NCI, DCPC
Chemoprevention Branch and Agent Development Committee

CLINICAL DEVELOPMENT PLAN:

N-ACETYL-*l*-CYSTEINE (NAC)

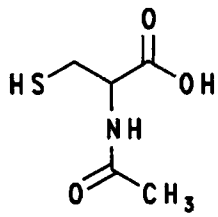
DRUG IDENTIFICATION

CAS Registry No.: 616-91-1

CAS Name (9CI): *N*-Acetyl-*l*-cysteine

Synonyms: *l*-α-Acetamido-β-mercaptopropionic Acid
N-Acetyl-3-mercaptoalanine
Fluimucil®
Mercapturic Acid
Mucomyst®
Parvolex®

Structure:



EXECUTIVE SUMMARY

N-Acetyl-*l*-cysteine (NAC) is a thiol-containing nucleophile which can detoxify electrophiles and free radicals by direct [1–4] and enzymatic conjugation [5]. More importantly, the agent also serves as a precursor of intracellular cysteine and glutathione (GSH) [3,6], and enhances the activities of glutathione-*S*-transferase (GST) [*e.g.*, 7,8], glutathione peroxidase [9,10], GSSG reductase, and NADH: and NAD(P)H:quinone reductase [11]. NAC has antiinflammatory properties [12], and inhibits prostaglandin synthesis [13] and ODC activity [7,9]. Finally, the agent may promote DNA repair by protecting ADP-ribosyl transferase activity [14]. Currently, NAC is an FDA-approved drug primarily used as an aerosolized mucolytic agent (Mucomyst®) in patients with bronchitis [15,16]. Parvolex®, the intravenous form of NAC, was introduced in 1979 as an antidote for liver toxicity resulting from overdose of acetaminophen (Tylenol®) [17]. In addition, administration of NAC has offered protection in chemotherapy [*e.g.*, 18] and radiotherapy [19] of cancer patients. Because of its antioxidant and detoxifying properties, as well as apparent safety and lack of major side effects [20,21], the development of NAC as a cancer chemopreventive drug was undertaken.

In preclinical efficacy studies, the chemopreventive activity of NAC was demonstrated in rat colon, intestines and mammary glands, hamster trachea, and mouse lung and bladder. The available studies are adequate to support the development of NAC as a chemopreventive drug. Addi-

tional studies in rat, mouse and hamster lung and mouse skin models of carcinogenesis are in progress. A study examining the modulation of intermediate biomarkers of carcinogenesis (K-*ras* in hamster pancreas) by NAC is also in progress.

Limited preclinical toxicology testing of NAC has been funded by the CB. The in-life phase of 90-day oral toxicity studies of the agent alone has been completed as part of studies on the combination of NAC with DFMO in rats and dogs; however, only one dose level was used. Acute, subacute, subchronic, and chronic oral toxicity studies in rats and dogs published in the literature indicate that NAC is not significantly toxic. Carcinogenicity studies will be required by the FDA before clinical trials with treatment periods in excess of one year are undertaken. Both carcinogenicity and necessary reproductive and teratogenicity studies of the oral formulation will be contracted, as required, after Phase II trials are completed.

A CB-funded Phase I trial of NAC has been completed. A well-tolerated daily dose of 800 mg/m^2 (*ca.* 0.12 mmol/kg-bw) was determined for 6 months of treatment. Minimal side effects were seen at daily doses of 1,600 mg/m^2 for 4 weeks, and the maximum tolerated dose was 6,400 mg/m^2 qd (*ca.* 1.0 mmol/kg-bw) in 6/10 subjects. Adverse effects (*e.g.*, heartburn, diarrhea, gas, cramps, bad taste) and pharmacokinetics (good absorption, and rapid metabolism and elimination) data are consistent with published reports. No other Phase I trials are anticipated.

