetylated in cells to yield *l*-cysteine, thereby promoting intracellular synthesis of GSH, which is catalyzed by γ-glutamylcysteine synthetase *(8)*. Moreover, NAC can increase GSH intracellular stores by inducing GSSG reductase activity, as shown both in vitro *(20)* and in humans *(21)*. In addition to acting as a GSH precursor, NAC is *per se* responsible for protective effects in the extracellular environment, mainly because of its nucleophilic and antioxidant properties *(8,9)*.

Administration of NAC can be particularly useful in the case of GSH depletion, which is known to occur under conditions of oxidative stress and exposure to toxic and carcinogenic agents *(10,11)*. Moreover, GSH stores appear to be depleted in chronic infections caused by at least three viruses (e.g., hepatitis B virus [HBV], hepatitis C virus [HCV;], and human immunodeficiency virus [HIV]) typically associated with cancer. In fact, both humans infected with HBV and woodchucks infected with woodchuck hepatitis virus display enhanced metabolic activation of chemical hepatocarcinogens, accompanied in woodchucks by GSH depletion related to the amounts of virus in hepatocytes *(22–24)*. GSH levels decreased in both plasma and peripheral-blood lymphocytes of patients chronically infected with HCV *(25)*. In HIV-infected healthy carriers or AIDS patients, GSH levels were decreased in plasma *(26,27)*, epithelial lining-fluid *(27)*, and peripheral-blood monocellular cells *(26)*. It is noteworthy that low thiol levels are associated with impaired survival *(28,29)*.

GSH depletion has also been shown to occur during physical exercise, which may induce oxidative stress. Blood GSH oxidation is a marker of exercise-induced oxidative stress in humans. Studies in GSH-deficient animals clearly indicate the central importance of having adequate tissue GSH to protect exercise-induced oxidative stress. Among the various thiol supplements studied, NAC and α-lipoic acid hold the most promise *(30)*.

### *2.2. Pharmacokinetics and Safety of NAC*

The apparent uptake of GSH occurs largely, if not entirely, through pathways involving prior breakdown to dipeptides and amino acids, transport of these, and intracellular synthesis of the tripeptide *(11)*. NAC easily penetrates cell membranes, and in humans, the intestinal absorption of NAC occurs rapidly. Although bioavailability is less than 10% *(31)*, this is much higher than absorption of GSH, which is not taken up intact to a significant extent into the portal blood from the gastrointestinal tract *(11)*. In the organism, NAC can be present in different forms, such as *N,N'*-diacetylcysteine or *N*-acetylcysteine-cysteine, and metabolism gives rise to a number of products, such as cysteine, cystine, methionine, GSH, and mixed disulphides, which are the predominant form in the plasma *(32)*. Therefore, the pharmacokinetics of NAC are difficult to evaluate, and contradictory results are reported in the literature. For instance, in one study oral NAC increased the levels of plasma cysteine and GSH in both plasma and bronchoalveolar lavage (BAL) fluid *(33)*, and another study found an increase of free cysteine but no change in total cysteine or GSH *(32)*. Still another one found no change of free or total cysteine or GSH in plasma *(21)*. These patterns hamper the evaluation of compliance in clinical trials using NAC, but also show that NAC has little influence on normal homeostasis.

NAC has very low toxicity in both experimental animals and treated humans. In fact, $LD_{50}$ by the oral route is greater than 10 g/kg body wt in both mice and rats, and $LD_{50}$ following intravenous administration is 4.6 g/kg in mice and 2.8 g/kg in rats *(34)*. A daily dose of 1 g/kg body wt given *per os* to rats for 18 consecutive mo had

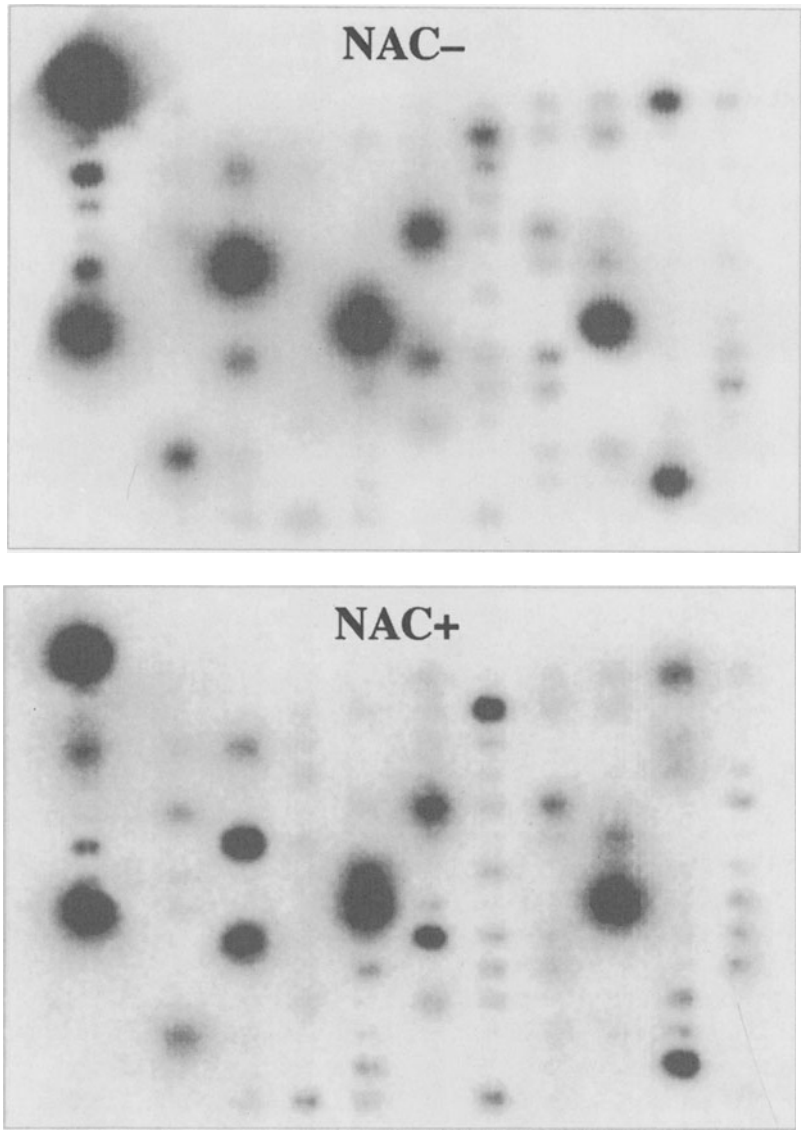


**Fig. 1.** Appearance of electronic autoradiographs of cDNA arrays (Atlas™ Mouse Stress Array) hybridized with $^{32}$P-labeled probes–obtained by reverse transcription of mRNA from the lung of A/J mice, either untreated (NAC) or receiving NAC in the drinking water, at the dose of 1 g/kg body wt, for five consecutive days (NAC+). None of the 36 expressed genes,from a total of 149 examined genes, varied more than twofold in the lungs of NAC-treated mice. The expressed genes could be classified into four main functional categories: (A) metabolism of xenobiotics, including a series of cytochrome P450 isoenzymes (CYP1A1, CYP2B29, CYP2E1, CYP2F2, CYP4B1, and CYP21A1), dimethylaniline oxidase 1, soluble epoxide hydrolase, DT-diaphorase, and nucleophosmin; (B) response to stress, including extracellular superoxide dismutase precursor, heme oxygenase 1, endoplasmic reticulum protein (ERP) 99, ER-60 precursor, ERP72, cyclophilin-40, vimentin, FK-506-binding protein and its precursor FKBP13; (C) protein removal and DNA repair, including T-complex protein 1 theta, eta and zeta sub-units, endonuclease III homolog 1, ATP-dependent DNA helicase II 70-kDa subunit rad52, DNA mismatch repair protein MSH3, mdm2 p53-associated protein, and BRCA2; (D) housekeeping genes, including ubiquitin, phospholipase A2, hypoxantine-guanine phosphoribosyl transferase, glyceraldehyde-3-phosphate dehydrogenase, myosin I alpha, cytoplasmic beta-actin, 45kDa calcium-binding protein precursor, and 405 ribosomal protein. The list of 113 genes whose electronic signal was less than twofold higher than background levels can be inferred, by exclusion, from the Atlas™ Mouse Stress Array gene list (available on the website http://atlas/info.clontech.com)

no detrimental effects *(34)*. In humans, doses of 0.5 g/kg body wt *per os (35)* or 0.3 g/kg body wt intravenously *(36)* were given when NAC was used as an antidote to acute intoxications. Other details regarding NAC safety in clinical trials are reported in Subheading 3.3.

All modulating effects of NAC described in the following sections were detected in a variety of situations leading to toxicity, imbalances of the redox state, or changes in homeostasis. At the molecular level, the safety of a chemopreventive agent can be evaluated by evaluating the lack of influence on gene expression under physiological conditions, especially regarding those genes involved in damage to macromolecules and stress response. For this purpose, we used cDNA array technology to investigate expression of multiple genes in the lung of A/J mice, either untreated or receiving NAC in the drinking water, at a dose of 1g/kg body wt, for five consecutive days. Regardless of treatment with NAC, 36 of the 149 genes included in the Atlas™ Mouse Stress Array (Clontech Laboratories, Inc., Palo Alto, CA) exhibited an electronic signal more than twofold higher than background levels. As shown in Fig. 1 and confirmed by software analysis (Atlas™ Image and Atlas™ Navigator 1.5, Clontech), none of the genes expressed varied more than twofold between the NAC-treated mice and untreated mice. Thus, even at the very high doses used in animal models to modulate intermediate biomarkers, prevent preneoplastic lesions and tumors, exert anti-angiogenic effects, and attenuate invasion and metastasis of cancer cells (as described in this chapter), oral NAC did not alter the background expression of a number of genes in the mouse lung. We plan to undertake similar studies in tissues of humans before and after treatment with NAC.

### *2.3. Pharmacological Applications of NAC*

NAC was introduced as a mucolytic agent in the early 1960s. Its capacity to reduce mucus viscosity depends on breakage of disulphide bridges and depolymerization of mucin molecules. This drug has found extensive clinical application in the therapy and prophylaxis of respiratory diseases. Aside from acute bronchitis, NAC was proposed for the treatment of chronic obstructive broncopulmonary disease *(37)* and its re-exacerbations, as documented by the meta-analysis of 15 clinical trials *(38)*, acute respiratory distress syndrome (ARDS), influenza-like syndromes *(39)*, and idiopathic pulmonary fibrosis *(40)*. Moreover, NAC has been proposed to treat a variety of conditions that share alterations of the redox status and GSH depletion as common pathogenetic determinants *(41)*. For instance, clinical trials have shown the ability of NAC to improve the renal function in hepatorenal syndrome *(42)*, prevent reduction in renal function induced by radiographic contrast agents *(43)*, decrease the risk of graft-vs-host disease (GVHD) in transplanted patients *(44)*, lower plasma homocysteine levels *(45)*, and limit the size of heart infarct *(46)*.

NAC has also been used as an antidote for acute intoxications. The prototype of this application is to treat overdosage of the analgesic drug acetaminophen (paracetamol) *(36)*, whose cytotoxicity is mediated by the reactive metabolite *N*-acetyl-*p*-benzoquinoneimine, a strongly electrophilic and oxidizing agent formed in the liver via cytochrome P450 monooxygenase, and in the kidney via prostaglandin synthetase system *(32)*. Other possible applications of NAC in clinical toxicology include protection against hepatorenal damage induced by the potent GSH-depleting toxins chloroform, carbon tetrachloride, 1,2-dichloropropane, and α-amanitine (contained in *Amanita phalloides*), bone-marrow toxicity of chloramphenicol, cardiorespiratory arrest caused by acrylonitrile, liver necrosis induced by bromobenzene, multiple lesions produced by mustard gas (dichlorodiethyl sulfide), and methemoglobinemia, hemolysis, and cataract induced by naphthalene *(32)*. Furthermore, NAC has been used to attenuate the side effects of cytostatic drugs—e.g., cardiomyopathy caused by doxorubicin (adriamycin) and the hemorrhagic cystitis caused by cyclophosphamide and iphosphamide *(32)*.

As described in this chapter, NAC has been extensively investigated for possible applications in the prevention of genotoxic damage and oxidative stress in cancer and other chronic degenerative diseases.

## 3. EFFICACY OF NAC AS A MODULATOR OF MUTAGENESIS AND CARCINOGENESIS

### *3.1. In Vitro Test Systems*

#### 3.1.1. Acellular Systems

NAC inhibited single-strand breakage in plasmid DNA exposed to aqueous extracts of cigarette smoke and a nitric oxide-releasing compound, which forms potent reactive species such as peroxynitrite *(47)*. NAC prevented oxidative damage to calf thymus DNA produced in Fenton-type reactions, which generate hydroxyl radicals (·OH). In particular, NAC inhibited DNA fragmentation and formation of $^{32}P$ postlabeled nucleotide modifications and 8-hydroxy-2'-deoxyguanosine

(8-OH-dG) in DNA exposed to either $H_2O_2$ or a mixture of $H_2O_2$ and $CuSO_4$ *(48)* as well as formation of the 8-OH-dG tautomer 8-oxo-2'-deoxyguanosine (8-oxo-dG) in DNA exposed to a mixture of $H_2O_2$, $CuSO_4$, nitrilotriacetic acid, and ascorbic acid *(49)*. NAC also inhibited formation of adducts to calf thymus DNA metabolically activated *N*-nitrosopyrrolidine *(50)*. Conversely, NAC did not inhibit the formation of $^{32}P$ postlabeled DNA adducts by metabolically activated benzo*[a]*pyrene (B*[a]*P) *(51)* and dibenzo*(a,l)*pyrene *(52)*. As discussed in Subheading 3.1.2., these findings are consistent with results of bacterial mutagenicity test systems using liver preparations from rats treated with enzyme inducers.

### 3.1.2. Bacteria

Several studies evaluated the ability of NAC to modulate genotoxicity of either direct-acting mutagens or promutagens in *Salmonella typhimurium his*$^-$ strains and, in some cases, in *Escherichia coli* strains with differential DNA repair capacities. Activity profiles for a variety of mutagens are available *(53)*.

Fifteen years ago we demonstrated that, like other thiols, NAC dose-dependently decreases "spontaneous" mutagenicity in strain TA104 *(54–56)*. This effect was confirmed by another laboratory *(57)*. Since inhibition of "spontaneous" revertants did not occur in TA102, which has different DNA repair capacities than TA104, we hypothesized that inhibition of "spontaneous" mutations by thiols depends not only on their antioxidant properties but also by possible effects on DNA repair mechanisms *(56)*.

NAC is known to act as a radioprotector *(58)*. However, NAC did not affect the mutagenicity, in strain TA102 and TA104, of ultraviolet (UV) radiation emitted either by monochromatic sources at 254 nm *(1)* or 365 nm *(8)* or by fluorescent *(8)* or halogen lamps *(59)*.

NAC attenuated the direct mutagenicity of peroxides, including cumene hydroperoxide *(1)* and hydrogen peroxide *(20)*, in TA102 and TA104 as well as DNA-damaging activity of hydrogen peroxide in *E. coli (60)*. Crocidolite, chrysotile fibers, and man-made vitreous fibre-21 increased 8-OH-dG levels in calf-thymus DNA and were mutagenic to TA100, and more potently, to its GSH-deficient derivatives TA100/NG-54 and TA100/NG-57. Pretreatment with NAC reduced the number of revertants to less than that of the parent strains *(61)*. Moreover, as reported in Subheading 4.2., NAC inhibited the bacterial genotoxicity of reactive oxygen species (ROS) generated in vitro by electron-transfer reactions *(55,62)* or produced by human phagocytic leukocytes *(63)*. With the exception of nitrofurantoin *(1)* and of sodium azide *(1,64)*, tested in strain TA100 and TA100NR, NAC was found to inhibit mutagenicity to *S. typhimurium his* strains of many direct mutagens that belong to a variety of chemical families and functional categories. The list includes the pesticides captan and folpet in TA100 *(8)*; the antitumor drugs 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]-acridine (ICR 191) in TA1537 *(8)*, and doxorubicin (adriamycin) in TA98 *(65)*; glutaraldehyde and formaldehyde, also in the form of vapors, in TA102 and TA104 *(54)*; β-propiolactone in TA100 *(8)*; hydralazine in TA98 *(8)*, quercetin in TA98 *(8)*; 4-nitroquinoline 1-oxide in TA100 *(20,66)*; *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) in TA1530 *(64)* and TA100 *(67)*, as well as in the differential DNA repair assay in *E. coli (67)*; 2-nitrofluorene in TA1538 and TA98 *(64)*; 1-nitropyrene, 1,8-dinitropyrene, and diesel extracts in TA98, TA100 and TA104 *(57)*; vinyl carbamate epoxide in TA1535 *(68)*; epichlorohydrin in TA1535 *(20)*; and sodium dichromate in TA102 *(20)*.

The effect of NAC on promutagens and S9-requiring complex mixtures is more difficult to interpret. Some data suggest a protective role of NAC—for instance, in the case of promutagens such as benzidine activated by hamster liver S9 *(69)*, aflatoxin $B_1$ activated by woodchuck liver regardless of infection with hepatitis virus *(23)*, and doxorubicin, whose mutagenicity was inhibited by NAC in the absence of—and even more efficiently, in the presence of—rat liver S9 *(65)*. In other cases, modulation by NAC was more uncertain, ranging from lack of inhibition to inhibition at high doses only to enhancement of mutagenicity. The tested compound included the aromatic amines 2-aminoanthracene, 2-acetylaminofluorene (2AAF) and 2-aminofluorene in TA1538 and TA98 *(20,64,70)*; aflatoxin $B_1$ in TA98 and TA100 *(20,70–72)*; cyclophosphamide in TA1535 *(20,70)*; 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) in TA98 *(20)*; B*[a]*P in TA1538, TA98 and TA100 *(20,64)*; and complex mixtures, including cigarette smoke *(66)* and cigarette smoke condensate (CSC) *(20)*. All uncertain or inconsistent results were observed when the promutagens were tested in the presence of rat liver S9, and the outcome depended on pretreatment of rats with enzyme inducers. As previously discussed in more detail *(8)*, these patterns occur because NAC

does not inhibit and may even stimulate metabolic activation.The resulting reactive metabolites are detoxified by coordinated blocking because of the nucleophilicity of this molecule and, to some extent, by stimulation of phase 2 enzymes (*see* Subheading 4.4.). In fact, the same promutagens were efficiently counteracted by NAC in animal models (*see* Subheading 3.2.1.) while, both in mutagenicity test systems (this Subheading) and acellular systems (*see* Subheading 3.1.1.), S9 mix cannot fully mimic the complexity of the in vivo situation. For instance, S9 mix lacks co-factors for phase II enzymes *(73)*.

#### 3.1.3. Cultured Mammalian Cells

NAC exerted protective effects in genotoxicity and cell-transformation assays in cultured mammalian cells. As evaluated by single-cell electrophoresis (COMET assay), NAC inhibited the genotoxicity of both acrolein and water-soluble cigarette smoke in human lymphoid cells containing Epstein-Barr episomes *(74)*. Using the micronucleus test, NAC attenuated the genotoxicities of arsenite in cultured human fibroblasts *(75)* and of the oxidizing agent paraquat in human endothelial cells *(76)*. Moreover, NAC inhibited induction of sister chromatid exchanges in Chinese hamster ovary cells co-cultivated with phagocytic leukocytes, which generate ROS *(77)*.

In particular cases, however, GSH contributes to metabolic activation of certain genotoxic agents. Accordingly, depending on the situation, NAC may exert adverse effects. This has been shown to be the case for MNNG, as assessed by evaluating either 6TG-resistant mutations in V79 Chinese hamster cells *(78)* or *his*$^+$ reversions in strain TA100 of *S. typhimurium (67)*. In both prokaryotic and eukaryotic cells, NAC enhanced mutagenicity when reacting intracellularly, although mutagenicity was enhanced when MNNG reacted intracellularly with NAC derivatives. NAC also enhanced DNA-strand breakage induced by potassium bromate in cultured human lymphocytes *(79)*. NAC did not affect formation of DNA adducts by dibenzo*(a,l)*pyrene in the human breast-cell line MCF-7 *(80)*. Tumor-promoting activity of $H_2O_2$ in rat liver-epithelial oval cells was decreased by NAC, which inhibited phosphorylation of connexin 43 and disruption of gap-junctional intercellular communications *(81)*. NAC attenuated the ability of B*[a]*P to induce transformation of cultured rat tracheal-epithelial cells. This system appears to identify chemopreventive compounds that act at early stages of the carcinogenesis process *(82)*.

In addition, a number of studies provided evidence that NAC displays anticytotoxic (Subheading 4.9.) and antiproliferative effects (Subheading 4.14.), and modulates apoptosis (Subheading 4.10.) in cultured mammalian cells.

