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A RANDOMIZED TRIAL OF THE USE OF N-ACETYLCYSTEINE FOR THE
PREVENTION OF TRIMETHOPRIM-SULFAMETHOXAZOLE HYPERSENSITIVITY
REACTIONS WHEN USED FOR THE PREVENTION OF PNEUMOCYSTIS CARINII PNEUMONIA
IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

By

SHARON L. WALMSLEY, MD, FRCPC

A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Community Health
University of Toronto

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ABSTRACT

Trimethoprim-sulfamethoxazole is the drug of choice for the prevention of Pneumocystis carinii pneumonia complicating human immunodeficiency virus infection. Use of this antibiotic combination is limited by the 20-30% incidence of hypersensitivity reactions. One hypothesis for this high rate of reactions in HIV-infected patients is their decreased levels of plasma and peripheral blood mononuclear cells glutathione, a substance required for the detoxification of reactive drug metabolites. N-acetylcysteine is converted enzymatically to glutathione. Two hundred and thirty-eight HIV infected patients were randomized into this controlled clinical trial. The primary objective was to compare the incidence of discontinuation of trimethoprim-sulfamethoxazole because of a hypersensitivity reaction in those randomized to receive trimethoprim-sulfamethoxazole alone (one single strength tablet twice daily) to that in those who received in addition N-acetylcysteine (15 cc of a 20% oral solution, 3 grams) twice daily, one hour prior to each dose of trimethoprim-sulfamethoxazole. In this study, 23% of patients had to discontinue the drug because of a hypersensitivity reaction. The difference in incidence between the treatment groups was 4% (95% CI -16%, +9%). This study was unable to demonstrate statistically that N-acetylcysteine could prevent, delay, or modify the features of the trimethoprim-sulfamethoxazole hypersensitivity reaction in this population.
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I. BACKGROUND

a) Objective

The objective of this study is to test the hypothesis that giving N-acetylcysteine (NAC or mucomyst) to patients infected with the human immunodeficiency virus (HIV) will decrease the extraordinary incidence of serious adverse reactions to trimethoprim-sulfamethoxazole (T/S) observed in this population.

b) Hypothesis

T/S is the drug of choice for the treatment and prevention of Pneumocystis carinii pneumonia (PCP), a major opportunistic infection in HIV. For unknown reasons, HIV-infected patients have an increased incidence of hypersensitivity to T/S. The incidence of serious reactions in HIV patients is approximately 30-50% as compared to less than 1% in other patient populations. The hypothesis that NAC will lower the incidence is based upon the following observations:

1. The major circulating thiol is cysteine and it is a precursor of glutathione which is the major intracellular thiol (Figure 1).

2. Conjugation with glutathione is a major route of detoxification of reactive metabolites of drugs (including T/S) and other chemicals (Figure 2).

3. Glutathione deficient lymphocytes from patients with a genetic defect in glutathione synthesis have an increased susceptibility to sulphonamide metabolites in vitro. Similarly, sensitive lymphocytes from patients who have had adverse reactions to sulphonamides can be protected in vitro by the addition of glutathione.
4. Patients with HIV have been found to have abnormally low levels of cysteine and glutathione in their blood and low glutathione levels in their peripheral blood mononuclear cells, a principal target of HIV. The mechanism of the depletion is uncertain and could include one or a combination of decreased synthesis, increased catabolism, increased conjugation or elimination, or increased oxidation.

5. N-acetylcysteine has been used clinically to regenerate glutathione in such circumstances as acetaminophen overdose where glutathione is depleted by a reactive metabolite.