A Phase III clinical trial (EUROSCAN) of oral NAC (600 mg qd) with or without retinyl palmitate (vitamin A) sponsored by the EORTC (European Organization for Research and Treatment of Cancer) is in progress. The study, carried out in patients curatively treated for laryngeal, oral cavity, and non-small cell lung carcinoma, examines the effect of NAC on survival, rate of recurrence of primary cancers or metastasis, and occurrence rate of second primaries in these individuals.

The NCI, DCPC Repository purchased NAC from Zambon Group S.P.A. (Vincenza, Italy) and formulated it into hard gelatin capsules or packets of dissolvable granules for the Phase I study. In future studies, only the capsule formulation will be used, which will also require manufacture of placebos. No difficulties are anticipated in procuring adequate supplies for future studies.

The primary target organs for clinical development of NAC are the bladder and colon. Based on existing preclinical (mouse) data, the dose necessary for inhibition of premalignant lung lesions (0.63 mmol/kg-bw/day) may be higher than the well-tolerated, chronic daily dose in humans (800 mg/m^2, or *ca.* 0.12 mmol/kg-bw). The results of the ongoing EUROSCAN trial may provide some relevant human data at low doses (600 mg qd, or *ca.* 0.05 mmol/kg-bw). For 1995, the CB is considering a Phase II trial of a higher NAC dose (1.4 g/m^2 qd, or *ca.* 0.2 mmol/kg-bw) to investigate modulation of squamous metaplasia/dysplasia and other intermediate biomarkers (ploidy, p53, PCNA, EGFR) in chronic smokers with or without prior smoking-related cancers. In contrast, attainment of an effective dose without adverse effects may be possible in the bladder. Thus, a second Phase II trial planned for 1995 involves prevention of bladder cancer in patients previously treated for superficial tumors with BCG. Future trials on prevention of colon cancer may also be considered for the same reason.

PRECLINICAL EFFICACY STUDIES

In studies sponsored by the CB, NAC has demonstrated chemopreventive efficacy in MNU-induced hamster trachea (6.4 g/kg diet, or *ca.* 4.7 mmol/kg-bw/day) [22], AOM-induced rat colon (600 mg/kg diet, or *ca.* 0.2 mmol/kg-bw/day), MNU-induced rat mammary gland (8 g/kg diet, or *ca.* 2.5 mmol/kg-bw/day) [22], and OH-BBN-induced mouse bladder (200 mg/kg diet, or *ca.* 0.2 mmol/kg-bw/day) carcinogenesis. In published studies, NAC was reported efficacious in the DMH-exposed rat colon and small intestine cancer model [23]. The results of *in vivo* studies are adequate to support the clinical development of NAC. The CB is funding additional animal efficacy studies in the NNK-induced rat and mouse lung, as well as the DMBA-induced rat mammary gland models. Studies of NAC in B(*a*)P-induced mouse skin and lung are also in progress because the agent inhibited B(*a*)P-induced morphological transformation of rat tracheal epithelial cells, an *in vitro* screening assay. The chemopreventive activity of NAC is also being examined in combinations with other agents, such as β-carotene (injectable), DFMO, fumaric acid, and oltipraz, in the MNU-induced hamster lung/trachea carcinogenesis model, and in combination with DFMO in the B(*a*)P-induced mouse skin model.

A significant effort in the CB program is to identify and validate intermediate biomarkers of cancer, and evaluate the potential for chemopreventive agents to modulate these markers [24]. The

chemopreventive activity of NAC is, therefore, being evaluated in *in vivo* intermediate biomarker assays including modulation of K-*ras* mutations in hamster pancreas. *In vitro*, NAC (1 μM) inhibited the formation of DMBA-induced hyperplastic alveolar nodules (HAN) in mouse mammary organ culture. In published studies, the agent delayed the development of 2-AAF-induced γ-glutamyl transpeptidase (GGT)-positive foci in rat liver [25,26], DMBA-induced/TPA-promoted papillomas in mouse skin [27], urethane-induced mouse lung adenomas [3,8], and AOM-induced foci of aberrant crypts in rat colon [28]. In related mechanistic studies, DNA adducts formed from 2-AAF in liver, from cigarette smoke in lung, and from B(*a*)P in various tissues were reduced by dietary administration of NAC to rats [29].