### 3.2. Animal Models

#### 3.2.1. Biomarkers and Preneoplastic Lesions

Many studies have provided evidence that, almost invariably, NAC exerts protective effects toward alterations of biomarkers induced in rodents by individual carcinogens and carcinogenic complex mixtures. The biomarkers that have been studied include adducts to hemoglobin and mitochondrial DNA, and adducts to nuclear DNA in various tissues; oxidative alterations of nucleotides; DNA fragmentation; DNA-protein crosslinks (DPXL); apoptosis; alterations of nuclear enzymes; cytogenetic damage; and preneoplastic lesions, such as altered foci in the liver, aberrant crypt foci (ACF) in the colon, and morphological and functional alterations in the respiratory tract.

**3.2.1.1. The Respiratory Tract** NAC was found to modulate a variety of biomarkers and/or preneoplastic lesions in isolated cells of the respiratory tract such as BAL cells, particularly pulmonary alveolar macrophages (PAM); and in tissues such as nasal, tracheal, bronchial, and bronchiolar epithelia, as well as in the mixed-cell population of the lung. Changes in the respiratory tract were induced in rodents exposed whole-body to cigarette smoke, either mainstream, sidestream or environmental, or receiving intratracheal instillations either of lung carcinogens, such as B*[a]*P or chromium(VI), or of complex mixtures, such as air-particulate extracts.

NAC decreased the levels of DNA adducts, detected by $^{32}$P postlabeling analysis in the dissected nasal epithelium of Sprague-Dawley rats exposed to mainstream cigarette smoke for 40 d (Izzotti A, Balansky R, unpublished data).

PAM, which are the predominant cells in BAL, are sentinel cells that phagocytize foreign particles and can both activate and detoxify carcinogens. Therefore, they are ideal for evaluating the modulation of biomarkers in both humans and experimental animals *(83)*. NAC given by gavage inhibited formation of DNA adducts detected by synchronous fluorescence spectrophotometry (SFS) in the lung of Sprague-Dawley rats exposed whole-body to mainstream cigarette smoke *(84)*. A similar effect detected by $^{32}$P postlabeling was observed in BAL cells of Sprague-Dawley

rats that were exposed whole-body to environmental cigarette smoke and received NAC in drinking water *(85)*. Formation of micronucleated and polynucleated PAM was significantly attenuated by oral NAC in $BDF_1$ mice that were exposed whole-body to mainstream cigarette smoke *(86)*, and in Sprague-Dawley rats that received intra-tracheal instillations of B*[a]*P *(87)* or air-particulate extracts *(88)* or exposed whole-body to mainstream cigarette smoke *(89)* or to environmental cigarette smoke *(85)*. In the last study, the effect of NAC, given in drinking water was enhanced by co-administration of oltipraz in the diet *(85)*. Moreover, NAC was capable of normalizing BAL cellularity, which in $BDF_1$ mice was still altered 11 wk after discontinuation of exposure to mainstream cigarette smoke because of an almost 10-fold increase of polymorphonucleates and a parallel decrease of PAM *(86)*.

A variety of biomarkers were evaluated in the lung mixed-cell population of Sprague-Dawley rats. Oral NAC suppressed formation of adducts to mitochondrial DNA in the lungs of rats exposed whole-body to mainstream cigarette smoke *(90)*, which may be a factor in the pathogenesis of several chronic degenerative diseases. NAC inhibited formation of SFS-positive adducts to nuclear DNA in the lungs of rats that received intra-tracheal instillations of B*[a]*P *(87,91)* or exposed whole-body to mainstream cigarette smoke *(84)*. NAC significantly decreased levels of $^{32}P$ postlabeled DNA adducts in the lungs of rats exposed to environmental cigarette smoke (85), yet in another study it had no effect on sidestream cigarette smoke *(92)*[a]. Interestingly, NAC interacted synergistically with oltipraz to decrease environmental cigarette smoke-induced DNA adducts in the lung *(85)*. NAC also decreased oxidative DNA damage (8-OH-dG) in the rat lung following exposure to environmental cigarette smoke *(85)*.

[a]Comparative experiments have provided evidence that the contrasting conclusions generated in these studies are methodological in nature, depending on the chromatographic conditions used to separate DNA adducts. In fact, the chromatographic system used in ref. *92*, employing isopropanol/ammonium hydroxide as developing buffer, yields much lower amounts of DNA adducts and fails to detect the massive diagonal radioactive zone (DRZ). The DRZ, which can be detected by using urea-containing developing buffer, expresses a multitude of DNA-binding agents that are present in cigarette smoke *(85,93)*.

In the lungs of rats that received intra-tracheal instillations of air-particulate extracts, both SFS and $^{32}P$ postlabeling analyses demonstrated the ability of NAC to decrease the levels of DNA adducts. Moreover, NAC counteracted the induction of micronuclei in PAM and epithelial cells of the respiratory tract, and the induction of polynucleated PAM. In the same animals' lungs, NAC attenuated stimulation of the nuclear enzyme poly(ADP ribose) polymerase (PARP), produced by the lung by exposure to air-particulate extracts *(88)*. NAC also affected genotoxic effects produced in vivo by chromium(VI), which is a lung carcinogen when inhaled at high doses *(94)*. In fact, NAC significantly decreased pre-genotoxic and genotoxic alterations produced by intra-tracheal instillations of sodium dichromate to rats, as shown by inhibition of $^{32}P$-postlabeled nucleotide modifications, DNA fragmentation, and DPXL *(95)*.

The histological structure of the rat trachea resembles that of the human bronchus, the major site of smoking-related cancer in humans *(96)*, and inhaled cigarette smoke induces preneoplastic changes in rat tracheal-epithelial cells *(97)*. In two separate experiments in Sprague-Dawley rats exposed to mainstream cigarette smoke, oral NAC inhibited formation of DNA adducts in the dissected tracheal epithelium *(98)*. Similarly, NAC diminished DNA adduct levels in the dissected tracheal epithelium of the same rat strain following exposure to environmental cigarette smoke *(85)*. However, in another laboratory, dietary NAC did not significantly influence the levels of most major and minor lipophilic DNA adducts formed in the whole trachea of rats exposed to sidestream cigarette smoke *(92)*[a]. Exposure to cigarette smoke, either mainstream or environmental, is a strong inducer of apoptosis in the respiratory tract, as evaluated both by morphological analysis and the TdT-mediated nick-end labeling (TUNEL) method *(99)*. Administration of NAC in drinking water significantly attenuated induction of apoptosis both in bronchial/bronchiolar epithelium of mainstream cigarette smoke-exposed rats and in PAM of environmental cigarette smoke-exposed rats. This effect was ascribed to the ability of NAC to inhibit the genotoxic effects and other events that trigger the apoptotic process *(99)*. Recently we demonstrated that the oral administration of NAC during pregency prevents birth-related genomic and transcriptional attentions in mouse lung *(100)*.

NAC was also shown to protect rat respiratory airways from morphological and functional alterations

produced by cigarette smoke. For instance, administration of NAC in drinking water significantly prevented epithelial secretory-cell hyperplasia, especially in the smallest bronchioli, as well as hypersecretion of mucus in the larynx and trachea of Wistar rats exposed whole-body to mainstream cigarette smoke *(101)*. In addition, treatment of Sprague-Dawley rats with NAC by gavage inhibited mainstream cigarette smoke-induced severe histopathological changes in terminal airways, including an intense inflammation of bronchial and bronchiolar mucosae accompanied by multiple hyperplastic and metaplastic foci of micropapillomatous growth, and a severe emphysema accompanied by extensive disruption of alveolar walls *(89)*. Oral NAC also prevented alterations in morphometry, consisting of airway-wall thickening of small, medium, and large bronchi, and alterations in ventilation distribution after exposure of rats to cigarette smoke for 10 wk *(102)*.

**3.2.1.2. The Digestive System** Oral NAC produced a significant shift from severe to mild preneoplastic lesions in the esophagus of $BD_6$ rats treated with *N,N*-diethylnitrosamine (DEN) and diethyldithiocarbamate (DEDTC) (*see* Subheading 3.2.2.2).

NAC was evaluated for the ability to inhibit the formation of ACF, putative precancerous lesions, in the colon of rats receiving subcutaneous(SC) injections of azoxymethane (AOM). In F344 rats, NAC (2 g/kg diet) significantly reduced the number of AOM-induced lesions from 228 to 151 ACF/animal *(103)*. However, this result was not confirmed in a further study carried out in the same laboratory under similar experimental conditions *(104)*. In F344 rats, the conjugate of NAC with phenylethyl isothiocyanate (PEITC), given by gavage, reduced the formation of total foci and multicrypt foci when administered during the post-initiation stage, but was ineffective as an anti-initiator *(105)*.

Administration of NAC in drinking water significantly decreased levels of adducts to mtDNA in the liver of Sprague-Dawley rats treated with 2AAF by gavage *(90)*. In Wistar rats that received the same carcinogen, co-administration of NAC in diet protected the nuclear enzyme PARP from the damage produced by 2AAF, either according to the Teebor and Becker protocol *(106)* or the Solt and Farber model *(8)*. Oral NAC significantly decreased the levels both of SFS-positive DNA adducts in the livers of Sprague-Dawley rats receiving intra-tracheal instillations of B*[a]*P *(87)*, and of $^{32}$P-postlabeled DNA adducts in the liver of Sprague-Dawley rats that received 7,12-dimethylbenz*(a)*anthracene (DMBA) by gavage (Izzotti A et al., unpublished data). NAC had a strong antagonistic effect on formation of hepatic lipid hydroperoxides in Sprague-Dawley rats treated with an intraperitoneal (ip) injection of the hepatocarcinogen safrole *(107)*. In a study on the protective role of thiols on induction of DNA damage in the liver of Sprague-Dawley rats, as determined by the alkaline elution technique, an ip injection of NAC greatly reduced the genotoxicity of MNNG, yet it did not affect the genotoxicity of *N*-methyl-*N*-nitrosourea (MNU) *(108)*.

The transplacental exposure of Swiss albino mice to environmental cigarette smoke resulted in the enhancement of bulky DNA adduct and 8-OH-dG levels, along with overexpression of a large number of genes in the fetus liver. Administration of NAC in drinking water to environmental cigarette smoke-exposed pregnant mice significantly attenuated these alterations (*109*).

In two separated studies, NAC inhibited and delayed the formation of γ-glutamyltranspeptidase (GGT)-positive foci in the liver of Wistar rats fed a 2AAF-supplemented diet for four consecutive cycles, according to the Teebor and Becker protocol *(110)*.

**3.2.1.3. Hair Follicle Cells** Oral NAC prevented the alopecia induced in C57BL/6 mice by doxorubicin, which typically causes oxidative DNA damage *(111)*. In the same mouse strain, whole-body exposure to environmental cigarette smoke induced grey hair and alopecia, accompanied by apoptosis of hair follicle cells. Administration of NAC in drinking water totally prevented these effects *(112)*.

**3.2.1.4. Mammary Glands** In a study using blind coded samples, administration of dietary NAC (10 g/kg diet) significantly decreased DNA adducts levels in mammary epithelial cells of Sprague-Dawley rats that received a single DMBA dose (12 mg/rat) by gavage (our unpublished data, in collaboration with the University of Alabama at Birmingham and the U.S. National Cancer Institute, Chemoprevention Branch).

**3.2.1.5. The Cardiovascular System** Oral NAC inhibited the formation of SFS-positive DNA adducts in the hearts of Sprague-Dawley rats that received intra-tracheal instillations of B*[a]*P *(87)* or exposed whole-body to mainstream cigarette smoke *(84)*. Similarly, NAC significantly decreased levels of DNA adducts in the heart of the same rat strain following exposure to environmental cigarette smoke, as assessed by $^{32}$P postlabeling *(85)*, but had no effect in

another study using rats exposed to sidestream cigarette smoke *(92)*.

In the aorta of Sprague-Dawley rats, NAC significantly reduced the level of DNA adducts, as detected by SFS in mainstream cigarette smoke-exposed rats *(91)* or by $^{32}$P-postlabeling in environmental cigarette smoke-exposed rats *(113)*.

**3.2.1.6. The Urogenital Tract** Administration of NAC by gavage significantly decreased the levels of DNA adducts in Sprague-Dawley rats exposed to mainstream cigarette smoke, as detected by SFS in kidney or by $^{32}$P postlabeling in the testis *(8)*.

NAC significantly reduced dominant lethal mutations in Sprague-Dawley rats treated with ethyl methanesulfonate *(114)*.

**3.2.1.7. Bone Marrow and Peripheral Blood** In Sprague-Dawley rats exposed whole-body to environmental cigarette smoke, treatment with NAC in drinking water strongly reduced levels of adducts to hemoglobin of 4-aminobiphenyl and B*(a)*P-7,8-diol-9,10-epoxide, typical constituents of cigarette smoke. This effect was further potentiated by combining NAC with oltipraz in diet *(85)*.

Several studies have investigated the ability of NAC to modulate systemic genotoxic effects in rodents, either untreated or treated with cigarette smoke, polycyclic aromatic hydrocarbons (PAH), aromatic amines, or urethane. These effects were usually determined by evaluating the frequency of micronucleated (MN) polychromatic erythrocytes (PCE), either in fetal liver or adult bone marrow, and of MN normochromatic erythrocytes (NCE) in the peripheral blood.

Lifetime administration of NAC in the drinking water, at 0.1 or 0.5 g/kg body wt, did not affect "spontaneous" levels of MN PCE in the peripheral blood of both male and female $BDF_1$ mice (Balansky R et al., unpublished data).

In $BDF_1$ mice exposed whole-body to mainstream cigarette smoke, NAC often decreased smoke-induced formation of MN PCE in bone marrow, but not to a significant extent, and significantly attenuated the formation of MN NCE in peripheral blood *(86)*. Following exposure of Sprague-Dawley rats to mainstream cigarette smoke, NAC decreased toxicity in bone marrow, as shown by normalization of the PCE:NCE ratio, but failed to attenuate the frequency of MN PCE *(89)*. Administration of NAC in drinking water and its combination with dietary oltipraz significantly decreased the frequency of MN PCE in bone marrow of Sprague-Dawley rats exposed to environmental cigarette smoke *(85)*. NAC, given in drinking water during pregnancy, significantly decreased frequency of MN PCE in the liver of fetuses of Swiss albino mice whose mothers had been exposed to environmental cigarette smoke during pregnancy (*109*).

NAC failed to inhibit the induction of MN PCE in the bone marrow of C57BL/6 mice treated with DMBA by gavage *(115)*. Conversely, the frequency of MN PCE was significantly attenuated by NAC in $BDF_1$ mice that received a single ip injection either of the aromatic amine 2AAF or of the chromium(VI) salt sodium dichromate, and in Balb/c mice that received a single ip injection of urethane (Balansky R et al., unpublished data). Moreover, the frequency of MN PCE, periodically monitored in Balb/c mice treated with ip injections of urethane, was significantly decreased by NAC in a dose-related fashion, which predicted the subsequent inhibition of urethane-induced lung tumors *(116)*.

### 3.2.2. Tumors

**3.2.2.1. The Respiratory Tract** In Fischer rats that received sc injections of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK),1.5 mg/kg body wt 3×/wk for 21 wk, the incidence of nasal cavity tumors was decreased from 78% of controls to 61% and 47% when the diet was supplemented with NAC at 6.5 and 13.1 g/kg diet, respectively *(117)*.

Dietary NAC (6.8 g/kg diet) significantly inhibited the formation of squamous carcinomas in the tracheas of Syrian golden hamsters that received a local application of 5% MNU once per wk for 15 wk *(118)*.

The effect of oral NAC on lung adenomas induced by urethane was evaluated in three mouse strains. Administration of NAC (2 g/kg diet) in the diet significantly decreased both tumor incidence and multiplicity in Swiss albino mice treated with a single ip administration of urethane at the dose of 1 g/kg body wt *(119)*. A significant and dose-dependent decrease of lung-tumor multiplicity was produced by NAC (0.1 or 0.5 g/kg body wt) in the drinking water of Balb/c mice treated with 10 daily ip injections (0.4 g/kg body wt each) of urethane *(116)*. A significant decrease of tumor multiplicity was observed when NAC was given (2 g/kg diet) to A/J mice treated with a single ip injection of urethane at 0.25 g/kg body wt, but no protective effect occurred when the carcinogen was dosed

at either 1 or 0.1 g/kg body wt *(120)*. In a further study of A/J mice treated with a single ip injection of urethane (1 g/kg body wt), administration of NAC in the drinking water (1 g/kg body wt) significantly decreased tumor multiplicity; this effect was further enhanced by combining NAC with ascorbic acid at the same dose *(121)*.

NAC failed to affect the lung tumor yield in A/J mice exposed whole-body to environmental cigarette smoke for 5 mo, followed by 4 mo of recovery in filtered air *(120)*. As confirmed by our laboratory *(122)*, this treatment results in a weak but significant increase of lung tumors. Notably, with the exception of combined treatment with *myo*-inositol and dexamethasone *(123,124)*, all tested chemopreventive agents, including PEITC *(120)* and its combination with benzyl isothiocyanate (BITC) *(123)*, decaffeinated green tea *(120)*, acetylsalicylic acid *(123)*, D-limonene, and 1,4-phenylenbis(methylene)selenoisocyanate *(124)*, were unsuccessful in attenuating the increase in lung tumors produced by environmental cigarette smoke in this experimental model.

Dietary administration of NAC, at either 6.5 or 13.1 g/kg diet, did not affect lung-tumor incidence in Fischer rats that received sc injections of NNK (1.5 mg/kg body wt) 3×/wk for 21 wk. Determination of lung-tumor multiplicity was not possible in this study *(117)*. Similarly, administration of NAC at either 13.1 or 26.2 g/kg diet did not affect the lung tumor yield 16 wk after a single (10 μmol) ip injection of NNK in A/J mice. However, incidence of NNK-induced lung adenocarcinomas significantly decreased by 26.2 g NAC/kg diet 52 wk after carcinogen administration, indicating that NAC retards malignant progression in the lungs of NNK-treated A/J mice *(125)*.

The fact that isothiocyanates are excreted as NAC conjugates via the mercapturic acid pathway has prompted studies intended to evaluate the ability of conjugates of NAC with either PEITC, 3-phenylhexyl isothiocyanate, or BITC to modulate the lung-tumor yield in NNK-treated A/J mice. Although the protective effects of isothiocyanate in this model were not potentiated following conjugation with NAC, it was concluded that use of NAC conjugates may be convenient because of their reduced toxicity compared to isothiocyanate alone and their higher lipophilicity, which facilitates absorption and gradual dissociation of NAC and isothiocyanates in the body *(126)*. These conjugates also significantly inhibited lung tumors in A/J mice when given after an injection of NNK *(127)*. Moreover, administration of the NAC conjugates of BITC and PEITC with the diet, after a single dose of B*[a]*P by gavage, significantly reduced the lung-tumor multiplicity *(128)*.

**3.2.2.2. The Digestive System** NAC given in drinking water (0.4 g/kg body wt) produced a significant shift from severe to mild preneoplastic lesions (hyperplasia and keratosis/acanthosis) and an inhibition of tumor multiplicity (papillomas and squamocellular carcinomas) in the esophagus in $BD_6$ rats treated with 8 weekly ip injections of DEN (50 mg/kg body wt) followed in 4 h with ip injections of DEDTC (50 mg/kg body wt) *(129)*.

The ability of NAC to attenuate chemically induced colon tumors was demonstrated in two rat models. The administration of NAC with drinking water (1 g/L) in Wistar rats treated with 15 weekly sc injections of 1,2-dimethylhydrazine (DMH) significantly decreased both the incidence and multiplicity of intestinal tumors *(130)*. In F344 rats treated with two weekly sc injections of AOM, the administration of NAC with the diet, at either 0.6 or 1.2 g/kg diet, significantly decreased the multiplicity of colon adenocarcinomas *(131)*. In C57BL/6J mice treated for 12 weeks with dextran sulfate sodium in the drinking water and iron-enriched diet, followed by a 10 day recovery period, administration of NAC with the diet (2 g/kg in the diet) significantly decreased both incidence and multiplicity of colorectal adenocarcinomas as well as chronic ulcerative colitis (*132*).

Conversely, NAC failed to inhibit liver tumor formation in rodents treated with DEN. This lack of protective effects was observed in C3H mice that received ip injections of NAC (30 mg/kg body wt) on d 13, 14, and 15, followed by administration of this agent in the diet (2 g/kg) starting at 21 d of age; DEN was administered ip, at 4 mg/kg body wt, on d 15 of age *(133)*. Moreover, under the same conditions reported for the esophagus, NAC did not affect the yield of benign and malignant liver tumors in $BD_6$ rats treated with DEN and DEDTC *(129)*.

**3.2.2.3. Skin** NAC inhibited the formation of skin papillomas in mice treated with DMBA and 12-*O*-tetradecanoyl-13-phorbol acetate (TPA) *(134)*. Unlike GSH, topical application of NAC to skin papillomas did not significantly inhibit progression to squamous cell carcinomas *(135)*. Benign and malignant skin tumors were produced by the topical application of B*[a]*P (64 μg/mouse), twice /wk for 7 wk, in *p53* haploinsufficient Tg.AC (v-Ha-*ras*) mice, which contain activated, carcinogen-inducible *ras* oncogene and an

inactivated *p53* tumor-suppressor gene. NAC, given at a dosage of 30 g/kg diet, significantly delayed the appearance of skin lesions, reduced tumor multiplicity by 43%, and improved survival by 5 wk. However, malignant spindle-cell tumors were only observed in 25% of NAC-fed mice, and not in controls *(136)*.