Thus it is reasonable to propose that the increased incidence of adverse reactions to T/S in patients with HIV infection is a consequence of low glutathione levels and an inability to adequately detoxify reactive drug metabolites. It follows that a maneuver to increase glutathione levels would decrease the incidence of T/S adverse reactions.

c) PCP Prophylaxis in HIV

PCP remains the commonest AIDS-defining opportunistic infection for patients living with the HIV virus and is an important cause of morbidity and mortality (Bennett et al, 1995; Wall et al, 1993; Schwarcz et al, 1997; Delmas et al, 1995; Hoover et al, 1993). For mild to moderate cases, early diagnosis and treatment with T/S, intravenous pentamidine, clindamycin with primaquine, atovaquone or trimetrexate with or without adjunctive steroids is successful in over 70% of cases (Davey et al, 1990; Masur, 1992; Haverkos, 1984; Winston et al, 1980; Small et al, 1985; Klein et al, 1992; Wharton et al, 1986; Toma et al, 1993; Sattler et al, 1994; Smith et al, 1992; Safrin et al, 1996; Medina et al, 1990; Hughes et al, 1993; Walmsley et al, 1995; Walmsley et al, 1988; Bozzette et al, 1990; Gagnon et al, 1990; Nielsen et al, 1992; NIH, 1990). However, mortality remains at 50-85% in patients with respiratory failure requiring intubation (Bennett et al, 1995; Bennett et al, 1993). Drug therapy is also associated with short term and long term toxicity including bone marrow suppression, rash, renal failure, pancreatitis and altered glucose metabolism. Parenchymal lung necrosis occurring during an acute PCP episode may permanently damage lung tissue, resulting in pneumatoceles and
bullae which in turn may rupture causing pneumothorax (Sepkowitz et al, 1991) or become colonized and invaded with aspergillus (Torrents et al, 1991).

Given the predictable occurrence of this infection with advancing immunosuppression, preventative strategies are important. *Pneumocystis carinii* is an ubiquitous pathogen (Stringer, 1996). Seroprevalence studies have revealed that 80-90% of the population is infected with this organism, usually during childhood. Provided there is normal cell-mediated immunity, infection is not accompanied by disease and the organism remains latent. With progressive damage to the CD₄ bearing T lymphocytes in persons with HIV, there is an increased risk of reactivation of PCP. The risk of developing PCP is approximately 18% at one year and 33% within three years for patients with CD₄ counts ≤ 200 x 10⁶/L (Phair et al, 1990) and thus, primary prophylaxis is currently recommended for these individuals. After one episode of PCP has occurred, approximately 60% will relapse by 12 months if specific secondary prophylaxis is not used (US Public Health, 1992).

Prophylaxis does not completely prevent PCP, but it reduces its incidence and prolongs the period of AIDS-free survival (Chaisson et al, 1992). Several drugs are currently available for prophylaxis of PCP. Those used most commonly are T/S, dapsone with or without pyrimethamine or trimethoprim and aerosolized pentamidine (Bucher et al). A large scale clinical trial (ACTG 081) comparing these three agents as primary prophylaxis has recently been completed by the AIDS Clinical Trial Group (Bozette et al, 1995). The results of this trial supports the use of T/S for first line prophylaxis if there is no history of serious adverse reactions to either sulphonamides or trimethoprim. However, only 21% of patients assigned to T/S completed the study receiving their originally assigned drug at the original dose due to adverse events. T/S was also found to be more effective than aerosolized pentamidine in two controlled trials on primary and secondary prophylaxis (Schneider et al, 1992; Hardy et al, 1992; May et al, 1994). For compliant patients breakthrough rates of less than 5% are reported (Fischl et al, 1988). In addition to its efficacy for preventing PCP, another potential advantage of using T/S is prevention against reactivation of toxoplasmosis and severe bacterial infection (Martin et al, 1992; Antinori et al, 1995; Girard et al, 1993; Heald et al, 1991; Mallolas et al,
1993; Rizzardi et al, 1996; Edge et al, 1996). Although aerosolized pentamidine is effective and recommended for patients who can not tolerate T/S, it has several disadvantages. These include the lack of prophylaxis against toxoplasmosis and bacterial infections, the cost, and the risk of aerosol spread of other organisms, i.e. *Mycobacterium tuberculosis*, which could result in outbreaks in patients attending administration centres (Castellano et al, 1991; Freedberg et al, 1991; CDC, 1989). The breakthrough rate of PCP is reported to be 20% with this agent. Further, as the effectiveness of aerosol pentamidine is limited to the lungs, cases of extrapulmonary PCP have been reported (Raviglione, 1990; Telzak et al, 1990). Dapsone is another potential systemic agent for the prevention of PCP (Antinori et al, 1995; Girard et al, 1993; Tocchetti et al, 1994; Mallolas et al, 1993; Blum et al, 1992; Lee et al, 1989). In a recent large trial, a 50 mg dose of dapsone administered twice daily conferred protection against PCP similar to that of T/S, however, the incidence of PCP was higher among persons who required dose reductions to 50 mg per day (Bozzette et al, 1995). Complications of dapsone use include hemolytic anemia (especially in patients deficient in the enzyme glucose-6-phosphate-dehydrogenase), methemoglobinemia and peripheral neuropathy. Approximately 25% of patients allergic to T/S will demonstrate cross hypersensitivity to this agent (Beaumont et al, 1996).