PRECLINICAL SAFETY STUDIES

Safety NCI has not funded toxicity studies on NAC because results are available from published reports. NAC was evaluated in a 60-day subacute study of male rats at oral doses of 100, 200, 400, and 800 mg/kg-bw/day (0.6, 1.2, 2.4, and 4.9 mmol/kg-bw/day) for 5 days per week [21]. After 2 weeks of dosing, 1,600 mg/kg-bw/day (9.8 mmol/kg-bw/day) was substituted for the lowest dose. No significant gross or microscopic pathologic changes were noted in the tissues examined (organs not specified). In other 60-day and 90-day studies, beagle dogs were administered NAC orally up to 320 mg/kg-bw/day (2.0 mmol/kg-bw/day), or intravenously injected with 10% NAC solution twice a day up to 400 mg/kg-bw/day (2.4 mmol/kg-bw/day) with no signs of significant toxicity [21].

Two-year carcinogenicity studies with NAC in laboratory animals have not yet been performed. However, published long-term oral studies of NAC in rats (12 months with 6 months of follow-up) at doses up to 1,000 mg/kg-bw/day (*ca.* 6.1 mmol/kgbw/day or 5.2 times the human dose) have provided no evidence of oncogenic activity [30,31]. Also, administration of NAC at doses up to 1,000 mg/kg-bw/day (6.1 mmol/kg-bw/day) for 18 consecutive months produced no significant pathological changes [21]. Finally, no carcinogenic effects were observed in dogs following treatment with NAC at doses up to 300 mg/kg-bw/day (1.8 mmol/kg-bw/day) for 12 months [30]. It should be noted that NAC was not mutagenic in a published Ames *Salmonella typhimurium* study with or without metabolic activation [30] and instead inhibits the activity of direct-acting and promutagens [*e.g.*, 2].

Reproductive and developmental toxicity studies have not been sponsored by the CB. A teratology study using a single dose level of NAC equivalent to 2.6 times the human dose was reported by Zambon Group S.P.A. [21,30]. Oral doses of 500 mg/kg-bw/day (3.1 mmol/kg-bw/day) were administered to pregnant Dutch Belted rabbits by intubation on days 6 through 16 of gestation. The agent was non-teratogenic under these conditions.

No reproductive toxicity studies with an oral formulation of NAC have been reported in the literature. Reproductive, developmental, and carcinogenicity studies of the oral formulation will be contracted by the CB as necessary after the proposed Phase II studies are completed.

The CB has concentrated on toxicity studies of NAC in combination with DFMO, and preliminary results are available from a 90-day oral toxicity study in rats and dogs. In rats, NAC administered ig at 2,000 mg/kg-bw/day (12.3 mmol/kg-bw/day) with or without DFMO (1,000 mg DFMO/kg-bw/day or 5.5 mmol/kg-bw/day) caused rough coat, and reduced body weight gain and food consumption, particularly in males. Treatment-related anemia and hepatotoxicity (portal triad and bile duct hyperplasia, hepatic necrosis, periportal fibrosis) were also observed. Since hepatic and forestomach lesions were also observed in the low dose combination group (1,000 mg NAC/kg-bw/day + 250 mg DFMO/kg-bw/day), a NOEL was not identified.

In male and female dogs, oral (capsule) administration of 640 mg NAC/kg-bw/day (3.9 mmol/kg-bw/day) in combination with 75 mg DFMO/kg-bw/day produced mild hepatic vacuolization in males and females and splenic hemosiderosis in males. Since the hepatoxicity was also observed at the low dose combination (320 mg NAC/kg-bw/day + 25 mg DFMO/kg-bw/day) in males, a NOEL was identified only for females at this dose.