**3.2.2.4. Mammary Glands** NAC, given with the diet (8 g/kg diet) inhibited the formation of mammary adenocarcinomas in rats treated with methylnitrosourea (MNU) *(118)*. NAC (1 μM) inhibited the formation of DMBA-induced hyperplastic alveolar nodules in mouse mammary-organ culture *(6)* and inhibited the formation of DNA adducts in both liver and mammary epithelial cells of DMBA-treated rats (A. Izzotti et al., unpublished data). However, given with the diet (4 or 8 g/kg diet), NAC failed to inhibit the formation of mammary adenocarcinomas in Sprague-Dawley rats treated with a single dose of DMBA (12 mg/rat) by gavage *(137)*.

**3.2.2.5. The Bladder** Dietary NAC (0.2 g/kg diet) significantly inhibited the formation of transitional-cell bladder carcinomas in mice treated with *N*-butyl-*N*-(hydroxybutyl)nitrosamine (OH-BBN) *(138)*.

**3.2.2.6. The Brain** Intravenous injection of ethylnitrosourea to pregnant rats on d 18 of gestation resulted in formation of gliomas in the offspring, which had a shortened life expectancy. Diet supplementation with NAC produced no effect on the formation of brain tumors, but life expectancy was significantly improved *(139)*. Interestingly, a pharmacokinetic study had shown that radiolabeled NAC was taken up by most of the investigated mouse tissues, except the brain and spinal cord *(140)*.

**3.2.2.7. Other Sites** In Wistar rats that received 2AAF with the diet (0.5 g/kg diet), according to the Teebor and Becker model, co-administration of NAC (1 g/kg diet) inhibited the formation of Zymbal gland squamous-cell carcinomas *(110)*.

In F344 rats receiving sc injections of NNK (1.5 mg/kg body wt) 3×/wk a wk for 21 wk, administration of NAC with the diet, at 13.1 g/kg, reduced the incidence of Leydig-cell tumors of the testis from 27/36 (75%) to 11/36 (30.5%) *(117)*.

In the same study, in the absence of NNK administration, no tumor was detected in the pancreas of NAC-treated rats, vs a 25% incidence recorded in untreated controls *(117)*.

Administration of NAC with drinking water at 0.055 g/kg body wt did not inhibit the formation of tumors (fibrosarcomas, osteosarcomas, and carcinomas) in the hind legs of Wistar rats treated with local X-irradiation (16 Gy) and hyperthemia (60 min at 43°C), either individually or in combination *(141)*.

### *3.3. Chemoprevention Studies in Humans*

#### 3.3.1. Phase I Clinical Trials

In addition to extensive clinical experience demonstrating the safety of NAC, even at very high doses (*see* Subheading 2.2.), a Phase I trial specifically evaluated the pharmacokinetics and pharmacodynamics of NAC as a potential cancer chemopreventive agent. This trial involved the escalation of both the NAC dose and the number of the treated subjects. The highest nontoxic dose was 800 mg/m$^2$/d (0.12 mmol/kg body wt) in most of the subjects. Minimal side effects were seen at daily doses of 1600 mg/m$^2$ for 4 wk; the maximum tolerated dose was 6400 mg/m$^2$/d (1 mmol/kg body wt), which caused bad taste and gastrointestinal disturbance in 40% of subjects *(21)*. The Euroscan trial randomized 2592 patients to NAC (600 mg/d), retinyl palmitate, a combination of both agents, or no treatment (*see* Subheading 3.3.3.). NAC was the least toxic, and 17.9% of patients failed to complete 2 y of treatment, vs 25.8% in the retinyl palmitate and 25.2% in the combined treatment arm *(142)*. In a Phase II trial, 21 smoking volunteers received 600 mg NAC in two daily doses for 6 mo (*see* Subheading 3.3.2.); all subjects tolerated the treatment and completed the study, although one participant experienced minor side effects *(143)*. Another Phase I/pharmacodynamic study evaluated the association of NAC (six smokers, 1,200 mg/d) with oltipraz at either 200 mg/d (four smokers) or 400 mg/d (nine smokers). The study was closed as a result of the side effects of treatments *(144)*.

#### 3.3.2. Phase II Clinical Trials

The urinary excretion of cigarette smoke genotoxic metabolites was evaluated in the *his*$^-$ strain YG1024 of *S. typhimurium* and in *E. coli* strains with distinctive DNA repair capacities. After treatment with oral NAC (600–800 mg/d), 7 out of 10 smokers significantly decreased excretion of genotoxic metabolites. This effect was already evident after 1 d of NAC administration, and was reversible upon withdrawal of treatment *(145)*.

Oral NAC, given for up to 142 d in three daily doses of 600 mg to 11 nonsmoking patients suffering from alveolar pulmonary fibrosis, significantly lowered levels of 4-aminobiphenyl-hemoglobin adducts *(146)*. Mechanistically, it is noteworthy that NAC is a good pre-

cursor of GSH in red blood cells *(70)*, and that GSH and hemoglobin compete for reaction with nitrosobiphenyl, a reactive metabolite of 4-aminobiphenyl *(147)*.

Agents such as NAC, which have antimutagenic activity, may protect against the numerous mutagenic events that occur throughout colon carcinogenesis *(148)*. Preliminary data suggested that NAC can decrease the recurrence rate of adenomatous polyps *(149)*. Moreover, a significant decrease in the proliferation index of colonic crypts occurred in 34 patients with previous adenomatous colonic polyps, after treatment with oral NAC (800 mg/d) for 12 mo. No variation of the same index was observed in 30 subjects who received a placebo *(150)*.

A battery of biomarkers was evaluated in healthy smoking volunteers in a double-blind Phase II chemoprevention trial in which 21 subjects were given a placebo and 20 subjects were treated with NAC (600 mg oral tablets/d), for a period of 6 mo. Although the placebo group had no significant variation in any of the investigated biomarkers, the NAC group showed a significant decrease in levels of both 8-OH-dG and lipophilic DNA adducts in BAL cells as well as in the frequency of micronuclei in the mouth floor and soft palate cells *(143)*.

Thus, several studies showed that oral NAC is capable of modulating certain biomarkers involved in carcinogenesis. Certainly, there is a broad interindividual variability in the response to treatment. As a first pharmacogenomic approach in chemoprevention research, we stratified the subjects involved in the previously mentioned Phase II trial *(143)* according to their metabolic genotypes. The decrease in micronucleus frequency in buccal cells of smokers after 6 mo of treatment with NAC became even more evident in subjects who were either *NAT2* slow acetylators or had a null *GSTM1* genotype, although there was no significant decrease either in *NAT2* fast acetylators or *GSTM1*-positive subjects (our unpublished data, in collaboration with van Schooten FJ et al.). These preliminary data suggest that metabolic genetic polymorphisms can influence responsiveness to NAC treatment in terms of reducing a genotoxicity biomarker. In particular, subjects who are slow acetylators or lack the *GSTM1* genotype thus have lower detoxifying capacities, and appear to derive greater benefit from NAC administration.

The "mutagen sensitivity" assay, which measures chromosomal damage induced by bleomycin in cultures of peripheral blood lympocytes, has been proposed as a method to evaluate the efficacy of chemopreventive agents *(151,152)*. However, it has also been shown that sensitivity to bleomycin is constitutional *(153)*. NAC, in doses ranging from 0.1–10 mmol/L, decreased by 23–73% the number of bleomycin-induced chromosomal breaks in freshly cultured lymphocytes from head and neck cancer patients *(151,152)*. However, sensitivity to bleomycin was not changed in lymphocytes from head and neck cancer patients who received oral NAC (600 mg/d for 3–9 mo), as compared to lymphocytes from untreated patients *(153)*.

### 3.3.3. Phase III Clinical Trials

A multicentric clinical trial (Euroscan) evaluated 2592 patients previously treated for head and neck cancer or lung cancer. The patients were mostly previous or current smokers, and were randomly assigned to four groups that received either no treatment, retinyl palmitate, NAC (600 mg/d), or both drugs for a period of 2 yr. After a median followup of 49 mo, 916 patients had experienced local/regional recurrences, second primary tumors, distant metastasis, and/or death. There was no significant difference among the four intervention arms *(142)*. Clearly, the target of this Phase III trial was different from that of the previously reported Phase II trial in smokers *(143)*, which evaluated the effects of NAC in healthy subjects, thereby reproducing a primary prevention setting.

## 4. PROTECTIVE MECHANISMS OF NAC IN MUTAGENESIS AND CARCINOGENESIS

In addition to evaluating the efficacy of putative chemopreventive agents, it is essential to understand their mechanisms of action. Inhibition of mutagenesis and carcinogenesis can be achieved by a variety of mechanisms, which are often interconnected and reiterated in various stages of carcinogenesis. During the last 15 years, we proposed several classifications of mechanisms of inhibitors and carcinogenesis, and provided examples of protective agents within each category (for instance, *see* refs. 154–157). Some chemopreventive agents have pleiotropic properties and have multiple points of intervention, which renders them effective in different stages of the pathogenetic process and efficacious toward a broader spectrum of mutagens and carcinogens. As discussed here, NAC is a typical example of an agent that acts via many different mechanisms, ranging from inhibition of mutation and cancer initiation to prevention of angiogenesis, invasion, and metastasis of cancer cells. In reviewing the protective mechanisms

of NAC it should be considered that as for any other chemopreventive agent, it is often difficult to distinguish whether modulation of a given end point is a genuine mechanism or rather a secondary effect—e.g., the epiphenomenon of other mechanisms acting upstream in the chain of events that ultimately regulate the observed end point. Another preliminary consideration is that many mechanisms of NAC depend on its ability to act as a precursor of intracellular *l*-cysteine and GSH. However, other mechanisms, especially in the extracellular environment, can be ascribed to NAC itself.

### *4.1. Nucleophilicity*

As discussed in the following subsection, the SH group of NAC and other aminothiols renders them highly reactive toward electrophilic compounds and metabolites, thereby preventing their binding to nucleophilic sites of DNA and other molecules that are critical targets in carcinogenesis. This property of NAC is broadly supported by results obtained in in vitro test systems with direct-acting mutagens (*see* Subheading 3.1.). The blocking of proximal or ultimate metabolites of carcinogens by NAC has also been demonstrated by analytical methods, as with aflatoxin $B_1$, whose difuran region is bound by NAC *(71)*, and of formic acid 2-[4-(5-nitro-furyl)-2-thiazolyl]-hydrazide (FNT), a renal carcinogen with a reactive intermediate produced by peroxidation via prostglandin H synthetase capable of forming 5-*(S)*-substituted thioether conjugates with GSH and NAC *(158)*.

### *4.2. Antioxidant Activity*

Together with nucleophilicity, antioxidant activity represents a crucial mechanism of NAC, to such an extent that this molecule is more and more extensively used as a prototype agent to evaluate the role of oxidative mechanisms in a wide range of physiological and pathological processess.

The main mechanism of NAC as an antioxidant is scavenging ROS. ROS are endogenously generated by all aerobic cells, and are known to participate in a wide variety of deleterious reactions *(159)* and to be closely associated with alterations in gene expression *(160)*. NAC has been reported to react rapidly with hydroxyl radical (•OH) and hypochlorous ion ($^-$OCl), and more slowly with superoxide anion radical ($O^-_2$) and hydrogen peroxide ($H_2O_2$) *(161,162)*. The reaction of NAC with $H_2O_2$ involves a non-enzymic reduction of the peroxide with concomitant formation of NAC disulfide *(163)*. NAC and other thiols react with singlet oxygen ($^1O_2$), presumably by formation of corresponding disulfides *(164)*. In bacterial test systems, NAC attenuated the genotoxicity of peroxides, such as $H_2O_2$ *(20,60)* and cumene hydroperoxide *(1)*. NAC also inhibited the mutagenicity of •OH released by human phagocytic leukocytes *(63)*. NAC exerted protective effects when ROS were generated by monoelectronic reduction of $O_2$ in the reaction of xanthine oxidase with hypoxanthine, which forms $H_2O_2$. Mutagenicity at this stage was ascribed to $O^-_2$, since catalase had no significant effect. A further enhancement of mutagenicity was observed by accelerating $O^-_2$ dismutation upon addition of superoxide dismutase (SOD), leading to the formation of $H_2O_2$. In any case, NAC efficiently decreased the mutagenicity of ROS *(55)*. Moreover, NAC attenuated the genotoxic effects produced by volatile ROS generated by illuminating the chromophore rose bengal, which results both in electron transfer reactions ($O^-_2$, $H_2O_2$, and •OH) and energy transfer reactions ($^1O^2$) *(62)*. By using $^{32}P$ postlabeling methods, NAC was found to inhibit the formation of 8-OH-dG *(48)* and 8-oxo-dG *(49)* in DNA exposed to •OH-generating Fenton-type reactions. In addition, NAC protected DNA from reactive nitrogen species (RNS), such as peroxynitrite resulting from the reaction between cigarette smoke and a nitric oxide-releasing compound *(47)*.

Lipid peroxidation is an important mechanism involved in the pathogenesis of diverse diseases. NAC inhibited the lipid peroxidation induced by inflammatory reactions of malondialdehyde in vivo *(165)*, and counteracted the effects of 4-hydroxy-2-nonenal *(166)*.

Recently, NAC has also been shown to inhibit cyclooxygenases (COX). For instance, it inhibited the expression of COX-2 in colorectal cancer (CRC) cells *(167)* and in vascular muscle cells *(168)*. NAC was found to be a strong inhibitor of NNK-induced prostaglandin $E_2$ ($PGE_2$) synthesis by decreasing COX-1 expression in cultured human macrophages. It was suggested that ROS, generated during pulmonary metabolism of NNK, could act as signal-transduction messengers and activate NFκB, which induces COX activity and increases $PGE_2$ synthesis *(169)*.

Although many studies have documented the antioxidant properties of NAC, a few studies show that, under certain conditions, this thiol may behave as a pro-oxidant. For instance, in the presence of Cu(II), NAC induced DNA damage by generating $H_2O_2$ *(170)*. A similar situation also occurs with other typical antioxidants, and has frequently been reported with

ascorbic acid (AsA) *(121)*. In this respect, it is noteworthy that GSH has been shown, both in vitro and in vivo, to maintain a reducing intracellular milieu that can reduce dehydroascorbic acid *(171,172)*. A similar effect was produced by combining AsA with NAC *(121)*.

### *4.3. Inhibition of the Nitrosation Reaction and Blocking of the Nitrosation Products*

The nitrosation reaction mainly occurs in the acidic stomach environment, and represents an important source of endogenously formed carcinogens. We comparatively evaluated the ability of NAC, GSH, and ascorbic acid to affect the nitrosation reaction and to block the nitrosation products of famotidine and ranitidine in a simulated acidic environment. Compared to ascorbic acid, a prototype inhibitor of nitrosation, the two thiols were much slower in reacting with sodium nitrite. However, they were much more efficient in blocking the mutagenicity of nitrosoderivatives of both famotidine and ranitidine *(173)*.

### *4.4. Modulation of Xenobiotic Metabolism*

NAC appears to behave as a weak bifunctional inducer of xenobiotic metabolism. In fact, it poorly influences phase 1 enzymes that are involved in the metabolic activation of procarcinogens, and stimulates phase 2 enzymes to some extent *(8)*.

In particular, NAC did not affect the levels of total cytochromes P450 when assayed either in vitro in chicken embryo hepatocytes *(174)* or in vivo in the rat liver and lung *(70)*. Dietary NAC slightly—but significantly—induced aryl hydrocarbon hydroxylase (AHH) activity in the liver of Wistar rats *(2)*, yet did not affect the increase of AHH in the lung and of 7-ethoxyresorufin *O*-deethylase (EROD) and epoxide hydrolase induced by the exposure of Sprague-Dawley rats to mainstream cigarette smoke *(175)*.

Several studies have provided evidence that NAC is capable of modulating cytosolic enzyme activities that are directly or indirectly related to the GSH cycle (*see* Subheading 2.1.). In fact, dietary NAC stimulated G6PD, 6PGD, GSSG reductase, and NAD(P)H-dependent diaphorases in the hepatic and pulmonary S105 fractions of Sprague-Dawley rats *(70)*. It also stimulated G6PD, 6PGD, NAD(P)H-dependent diaphorases, chromium(VI) reduction, and GST in PAM S12 fractions from the same animals *(176)*. Stimulation of GSSG reductase was also found to occur in peripheral-blood lymphocytes of human volunteers who took oral NAC *(21)*.

GST, a crucial phase 2 enzyme, has been proposed as a marker for potential inhibitors of chemical carcinogenesis *(177)*. GST was slightly but significantly induced in the liver of Swiss albino mice *(119)* and F344 rats *(132)* that received NAC with the diet. NAC is an inducer of *GSTP1* gene, an effect largely mediated by the activator protein-1 (AP-1) site *(178)*. Interestingly, from a total of 688 tested genes by cDNA array technology, *GSTM2* and *GSTP1* were the only genes overexpressed in the liver of Swiss albino fetuses whose mothers had received NAC in drinking water (Izzotti A et al., manuscript in preparation).

### *4.5. Effects in Mitochondria*

NAC has shown protective effects in mitochondria. As discussed in Subheading 3.2.1.1., its oral administration inhibited the formation of adducts to mtDNA in the lungs of rats exposed to mainstream cigarette smoke *(90)*. In mice, NAC protected against the age-related increase in oxidized proteins in synaptic mitochondria *(179)* and the decline of oxidative phosphorylation in liver mitochondria, whereas NAC significantly increased the specific activity of complex I, IV, and V *(180)*. In vitro, NAC enhanced adenosine 5'triphosphate (ATP) levels in bovine retinal pigment epithelium cells subjected to oxidative stress *(181)* and prevented the tobacco-smoke-induced disruption of deltapsim (mitochondrial membrane potential) that resulted in apoptotic death of human monocytes *(182)*.

### *4.6. Decrease of Biologically Effective Carcinogen Dose*

Adducts to macromolecules are crucial steps in carcinogenesis. Accordingly, in principle, inhibition of their formation in treated animals or exposed humans would be expected to predict a decreased risk of developing the disease *(183)*. As reported in greater detail in Subheading 3.2.1. and in the references cited therein, oral NAC significantly decreased the levels of adducts to hemoglobin and adducts to nuclear DNA in isolated cells (BAL cells, mammary epithelial cells), tissues (tracheal epithelium), and organs (lung, liver, heart, kidney, testis) of rodents exposed to carcinogens, such as B[*a*]P and DMBA, and complex mixtures, such as air-particulate extracts, mainstream cigarette smoke, and environmental cigarette smoke. In humans, oral NAC significantly decreased hemoglobin adduct levels in nonsmokers and DNA adduct levels in BAL cells of smokers (*see* Subheading 3.3.2.).

### *4.7. Effects on DNA Repair*

The differential ability of NAC and other thiols to decrease the "spontaneous" mutagenicity in strain TA104 of *S. typhimurium* but not in TA102, which has a deletion of the *uvrB* gene encoding for an error-free DNA excision-repair system, suggests that NAC may influence DNA-repair processes *(56)*. Using three different hepatocarcinogenesis models (Teebor and Becker, Solt and Faber, and Druckey) in Wistar rats, dietary NAC prevented the 2AAF-induced depletion of PARP, a nuclear enzyme that modulates changes in chromatin architecture during DNA replication or DNA excision repair *(106)*. Depletion of GSH is known to lead to methionine deficiency thereby impairing the methylation process *(184)*.

### *4.8. Modulation of Gene Expression and Signal-Transduction Pathways*

As discussed in Subheading 2.2., even high doses of NAC have little effect on the expression of a number of genes in the mouse lung under physiological conditions. However, in recent years, an impressive number of studies have documented that NAC is capable of modulating a variety of signal-transduction pathways altered by toxic agents, carcinogens, cytokines, and changes of the redox potential. For example, the following effects were produced by NAC: the post-transcriptional increase of *p53* expression *(185)*; decrease of retinoblastoma (RB) protein phosphorylation which leads to reversal of RB-mediated growth inhibition *(186)*; decrease of c-*fos* and c-*jun* induction *(187)*; inhibition of activation *(188)* and binding activity *(189)* of the transcription factor activator protein-1 (AP-1); inhibition of activation *(188,190)*; and nuclear translocation *(191)* of the transcription factor NFκB; inhibition of activity of the transcription factor-signal-transducer and activator of transcription (STAT1) (192); inhibition of overexpression of NFκB-inducing kinase (NIK) and IκB kinases (IKK-α and IKKβ) *(190)*; inactivation of proteine kinase C (PKC) *(193)*; blocking the expression of *GADD153* gene *(194)*; uncoupling signal transduction from *ras* to mitogen-activated protein kinase (MAPK) *(185)*; activation of phosphorylation of extracellular signal-regulated kinase (ERK)-MAPK *(195)*; induction of *p16 (INK4a)* and *p21 (WAF/CIP1)* expression and prolongation of cell-cycle transition through the $G_1$ phase *(196)*; inhibition of tumor necrosis factor-α (TNFα) release *(191)* and TNFα induced sphingomyelin hydrolysis and ceramide generation *(197)*; decrease of the biological activity of transforming growth factor-β (TGFβ) as a result of a direct effect on the TGFβ molecule *(198)*; suppression of epidermal growth factor (EGF) dimerization, activation of the EGF cellular receptor and EGF-induced activation of c-*ras (199)*; inhibition of c-Ha-*ras* expression *(200)*.