There is some controversy as to the optimal dose of T/S for prophylaxis (Ruskin et al, 1991; Nielsen et al, 1993; Wormser et al, 1991; Podzamczer et al, 1993; Stein et al, 1991; Schneider et al, 1995). Randomized trials have evaluated one double strength tablet twice daily, one double strength tablet once daily and one double strength tablet every Monday, Wednesday and Friday. Based upon the current evidence, the United States Public Health Service Task Force of the Centers for Disease Control has recommended one double strength tablet (160 mg trimethoprim, 800 mg sulfamethoxazole) once per day (US Public Health Service, 1992).

d) Sulphonamide Hypersensitivity

Although T/S is the preferred drug for prophylaxis against PCP, many persons cannot tolerate it (Ionnides et al, 1996). Adverse effects have been reported in as many as 65% of patients
with HIV in contrast to only 2-8% of the general population (Schneider et al, 1992, Hardy et al, 1992; Fischl et al, 1988). These adverse effects include hypersensitivity, GI intolerance and hematologic toxicity (Gordin et al, 1984; Hughes et al, 1995). A major dose limiting toxicity is a hypersensitivity reaction characterized primarily by fever, morbilliform eruption and pruritus which has been observed in 8-65% of patients (van der Ven et al, 1996; Bayard et al, 1992). Severe exfoliative rashes, such as Stevens-Johnson syndrome, occur only rarely (Kelly et al, 1992; Walsh et al, 1993). The hypersensitivity reaction can in addition involve almost every organ, including the liver, bone marrow, kidneys, lungs, heart, central nervous system, thyroid, pancreas, muscles and gonads (Bayard et al, 1992; Gupta et al, 1992). Also reported are hyperthermia, hypotension and new pulmonary infiltrates mimicking the respiratory distress syndrome (Johnson et al, 1990). Hypersensitivity reactions occur more commonly in HIV-infected patients than in the general population where they are observed in less than 5% of patients treated with sulphonamides. Most serious adverse reactions to T/S are idiosyncratic in nature and the majority are thought due to the sulphonamide component of the combination (Shear et al, 1986). The delay of approximately seven days between the initiation of treatment and the development of the reaction (although immediate reactions do occur upon rechallenge) suggest that the immune system is involved in this reaction (van der Ven et al, 1991). Mediators of inflammation, such as cytokines, may have a role in the pathogenesis. IgE or atopy have not been found to be involved (Koopmans et al, 1995).

The incidence of reported hypersensitivity reactions to T/S varies widely in the literature (Table 1,2). These differences may reflect patient population, drug dose and duration and concurrent therapies. They are reported to occur more frequently in patients receiving treatment for PCP (15-20 mg/kg of trimethoprim component) than in the patient on prophylactic doses. Many patients who develop reactions during treatment of PCP may subsequently receive the drug as secondary prophylaxis without difficulty (Carr et al, 1993; Shafer et al, 1989). In mild cases, fever and rash may be treated symptomatically with acetaminophen, aspirin, anti-histamines, diphenhydramine or anti-pruritics. The use of adjunctive corticosteroids for moderate to severe PCP has also been associated with a decreased risk of hypersensitivity reactions (Walmsley SL et al, 1995; Caumes et al, 1994). As
T/S is a preferred agent for PCP prophylaxis, various protocols for desensitization with or without corticosteroids following hypersensitivity have been developed (Quirino et al, 1996; Bissuel et al, 1995; Bachmeyer et al, 1995; Nguyen et al, 1995; Gluckstein et al, 1995; Piketty et al, 1995). In these protocols, the dose of T/S is started low and gradually increased over hours to days. Although the short term (days-weeks) efficacy of desensitization is good (approximately 60-80%) they are less successful in the longer term (30-70%). For many of these studies, the number of patients studied over time is limited. The mechanisms for the efficacy of desensitization is unknown and no predictors of success have been discerned.