ADME In published literature, NAC was readily and completely absorbed following oral administration to rats and dogs [30]. It was shown to be stable in simulated gastric and intestinal fluids. Maximum plasma (t_{max}) radioactivity was obtained approximately 1–2 hours after a 100 mg/kg-bw dose of ^{35}S-labeled NAC to both species. The drug distributed mainly to the kidneys, liver and lungs. In plasma and tissues, NAC and its metabolites were present in both free and protein-bound forms. In rats, 36% of an oral 100 mg/kg-bw dose of NAC was excreted within 96 hours.

Following a dose of 100 mg/kg-bw in rats, radiolabeled, protein-bound NAC disulfide was eliminated rapidly in the first four hours, and more slowly in the following 44 hours [30]. In contrast, this form of NAC decreased only from approximately 50 to 20 nmol/g tissue in the lung between 4 and 48 hours post-administration, respectively. The demonstrated presence of the drug in this tissue is why chemopreventive properties should be evaluated in several models of animal lung and tracheal carcinogenesis. It is unknown if the GSH pool or related enzymes are affected in this tissue.

CLINICAL SAFETY: PHASE I STUDIES

A CB-sponsored Phase I study (Dr. P. Creaven, Roswell Park Cancer Institute) was carried out in 13 patients at high risk for cancer, *e.g.*, heavy smoker, prior smoking-related malignancy, familial adenomatous polyposis (FAP) patient or relative, or presence of a premalignant skin lesion (actinic keratosis). The first step in the trial was a dose-titration study to select a nontoxic oral dose for 6-month chronic administration. In Step 1, doses from 200–3,200 mg/m^2 bid (*ca.* 0.06–1.0 mmol/kg-bw daily) were administered to selected subjects for 4 weeks. The maximum tolerated daily dose was 3,200 mg/m^2 bid (*ca.* 1.0 mmol/kg-bw daily) for 6/10 subjects. Adverse effects (*e.g.*, heart-burn, diarrhea, gas, cramps, bad taste) were consistent with published reports. All 13 patients tolerated a dose of 400 mg/m^2 bid (*ca.* 0.12 mmol/kg-bw daily), and minimal side effects were seen at 800 mg/m^2 bid (*ca.* 0.24 mmol/kg-bw/day). When administered for 6 months, a dose of 800 mg/m^2 qd was extremely well-tolerated. No additional Phase I studies are anticipated.

Drug Effect Measurement Total and free GSH and cysteine levels in plasma, GSH in erythrocytes and white blood cells, and GST-μ, GSSG reductase and glutathione peroxidase activity in white blood cells were investigated as drug effect measurements in the Phase I study. Although formation of cysteine is the rate-limiting step in GSH synthesis, results suggest that cysteine and GSH measurements appear to be variable and lack sensitivity to change. Also, GSH peroxidase and GST activities were unrelated to NAC dose. GSSG reductase activity, as well as that of NADH: and NAD(P)H: quinone reductase, glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD), have been observed to increase in rat liver preparations *in vitro* in response to NAC exposure. However, Phase I data suggest that only GSSG reductase activity measured in peripheral lymphocytes increased with NAC dose.

Inhibition of unscheduled DNA synthesis (UDS) was also evaluated as a drug effect measurement due to published evidence of inhibition of direct-acting mutagens, as well as protection of DNA repair enzymes, by NAC. However, [^{3}H]-thymidine incorporation induced by UV-irradiation or *cis*-platinum in lymphocytes from Phase I subjects was not related to agent dose.

Safety In the Phase I study, the dose-escalation step evaluated two divided doses for 4 weeks up to a maximum daily dose of 6,400 mg/m^2 in 13 subjects. Adverse effects observed at the highest dose included gastrointestinal complaints (heartburn, abdominal pain, gas with foul odor, indigestion), fatigue, bad taste and aftertaste. A daily 1,600 mg/m^2 (*ca.* 0.24 mmol/kg-bw) dose was tolerated with minimal side effects; thus, 800 mg/m^2 qd was selected for the subsequent 6-month chronic study.