### *4.9. Anticytotoxic Activity*

In addition to the studies which specifically deal with modulation of apoptosis (*see* Subheading 4.10.), many reports have examined the anticytotoxic activity of NAC, to such an extent that this molecule has been defined as a pluripotent protector against cell death *(201)*. To give a few examples, NAC prevented the GSH-depleting effects of cigarette smoke in the isolated perfused rat lung *(202)* and counteracted the toxicity of cigarette smoke in cultured rat hepatocytes and lung cells *(203)*, human bronchial cells *(203)*, alveolar macrophages *(204)*, and monocytes *(205)*. NAC was by far the most effective of the agents studied in protecting rat cardiomyocytes from the toxicities of the *N*-hydroxyl amino metabolites of the heterocyclic amines 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-2-methyl-6-phenylimidazo[4,5-*b*]pyridine (PHiP) *(206)*. NAC inhibited the toxicity to endothelial cells of cigarette smoke extracts *(205)*, hyperoxia *(207)*, and ROS generated in the hypoxanthine-xanthine oxidase system *(208)*. NAC resulted in a significant inhibition of chromium(VI)-induced cell death in NFκB-inhibited cells, which suggested that NFκB is essential for inhibiting ROS-dependent cytotoxicity *(209)*.

### *4.10. Modulation of Apoptosis*

More than 100 data dealing with modulation of apoptosis by NAC were selected from MEDLINE in a survey carried out in November 2000. Test cells included both normal cells from different systems (respiratory, cardiovascular, haematopoietic, digestive, nervous, and endocrine) and tumor cells (carcinomas, sarcomas, and tumors of the hematopoietic system) from a variety of species, including humans, bovines, felines, and rodents. Many different inducers were used in order to elicit apoptosis via ROS-mediated mechanisms. They included peroxides, such as $H_2O_2$ (210–215) and *tert*-butylhydroperoxide *(216)*; ROS donors (215,217–221); NO *(222,223)* and $NO_x$ donors *(220,222,224)*; GSH depletors *(225–227)*; modulators of metabolism, such as reducing sugars *(228–230)*, glutamate *(231,232)*, methylglyoxal, and 3-deoxyglu-

cosone *(233,234)*; L-DOPA *(235)*; dopamine *(236–240)*; 7-ketocholesterol *(241)*; neopterin, biopterin, and folate *(242)*, complex I (NADH dehydrogenase) inhibitors *(243)*, glucose deprivation *(244)*, and trophic factor deprivation *(209)*; signal-trasduction modulators, such as NGF deprivation *(245,246)*, TNF-α(247–250), TNFα plus HIV-1 Tat *(251)*, TGF-α1 *(214)*, HGF *(252)*, antagonist G *(253)*, overexpression of *c-myc (254)*, antibodies toward *fas (221,255,256)* or CD3 *(246)*, and the lipid-signalling molecule ceramide *(257–259)*; a variety of chemical agents such as bleomycin *(260)*, actinomycin D *(261)*, daunorubicin *(262,263)*, doxorubicin *(264)*, nitrogen mustard *(265)*, pyrrolo-1,5-benzoxazepines *(266)*, retinoids (267–269), etoposide *(241,270,271)*, cycloheximide *(241)*, butyl hydroxytoluene *(272)*, and vanilloid compounds *(273)*; caffeic acid derivatives *(274)*; nordihydroguaiaretic acid *(275)*, selenite *(276)*, arsenite *(277,278)*, copper *(279)*, manganese *(280)*, and cadmium *(281)*; complex mixtures, such as cigarette smoke *(99)* and diesel exhaust particles *(282,283)*; mitogenic stimuli *(284)*; toxins, such as ricin *(285)*, endotoxin *(286)*, and 1-methyl-4-(2'-ethylphenyl)-1,2,3,6-tetrahydropyridine *(280)*; hormones and analogs or modulators, such as tamoxifen and derivatives *(287)*, glucocorticoid hormones *(288)*, methylprednisolone *(289)*, and dexamethasone *(271)*; viral infections caused by HIV *(290,291)*, feline immunodeficiency virus *(292)*, Sindbis virus *(293)*, and bovine viral diarrhea virus *(294)*; physical agents, such as asbestos *(295)*; and suboptimal growth temperature *(296)*. Actually, all cited agents work via a variety of mechanisms, which are often interconnected and share the common property of causing an imbalance of redox potential that leads to apoptosis. In several cases, the authors of the studies mentioned here have specified that certain effects of NAC were independent of its antioxidant properties and ability to replenish GSH stores.

On the whole, 91 of the 104 data (89.5%) reported with in vitro studies showed that NAC inhibits apoptosis. Ten data (9.6%) were consistent with lack of effect of NAC on apoptosis, and three data only (2.9%) showed an enhancement of apoptosis by NAC. Interestingly, in one of these three studies, NAC induced apoptosis in several transformed cell lines and transformed primary cultures, but not in normal cells *(297)*. It is important to consider that modulation of apoptosis has multiple meanings. Thus, stimulation of apoptosis is a mechanism shared by certain chemopreventive agents and a number of chemotherapeutical drugs *(298)*. In the case of chemopreventive agents, however, one should discriminate between the induction of apoptosis as an anticarcinogenic mechanism and inhibition of apoptosis as an epiphenomenon of protective mechanisms that modulate apoptosis signalling *(156)*. In other degenerative diseases, especially those that affect post-mitotic tissues, the inhibition of apoptosis is clearly a protective mechanism *(299)*.

In the in vivo studies, oral NAC protected the respiratory tract of Sprague-Dawley rats from cigarette smoke-induced apoptosis, as shown by inhibition of apoptosis in the bronchial and bronchiolar epithelia of mainstream cigarette smoke-exposed rats and in the PAM of environmental cigarette smoke-exposed rats *(99)*. Moreover, oral NAC prevented hair follicle-cell apoptosis and alopecia in C57BL/6 mice exposed to environmental cigarette smoke *(112)*. The protective effect of NAC toward smoke-induced apoptosis in the respiratory tract may be relevant in lung carcinogenesis as well as in other pulmonary diseases *(300–302)*. Further studies demonstrated the ability of NAC to inhibit ROS-mediated apoptosis in situations which may play a role in other pathological conditions, such as experimental diabetes in pancreatic β cells of mice *(303)*, balloon-catheter injury in the rabbit carotid artery *(304)*, and tectal-cell lesions in the eye retinal ganglion of chicken embryos *(305)*.

### *4.11. Antiinflammatory Activity*

It is well-known that NAC has antiinflammatory properties which act through mechanisms which are also involved in carcinogenesis. For instance, oral NAC normalized BAL cellularity after cessation of exposure of mice to mainstream cigarette smoke *(86)*. Its administration to rats decreased lung NFκB activation, cytokine-induced neutrophil chemoattractant mRNA expression in lung tissue, and endotoxin-induced neutrophilic alveolitis *(306)*. In another rat model of lung injury, NAC decreased all tested parameters of lung injury, such as lipid peroxidation, production of nitrite/nitrate, TNFα, and interleukin (IL) 1β *(307)*. Interestingly, the infusion of NAC at high doses reduced respiratory burst, but augmented neutrophil phagocytosis in intensive care unit patients *(308)*. NAC was consistently found to inhibit either gene expression, production, or secretion of variously induced pro-inflammatory cytokines, such as TNFα *(309)* and IL-8 *(310–312)*, and intracellular adhesion molecule-1 (ICAM-1) *(309)*. Another antiinflammatory mechanism of NAC involves the previously discussed inhibition of COX-2 *(167,168)*.

### *4.12. Protective Effects in Tumor-Associated Viral Diseases*

As discussed in Subheading 2.1., GSH depletion occurs in viral diseases associated with tumors in humans. NAC has been shown to exert protective effects in infections caused by HBV and HCV, which are associated with primary hepatocellular carcinoma; in HIV infection, which is associated with Kaposi's sarcoma (KS) and other tumors; and in Epstein-Barr virus (EBV) infection, which is associated with Burkitt's lymphoma, sinonasal angiocentric T-cell lymphoma, immunosuppression-related lymphoma, Hodgkin's disease, and nasopharyngeal carcinoma.

NAC was found to inhibit HBV replication by disturbing the virus assembly *(313)*. It counteracted the induction of the transcription factors STAT-3 and NFκB by the HBV X (HBx) protein, which plays vital roles in viral replication and in the generation of hepatocellular carcinoma *(314)*. NAC potently suppressed the induction of κB-controlled reporter genes by the HBV middle surface (MHBs) antigen *(315)*. Moreover, NAC inhibited activation of aflatoxin $B_1$ to mutagenic metabolites in the presence of liver preparations from woodchucks, either uninfected or infected with woodchuck hepatisis virus or also bearing primary hepatocellular carcinoma *(23)*.

Oral NAC increased the levels of GSH in plasma and peripheral blood lymphocytes of patients who were chronically infected with HCV *(25)*. NAC eliminated the activation of STAT-3 and NFκB by the HCV nonstructural protein 5A (NS5A) *(316)*. However, NAC did not enhance the benefit of conventional therapy with interferon-α in chronic HCV patients *(317)*.

NAC displayed several beneficial effects in HIV infection, which correlates with the clinical finding that its oral administration combined with antiretroviral therapy significantly reduced mortality in AIDS patients *(28,29)*. NAC replenished GSH levels in plasma and T lymphocytes of HIV carriers *(318)*, and inhibited HIV reverse transcriptase in chronically infected T lymphocytes *(319)*, monocellular cells *(320,321)*, and macrophages *(322)*, which resulted in the suppression of HIV replication in the absence of cytotoxic or cytostatic effects. HIV production was also inhibited by NAC thrugh the inhibition of cytokines *(323)*. It is noteworthy that, as reported in Subheading 4.18., oral NAC strongly inhibited the growth of KS in nude mice *(324)*.

NAC has been proposed as a good candidate for controlling EBV infection, based on its ability to suppress CD21, a receptor for EBV *(325)*.

### *4.13. Protection of Gap-Intercellular Communications*

Gap-junctional intercellular communications (GJIC) play a role in carcinogenesis, especially in tumor promotion. NAC was found to inhibit disruption of GJIC and hyperphosphorylation of connexin 43 in cultures of rat-liver epithelial cells initiated with MNNG and further treated with $H_2O_2$ as a tumor promoter *(81)*.

### *4.14. Inhibition of Proliferation*

A number of studies have pointed out the possible antiproliferative effects of NAC in cultured mammalian cells via a variety of mechanisms. For instance, NAC inhibited the TPA-mediated induction of cyclin $D_1$ and DNA synthesis *(326)*, and inhibited the abnormal cell-cycle progression mediated by the *p38* MAPK cascade *(327)*. NAC reduced the elevation of *c-fos* and decreased the proliferation of rat tracheal smooth-muscle cells *(328)*. It induced *p16* (INK4a) and *p21* (WAF/CIP1) gene expression, and prolonged cell-cycle transition through $G_1$ phase in various types of mammalian cells. This finding suggested a potentially novel molecular basis for chemoprevention by NAC, as increased intracellular GSH was not required for $G_1$ arrest, and other antioxidants whose action is limited to scavenging radicals did not induce $G_1$ arrest *(195)*. Both L-NAC (GSH precursor) and D-NAC (non-precursor of GSH) slowed cell-cycle progression by inhibiting topoisomerase-IIα activity *(329)*. The remarkable decrease of cyclin $D_1$ expression and increase of pancreatic carcinoma cells in $G_1$ phase suggest the possible utility of NAC as an antitumor agent *(330)*.

There are also indications that NAC can exert antiproliferative effects in humans. For instance, oral NAC attenuated the proliferative index in the colon in patients with previous adenomatous colonic polyps *(149)*, and its topical application was successfully used for the treatment for lamellar hycthyosis, based on the observed inhibition of proliferation in cultured human keratinocytes *(331)*.

### *4.15. Stimulation of Differentiation*

The prolonged culture of mesangial cells, derived from isolated rat glomeruli, produced multifocal nodule structures, known as "hillocks," which consist of cells and the extracellular matrix. Exposure of

mesangial cells to NAC dramatically facilitated hillock formation. These results indicated that, through a redox-sensitive mechanism, NAC induces mesangial cells to create a three-dimensional cytoarchitecture that underlies cellular differentiation *(332)*.

### *4.16. Modulation of Tumor Progression*

In addition to its effects on cell proliferation (Subheading 4.14.) and angiogenesis (Subheading 4.18), as previously reported (Subheading 3.2.2.1.), NAC retarded malignant progression in the lungs of NNK-treated A/J mice *(125)*.

### *4.17. Modulation of Immune Functions*

A number of studies have evaluated the immunological effects of NAC. To give a few examples, NAC enhanced natural killer (NK) function in cells of aging mice, which is a favorable response because aging mice have decrease NK function and a higher incidence of neoplasia *(333,334)*.Administration of NAC to mice enhanced the immunogenic potential of tumor cells *(335)*. The topical application of NAC prevented suppression of contact hypersensitivity in UVB-exposed mice at a site distant from the site of application, which demonstrated the ability of NAC to prevent systemic immunosuppression *(336)*. In randomized clinical trials, NAC was found to improve immune functions in asymptomatic HIV-infected subjects *(337)*.

### *4.18. Inhibition of Angiogenesis*

The "angiogenic switch" refers to acquisition of an angiogenic phenotype by tumor cells, generally triggered by the release of angiogenic factors such as vascular endothelial growth factor (VEGF), interleukin 8 (IL-8),TGFβ),and basic-fibroblast-like growth factor (bFGF). Tumor angiogenesis is known to play a critical role in tumor progression, but is also an early event in tumorigenesis that favors the expansion of hyperplastic foci and subsequent tumor development *(338)*. Diverse cancer chemopreventive agents appear to interfere with common pathways in the angiogenic cascade by blocking the angiogenic switch and maintaining tumor dormancy. Several chemopreventive agents share this mechanism, suggesting that it may be a key factor; we thus propose the novel concept of "angioprevention" *(338)*.

Several in vitro and in vivo studies have provided evidence that NAC is a potent antiangiogenic agent *(338)*. NAC strongly inhibits expression or production of VEGF, either secreted under basal conditions by human cancer cells, such as melanoma *(339)* and KS *(324)*, or induced by a variety of agents such as $H_2O_2$ *(340)*, IL-1 *(341)*, arsenite *(342)*, hypoxia-inducible factor 1α (HIF-1) *(342)*, epithelial growth factor, and TPA *(343)*. NAC also inhibited adverse effects of VEGF, such as the induction of expression of monocyte chemoattractant protein-1 (MCP-1), a chemochine that has been proposed to recruit leukocytes to sites of inflammation, neovascularization, and vascular injury *(344)*. NAC also inhibited invasion, chemotaxis, and gelatinolytic activity of human endothelial cell lines EAhy926 and HUVE cells *(345)* and highly vascularized KS-Imm cells *(324)*. At the same time, NAC inhibited TGFβ-induced apoptosis and genotoxic damage produced by the oxidizing agent paraquat in EAhy926 cells *(76)*.

Antiangiogenic activity of NAC was further supported by two in vivo studies in mice. In the first, sponges of Matrigel (a reconstituted basement membrane matrix) containing heparin and highly angiogenic KS-cell supernatants were implanted subcutaneously in C57BL/6 mice. After 72 h, these sponges became highly vascularized and hemorragic. Administration of NAC in drinking water (2 g/kg body wt) significantly reduced the formation of new blood vessels, by an average of 70%, and reduced hemoglobin content in the Matrigel implants *(345)*. In another study, KS-Imm cells were injected subcutaneously in the flank region of (CD-1)BR nude mice. After established tumor masses were detectable in all mice, NAC was given in the drinking water (2 g/kg body wt). Consistent with the higher KS frequency in males as compared to females, the tumor volume in control mice at the end of the experiment was significantly higher in males ($3.2 \pm 0.5$ $cm^3$) than in females ($1.0 \pm 0.3$ $cm^3$). NAC-treated mice had significantly reduced growth in both genders, and the tumor volume was $1.1 \pm 1.0$ $cm^3$ in males and $0.3 \pm 0.2$ $cm^3$ in females. Most NAC-treated females and one-half of the male animals showed an evident trend of tumor mass regression *(324)*. In a survival experiment, NAC again significantly reduced tumor volume ($9.8 \pm 6.2$ $cm^3$ in control mice as opposed to $1.3 \pm 1.1$ $cm^3$ in NAC-treated mice when all mice were still alive) and increased median survival time (45 d in controls vs 108 d in treated animals). Two-thirds of treated mice showed regression of the neoplastic mass after 15–55 d, which in one-half of the responders appeared to be complete. These mice showed a high and significant correlation between tumor volume and either VEGF or proliferation markers PCNA

and Ki-67, which accordingly were significantly lower in NAC-treated mice than in controls. This correlated with reduced VEGF production in NAC-treated cultures *(324)*.

### *4.19. Inhibition of Invasion and Metastasis of Cancer Cells*

Inhibition of angiogenesis by NAC, as discussed in Subheading 4.18., interferes with growth of the neoplastic mass and prevents possible contact between cancer cells and vessels, a prerequisite for invasion of blood and lymphatic streams by cancer cells and their subsequent colonization in distant tissues to form metastases. Several additional lines of evidence from both in vitro and in vivo experiments provide evidence for NAC's ability to prevent invasion and metastasis.

A crucial role in invasion is played by degradation of type IV collagen in vessel basement membranes. This process is accomplished by enzymes that are produced by cancer cells, particularly matrix metalloproteases (MMP) known as type IV collagenases, such as MMP-2 (gelatinase A) and MMP-9 (gelatinase B) *(346)*. These enzymes are thus key targets for inhibitors of invasion and metastasis. In agreement with preliminary data reported by another laboratory *(347)*, we showed that NAC completely inhibits both MMP-2 and MMP-9 produced by murine melanoma cells (K1735 and B16-BL6) and Lewis lung carcinoma cells (C87) *(348)*. Furthermore, it has been shown that NAC inhibited the invasion of T24 human bladder cancer cells by decreasing MMP-9 production *(349)*. Inhibition of type IV collagenases by NAC can be ascribed to the fact that sulfhydryl groups chelate a zinc ion that is essential for the activity of these enzymes *(350)*. In addition, as reported in Subheading 4.8., NAC inhibits activation *(188)* and binding activity *(189)* of AP-1, a heterodimeric complex encoded by proto-oncogenes c-*jun* and c-*fos* that is involved in collagenase gene transcription *(189)*.

Additional protective effects of NAC may result from its ability to attenuate oxidative mechanisms (Subheading 4.2.) and inflammation (Subheading 4.11.), which have been shown to play a role in invasion and metastasis. For instance, $H_2O_2$ may contribute to retention or extravasion of circulating tumor cells, and $O_2^-$ released by tumor cells may be involved in basement membrane degradation *(351)*. Similar observations have also been made in studies on noncancerous cells. NAC inhibited intrinsic $O_2^-$ generation as a byproduct of macrophages and neutrophils in human fetal membranes, and dramatically suppressed MMP-9 activity *(352)*. During acute endotoxemia, NAC blocked MMP-2 and MMP-9 activation in rat lung *(353)*. In normal human bronchial cells, NAC inhibited expression of MMP-9 induced by TNFα via the NFκB-mediated pathway *(354)*. Moreover, NAC inhibited MMP production in macrophage-derived foam cells *(355)* and alveolar macrophages *(356)* via oxidant-sensitive pathways.

Using the Boyden chamber assay, which evaluates in vitro the ability of cancer cells to invade a reconstituted basement membrane (Matrigel), provides information on three properties of malignant cells—e.g., adhesion to basement membranes, degradation, and invasion. At nontoxic concentrations, NAC inhibited chemotactic and invasive activities of five cancer cell lines of either human or murine origin in a dose-dependent manner *(348)*.