Other factors have been associated with an increased risk of hypersensitivity reaction (Carr et al, 1993). One group has reported that patients with lower CD4 counts have decreased risk of T/S hypersensitivity reactions during treatment of acute PCP. It is hypothesized that HIV infection of CD4 lymphocytes or monocytes enhances T-lymphocyte sensitivity to T/S or its metabolites. As the CD4 counts decrease, insufficient lymphocytes are available to protect against this response. The role of viral infections as a stimulator of drug reactions is poorly understood, however, there is some similarity between T/S-associated hypersensitivity in HIV and the ampicillin-associated rash of acute Epstein-Barr virus or CMV infections. This data suggests that a patient may be less likely to develop hypersensitivity with re-challenge or desensitization if their immune function deteriorates between the initial hypersensitivity and re-challenge. In support of the hypothesis, one study demonstrated that only 15/26 or 58% of patients re-challenged with T/S developed hypersensitivity reactions including 12 in which it was severe enough to cause the discontinuation of T/S (Carr et al, 1993). Rechallenge was more likely to be successful in patients with low CD4 counts. This contrasts with other data in which 66% of 130 patients were successfully crossed over from aerosolized pentamidine to T/S for PCP prophylaxis. Of patients with CD4 ≤ 200/mm³, 57% developed rashes after cross-over compared with only 27% of patients with higher CD4 cell counts (Kennedy et al, 1993).
Role of Acetylator Phenotype

Sulfamethoxazole is metabolized predominantly by the N-acetyltransferase (50-70%) to N₄-acetyl sulphamethoxazole and by the cytochrome p450 enzyme system (10-15%) to 5-hydroxysulphamethoxazole (Figure 2) (van der Ven et al, 1991). Sulfamethoxazole can also be oxidized on the N₄ position to form a hydroxylamine derivative. The rate of production of this reactive intermediate may be influenced by the rate of acetylation of the parent compound (Rieder et al, 1991). Individuals differ markedly in the rate at which drugs are acetylated and there is a bimodal distribution of the population into “rapid acetylators” and “slow acetylators”. The rate of acetylation is under genetic control with rapid acetylation, an autosomal dominant trait. This suggests that acetylator status may be an important risk factor for T/S hypersensitivity. In other settings, slow acetylators have been shown to have an increased risk of cutaneous hypersensitivity to drugs metabolized primarily by acetylation including isoniazid. One study (Carr et al, 1994) demonstrated in a small group of HIV infected patients that those with a history of T/S hypersensitivity reaction were more likely to demonstrate a slow acetylator phenotype. Others have not confirmed this observation and slow acetylation phenotype alone is probably insufficient to cause hypersensitivity. Acetylator genotype was not determined in this study, therefore, it is unclear whether these patients had an increased genetic risk for the reaction, or whether the phenotypic expression was altered by the underlying HIV infection.

e) Glutathione Deficiency in HIV

Glutathione (GSH), an important antioxidant, is the major low molecular weight thiol in mammalian cells (Gilbert, 1988; Larsson et al, 1983). In its reduced form, GSH plays a major role in protecting cellular macromolecules from irreversible damage produced by toxic reactive intermediates (oxidative stress) generated during xenobiotic metabolism (including the electrophilic hydroxylamine reactive intermediates of T/S). These intermediates are detoxified by conjugation with GSH, further metabolized and excreted in the urine. In the detoxification process, glutathione disulfide (GSSH) is produced and there is a decrease in reduced GSH.
Under normal conditions, the body can maintain normal GSH concentrations through reduction of GSSH to GSH by glutathione reductase or by increased synthesis of GSH. With inadequate intracellular GSH, the intermediates can bind irreversibly to cellular macromolecules, initiating immune mediated hypersensitivity reactions (Figure 1).