In the 6-month study, only one case of drug-related withdrawal was reported (among 13 subjects); it appeared to be an idiosyncratic reaction manifested by elevated creatinine and feelings of "unusual heaviness and fatigue."

A safety study was conducted in normal volunteers by Mead Johnson Research Center [32]. In this study, oral administration of NAC was reportedly well-tolerated at daily doses up to 11.2 g for 9 months; a few cases of vomiting (1/12) and diarrhea (4/12) were noted in individuals at 5.6 g only. Other side effects reported in the literature are headache and skin rash, including urticaria, in 1–5% of patients treated with NAC for other indications [20].

ADME After oral intake, the maximum plasma concentration is reached 1–2 hours after ingestion. NAC rapidly binds to plasma proteins, making it difficult to establish pharmacokinetics and bioavailability. The parent drug undergoes extensive metabolism to cysteine and GSH, which are also present in plasma in both free and bound forms. Pharmacokinetic data (good absorption and rapid metabolism and elimination) from the CB-funded Phase I trial are consistent with published reports.

Single-dose plasma pharmacokinetics of NAC were studied after the first day in 9/13 patients entered into the 6-month treatment step of the Phase I study (800 mg/m^2 daily). NAC was absorbed rapidly in these subjects with a plasma t_{max}=1 hour. Significant interindividual variation was observed in the remaining pharmacokinetic values: C_{max}=1.7–14.2 μg/ml, $AUC_{(0-24\ hours)}$=9.2–

57.2 μg/ml·hr, and plasma $t_{½}$=1.1–4.6 hr.

Results from Step 2 of the Phase I study suggest that the plasma pharmacokinetics of NAC (800 mg/m^2 qd for 6 months) alter with chronic administration. Decreased absorption of the drug is indicated, since the C_{max}, $t_{½}$ and AUC values decreased.

A number of pharmacokinetics studies have been reported in the literature [33–35]. Upon oral administration, NAC is well absorbed, rapidly metabolized to *l*-cysteine and GSH, and excreted in the urine. Four different oral 600 mg formulations of NAC have been compared (dissolvable granules, effervescent tablets, slow-release tablets, dissolving tablets); the slow-release tablets produced a much flatter dose-plasma concentration curve (C_{max}= 4.7 μM and t_{max}=1.9 hr) than the other three formulations (C_{max}=15.0–16.9 μM and t_{max}=0.65–0.75 hr) [36]. In a bioavailability study, a 400 mg dose as suckable tablets or granules was found to be comparable in healthy volunteers [15]. In a study using the gelatin capsule formulation, a single oral dose of 250 mg NAC/m^2 resulted in C_{max}= 1.75 μg/ml, t_{max}=0.72 hr, AUC=3.74 μg/ml·hr, and $t_{1/2}$=2.1 hr [35]. After multiple oral dosing of NAC at 600 mg qd for 10 days, pharmacokinetics parameters were altered (C_{max}=0.33 μg/ml, t_{max}=1.5 hr), indicating reduced absorption [34].

CLINICAL EFFICACY: PHASE II/III STUDIES

A Phase III study sponsored by the EORTC was initiated in June, 1988 [37,38]. This study, EUROSCAN, was designed to evaluate the effect of retinyl palmitate (vitamin A), NAC, or a combination of both agents in preventing or delaying the recurrence of primary cancers (local/regional or distant metastases) or the occurrence of second primary cancers in patients curatively treated for laryngeal, oral cavity, and non-small cell lung carcinoma. NAC is being administered orally at 600 mg qd for 2 years, and will involve 2,000 patients. As of January 1, 1993, 372 patients had received the agent, and 1,821 patients had been accrued [39]. From the limited data available, this dose has been found to be well-tolerated and devoid of any major side effects.

Dr. L. Wattenberg (University of Minnesota) has initiated an independent Phase II trial to evaluate modulation of intermediate biomarkers in the colon. In this double-blinded, placebo-controlled study, NAC is being administered at a dose of 400 mg bid for 60 days. Drug supply is being formulated by the NCI, DCPC Repository.