A number of experiments have provided evidence for the in vivo modulation of tumor- and malignant-cell metastasis by NAC. Particularly in experimental metastasis assays, in which formation of a primary tumor is bypassed by intravenous (iv) injection of B16-F10 melanoma cells in (CD-1)BR nude mice resulting in development of lung metastases, NAC reduced the metastatic burden. In spontaneous metastasis assays, B16-BL6 melanoma cells were injected subcutaneously into the footpad of C57BL/6 mice, resulting in local primary tumors and the subsequent spread of lung metastases. Oral administration of NAC (0.5, 1, and 2 g/kg body wt) resulted in a sharp decrease of experimental metastases, in a dose-related decrease of the weight of locally formed primary tumors, and in the reduction of spontaneous metastases *(348)*. Using the same murine models, six separate experiments showed that NAC given with drinking water and doxorubicin given parenterally behaved synergistically in reducing the weight and frequency of primary tumors and local recurrences, and the number of both experimental and spontaneous metastases *(357)*. This conclusion was further supported by an additional study in which administration of NAC alone and in combination with doxorubicin was found to significantly improve survival rates of mice treated with cancer cells *(111)*. These experiments found that oral NAC prevents alopecia induced by doxorubicin in C57BL/6 mice *(111)*. Therefore, NAC appears to exert several protective effects toward important side effects of doxorubicin, including ROS-dependent congestive cardiomyopathy *(358)*, genotoxicity in vitro *(65)*, and

alopecia in mice *(111)*. At the same time, NAC and doxorubicin appear to work synergistically to prevent metastases in mice *(111,357)*.

Several independent studies have confirmed the antitumoral and antimetastatic properties of NAC. For instance, oral NAC inhibited tumor appearance in more than one-third (18 of 50) of B6D2F1 mice injected with L1210 lymphoma cells. The same animals were resistant to a second inoculation of L1210 cells with no further treatment with NAC *(359)*. The iv administration of NAC, coupled with high hydrostatic pressure, provoked an anti-tumor response capable of eradicating metastatic nodules formed by Lewis lung carcinoma cells in C57BL/6 and Balb/c mice *(335)*. In spontaneous hypertensive (SHR) rats, ER-1 cells treated for 1 mo with EGF produced tumor nodules in the peritoneum after intraperitoneal (ip) injection, and formed lung metastases after iv injection. Both peritoneal tumors and lung metastases were significantly reduced when ER-1 cells were co-treated with EGF and 5 or 10 m*M* NAC *(360)*.

### *4.20. Inhibition of Multidrug Resistance Genes*

Overexpression of multidrug resistance *(mdr)* genes and their encoded P-glycoproteins mediates the adenosine 5' triphosphate (ATP)-efflux of lipophilic xenobiotics and anticancer drugs, and is a major mechanism for the development of resistance of cancer cells to chemotherapy. The addition of $H_2O_2$ to the culture medium of primary rat hepatocytes resulted in *mdrb-1* mRNA and P-glycoprotein overexpression, which was markedly suppressed by NAC *(361)*. Similarly, NAC was found to inhibit the NFκB-mediated activation of *mdrb-1* by the hepatocarcinogen 2AAF *(362)*.

### *4.21. Extension of Lifespan in Experimental Test Systems*

Because of mechanisms such as antioxidant activity (Subheading 4.2.), effects in mitochondria (Subheading 4.5.), and stimulation of immune functions during aging (Subheading 4.17.), NAC would be expected to have anti-aging properties. In fact, under in vitro conditions, NAC markedly extended the lifespan of human diploid fibroblasts. Since the GSH depletor L-buthionine-(R,S)-sulfoximine (BSO) had an opposite effect, it was concluded that cellular GSH level is a determinant of the lifespan of these cells *(363)*. A study in *Drosophila melanogaster* showed that dietary NAC resulted in a significant and dose-dependent increase of the lifespan. The largest effect was obtained at a NAC concentration of 10 mg/mL food and corresponded to a 27% extension of the lifespan. At this dose, NAC treatment did not influence the feeding behavior or the physical activity of the flies *(364)*. These results corroborate the finding that aging in the mosquito is correlated with a GSH loss that may be caused by cysteine deficiency *(365)*.

### *4.22. Improvement of Quality of Life for Cancer Patients*

Studies in humans have led to the conclusion that NAC may improve the quality of life of cancer patients. In fact, NAC increased the body-cell mass both in healthy subjects with high plasma cysteine/thiol ratios and in cancer patients. The body-cell mass is the sum of the oxygen-consuming, potassium-rich, and glucose-oxidizing cells and—in practical terms—is the total body mass minus body fat and extracellular mass. In addition, NAC increased albumin level and functional capacity in cancer patients, in the absence of detectable changes in the GSH status. These studies showed a substantial change in the plasma thiol/disulfide redox state in human senescence and wasting, and suggested that this change may be a causative factor and a potential target for clinical intervention. The effect of NAC treatment on the albumin level was considered particularly important because of earlier unsuccessful attempts to improve this parameter by nutritional therapy, and because the albumin level is a strong predictor of hospital survival and the cost of hospitalization *(366)*.

## 5. CONCLUSIONS

Cancer chemopreventive agents should have certain essential requirements, including low cost, practicality of use, lack of side effects, and efficacy *(183)*. There is no doubt that NAC meets the first three requirements. As to efficacy, NAC showed an impressive variety of protective effects and mechanisms in vitro, in animal models, and also in humans at the level of intermediate biomarkers. As for most drugs assayed in intervention trials, its efficacy as a cancer chemopreventive agent has not yet been established in humans. Studies designed to evaluate whether regular NAC intake in healthy individuals can actually lower the risk of developing cancer are particularly imortant. Moreover, the anti-angiogenic and anti-invasive properties of NAC, which have a solid mechanistic foundation, warrant studies aimed at evaluating whether this molecule—possibly at high doses and in combination with

cytostatic drugs—could help to inhibit the progression of cancer in humans.

## ACKNOWLEDGMENTS

The studies reported in this article were supported by grants from the US National Cancer Institute, the Italian Association for Cancer Research (AIRC), and the Italian Ministry of University and Research (MIUR).

We thank Dr. Sofia Ceravolo for her skillful assistance in editing the manuscript.

## REFERENCES

1. De Flora S, Bennicelli C, Serra D, et al. Role of glutathione and *N*-acetylcysteine as inhibitors of mutagenesis and carcinogenesis, in *Absorption and Utilization of Amino Acids, Vol. III.* Friedman M, ed. CRC Press, Boca Raton, FL, 1989, pp.19–53.
2. De Flora S, Camoirano A, Izzotti A, et al. Antimutagenic and anticarcinogenic mechanisms of aminothiols, in *Anticarcinogenesis and Radiation Protection III.* Nygaard F, Upton AC, eds. Plenum Press, New York, 1991, pp.275–285.
3. De Flora S, Izzotti A, D'Agostini F, Cesarone CF. Antioxidant activity and other mechanisms of thiols in chemoprevention of mutation and cancer. *Am J Med* 1991;91 suppl 3C:122–130.
4. De Flora S, Izzotti A, D'Agostini F, et al. Chemopreventive properties of *N*-acetylcysteine and other thiols, in *Cancer Chemoprevention.* Wattenberg L, Lipkin M, Boone CW, Kelloff GJ, eds. CRC Press, Boca Raton, FL, 1992, pp.183–194.
5. De Vries N, De Flora S. *N*-Acetyl-L-cysteine. *J Cell Biochem* 1993; 17F suppl:270–278.
6. Kelloff GJ, Crowell JA, Boone CW, et al. Clinical development plans for cancer chemopreventive agents: *N*-acetylcysteine. *J Cell Biochem* 1994;20:63–73.
7. De Flora S, Cesarone CF, Balansky RM, et al. Chemopreventive properties and mechanisms of *N*-acetylcysteine. The experimental background. *J Cell Biochem* 1995;58 suppl. 22:33–41.
8. De Flora S, Balansky R, Bennicelli C, et al. Mechanisms of anticarcinogenesis: The example of *N*-acetylcysteine, in *Drugs, Diet and Disease, Vol. 1. Mechanistic Approaches to Cancer.* Ioannides C, Lewis DFV, eds. Ellis Horwood, Hemel Hempstead, 1995, pp.151–203.
9. De Flora S, Izzotti A, D'Agostini F, Balansky RM. Mechanisms of *N*-acetylcysteine in the prevention of DNA damage and cancer, with special reference to smoking-related endpoints. *Carcinogenesis* 2001;22:999–1013.
10. Harington JS. The sulfhydryl group and carcinogenesis. *Adv Cancer Res* 1967;10:247–309.
11. Meister A. Metabolism and function of glutathione, in *Glutathione: Chemical, Biochemical and Medical Aspects.* Dolphin D, Poulson R, Avramovis O, eds. John Wiley, New York, 1989, pp.367–374.
12. Cohen G, Hochstein P. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry* 1963;2:1420–1428.
13. Chasseaud LF. The role of glutathione and glutathione *S*-transferases in the metabolism of chemical carcinogens and other electrophilic agents. *Adv Cancer Res* 1979;29:175–274.
14. Ketterer B, Harris JM, Talaska G, et al. The human glutathione *S*-transferase supergene family, its polymorphism, and its effect on susceptibility to lung cancer. *Environ Hlth Perspect* 1992;98:87–94.
15. Hayes JD, Pulford DJ. The glutathione-*S*-transferase supergene family: regulation of GST and the contribution of isoenzyme to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995;30:445–600.
16. Seidegård J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 1988;85:7293–7297.
17. Tanningher M, Malacarne D, Izzotti A, et al. Drug metabolism polymorphisms as modulators of cancer susceptibility. *Mutat Res* 1999;436:227–261.
18. Izzotti.A, Saccà SC, Cartiglia C, De Flora S. Oxidative DNA damage in the eye of glaucoma patients. *Am J Med,* 2003;114:638–646.
19. Izzotti A, Cartiglia C, Lewtas J, De Flora S. Increased DNA alterations in atherosclerotic lesions of individuals lacking the *GSTM1* genotype. *FASEB J* 2001;15:752–757.
20. De Flora S, Bennicelli C, Zanacchi P, et al. In vitro effects of *N*-acetylcysteine on the mutagenicity of direct-acting compounds and procarcinogens. *Carcinogenesis* 1984;5:505–510.
21. Pendyala L, Creaven PJ. Pharmacokinetic and pharmacodynamic studies of *N*-acetylcysteine, a potential chemopreventive agent during a phase I trial. *Cancer Epidemiol Biomark Prev* 1995;4:245–251.
22. De Flora S, Hietanen E, Bartsch H, et al. Enhanced metabolic activation of chemical hepatocarcinogens in woodchucks infected with hepatitis B virus. *Carcinogenesis* 1989;10:1099–1106.
23. De Flora S, Bennicelli C, Camoirano A, et al. Metabolic activation of food hepatocarcinogens in hepatitis B virus-infected humans and animals, in *Mutagens and Carcinogens in the Diet.* Pariza MW, Aeschbacher H-U, Felton JS, Sato S, eds. New York, Wiley-Liss, 1990, pp.167–172.
24. Izzotti A, Scatolini L, Lewtas J, et al. Enhanced levels of DNA adducts in the liver of woodchucks infected with hepatitis virus. *Chem-Biol Inter* 1995;97:273–285.
25. Suàrez M, Beloqui O, Prieto J. GSH and chronic hepatitis C virus (Abstract), in *Preventive and Therapeutic Strategies for Lung Protection: The Role of Glutathione System.* ESI Stampa Medica, San Donato Milanese, 1992, p.20.
26. Eck HP, Gmünder H, Hartmann M, et al. Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients. *Biol Chem Hoppe-Seyler* 1989;370:101–108.
27. Buhl R, Holroyd KJ, Mastrangeli A, et al. Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* 1989; ii:1294–1298.
28. Herzenberg LA, De Rosa SC, Dubs JG, et al. Glutathione deficiency is associated with impaired survival in HIV disease. *Proc Natl Acad Sci USA* 1997;94:1967–1972.
29. Marmor M, Alcabes P, Titus S, et al. Low serum thiol levels predict shorter times-to-death among HIV-infected injecting drug users. *AIDS* 1997;11:1389–1393.
30. Sen CK, Packer L. Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr* 2000;72 Suppl 2:653S–669S.

31. Burgunder JM, Varriale A, Lauterberg BH. Effect of *N*-acetylcysteine and glutathione following paracetamol administration. *Eur J Clin Pharmacol* 1989;36:127–131.
32. Flanagan RJ, Meredith TJ. Use of *N*-acetylcysteine in clinical toxicology. *Am J Med* 1991;91:131–139.
33. Bridgeman MME, Marsden M, MacNee W, et al. Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with *N*-acetylcysteine. *Thorax* 1991;46:39–42.
34. Johnston RE, Hawkins HC, Weikel JH Jr. The toxicity of *N*-acetylcysteine in laboratory animals. *Semin Oncol* 1983;10 suppl. 1:17–24.
35. Mulvaney W, Quilter T, Mortera A. Experiences with acetylcysteine in cystinuric patients. *J. Urol* 1975;114:107–108.
36. Prescott LF, Illingworth RN, Critchley JAJH, et al. Intravenous *N*-acetylcysteine: the treatment of choice for paracetamol poisoning. *Br Med J* 1979;2:1097–1100.
37. Repine JE, Bast A, Lankhorst I, The Oxidative Stress Study Group. Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1997;56:341–357.
38. Stey C, Steurer J, Bachmann S, et al. The effect of oral *N*-acetylcysteine in chronic bronchitis: a quantitative systematic review. *Eur Respir J* 2000;16:253–262.
39. De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term *N*-acetylcysteine treatment. *Eur Respir J* 1997;10:1535–1541.
40. Meyer A, Buhl R, Kampf S, Magnussen H. Intravenous *N*-acetylcysteine and lung glutathione of patients with pulmonary fibrosis and normals. *Am J Crit Care Med* 1995;152:1055–1060.
41. Crystal RG, Bast A (eds). Oxidants and antioxidants: pathophysiologic determinants and therapeutic agents. *Am J Med* 1991;91:1–145.
42. Holt S, Goodier D, Marley R, et al. Improvement in renal function in hepatorenal syndrome with *N*-acetylcysteine. *Lancet* 1999;353:294–295.
43. Tepel M, van der Giet M, Schwarzfeld C, et al. Prevention of radiographic-contrast-agent-induced reductions in renal function by acetylcysteine. *N Engl J Med* 2000;343:180–184.
44. Colombo AA, Alessandrino EP, Bernasconi P, et al. *N*-acetylcysteine in the treatment of steroid-resistant acute graft-versus-host-disease: preliminary results. Gruppo Italiano Trapianto di Midollo Osseo (GITMO). *Transplantation* 1999;68:1414–1416.
45. Wiklund O, Fager G, Anderson A, et al. *N*-acetylcysteine treatment lowers plasma homocysteine but not serum lipoprotein(a) levels. *Atherosclerosis* 1996;119:99–106.
46. Sochman J, Vrbska J, Musilova B, Rocek M. Infarct size limitation: acute *N*-acetylcysteine defense (ISLAND trial): preliminary analysis and report after the first 30 patients. *Clin Cardiol* 1996;19:94–100.
47. Yoshie Y, Ohshima H. Synergistic induction of DNA strand breakage by cigarette tar and nitric oxide. *Carcinogenesis* 1997;18:1359–1363.
48. Izzotti A, Orlando M, Gasparini L, et al. In vitro inhibition by *N*-acetylcysteine of oxidative DNA modifications detected by $^{32}$P postlabeling. *Free Radic Res* 1998;28:165–178.
49. Srinivasan P, Vadhanan MV, Arif JM, Gupta RC. Evaluation of antioxidant potential of natural and synthetic agents *in vitro* (Abstract). *Proc Am Ass Cancer Res* 2000;41:663–664.
50. Wang M, Nishikawa A, Chung FL. Differential effects of thiols on DNA modifications via alkylation and Michael addition by alpha-acetoxy-*N*-nitrosopyrrolidine. *Chem Res Toxicol* 1992;5:528–531.
51. Smith WA, Gupta RC. Use of a microsome-mediated test system to assess efficacy and mechanisms of cancer chemopreventive agents. *Carcinogenesis* 1996;17:1285–1290.
52. Smith WA, Arif JM, Gupta RC. Effect of cancer chemopreventive agents on microsome-mediated DNA adduction of the breast carcinogen dibenzo*[a,l]*pyrene. *Mutat Res* 1998;412:307–314.
53. Waters MD, Stack HF, Jackson MA, et al. Activity profiles of antimutagens. In vitro and in vivo data. *Mutat Res* 1996;350:109–129.
54. De Flora S, Bennicelli C, Camoirano A, et al. Inhibition of mutagenesis and carcinogenesis by *N*-acetylcysteine, in *Anticarcinogenesis and Radiation Protection*. Cerutti PA, Nygaard O, Simic MG, eds. Plenum Press, New York and London, 1987, pp.373–379.
55. De Flora S, Bennicelli C, Zanacchi P, et al. Mutagenicity of active oxygen species in bacteria and its enzymatic or chemical inhibition. *Mutat Res* 1989;214:153–158.
56. De Flora S, Bennicelli C, Rovida A, et al. Inhibition of the "spontaneous" mutagenicity in *Salmonella typhimurium* TA102 and TA104. *Mutat Res* 1994;307:157–167.
57. Barale R, Micheletti R, Sbrana C, et al. *N*-acetylcysteine inhibits diesel extract mutagenicity in the Ames test and SCE induction in human lymphocytes in *Antimutagenesis and Anticarcinogenesis Mechanisms III*. Bronzetti G, Hayatsu H, De Flora S, et al., eds. Plenum Press, New York, 1993, pp.149–160.
58. Selig C, Nothdurft W, Fliedner TM. Radioprotective effect of *N*-acetylcysteine on granulocyte/macrophage colony-forming cells of human bone marrow. *J Cancer Res Clin Oncol* 1993; 115:346–349.
59. De Flora S, Camoirano A, Izzotti A, Bennicelli C. Potent genotoxicity of halogen lamps, compared to fluorescent light and sunlight. *Carcinogenesis* 1990;11:2171–2177.
60. De Flora S. Detoxification of genotoxic compounds as a threshold mechanism limiting their carcinogenicity. *Toxicol Pathol* 1984;12:337–343.
61. Howden PJ, Faux SP. Glutathione modulates the formation of 8-hydroxydeoxy-guanosine in isolated DNA and mutagenicity in *Salmonella typhimurium* TA100 induced by mineral fibres. *Carcinogenesis* 1996;17:2275–2277.
62. Camoirano A, De Flora S, Dahl T. Genotoxicity of volatile and secondary reactive oxygen species generated by photosensitization. *Env Mol Mutag* 1993;21:219–228.
63. Weitzman SA, Stossel TP. Effects of oxygen radical scavengers and antioxidants on phagocyte-induced mutagenesis. *J Immunol* 1982;128:2770–2772.
64. Wilpart M, Mainguet P, Geeroms D, Roberfroid M. Desmutagenic effects of *N*-acetylcysteine on direct and indirect mutagens. *Mutat Res* 1985;142:169–177.
65. De Flora S, Camoirano A, Izzotti A, et al. Antimutagenic and anticarcinogenic mechanisms of aminothiols, in *Anticarcinogenesis and Radiation Protection III*. Nygaard F, Upton AC eds. Plenum Press, New York, 1991, pp.275–285.
66. Camoirano A, Balansky RM, Bennicelli C, et al. Experimental databases on inhibition of the bacterial mutagenicity of 4-nitroquinoline 1-oxide and cigarette smoke. *Mutat Res* 1994;317: 89–109.