One Phase II trial of NAC in the lung has been proposed for 1995 by the CB. Modulation of squamous metaplasia/dysplasia and other intermediate biomarkers (ploidy, p53, PCNA, EGFR) will be investigated in chronic smokers with or without prior smoking-related cancers. Preclinical efficacy studies have demonstrated inhibition of cancers in this organ in animal models. In addition, NAC binds to rat and dog lung tissue. A second Phase II trial in bladder is also being considered for 1995. Any additional Phase II trials proposed will be evaluated critically for relevance, priority, and need.

PHARMACODYNAMICS

Based on efficacy and toxicity data in the rat, attainment of a cancer inhibitory dose in the human bladder should be possible without toxicity. The lowest effective dose of *ca.* 0.16 mmol/kg-bw/day in the OH-BBN-induced mouse bladder carcinogenesis model is similar to the well-tolerated human dose of 800 mg/m^2 qd (*ca.* 0.12 mmol/kg-bw qd) for 6 months. The effective mouse dose was 4-fold lower than the 6-week MTD in the same species (625 mg/kg diet/day, or *ca.* 0.5 mmol/kg-bw/day). In the rat colon model, a similar effective dose (0.18 mmol/kg-bw/day) was over 30-fold lower than the 6-month NOEL (1 g/kg-bw/day, or 6.1 mmol/kg-bw/day) in the same species; this suggests that non-toxic doses of NAC may be attainable in clinical trials evaluating prevention of colon and bladder cancer. In the lung, however, the dose range between efficacy and toxicity may overlap. In a published study in the mouse, a dose of 120 mg/kg-bw/day (0.74 mmol/kg-bw/day) inhibited lung adenoma incidence and multiplicity [3,8]; however, this dose is approximately 6-fold higher than the MTD in the same species. The fact that the disulfide remains bound to lung tissue in rats suggests that lower doses administered over a longer time period may attain effective tissue levels in clinical trials evaluating prevention of lung cancer. The results of the EUROSCAN trial may provide relevant human data.

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement Issues

Large individual variations in blood levels of NAC were observed in the Phase I study. NAC increases both GSH levels and enzyme activities

involved in GSH production and conjugation. Increases in these parameters in urine, peripheral blood, or tissue may serve as appropriate drug effect measurements for clinical trials, and several have been investigated in the current Phase I clinical trial of NAC—free and bound GSH in plasma, and GSH levels and GST (μ isozyme) and GSSG reductase activities in white blood cells. Preliminary data indicate GSH and GST measurements appeared to start to return to baseline with 6-month administration, and thus showed tolerance. GSH levels were highly variable. In contrast, GSSG reductase activity in peripheral lymphocytes seems to increase with NAC dose.

Safety Issues

There are no specific toxicity issues for NAC other than determining preclinical testing needed to complete FDA requirements (see **Regulatory Issues**).

Pharmacodynamics Issues

The primary target organs for clinical development of NAC are the bladder and colon. Based on efficacy and toxicity data in the mouse and rat, attainment of an effective dose in these human tissues should be possible without toxicity. The lowest inhibitory dose (*ca.* 0.16 mmol/kg-bw/day) in the OH-BBN-induced mouse bladder carcinogenesis model is similar to the well-tolerated, chronic human dose of 800 mg/m^2 daily for 6 months (*ca.* 0.12 mmol/kg-bw). The effective mouse dose was 4-fold lower than the 6-week MTD in the same species (625 mg/kg diet/day, or *ca.* 0.5 mmol/kg-bw/day). In the rat colon model, a similar effective NAC dose (0.18 mmol/kg-bw/day) was over 30-fold lower than the 6-month NOEL (1 g/kg-bw/day, or 6.1 mmol/kg-bw/day) in the same species.

It is unknown if the doses necessary for inhibition of lung carcinogenesis in humans are attainable without toxicity. In the mouse, a dose of 0.74 mmol/kg-bw/day inhibited lung adenoma incidence and multiplicity, but this dose is approximately 6-fold higher than the MTD in the same species. It is possible, however, that lower doses over a long period may be effective because of the demonstrated presence of NAC in lung tissue up to 48 hours after oral administration to rats. The results of the EUROSCAN trial may provide relevant human data at lower NAC doses (*ca.* 0.05 mmol/kg-bw). In the interim (1995), the CB has proposed a Phase II trial of a higher NAC dose (1,400 mg/m^2 qd, or *ca.* 0.2 mmol/kg-bw) to investigate modulation of squamous metaplasia/dysplasia and other intermediate biomarkers (p53, ploidy, PCNA, EGFR) in chronic smokers with or without prior smoking-related cancers. Only minimal side effects were seen at a slightly higher dose in the Phase I trial (*i.e.*, 1,600 mg/m^2 daily, or *ca.* 0.24 mmol/kg-bw).

In the Phase I clinical trial, alterations in GSH and GST measurements in plasma and cells were not related to daily doses up to 6,400 mg/m^2 (*ca.* 1.0 mmol/kg-bw), suggesting operation of a feedback system. After several days on a single dose level, these same measurements increased, but 6 months of treatment resulted in return of GSH and GST toward baseline values. GSSG reductase activity in lymphocytes seems to show a dose-related increase. It is unknown which of the activities of NAC may be related to inhibition of carcinogenesis in specific tissues and how this relates to plasma measurements. The drug effects of NAC relevant to chemoprevention will most probably require further characterization during the development of the agent as a chemopreventive drug.

Regulatory Issues

Preclinical toxicity tests of NAC alone have not been sponsored by the CB. Carcinogenicity assays will need to be performed after Phase II trials, if further development of this agent is desired. Finally, Segment I, II and III reproductive toxicity assays will need to be performed only if the manufacturer's studies are inadequate.

The CB has concentrated on toxicity studies of NAC in combination with DFMO in rats and dogs. A NOEL was established only in female dogs. Further toxicity testing will be necessary if clinical development of this combination is pursued.

Supply and Formulation Issues

NAC use patents appear to have expired and it is available from several manufacturers. The agent is purchased from Zambon Group S.P.A. (Vincenza, Italy) and formulated by NCI, DCPC for CB-sponsored trials. The formulations are either gel capsules (50, 200, 500 mg with 2% ascorbic acid) or powder packets (700, 1000, 2500 mg with no inert ingredients). The latter is diluted in sufficient fruit juice to disguise the taste (≤1%). The gelatin capsules are used for subjects unable to tolerate the

taste. In future trials, only the capsule formulation will be used. It should be noted that the lead time necessary for manufacturing supplies of currently available dosage forms for a new study is 4–6 months, and this interval should be incorporated in the design of new studies. No supply problems are foreseen.

Intermediate Biomarker Issues

NAC (0.31, 0.61 mmol/kg-bw) did not reduce the formation of carcinogen-induced colonic aberrant crypt foci in the rat. A study of K-*ras* in hamster pancreas is underway. The proposed Phase II trial using regression of bronchial squamous metaplasia/dysplasia will provide information regarding this aspect of the chemopreventive efficacy of the agent. Histological regression will also be correlated with additional genetic, proliferation and differentiation biomarkers, *e.g.*, ploidy, EGFR, PCNA, and p53 mutations. It would be of interest to investigate modulation of similar biomarkers by NAC in the strain A mouse lung carcinogenesis model.

Clinical Issues

Compliance may be an important issue in future long-term clinical trials; this concern arises from the strong sulfur odor and potential GI effects of NAC. These aspects should be monitored in ongoing or upcoming clinical trials. Also, similar information from the EUROSCAN trial would be helpful.

Clinical development of NAC as a cancer chemopreventive drug by the CB is targeted at the bladder and colon. Attainment of an effective dose without adverse effects may be possible in both of these tissues. Thus, a Phase II trial planned for 1995 involves prevention of bladder cancer in patients previously treated for superficial tumors with BCG. Future trials on prevention of colon cancer may also be considered for the same reason.