67. Camoirano A, Badolati GS, Zanacchi P, et al. Dual role of thiols in *N*-methyl-*N*-nitro-*N*-nitrosoguanidine genotoxicity. *Life Science Adv-Exp Oncol* 1988;7:21–25.
68. Park K, Liem A, Stewart BC, Miller JA. Vinyl carbamate epoxide, a major strong elecrophilic, mutagenic and carcinogenic metabolite of vinyl carbamate and ethyl carbamate (urethane). *Carcinogenesis* 1993;14:441–450.
69. Josephy PD, Carter MH, Goldberg MT. Inhibition of benzidine mutagenesis by nucleophiles: a study using the Ames test with hamster hepatic activation. *Mutat Res* 1985;143:5–10.
70. De Flora S, Bennicelli C, Camoirano A, et al. In vivo effects of *N*-acetylcysteine on glutathione metabolism and on the biotransformation of carcinogenic and/or mutagenic compounds. *Carcinogenesis* 1985;6:1735–1745.
71. Friedman M, Wehr CM, Schade JE, MacGregor JT. Inactivation of aflatoxin $B_1$ mutagenicity by thiols. *Food Chem Toxicol* 1982;20:887–892.
72. Shetty TK, Francis AR, Bhattacharya RK. Modifying role of dietary factors on the mutagenicity of aflatoxin $B_1$: in vitro effect of sulphur-containing amino acids. *Mutat Res* 1989;222:403–407.
73. Ioannides C, Ayrton AD, Lewis DFW, Walker R. Extrapolation of in vitro antimutagenicity to the in vivo situation: the case for anthraflavic acid. *Basic Life Sci* 1993;61:103–110
74. Yang Q, Hergenhahn M, Weninger A, Bartsch H. Cigarette smoke induces direct DNA damage in the human B-lymphoid cell line Raji. *Carcinogenesis* 1999;20:1769–1775.
75. Yih LH, Lee TC. Effects of exposure protocols on induction of kinetochore-plus and -minus micronuclei by arsenite in diploid human fibroblasts. *Mutat Res* 1999;440:75–82.
76. Aluigi MG, De Flora S, D'Agostini F,et al. Antiapoptotic and antigenotoxic effects of *N*-acetylcysteine in human cells of endothelial origin. *Anticancer Res* 2000;20:3183–3187.
77. Weitberg AB, Weitzman SA, Clark EP, Stossel TP. Effects of antioxidants on oxidant-induced sister chromatid exchange formation. *J Clin Investig* 1985;75:1835–1841.
78. Romert L, Jenssen D. Mechanism of *N*-acetylcysteine (NAC) and other thiols as both positive and negative modifiers of MNNG-induced mutagenicity in V79 Chinese hamster cells. *Carcinogenesis* 1987;8:1531–1535.
79. Parsons JL, Chipman JK. The role of glutathione in DNA damage by potassium bromate in vitro. *Mutagenesis* 2000;15:311–316.
80. Smith WA, Freeman JW, Gupta RC. Effect of chemopreventive agents on DNA adduction induced by the potent mammary carcinogen dibenzo*[a,l]*pyrene in the human breast cells MCF-7. *Mutat Res* 2001;480-481:97–108.
81. Huang R-P, Peng A, Hossain MZ, et al. Tumor promotion by hydrogen peroxide in rat liver epithelial cells. *Carcinogenesis* 1999;20:485–492.
82. Steele VE, Kelloff GJ, Wilkinson BP, Arnold JT. Inhibition of transformation in cultured rat tracheal epithelial cells by potential chemopreventive agents. *Cancer Res* 1990;50:2068–2074.
83. De Flora S, Izzotti A, D'Agostini F, et al. Pulmonary alveolar macrophages in molecular epidemiology and chemoprevention of cancer. *Environ Health Perspect* 1993;99:249–252.
84. Izzotti A, Balansky R, Coscia N, et al. Chemoprevention of smoke-related DNA adduct formation in rat lung and heart. *Carcinogenesis* 1992;13:2187–2190.
85. Izzotti A, Balansky RM, D'Agostini F, et al. Modulation of biomarkers by chemopreventive agents in smoke-exposed rats. *Cancer Res* 2001;61:2472–2479.
86. Balansky R, D'Agostini F, De Flora S. Induction, persistence and modulation of cytogenetic alterations in cells of smoke-exposed mice. *Carcinogenesis* 1999;20:1491–1497.
87. De Flora S, D'Agostini F, Izzotti A, Balansky R. Prevention by *N*-acetylcysteine of benzo*(a)*pyrene clastogenicity and DNA adducts in rats. *Mutat Res* 1991;250:87–93.
88. Izzotti A, Camoirano A, D'Agostini F, et al. Biomarker alterations produced in rat lung by intratracheal instillations of air particulate extracts, and chemoprevention with oral *N*-acetylcysteine. *Cancer Res* 1996;56:1533–1538.
89. Balansky R, D'Agostini F, De Flora S. Protection by *N*-acetylcysteine of the histopathological and cytogenetical damage produced by exposure of rats to cigarette smoke. *Cancer Lett* 1992;64:123–131.
90. Balansky R, Izzotti A, Scatolini L, et al. Induction by carcinogens and chemoprevention by *N*-acetylcysteine of adducts to mitochondrial DNA in rat organs. *Cancer Res* 1996;56:1642–1647.
91. Izzotti A, D'Agostini F, Bagnasco M, et al. Chemoprevention of carcinogen-DNA adducts and chronic degenerative diseases. *Cancer Res* 1994;54 suppl:1994s–1998s.
92. Arif JM, Gairola CG, Glauert HP, et al. Effects of dietary supplementation of *N*-acetylcysteine on cigarette smoke-related DNA adducts in rat tissues. *Int J Oncol* 1997;11:1227–1233.
93. Izzotti A, Bagnasco M, D'Agostini F, et al. Formation and persistence of nucleotide alterations in rats exposed whole-body to environmental cigarette smoke. *Carcinogenesis* 1999;20:1499–1505.
94. De Flora S. Threshold mechanisms and site specificity in chromium(VI) carcinogenesis. *Carcinogenesis* 2000;21:533–541.
95. Izzotti A, Orlando M, Bagnasco M, Camoirano A, De Flora S. DNA fragmentation, DNA-protein crosslinks, $^{32}P$ postlabeled modifications and formation of 8-hydroxy-2'-deoxyguanosine in the lung but not in the liver of rats receiving intratracheal instillations of chromium(VI). Chemoprevention by *N*-acetylcysteine. *Mutat Res* 1998;400:233–244.
96. Kendrick J, Nettesheim P, Hammos AS. Tumor induction in tracheal grafts. A new experimental model for respiratory carcinogenesis studies. *J Natl Cancer Inst* 1974;52:1317–1325.
97. Thomassen DG, Chen BT, Mauderly JL, et al. Inhaled cigarette smoke induces preneoplastic changes in rat tracheal epithelial cells. *Carcinogenesis* 1989;10:2359–2361.
98. Izzotti A, Balansky RM, Scatolini L, et al. Inhibition by *N*-acetylcysteine of carcinogen-DNA adducts in the tracheal epithelium of rats exposed to cigarette smoke. *Carcinogenesis* 1995;16:669–672.
99. D'Agostini F, Balansky RM, Izzotti A, et al. Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. *Carcinogenesis* 2001;22:375–380.
100. Izzotti A, Balansky RM, Camoirano A, et al. Birth-related genomic and transcripional changes in mouse lung. Modulation by transplacental *N*-acetylcysteine. *Mutat. res (rev Mutat Res)* 2003;544:441–449.
101. Rogers DF, Turner NC, Marriot C, Jeffery PK. Oral *N*-acetylcysteine or *S*-carboxymethylcysteine inhibit cigarette smoke-induced hypersecretion of mucus in rat larynx and trachea in situ. *Eur Respir J* 1989;2:955–960.

102. Rubio ML, Sanchez-Cifuentes MV, Ortega M, et al. *N*-acetylcysteine prevents cigarette smoke induced small airways alterations in rats. *Eur Resp J* 2000;15:505–511.
103. Pereira MA, Khoury MD. Prevention by chemopreventive agents of azoxymethane-induced foci of aberrant crypts in rat colon. *Cancer Lett* 1991;61:27–33.
104. Pereira MA, Barnes LH, Rassman VL, et al. Use of azoxymethane-induced foci of aberrant crypts in rat colon to identify potential cancer chemopreventive agents. *Carcinogenesis* 1994;15:1049–1054.
105. Chung FL, Conaway CC, Rao CV Reddy BS. Chemoprevention of colonic aberrant crypt foci in Fisher rats by sulforaphane and phenethyl isothiocyanate. *Carcinogenesis* 2000;21:2287–2291.
106. Cesarone CF, Menegazzi M, Scarabelli L, et al. Protection of molecular enzymes by aminothiols, in *Anticarcinogenesis and Radiation Protection, Vol. 2*. Nygaard F, Upton AC, eds. Plenum Press, New York, 1991, pp.261–268.
107. Liu TY, Chen CC, Chi CW. Safrole-induced oxidative damage in the liver of Sprague-Dawley rats. *Food Chem Toxicol* 1999;37:697–702.
108. Chan JYH, Stout DL, Becker FK. Protective role of thiols in carcinogen-induced DNA damage in rat. *Carcinogenesis* 1986;7:1621–1624.
109. Izzotti A, Balansky RM, Cartiglia C, et al. Genomic and transcriptional alterations in mouse fetus liver after transplacental exposure to cigarette smoke. *FASEB J* 2003;17:1127–1129.
110. Cesarone C F, Scarabelli L, Orunesu M, et al. Effects of aminothiols in 2-acetylaminofluorene-treated rats. I. Damage and repair of liver DNA, hyperplastic foci, and Zymbal gland tumors. *In Vivo* 1987;1:85–91.
111. D'Agostini F, Bagnasco M, Giunciuglio D, et al. Oral *N*-acetylcysteine inhibition of doxorubicin-induced clastogenicity and alopecia: Interaction between the two drugs in preventing primary tumors and lung micrometastases in mice. *Int J Oncol* 1998;13:217–224.
112. D'Agostini F, Balansky R, Pesce CM, et al. Alopecia and hair follicle cell apoptosis in mice exposed to environmental cigarette smoke. *Toxicol Lett* 2000;114:117–123.
113. Izzotti A, Camoirano A, Cartiglia C, et al. Formation of DNA adducts in the aorta of smoke-exposed rats. Modulation by five chemopreventive agents. *Mutat Res* 2001;494:97–106.
114. Gandy J, Bates HK, Conder LA, Harbison RD. Effects of reproductive tract glutathione enhancement and depletion on ethyl methanesulfonate-induced dominant lethal mutations in Sprague-Dawley rats. *Teratog Carcinog Mutagen* 1992;12:61–70.
115. Doyle CE, Mackay JM, Ashby J. Failure of *N*-acetylcysteine to protect the mouse bone marrow against the clastogenicity of 7,12-dimethylbenzanthracene. *Mutagenesis* 1993;8:583–584.
116. Balansky R, De Flora S. Chemoprevention by *N*-acetylcysteine of urethane-induced lung tumors in mice, as related to the time-course monitoring of micronuclei in peripheral blood erythrocytes. *Int J Cancer* 1998;77:302–305.
117. Chung FL, Kelloff GJ, Steele VE, et al. Chemopreventive efficacy of arylalkylisothiocyanates and *N*-acetylcysteine for lung tumorigenesis in Fischer rats. *Cancer Res* 1996;56:772–778.
118. Boone CW, Steele VE, Kelloff GJ. Screening for chemopreventive (anticarcinogenic) compounds in rodents. *Mutat Res* 1992;267:251–255.
119. De Flora S, Astengo M, Serra D, Bennicelli C. Inhibition of urethan-induced lung tumors in mice by dietary *N*-acetylcysteine. *Cancer Lett* 1986;32:235–241.
120. Witschi H, Espiritu I, Yu M, Willits NH. The effects of phenethyl isothiocyanate, *N*-acetylcysteine and green tea on tobacco smoke-induced lung tumors in strain A/J mice. *Carcinogenesis* 1998,19:1789–1794.
121. D'Agostini F, Balansky R, Camoirano A, De Flora S. Interactions between *N*-acetylcysteine and ascorbic acid in modulating mutagenesis and carcinogenesis. *Int J Cancer* 2000;88:702–707
122. D'Agostini F, Balansky RM, Bennicelli C, et al. Pilot studies evaluating the lung tumor yield in cigarette smoke-exposed mice. *Int J Oncol* 2001,18:607–615.
123. Witschi H, Espiritu I, Uyeminami D. Chemoprevention of tobacco smoke-induced lung tumors in A/J strain mice with dietary *myo*-inositol and dexamethasone. *Carcinogenesis* 1999;20:1375–1378.
124. Witschi H, Uyeminami D, Moram D, Espiritu I. Chemoprevention of tobacco smoke lung carcinogenesis in mice after cessation of smoke exposure. *Carcinogenesis* 2000;21:977–982.
125. Conaway CC, Jiao D, Kelloff GJ, et al. Chemopreventive potential of fumaric acid, *N*-acetylcysteine, *N*-(4-hydroxyphenyl) retinamide and beta-carotene for tobacco-nitrosamine-induced lung tumors in A/J mice. *Cancer Lett* 1998;124:85–93.
126. Jiao D, Smith TJ, Yang C-S, et al. Chemopreventive activity of thiol conjugates of isothiocyanates for lung tumorigenesis. *Carcinogenesis* 1997;18:2143–2147.
127. Conaway CC, Krzeminski J, Amin S, et al. *N*-acetylcysteine conjugates of isothiocyanates inhibit lung tumors induced by a tobacco carcinogen at post-initiation stages (Abstract). *Proc Am Assoc Cancer Res* 2000;41:660.
128. Yang Y-M, Conaway CC, Chiao JW, et al. Inhibition of benzo*(a)*pyrene-induced lung tumorigenesis in A/J mice by dietary *N*-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res* 2002;62:2–7.
129. Balansky RM, Ganchev G, D'Agostini F, De Flora S. Effects of *N*-acetylcysteine in an oesophageal carcinogenesis model in rats treated with diethylnitrosamine and diethyldithiocarbamate. *Int J Cancer* 2002;98:493–497.
130. Wilpart M, Speder A, Roberfroid M. Anti-initiation activity of *N*-acetylcysteine in experimental colonic carcinogenesis. *Cancer Lett* 1986;31:319,324.
131. Reddy BS, Rao CV, Rivenson A, Kelloff GJ. Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res* 1993;53:3493–3498.
132. Seril DN, Liao J, Ho KLK et al. Inhibition of chronic ulcerative colitis-associated colorectal adenocarcinoma development in a murine model by *N*-acetylcysteine. *Carcinogenesis* 2002;23:993–1001.
133. Pereira MA. Chemoprevention of diethylnitrosamine-induced liver foci and hepatocellular adenomas in C3H mice. *Anticancer Res* 1995;15:1953–1956.
134. Rotstein JB, Slaga TJ. Anticarcinogenic mechanisms, as evaluated in the multistage mouse skin model. *Mutat Res* 1988;202:421–427.

135. Rotstein JB, Slaga TJ. Effect of exogenous glutathione on tumor progression in the murine skin multistage carcinogenesis model. *Carcinogenesis* 1988;9:1547–1551.
136. Martin KR, Trempus C, Saulnier M, et al. Dietary *N*-acetyl-L-cysteine modulates benzo*(a)*pyrene-induced skin tumors in cancer-prone p53 haploinsufficient Tg.AC *(v-Ha-ras)* mice. *Carcinogenesis* 2001;22:1373–1378.
137. Lubet RA, Steele VE, Eto I, et al. Chemopreventive efficacy of anethole trithione, *N*-acetyl-L-cysteine, miconazole and phenethyl isothiocyanate in the DMBA-induced rat mammary cancer model. *Int J Cancer* 1997;72:95–101.
138. Kelloff GJ, Boone CW, Malone WF, et al. Development of chemopreventive agents for bladder cancer *J Cell Biochem* 1992;161:1–12.
139. Ross DA, Kish P, Muraszko KM, et al. Effect of dietary vitamin A or *N*-acetylcysteine in ethylnitrosourea-induced rat gliomas. *J Neurooncol* 1998;40:29–38.
140. McLellan LI, Lewis AD, Holl DJ, et al. Uptake and distribution of *N*-acetylcysteine in mice: tissue-specific effects on glutathione concentrations. *Carcinogenesis* 1995; 16:2099–2106.
141. Sminia P, van der Kracht AHW, Frederiks WM, Jansen W. Hyperthermia, radiation carcinogenesis and the protective potential of vitamin A and *N*-acetylcysteine. *J Cancer Res Clin Oncol* 1996;122:343–350.
142. van Zandwijk N, Dalesio O, Pastorino U, et al. EUROSCAN, a randomized trial of vitamin A and *N*-acetylcysteine in patients with head and neck cancer or lung cancer. *J Natl Cancer Inst* 2000;92:977–986.
143. Van Schooten FJ, Nia AB, De Flora S, et al. Effects of oral *N*-acetylcysteine: a multi-biomarker study in smokers. *Cancer Epidemiol Biomark Prev* 2002;11:176–175.
144. Pendyala L, Schwartz G, Bolanowska-Higdon W, et al. Phase I/pharmacodynamic study of *N*-acetylcysteine/oltipraz in smokers. Early termination due to excessive toxicity. *Cancer Epidemiol Biomark Prev* 2001;10:269–272.
145. De Flora S, Camoirano A, Bagnasco M, et al. Smokers and urinary genotoxins. Implications for selection of cohorts and modulation of endpoints in chemoprevention trials. *J Cell Biochem* 1996;25 suppl:92–98.
146. Rösler S, Behr J, Richter E. *N*-acetylcysteine treatment lowers 4-aminobiphenyl haemoglobin adduct levels in non-smokers. *Eur J Cancer Prev* 1999;8:469–472.
147. Bryant MS, Skipper PL, Tannenbaum SR, Maclure M. Haemoglobin adducts of 4-aminobiphenyl in smokers and nonsmokers. *Cancer Res* 1987;47:602–608.
148. Greenwald P, Kelloff GJ, Boone CW, McDonald SS. Genetic and cellular changes in colorectal cancer: proposed targets of chemopreventive agents. *Cancer Epidemiol Biomark Prev* 1995;4:691–702.
149. Ponz de Leon M, Roncucci L. Chemoprevention of colorectal tumors: role of lactulose and of other agents. *Scand J Gastroenterol* 1997;222:72–75.
150. Estensen RD, Levy M, Klopp SJ, et al. *N*-acetylcysteine suppression of the proliferative index in the colon of patients with previous adenomatous colonic polyps. *Cancer Lett* 1999;147:109–114.
151. Trizna Z, Schants SP, Hsu TC. Effects of *N*-acetyl-L-cysteine and ascorbic acid on mutagen-induced chromosomal sensitivity in patients with head and neck cancers. *Am J Surg* 1991;162:294–298.
152. Trizna Z, Schants SP, Lee JJ, et al. In vitro protective effects of chemopreventive agents against bleomycin-induced genotoxicity in lymphoblastoid cell lines and peripheral blood lymphocytes of head and neck cancer patients. *Cancer Detect Prev* 1993;17:575–583.
153. Cloos J, Bongers V, Lubsen H, et al. Lack of effect of daily *N*-acetylcysteine supplementation on mutagen sensitivity. *Cancer Epidemiol Biomark Prev* 1996;5:941–944.
154. De Flora S, Ramel C. Mechanisms of inhibitors of mutagenesis and carcinogenesis. Classification and overview. *Mutat Res* 1988;202:285–306.
155. De Flora S, Izzotti A, Bennicelli C. Mechanisms of antimutagenesis and anticarcinogenesis. Role in primary prevention, in *Antimutagenesis and Anticarcinogenesis Mechanisms III.* Bronzetti G, Hayatsu H, De Flora S, et al., eds. Plenum Press, New York, 1993, pp.1–16.
156. De Flora S. Mechanisms of inhibitors of mutagenesis and carcinogenesis. *Mutat Res* 1998;402:151–158.
157. De Flora S, Izzotti A, D'Agostini F, et al. Multiple points of intervention in the prevention of cancer and other mutation related diseases. *Mutat Res* 2001;480–481:9–22.
158. Walters FP, Wise RW, Lakshmi VM, et al. Metabolism of the renal carcinogen FNT by peroxidases. *Carcinogenesis* 1986;7:1411–1414.
159. Allen RG. Oxidative stress and superoxide dismutase in development, aging and gene regulation. *Age* 1998;21:47–76.
160. Allen RG, Tresini M. Oxidative stress and gene regulation. *Free Radic Biol Med* 2000;28:463–499.
161. Aruoma OI, Halliwell B, Hoey B M, Butler J. The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* 1989,6, 593–597.
162. Doelman CJ, Bast A. Oxygen radicals in lung pathology. *Free Radic Biol Med.* 1990;9:381–400.
163. Moldéus P, Cotgreave IA, Berggren M. Lung protection by a thiol-containing antioxidant: *N*-acetylcysteine. *Respiration* 1986;50 Suppl 1: 31–42.
164. Rougée M, Bensasson RV, Land EJ, Pariente R. Deactivation of singlet molecular oxygen by thiols and related compounds, possible protectors against skin photosensitivity. *Photochem Photobiol* 1988;47:485–489.
165. Christen S, Schaper M, Lykkesfeldt J, et al. Oxidative stress in brain during experimental bacterial meningitis: differential effects of α-phenyl-*tert*-butyl nitrone and *N*-acetylcysteine treatment. *Free Radic Biol Med* 2001;31:754–762.
166. Watanabe T, Pakala R, Katagiri T, Benedict CR. Lipid peroxidation product 4-hydroxy-2-nonenal acts synergistically with serotonin in inducing vascular smooth muscle cell proliferation. *Atherosclerosis* 2001;155:37–44.
167. Chinery R, Beauchamp RD, Shyr Y, et al. Antioxidants reduce cyclooxygenase-2 expression, prostaglandin production, and proliferation in colorectal cancer cells. *Cancer Res* 1998;58:2323–2327.
168. Yan Z, Subbaramaiah K, Camilli T, et al. Benzo*(a)*pyrene induces the transcription of cyclooxygenase-2 in vascular smooth muscle cells. Evidence for the involvement of extracellular signal-regulated kinase and NF-κB. *J Biol Chem* 2000;18:4949–4955.
169. Rioux N, Castonguay A. The induction of cyclooxygenase-1 by a tobacco carcinogen in U937 human macrophages is