In contrast, it is unknown if there is an effective dose to inhibit respiratory tract cancers. Based on existing preclinical (mouse) data, the dose necessary for cancer chemoprevention in this tissue (0.74 mmol/kg-bw/day) may be higher than the well-tolerated 6-month dose in humans (0.12 mmol/kg-bw daily). The results of the ongoing EUROSCAN trial may provide some of the necessary human data at lower doses. The CB is considering a second Phase II trial in 1995 at a higher dose (0.2 mmol NAC/kg-bw qd) to investigate modulation of squamous metaplasia/dysplasia and other intermediate biomarkers (ploidy, p53 mutations, PCNA, EGFR) in chronic smokers with or without prior smoking-related cancers.

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Table I. Clinical Trials of NAC Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase I (Safety, ADME)					
NO1-CN-85102-02 Tolerance to Long Term Administration of NAC in Subjects at Increased Risk of Malignancy: A Phase I, Pharmacokinetic and Biochemical Study (Dr. Patrick J. Creaven, Roswell Park Cancer Institute) 7/90–12/93 IND 38,890	---	High-risk individuals: prior malignancy, actinic keratosis, familial adenomatous polyposis, heavy smokers 13 subjects (M,F) >18 years for each step	Step 1: Oral dose-titration study from 200–3,200 mg/m^2 bid for 4 weeks/dose Step 2: Oral 800 mg/m^2 qd for 6 months 3.5 years	Drug effect measurements: Free and total GSH (plasma, RBC, WBC), cysteine (plasma), GST-μ (WBC), GSSG reductase (WBC), UDS (WBC) Results: GSSG reductase in WBC appears to increase with dose; however, GST, GSH, and UDS activity in WBC, plasma and RBC GSH, and plasma cysteine measurements not useful Safety: EKG, hematology, urinalysis	Safety, dose titration, and pharmacokinetics study completed Single-dose plasma pharmacokinetics: C_{max}=1.7–14.2 μg/ml, t_{max}=1 hr, $t_{1/2}$=1.1–4.6 hours, $AUC_{(0-24)}$=9.2–57.2 μg/ml·hr. Note significant interindividual variation Multi-dose pharmacokinetics: C_{max}=1.57–9.36 μg/ml and $t_{1/2}$=1.02–5.1 hours after 3 months. C_{max}, $t_{½}$ and AUC decrease after chronic dosing Adverse effects: heartburn, nausea, gas with foul odor, abdominal cramps, diarrhea, fatigue, vertigo, bad taste and aftertaste at the highest dose in Step 1. In Step 2, one withdrawal due to high creatinine, fatigue, and heaviness Published report: [40]

Study No. Title (PI) Period of Performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Study Duration	Endpoints	Remarks
Phase II (Dose titration, efficacy, intermediate biomarkers)					
Planned Study Phase II Chemoprevention Trial of *N*-Acetylcysteine (NAC) in Chronic Smokers. Modulation of Surrogate Endpoint Biomarkers (SEBs) of the Bronchial Epithelium. 1995 IND 38,890	Lung	Chronic smokers with or without a previously resected carcinoma of the respiratory tract. 50 smokers/arm	Oral 1,400 mg/m^2 qd for 24 months	Efficacy: Histological regression Other intermediate biomarkers: Micronucleated cell frequency, ploidy, p53 mutation, PCNA, EGFR, mutagen sensitivity	Proposed efficacy and evaluation of intermediate biomarkers
Planned Study Phase II Chemoprevention Trial of *N*-Acetylcysteine (NAC) in Patients Previously Treated with BCG for Superficial Bladder Cancer 1995	Bladder	Resected superficial bladder cancer patients treated with BCG Subchronic: 12 patients Chronic: 100 patients	Oral Subchronic: 1 month Chronic: 1 year		Study not yet designed

NAC DEVELOPMENT STATUS