correlated to the activation of NF-κB. *Carcinogenesis* 2000;21:1745–1751.
170. Oikawa S, Yamada K, Yamashita N, et al. *N*-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. *Carcinogenesis* 1999;20:1485–1490.
171. Meister A. Glutathione, ascorbate, and cellular protection. *Cancer Res* 1994;54 Suppl:1969s–1975s.
172. Winkler BS, Orselli SM, Rex TS. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radic Biol Med* 1994;17:339–349.
173. De Flora S, Cesarone CF, Bennicelli C, et al. Antigenotoxic and anticarcinogenic effects of thiols. In vitro inhibition of the mutagenicity of drug nitrosation products and protection of rat liver ADP-ribosyl transferase activity, in *Chemical Carcinogenesis: Models and Mechanisms*. Feo F, Pani P, Columbano A, Garcea R, eds. Plenum Press, New York, 1988, pp.75–86.
174. Cupo DJ, Wetterhahn KE. Modification of chromium(VI)-induced DNA damage by glutathione and cytochromes P-450 in chicken embryo hepatocytes. *Proc Natl Acad Sci USA* 1985;82:6755–6759.
175. Bagnasco M, Bennicelli C, Camoirano A, et al. Metabolic alterations produced by cigarette smoke in rat lung and liver, and their modulation by oral *N*-acetylcysteine. *Mutagenesis* 1992;7:295–301.
176. De Flora S, Romano M, Basso C, et al. Detoxifying activities in alveolar macrophages of rats treated with acetylcysteine, diethyl maleate and/or Aroclor. *Anticancer Res* 1986;6:1009–1012.
177. Wattenberg LW. Inhibition of neoplasia by minor dietary constituents. *Cancer Res* 1983;43:2448s–2453s.
178. Xia C, Hu J, Ketterer B, Taylor JB. The organization of the human *GSTP1-1* gene promoter and its response to retinoic acid and cellular redox status. *Biochem J* 1996;313:155–161.
179. Banaclocha MM, Hernandez AI, Martinez N, Ferrandiz ML. *N*-acetylcysteine protects against age-related increase in oxidized proteins in mouse synaptic mitochondria. *Brain Res* 1997;762:256–258.
180. Miquel J, Ferrandiz ML, De Juan E, et al. *N*-acetylcysteine protects against age-related decline of oxidative phosphorylation in liver mitochondria. *Eur J Pharmacol* 1995;292:333–335.
181. Palmero M, Bellot JL, Castillo M, et al. An in vitro model of ischemic-like stress in retinal pigmented epithelium cells: protective effects of antioxidants. *Mech Ageing Dev* 2000;114:185–190.
182. Banzet N, Franáois D, Polla BS. Tobacco smoke induces mitochondrial depolarization along with cell death: effects of antioxidants. *Redox Rep* 1999;4:229–236.
183. De Flora S, Balansky R, Scatolini L, et al. Adducts to nuclear DNA and mitochondrial DNA as biomarkers in chemoprevention, in *Principles of Chemoprevention*. Stewart BW, McGregor D, Kleihues P, eds. IARC Sci. Publ. No. 139. International Agency for Research on Cancer, Lyon, France, 1996, pp.291–301.
184. Lertratanangkoon K, Orkiszewski RS, Scimeca JM. Methyl-donors deficiency due to chemically induced glutathione depletion. *Cancer Res* 1996;56:995–1005.
185. Liu M, Pelling JC, Ju J, et al. Antioxidant action *via p53*-mediated apoptosis. *Cancer Res* 1998;58:1723–1729.
186. Nargi JL, Ratan RR, Griffin DE. p53-independent inhibition of proliferation and p21(WAF1/Cip1)-modulated induction of cell death by the antioxidants *N*-acetylcysteine and vitamin E. *Neoplasia* 1999;1:544–556.
187. Janssen YM, Heintz NH, Mosman BT. Induction of c-*fos* and c-*jun* proto-oncogene expression by asbestos is ameliorated by *N*-acetyl-L-cysteine in mesothelial cells. *Cancer Res* 1995;55:2085–2089.
188. Kamata H, Tanaka C, Yagisawa H, et al. Suppression of nerve growth factor-induced neuronal differentiation of PC12 cells. *J Biol Chem* 1996;271:33,018–33,025.
189. Bergelson S, Pinkus R, Daniel V. Intracellular glutathione levels regulate *Fos/Jun* induction and activation of glutathione *S*-transferase gene expression. *Cancer Res* 1994;54:36–40.
190. Ho E, Chen G, Bray TM. Supplementation of *N*-acetylcysteine inhibits NFκB activation and protects against alloxan-induced diabetes in CD-1 mice. *FASEB J* 1999;13:1845–1854.
191. Oka S, Kamata H, Kamata K, et al. *N*-acetylcysteine suppresses TNF-induced NF-κB activation through inhibition of IκB kinases. *FEBS Lett* 2000;472:196–202.
192. Maziere C, Dantin F, Dubois F, et al. Biphasic effects of UVA radiation on STAT1 activity and tyrosine phosphorylation in cultured human keratinocytes. *Free Radic Biol Med* 2000;28:1430–1437.
193. Ward NE, Pierce DS, Chung SE, et al. Irreversible inactivation of protein kinase C by glutathione. *J Biol Chem* 1998;273:12,558–12,566.
194. Luethy JD, Holbrook NJ. The pathway regulating *GADD153* induction in response to DNA damage is independent of protein kinase C and tyrosine kinases. *Cancer Res* 1994;54 Suppl:1902s–1906s.
195. Li WQ, Dehnade F, Zafarullah M. Thiol antioxidant, *N*-acetylcysteine, activates extracellular signal-regulated kinase signaling pathway in articular chondrocytes. *Biochem Biophys Res Commun* 2000;275:789–794.
196. Liu M, Wikonkal NM, Brash D. Induction of cyclin-dependent kinase inhibitors and $G_1$ prolongation by the chemoprevenive agent *N*-acetylcysteine. *Carcinogenesis* 1999;20:1869–1872.
197. Liu B, Andrieu-Abadie N, Levade T, et al. Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death. *J Biol Chem* 1998;273:11,313–11,320.
198. White AC, Maloney EK, Lee SL, et al. Reduction of endothelial cell related TGF-α activity by thiols. *Endothelium* 1999;6:231–239.
199. Kamata H, Shibukawa Y, Oka SI, Hirata H. Epidermal growth factor receptor is modulated by redox through multiple mechanisms. Effects of reduction and $H_2O_2$. *Eur J Biochem* 2000;267:1933–1944.
200. Kerzee JK, Ramos KS. Activation of c-Ha-*ras* by benzo*(a)*pyrene in vascular smooth muscle cells involves redox stress and aryl hydrocarbon receptor. *Mol Pharmacol* 2000;58:152–158.
201. Mayer M, Noble M. *N*-acetyl-L-cysteine is a pluripotent protector against cell death and enhancer of trophic factor-mediated cell survival *in vitro*. *Proc Natl Acad Sci USA* 1994;91:7496–7500.
202. Moldéus P, Cotgreave IA, Berggren M. Lung protection by a thiol-containing antioxidant: *N*-acetylcysteine. *Respiration* 1986;50 suppl:31–42.

203. Moldéus P, Berggren M, Grafstrom R. *N*-acetylcysteine protection against the toxicity of cigarette smoke and cigarette smoke condensates in various tissues and cells *in vitro*. *Eur J Respir Dis* 1985;139 suppl:123–129.
204. Voisin C, Aerts C, Wallaert B. Prevention of in vitro oxidant-mediated alveolar macrophage injury by cellular glutathione and precursors. *Bull Eur Physiopath Respir* 1987;23:309–313.
205. Pinot F, el Yaagoubi A, Christie P, et al. Induction of stress proteins by tobacco smoke in human monocytes: modulation by antixodants. *Cell Stress Chaperones* 1997;2:156–161.
206. Davis CD, Snyderwine EG. Protective effect of *N*-acetylcysteine against heterocyclic amine-induced cardiotoxicity in cultured myocyets and in rats. *Food Chem Toxicol* 1995;33:641–651.
207. Ota Y, Kugiyama K, Sugiyama S, et al. Impairment of endothelium-dependent relaxation of rabbit aortas by cigarette smoke extract. Role of free radicals and attenuation by captopril. *Atherosclerosis* 1997;131:195–202.
208. Junod AF, Jornot L, Grichting G. Comparative study on the selenium- and *N*-acetylcysteine-related effects on the toxic action of hyperoxia, paraquat and the enzyme reaction hypoxanthine-xanthine oxidase in cultured endothelial cells. *Agents Actions* 1987;22:176–183.
209. Chen F, Bower J, Leonard SS, et al. Protective roles of NF-κB for chromium(VI)-induced cytotoxicity is revealed by expression of IκB kinase-b mutant. *J Biol Chem* 2001;277:3342–3349.
210. Kurata S. Selective activation of p38 MAPK cascade and mitotic arrest caused by low level oxidative stress. *J Biol Chem* 2000;275:23413–23416.
211. Kanno S, Ishikawa M, Takayanagi M, et al. Exposure to hydrogen peroxide induces cell death via apoptosis in primary cultured mouse hepatocytes. *Biol Pharm Bull* 1999;22:1296–1300.
212. Kanno S, Ishikawa M, Takayanagi M, et al. Characterization of hydrogen peroxide-induced apoptosis in mouse primary cultured hepatocytes. *Biol Pharm Bull* 2000;23:37–42.
213. Rimpler MM, Rauen U, Schmidt T, et al. Protection against hydrogen peroxide cytotoxicity in rat-1 fibroblasts provided by the oncoprotein *Bcl-2*: maintenance of calcium homoeostasis is secondary to the effect of *Bcl-2* on cellular glutathione. *Biochem J* 1999;340:291–297.
214. Lafon C., Mathieu C, Guerrin M, et al. Transforming growth factor beta 1-induced apoptosis in human ovarian carcinoma cells: protection by the antioxidant *N*-acetylcysteine and *bcl-2*. *Cell Growth Differ* 1996;7:1095–1104.
215. Suhr SM, Kim DS. Comparison of the apoptotic pathways induced by L-amino acid oxidase and hydrogen peroxide. *J Biochem Tokyo* 1999;125:305–309.
216. Jiang S, Wu MW, Stemberg P, Jones DP. *Fas* mediates apoptosis and oxidant-induced cell death in cultured hRPE cells. *Investig Ophthalmol Vis Sci* 2000;41:645–655.
217. Chen SH, Liu SH,, Liang YC, et al. Death signaling pathway induced by pyrrolidine dithiocarbamate-Cu(2+) complex in the cultured rat cortical astrocytes. *Glia* 2000;31:249–261.
218. Galle J, Heermeier, Wanner C. Atherogenic lipoproteins, oxidative stress, and cell death. *Kidney Int* 1999;56 Suppl 71:S62–S65.
219. Ma Y, Ogino T, Kawabata T, et al. Cupric nitrilotriacetate-induced apoptosis in HL-60 cells association with lipid peroxidation, release of cytochrome c from mitochondria, and activation of caspase-3. *Free Radic Biol Med* 1999;27:227–233.
220. Foresti R, Sarathchandra P, Clark JE, et al. Peroxynitrite induces haem oxygenase-1 in vascular endothelial cells: a link to apoptosis. *Biochem J* 1999;339:729–736.
221. Lepri E, Gambelunghe C, Fioravanti A, et al. *N*-acetylcysteine increases apoptosis induced by $H_2O_2$ and mo-antiFas triggering in a 3DO hybridoma cell line. *Cell Biochem Funct* 2000;18:201–208.
222. Khan S, Kayahara M, Joashi U, et al. Differential induction of apoptosis in Swiss 3T3 cells by nitric oxide and the nitrosonium cation. *J Cell Sci* 1997;110:2315–2322.
223. Umansky V, Rocha, M. Breitkreuz R, et al. Glutathione is a factor of resistance of Jurkat leukemia cells to nitrite oxide-mediated apoptosis. *J Cell Biochem* 2000;78:578–587.
224. Lee JW, Beckham C, Michel BR, et al. HLA-DR-mediated signals for hematopoiesis and induction of apoptosis involve but are not limited to a nitric oxide pathway. *Blood* 1997;90:217–225.
225. Dvorakova K, Payne CM, Tome ME, et al. Induction of oxidative stress and apoptosis in myeloma cells by the aziridine-containing agent imexon. *Biochem Pharmacol* 2000;60:749–758.
226. Haddad JJ, Land SC. The differential expression of apoptosis factors in the alveolar epithelium is redox sensitive and requires NF-kB (RelA)-selective targeting. *Biochem Biophys Res Commun* 2000;271:257–267.
227. Watson RW, Rotstein OD, Nathens AB, et al. Thiol-mediated redox regulation of neutrophil apoptosis. *Surgery* 1996;120:150–158.
228. Kletsas D, Barbieri D, Stathakos D, et al. The highly reducing sugar 2-deoxy-D-ribose induces apoptosis in human fibroblasts by reduced glutathione depletion and cytoskeletal disruption. *Biochem Biophys Res Commun* 1998;243: 416–425.
229. Kaneto H, Fujii, J, Myint T, et al. Reducing sugars trigger oxidative modification and apoptosis in pancreatic beta-cells by provoking oxidative stress through the glycation reaction. *Biochem J* 1996;320:855–863.
230. Barbieri D, Grassilli E, Monti D, et al. D-Ribose and deoxy-D-ribose induce apoptosis in human quiescent peripheral blood mononuclear cells. *Biochem Biophys Res Commun* 1994;201:1109–1116.
231. Higuchi Y, Matsukawa S. Active oxygen-mediated chromosomal 1-2 Mbp giant DNA fragmentation into internucleosomal DNA fragmentation in apoptosis of glioma cells induced by glutamate. *Free Radic Biol Med* 1998;24:418–426.
232. Froissard P, Monrocq H, Duval D. Role of glutathione metabolism in the glutamate-induced programmed cell death of neuronal-like PC12 cells. *Eur J Pharmacol* 326:93–99.
233. Kikuchi S, Shinpo K, Moriwaka F, et al. Neurotoxicity of methylglyoxal and 3-deoxyglucosone on cultured cortical neurons: synergism between glycation and oxidative stress, possibly involved in neurodegenerative diseases. *J Neurosci Res* 1999;57:280–289.
234. Okado A, Kawasaki Y, Hasuike Y, et al. Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in

macrophage-derived cell lines. *Biochem Biophys Res Commun* 1996;225:219–224.
235. Migheli R, Godani C, Sciola L, et al. Enhancing effect of manganese on L-DOPA-induced apoptosis in PC12 cells: role of oxidative stress. *J Neurochem* 1999;73:1155–1163.
236. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res* 2000;47:446–456.
237. Luo Y. Umegaki H, Wang X, et al. Dopamine induces apoptosis through an oxidation-involved SAPK/JNK activation pathway. *J Biol Chem* 1998;273:3756–3764.
238. Hoyt KR, Reynolds IJ, Hastings TG. Mechanisms of dopamine-induced cell death in cultured rat forebrain neurons: interactions with and differences from glutamate-induced cell death. *Exp Neurol* 1997;143:269–281.
239. Masserano JM, Gong L, Kulaga H, et al. Dopamine induces apoptotic cell death of a catecholaminergic cell line derived from the central nervous system. *Mol Pharmacol* 1996;50:1309–1315.
240. Offen D, Ziv I, Sternin H, et al. Prevention of dopamine-induced cell death by thiol antioxidants: possible implications for treatment of Parkinson's disease. *Exp Neurol* 1996;141:32–39.
241. Lizard G, Gueldry S, Sordet O, et al. Glutathione is implied in the control of 7-ketocholesterol-induced apoptosis, which is associated with radical oxygen species production. *FASEB J* 1998;12:1651–1663.
242. Spottl N, Wirleitner B, Bock G, et al. Reduced pteridine derivatives induce apoptosis in human neuronal NT2/HNT cells. *Immunobiology* 2000;201: 478–491.
243. Seaton TA, Cooper *JM, Sch*apira AH. Free radical scavengers protect dopaminergic cell lines from apoptosis induced by complex 1 inhibitors. *Brain Res* 1997;777:110–118.
244. Song SH, Lee KH, Kang MS, Lee,Y.J. Role of paxillin in metabolic oxidative *stress-induced cy*toskeletal reorganization involvement of SAPK signal transduction pathway and PTP-PEST gene expression. *Free Radic Biol Med* 2000;29:61–70.
245. *Fer*rari G, Yan CY, Greene LA. *N*-acetylcysteine (D- and L-stereoisomers) prevents apoptotic death of neuronal cells. *J Neurosci 1995*;15:2857–2866.
246. Schultz JB, Bremen D, Reed JC, et al. Cooperative interaction of neuronal apoptosis by *BCL-2* and *BAG-1* expression: prevention of caspase activation and reduced production of reactive oxygen species. *J Neurochem* 1997; 69:2075–2086.
247. Singh I, Pahan K, Khan M, Singh AK Cytokine-mediated induction of ceramide production is redox-sensitive. Implication to proinflammatory cytokine-mediated apoptosis in demyelinating diseases. *J Biol Chem* 1998;23:20,354–20,362.
248. Liu B, Andrieu-Abadie N, Levade T, et al. Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death. *J Biol Chem* 1998;273:11,313–11,320.
249. Cossarizza A, Franceshi C, Monti D, et al. Prote*ctive effect* of *N*-acetylcysteine in tumor necrosis factor-alpha-induced apoptosis in U937 cells: the role of mitochondria. *Exp Cell Res* 1996;220:232–240.
250. Giardina C, Boulares H, Inan MS. NSAIDs and butyrate sensitize a human colorectal cancer cell line to TNF-alpha and *Fas* ligation: the role of reactive oxygen species. *Biochim Biophys Acta* 1999;1448:425–438.
251. Shi B, Raina J, Lorenzo A, et al. Neuronal apoptosis induced by HIV-1 *Tat protein* and TNF-alpha: potentiation of neurotoxicity mediated by oxidative stress and implcations for HIV-1 dementia. *J Neurovirol* 1998;4:281–290.
252. Arakaki N, Kajihara T, Arakaki R, et al. Involvement of oxidative stress in tumor cytotoxic activity of hepatocyte growth factor/scatter factor. *J Biol Chem* 1999;274:13,541–13,546.
253. MacKinnon AC, Waters C, Rahman I, et al. [Arg(6),D-Trp(7,9),*N*(me)Phe(8)]-substance P (6–11) (antagonist G) induces AP-1 transcription and sensitizes cells to chemotherapy. *Br J Cancer* 2000;83: 941–948.
254. Yildiz D, Ercal N, Frank RL, Matthews RH Effect of 4-hydroxynonenal and *N*-acetyl-L-cysteine on *Myc*-induced apoptosis. *Toxicol Lett* 1996;89:215–221.
255. Furuke K, Bloom ET. Redox-sensi*tive* events in *Fas*-induced apoptosis in human NK cells include ceramide generation and protein tyrosine dephosphorylation. *Int Immunol* 1998;10:1261–1272.
256. Um HD, Orenstein JM, Wahl SM. *Fas* mediates apoptosis in human monocytes by a reactive oxygen intermediate dependent pathway. *J Immunol* 1996;156:3469–3477.
257. Cao LC, Honeyman T, Jonassen J, Scheid C. Oxalate-induced ceramide accumulation in Madin-Darby canine kidney and LLC-PK1 cells. *Kidney Int* 2000;57: 2403–2411.
258. Davis MA, Flaws JA, Young M, et al. Effect of ceramide on intracellular glutathione determines apoptotic or necrotic cell death of JB6 tumor cells. *Toxicol Sci* 2000;53:48–55.
259. Quillet-Mary A, Jaffrezou JP, Mansat V, et al. Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis. *J Biol Chem* 1997;272:21,388–21,395.
260. Hug H, Strand S, et al. Reactive oxygen intermediates are involved in the induction of CD95 ligand mRNA expression by cytostatic drugs in hepatoma cells. *J Biol Chem* 1997;272:28,191–28,193.
261. Ando K, Hagiwara T, Beppu M, Kikugawa K. Naturally ocurring anti-band 3 antibody binds to apoptotic human T-lymphoid cell line Jurkat through sialylated poly-*N*-acetyllactosaminyl saccharide chains on the cell surface. *Biochem Biophys Res Commun* 2000;275:412–417.
262. Sawyer DB, Fukazawa R, Arstall MA, Kelly RA. Daunorubicin-induced apoptosis in rat cardiac myocytes is inhibited by dexrazoxane. *Circ Res* 1999;84:257–265.
263. Quillet-Mary A, Mansat V, Duchayne E, et al. Daunorubicin-induced internucleosomal DNA fragmentation in acute myeloid cell lines. *Leukemia* 1996;10:417–425.
264. Friesen C, Fulda S, Debatin KM Induction of CD95 ligand and apoptosis by doxorubicin is modulated by the redox state in chemosensitive- and drug-resistant tumor cells. *Cell Death Differ* 1999;6:471–480.
265. Weltin D, Aupeix K, Iltis C, et al. *N*-acetylcysteine protects lymphocytes from nitrogen mustard-induced apoptosis. *Biochem Pharmacol* 1996;51:1123–1129.
266. Zisterer DM, Campiani G, Nacci V, Williams DC. Pyrrolo-1,5-benzoxazepines induce apoptosis in HL-60, Jurkat, and Hut-78 cells: a new class of apoptotic agents. *J Pharmacol Exp Ther* 2000;293:48–59.
267. Shen JC, Wang TT, Chang S, Hursting SD. Mechanistic studies of the effects of the retinoid *N*-(4-hydroxyphenyl)reti-

namide on prostate cancer cell growth and apoptosis. *Mol Carcinog* 1999;24:160–168.
268. Furuke K, Sasada T, Ueda-Taniguchi Y, et al. Role of intracellular redox status in apoptosis induction of human T-cell leukemia virus type I-infected lymphocytes by 13-*cis*-retinoic acid. *Cancer Res* 1997;57:4916–4923.
269. Delia D, Aiello A, Meroni L, et al. Role of antioxidants and intracellular free radicals in retinamide-induced cell death. *Carcinogenesis* 1997;18:943–948.
270. Eymin B, Dubrez L, Allouche M, Solary E. Increased *gadd153* messenger RNA level is associated with apoptosis in human leukemic cells treated with etoposide. *Cancer Res* 1997;57:686–695.
271. Fésüs L, Szondy Z, Uray I. Probing the molecular program of apoptosis by cancer chemopreventive agents. *J Cell Biochem* 1995;22 Suppl:151–161.
272. Palomba L., Sestili P, Cantoni O. The antioxidant butylated hydroxytoluene induces apoptosis in human U937 cells: the role of hydrogen peroxide and altered redox state. *Free Radic Res* 1999;31:93–101.
273. Macho A, Blazquez MV, Navas P, Munoz E. Induction of apoptosis by vanilloid compounds does not require *de novo* gene transcription and activator potein 1 activiy. *Cell Growth Differ* 1998;9:277–286.
274. Chiao C., Carothers AM, Grunberger D, et al. Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells. *Cancer Res* 1995;55:3576–3583.
275. Biswal SS, Datta K, Shaw SD, et al. Glutathione oxidation and mitochondrial depolarization as mechanisms of nordihydroguaiaretic acid-induced apoptosis in lipoxygenase-deficient FL5.12 cells. *Toxicol Sci* 2000; 53:77–83.
276. Shen H, Yang, C, Liu J, Ong C. Dual role of glutathione in selenite-induced oxidative stress and apoptosis in human hepatoma cells. *Free Radical Biol Med* 2000;28:1115–1124.
277. Wedi B, Straede J, Wieland B, Kapp A. Eosinophil apoptosis is mediated by stimulators of cellular oxidative metabolism and inhibited by antioxidants: involvement of a thiol-sensitive redox regulation in eosinophil cell death. *Blood* 1999;94:2365–2373.
278. Watson RW, Redmond HP, Wang JH, et al. Mechanisms involved in sodium arsenite-induced apoptosis of human neutrophils. *J Leukoc Biol* 1996;60:625–632.
279. Pang JH., Chau LY. Copper-induced apoptosis and immediate early gene expression in macrophages. *Atherosclerosis* 1999;146:45–52.
280. Desole MS, Sciola L, Delogu MR, et al. Role of oxidative stress in the manganese and 1-methyl-4-(2'-ethylphenyl)-1,2,3,6-tetrahydropyridine-induced apoptosis in PC12 cells. *Neurochem Int* 1997;31:169–176.
281. Thevenod F, Friedmann JM, Katsen AD, Hauser IA. Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-κB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. *J Biol Chem* 2000;275:1887–1896.
282. Hiura TS, Li N, Kaplan R, et al. The role of a mitochondrial pathway in the induction of apoptosis by chemicals extracted from diesel exhaust particles. *J Immunol* 2000;165:2703–2711.
283. Hiura TS, Kaszubowski MP, Li N, Nel AE. Chemicals in diesel exhaust particles generate reactive oxygen radicals and induce apoptosis in macrophages. *J Immunol* 1999;163:5582–5591.
284. Sandstrom PA, Mannie MD, Buttke TM. Inhibition of activation-induced death in T cell hydridomas by thiol antioxidants: oxidative stress as a mediator of apoptosis. *J Leukoc Biol* 1994;55:221–226.
285. Oda T, Iwaoka J, Komatsu N, Muramatsu T. Involvement of *N*-acetylcysteine-sensitive pathways in ricin-induced apoptotic cell death in U937 cells. *Biosci Biotechnol Biochem* 1999;63:341–348.
286. Abello PA, Fidler SA, Buchman TG. Thiol reducing agents modulate induced apoptosis in porcine endothelial cells. *Shock* 1994;2:79–83.
287 Hayon T, Dvilansky A, Oriev L, Nathan I. Non-steroidal antiestrogens induce apoptosis in HL-60 and MOLT3 leukemic cells: involvement of reactive oxygen radicals and protein kinase C. *Anticancer Res* 1999;19:2089–2093.
288. Fernandez A, Kiefer J, Fosdick L, McConkey DJ. Oxygen radical production and thiol depletion are required for Ca(2+)-mediated endogenous endonuclease activation in apoptotic thymocytes. *J Immunol* 1995;155:5133–5139
289. Jones DP, Maellaro E, Jiang S, et al. Effects of *N*-acetyl-L-cysteine on T-cell apoptosis are not mediated by increased cellular glutathione. *Immunol Lett* 1995;45:205–209.
290. Sandstrom PA, Roberts B, Folks TM, Buttke TM. HIV gene expression enhances T cell susceptibility to hydrogen peroxide-induced apoptosis. *AIDS Res Hum Retroviruses* 1993;9:1107–1113.
291. Malorni W, Rivabene R, Santini MT Donelli G. *N*-acetylcysteine inhibits apoptosis and decreases viral particles in HIV-chronically infected U937 cells. *FEBS Lett* 1993;327:75–78.
292. Mortola E, Okuda M, Ohno K, et al. Inhibition of apoptosis and virus replication in feline immunodeficiency virus-infected cells by *N*-acetylcysteine and ascorbic acid. *J Vet Med Sci* 1998;60:1187–1193.
293. Lin KI, Lee SH, Narayanan R, et al. Thiol agents and *Bcl-2* identify an alphavirus-induced apoptotic pathway that requires activation of the transcription factor NF-kB. *J Cell Biol* 1995;131:1149–1161.
294. Schweizer M, Peterhans E. Oxidative stress in cells infected with bovine viral diarrhoea virus: a critical step in the induction of apoptosis. *J Gen Virol* 1999;80:1147–1155.
295. Jimenez LA, Zanella C, Fung H, et al. Role of extracellular signal-regulated protein kinases in apoptosis by asbestos and $H_2O_2$. *Am J Physiol* 1997;273:L1029–L1035.
296. Shimura M, Osawa Y, Yuo A, et al. Oxidative stress as a necessary factor in room temperature-induced apoptosis in HL-60 cells. *J Leukoc Biol* 2000;68:87–96.
297. Liu M, Pelling JC, Ju J, et al. Antioxidant action via *p53*-mediated apoptosis. *Cancer Res* 1998;58:1723–1729.
298. Thompson CB Apoptosis in the pathogenesis and treatment of disease. *Science* 1995;267:1456–1462.
299. Kinscherf R, Deigner HP, Haberkorn,U. Apoptosis modulators in the therapy of neurodegenerative diseases. *Expert Opin Investig Drugs* 2000;9:747–764.
300. Ortiz LA, Moroz K, Liu J-Y, et al. Alveolar macrophage apoptosis and TNF-alpha, but not p53, expression correlate with murine response to bleomycin. *Am J Physiol* 1998;275:L1208–L1218.

301. Kuwano K, Miyazaki H, Hagimoto N, et al. The involvement of Fas-Fas ligand pathway in fibrosis lung diseases. *Am J Resp Cell Mol Biol* 1999;20: 53–60.
302. Guinee D Jr, Brambilla E, Fleming M, et al. The potential role of BAX and BCL-2 expression in diffuse alveolar damage. *Am J Pathol* 1997;151:999–1007.
303. Kaneto H, Kajimoto Y, Miyagawa J, et al. Beneficial effects of antioxidants in diabetes: possible potection of pancreatic beta-cells against glucose toxicity. *Diabetes* 1999;48:2398–2406.
304. Pollman MJ, Hall JL, Gibbons GH. Determinants of vascular smooth muscle cell apoptosis after balloon angioplasty injury. Influence of redox state and cell phenotype. *Circ Res* 1999;84:113–121.
305. Castagne V, Clarke PG. Axotomy-induced retinal ganglion cell death in development: its time-course and its diminution by antioxidants. *Proc R Soc Lond B Biol Sci* 1996;263:1193–1197.
306. Blackwell TS, Blackwell TR, Holden EP, et al. In vivo antioxidant treatment suppresses nuclear factor-κB activation and neutrophilic lung inflammation. *J Immunol* 1996;157:1630–1637.
307. Cuzzocrea S, Mazzon E, Dugo L, et al. Protective effects of *N*-acetylcysteine on lung injury and red blood cell modification induced by carrageenan in the rat. *FASEB J* 2001;15:1187–1200.
308. Heller AR, Groth G, Heller SC, et al. *N*-acetylcysteine reduces respiratory burst but augments neutrophil phagocytosis in intensive care unit patients. *Crit Care Med* 2001;29:272–276.
309. Sakurada S, Kato T, Okamoto T. Induction of cytokines and ICAM-1 by proinflammatory cytokines in primary rheumatoid synovial fibroblasts and inhibition by *N*-acetyl-L-cysteine and aspirin. *Int Immunol* 1996; 8:1483–1493.
310. Matsumoto K, Hashimoto S, Gon Y, et al. *N*-acetylcysteine inhibits IL-1 alpha-induced IL-8 secretion by bronchial epithelial cells. *Respir Med* 1998;92:512–515.
311. Sato M, Miyazaki T, Nagaya T, et al. Antioxidants inhibit tumor necrosis factor-alpha mediated stimulation of interleukin-8, monocyte chemoattractant protein-1, and collagenase expression in cultured human synovial cells. *J Rheumatol* 1996;23:432–438.
312. Kawasaki S, Takizawa H, Takami K, et al. Benzene-extracted components are important for the major activity of diesel exhaust particles: effect of interleukin-8 gene expression in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2001;24:419–426.
313. Weiss L, Hildt E, Hofschneider PH. Anti-hepatitis B virus activity of *N*-acetyl-L-cysteine (NAC): new aspects of a well-established drug. *Antiviral Res* 1996;32:43–53.
314. Waris G, Huh KW, Siddiqui A. Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-κB via oxidative stress. *Mol Cell Biol* 2001;21:7721–7730.
315. Meyer M, Caselmann WH, Schluter V, et al. Hepatitis B virus transactivator MHBst: activation of NF-κB, selective inhibition by antioxidants and integral membrane localization. *EMBO J* 1992;11:2991–3001.
316. Gong G, Waris G, Tanveer R, Siddiqui A. Human hepatitis C virus NS5A protein alters intracellular calcium levels, induces oxidative stress, and activates STAT-3 and NF-κB. *Proc Natl Acad Sci USA* 2001;98:9599–9604.
317. Grant PR, Black A, Garcia N, et al. Combination therapy with interferon-alpha plus *N*-acetylcysteine for chronic hepatitis C: a placebo controlled double-blind multicentre study. *J Med Virol* 2000;61:439–442.
318. De Rosa SC, Zaretsky MD, Dubs JG, et al. *N*-acetylcysteine replenishes glutathione in HIV infection. *Eur J Clin Invest* 2000;30:915–929.
319. Harakeh S, Jariwalla R.J. Comparative study of the anti-HIV activities of ascorbate and thiol-containing reducing agents in chronically HIV-infected cells. *Am J Clin Nutr* 1991;54 Suppl 6:1231S–1235S.
320. Ho WZ, Douglas SD. Glutathione and *N*-acetylcysteine suppression of human immunodeficiency virus replication in human monocyte/macrophages in vitro. *AIDS Res Hum Retroviruses* 1992;8:1249–1253.
321. Lioy J, Ho WZ, Cutilli JR, et al. Thiol suppression of human immunodeficiency virus type 1 replication in primary cord blood monocyte-derived macrophages in vitro. *J Clin Investig* 1993;91:495–498.
322. Kalebic T, Kinter A, Poli G, et al. Suppression of human immunodeficiency virus expression in chronically infected monocytic cells by glutathione, glutathione ester, and *N*-acetylcysteine. *Proc Natl Acad Sci USA* 1991; 88:986–990.
323. Roederer M, Staal FJ, Raju PA, et al. Cytokine-stimulated human immunodeficiency virus replication is inhibited by *N*-acetyl-L-cysteine. *Proc Natl Acad Sci USA* 1990;87:4884–4888.
324. Albini A, Morini MD, D'Agostini F, et al. Inhibition of angiogenesis-driven Kaposi's sarcoma tumor growth in nude mice by oral *N*-acetylcysteine. *Cancer Res* 2001;61:8171–8178.
325. Nishinaka Y, Nakamura H, Okada N, et al. Redox control of EBV infection: prevention by thiol-dependent modulation of functional CD21/EBV receptor expression. *Antiox Redox Signal* 2001;3:1075–1087.
326. Huang TS, Duyster J, Wang JY. Biological response to phorbol ester determined by alternative G1 pathways. *Proc Natl Acad Sci USA* 1995;92:4793–4797.
327. Kurata S. Selective activation of p38 MAPK cascade and mitotic arrest caused by low level oxidative stress. *J Biol Chem* 2000;275:23,413–23,416.
328. Brar SS, Kennedy TP, Whorton AR, et al. Requirement for reactive oxygen species in serum-derived growth factor-induced growth of airway smooth muscle. *J Biol Chem* 1999;274:20,017–20,026.
329. Grdina DJ, Murley JS, Roberts JC. Effects of thiols on topoisomerase-II α activity and cell cycle progression. *Cell Prolif* 1998;31:217–229.
330. Kusano C, Takao S, Noma H, et al. *N*-acetylcysteine inhibits cell cycle progression in pancreatic carcinoma cells. *Hum Cell* 2000;13:213–220.
331. Redondo P, Bauza A. Topical *N*-acetylcysteine for lamellar ichthyosis [Letter]. *Lancet* 1999;354:1880.
332. Kitamura M. The antioxidant *N*-acetylcysteine induces mesangial cells to create three-dimensional cytoarchitecture that underlies cellular differentiation. *J Am Soc Nephrol* 1999;10:746–751.
333. Breithaupt TB, Vazquez A, Baez I, Eylar EH. The suppression of T cell function and NF-κB expression by serine protease inhibitors is blocked by *N*-acetylcysteine. *Cell Immunol* 1996;173:124–130.

334. Ferrandez MD, Correa R, Del Rio M, De la Fuente M. Effects in vitro of several antioxidants on the natural killer function of aging mice. *Exp Gerontol* 1999;34:675–685.
335. Goldman Y, Peled A, Shinitzky M. Effective elimination of lung metastases induced by tumor cells treated with hydrostatic pressure and *N*-acetyl-L-cysteine. *Cancer Res* 2000;60:350–358.
336. Van den Broeke LT, Beijersbergen Van Henegouwen GMJ. Topically applied N-acetylcysteine as a protector against UVB-induced systemic immune suppression. *J Photochem Photobiol B*: 1995;27:61–65.
337. Breitkreutz R, Pittack N, Nebe CT, et al. Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. *J Mol Med* 2000;78:55–62.
338. Tosetti F, Ferrari N, De Flora S, Albini A. "Angioprevention:" Angiogenesis is a common and key target for cancer chemopreventive agents. *FASEB J* 2002;16:2–14.
339. Redondo P, Bandres E, Solano T, et al. Vascular endothelial growth factor (VEGF) and melanoma. *N*-acetylcysteine downregulates VEGF production in vitro. *Cytokine* 2000;12:374–378.
340. Chua CC, Hamdy RC, Chua BH. Upregulation of vascular endothelial growth factor by $H_2O_2$ in rat heart endothelial cells. *Free Radic Biol Med* 1998;15:891–897.
341. Tsuji F, Matsuoka H, Aono H, et al. Effects of sulfhydryl compounds on interleukin-1-induced vascular endothelial growth factor production in human synovial stromal cells. *Biol Pharm Bull* 2000;23:663–665.
342. Duyndam MC, Hulscher TM, Fontijin D, et al. Induction of vascular endothelial growth factor expression and hypoxia-inducible factor 1 alpha protein by the oxidative stressor arsenite. *J Biol Chem* 2001;276:48,066–48,076.
343. Redondo P, Jimenez E, Perez A, Garcia-Foncillas J. *N*-acetylcysteine downregulates vascular endothelial growth factor production by human keratinocytes in vitro. *Arch Dermatol Res* 2000; 292:621–628.
344. Marumo T, Schini-Kert VB, Busse R. Vascular endothelial growth factor activates nuclear factor-κB and induces monocyte chemoattractant protein-1 in bovine retinal endothelial cells. *Diabetes* 1999;48:1131–1137.
345. Cai T, Fassina GF, Giunciuglio D, et al. *N*-Acetylcysteine inhibits endothelial cell invasion and angiogenesis while protecting from apoptosis. *Lab Investig* 1999;79:1151–1159.
346. Morini M, Cai T, Aluigi MG, et al. The role of the thiol *N*-acetylcysteine in the prevention of tumor invasion and angiogenesis. *Int J Biol Markers* 1999;14:268–271.
347. Masiero L, Mazzieri R; Kalebic T, et al. Modulation of expression and activity of MMP-2 by reducing agents and uPA plasmin (Abstract). *Eur J Histochem* 1993; 37 Suppl:60–61.
348. Albini A, D'Agostini F, Giunciuglio D, et al. Inhibition of invasion, gelatinase activity, tumor take and metastasis of malignant cells by *N*-acetylcysteine. *Int J Cancer* 1995;61:121–129.
349. Kawakami S, Kageyama Y, Fujii Y, et al. Inhibitory effect of *N*-acetylcysteine on invasion and MMP-9 production of T24 human bladder cancer cells. *Anticancer Res* 2001;21:213–219.
350. Stetler-Stevenson GW, Talano J, Gallagher M, et al. Inhibition of human type IV collagenase by a highly conserved peptide sequence derived from its prosegment. *Am J Med Sci* 1991;302:163–170.
351. Nonaka Y, Iwagaki H, Kimura T, et al. Effect of reactive oxygen intermediates on the *in vitro* invasive capacity of tumor cells and liver metastasis in mice. *Int J Cancer* 1993;54:983–986.
352. Buhimschi IA, Kramer WB, Buhimschi CS, et al. Reduction-oxidation (redox) state regulation of matrix metalloproteinase activity in human fetal membranes. *Am J Obstet Gynecol* 2000;182:458–464.
353. Lois M, Brown LA, Moss IM, et al. Ethanol ingestion increases activation of matrix metalloproteinases in rat lungs during acute endotoxemia. *Am J Respir Crit Care Med* 1999;160:1354–1360.
354. Hozumi A, Nishimura Y, Nishiuma T, et al. Induction of MMP-9 in normal human bronchial epithelial cells by TNF-alpha via NF-κB-mediated pathway. *Am J Physiol Lung Cell Mol Physiol* 2001;281:L1444–L1452.
355. Galis ZS, Asanuma K, Godin D, Meng X. *N*-acetylcysteine decreases the matrix-degrading capacity of macrophage-derived foam cells; new target for antioxidant theraphy? *Circulation* 1998;97:2445–2453.
356. Yoshida M, Korfhagen TR, Whitsett JA. Surfactant protein D regulates NF-κB and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. J Immunol 2001;166:7514–7519.
357. De Flora S, D'Agostini F, Masiello L, et al. Synergism between *N*-acetylcysteine and doxorubicin in the prevention of tumorigenicity and metastasis in murine models. *Int J Cancer* 1996;62:842–848.
358. Doroshow JH, Locker JY, Ifrim I, Myers CE. Prevention of doxorubicin cardiac toxicity in the mouse by *N*-acetylcysteine. *J Clin Investig* 1981;68:1053–1064.
359. Delneste Y, Jeannin P, Potier L, et al. *N*-acetyl-L-cysteine exhibits antitumoral activity by increasing tumor necrosis factor alpha-dependent T-cell cytotoxicity. *Blood* 1997;90:1124–1132.
360. Hamada J-i, Nakata D, Nakae D, et al. Increased oxidative DNA damage in mammary tumor cells by continuous epidermal growth factor stimulation. *J Natl Cancer Inst* 2001;93:214–219.
361. Ziemann C, Bürkle A, Kahl GF, Hirsch-Ernst KI. Reactive oxygen species participate in mdr1b mRNA and P-glycoprotein overexpression in primary rat hepatocytes cultures. *Carcinogenesis* 1999;20:407–414.
362. Deng L, Lin-Lee YC, Claret FX, Kuo MT. 2-Acetylaminofluorene up-regulates rat mdr1b expression through generating reactive oxygen species that activate NF-κB pathway. *J Biol Chem* 2001;276:413–420.
363. Honda S, Matsuo M. Relationships between the cellular glutathione level and *in vitro* life span of human diploid fibroblasts. *Exp Gerontol* 1988;23:81–86.
364. Brack C, Bechter-Thuring E, Labuhn M. *N*-acetylcysteine slows down ageing and increases the life span of *Drosophila melanogaster*. *Cell Mol Life Sci* 1997;53:960–966.
365. Richie JPJ, Lang CA. A decrease in cysteine levels causes the glutathione deficiency of aging in the mosquito. *Proc Soc Exp Biol Med* 1988;187:235–240.
366. Hack V, Breitkreutz R, Kinscherf R, et al. The redox state as a correlate of senescence and wasting and as a target for therapeutic intervention. *Blood* 1998;92:59–67.