In vitro studies have shown that hydroxylamine metabolites lead to an increased cytotoxicity towards lymphocytes and peripheral blood mononuclear cells of patients with a clinical history of sulphonamide hypersensitivity compared with control lymphocytes of non-allergic individuals (Rieder et al, 1985; Rieder et al, 1989). The cytotoxicity of these hydroxylamine derivatives was decreased after co-incubation with glutathione or NAC. Reider has also shown that HIV-infected MOLT-III lymphoblasts were more sensitive than uninfected cells to cytotoxicity induced by the hydroxalamine metabolite of T/S (Rieder et al, 1994). The HIV infected cells had lower initial glutathione content. In vivo, sulphonamide hypersensitivity could be due to increased production of a reactive metabolite together with the relative inability of tissues to detoxify such a substance.

Multiple studies in the literature, including our own, have shown that plasma GSH and cysteine (a precursor of GSH), lymphocytic GSH and lung epithelial lining fluid GSH are reduced in HIV and AIDS patients relative to seronegative controls (Staal et al, 1992; Buhl et al, 1989; Aukrust et al, 1995; Eck et al, 1989; Staal et al; 1992; Roederer et al, 1993; Walmsley et al, 1997; Holroyd et al, 1993). This depletion has not been observed in all studies (Pirmohamed et al, 1996). Some studies have shown that glutathione levels are lower in patients with more advanced disease than seropositive asymptomatic patients although we have not confirmed this in our work. The mechanism for the decreased thiol concentration in HIV patients, if true, is unknown and could involve one or all of a combination of decreased synthesis, increased catabolism, increased conjugation and elimination or increased oxidation.

The deficiency of glutathione observed in HIV infected patients could theoretically reduce the capacity to scavenge the reactive metabolites of T/S and lead to an increased rate of hypersensitivity reactions as is observed in this population (van der Ven et al, 1991). One
group has found that low whole blood glutathione levels prior to commencing T/S as PCP therapy or prophylaxis were predictive of the development of hypersensitivity.

Thus, if glutathione levels could be increased in HIV-infected patients, it might decrease the incidence of T/S reactions.

f) **N-acetylcysteine**

Glutathione is a tripeptide of cysteine, glycine and glutamic acid and is found in all body tissues. Absorption of glutathione from the intestine is poor (Witschi et al, 1992). Glutathione is synthesized from cysteine by a series of enzymatic reactions. NAC is a form of cysteine that is modified by N-acetylation to improve absorption and cellular penetration (Figure 1). It is the treatment of choice for the reactive intermediate-mediated hepatotoxicity seen in acetaminophen overdose. In this situation, hepatic necrosis results from excess acetaminophen after depleting glutathione is converted into alkylating compounds that bind to proteins and enzymes damaging hepatic cell membranes. NAC is used to prevent this poisoning by acting as a precursor for glutathione synthesis and forming complexes with toxic reactive metabolites of acetaminophen, thus preventing hepatic cell necrosis (Holdiness, 1991). Patients usually receive 140 mg per day of loading dose and 17 maintenance doses of 70 mg/kg given at four hour intervals. The protective effects of NAC are thought to result from its ability to increase GSH concentrations and directly or indirectly scavenge electrophilic reactive intermediates (via the sulfhydryl group). NAC is also known to promote the synthesis of glutathione in cell culture.

Because the mechanism of decreased glutathione levels in HIV is unknown, there is no guarantee that NAC will increase glutathione levels. While NAC is potentially useful, a problem is its poor oral bioavailability (approximately 10%). This is because NAC undergoes rapid and extensive first pass metabolism (Holdiness 1991; Borgstrom et al, 1980; Walker et al, 1992). When NAC was given to healthy volunteers at a single oral dose of 30 mg/kg, the median peak serum concentration was only 66 μM (Borgstrom et al, 1980). After continued
use, peak plasma levels of 0.28-2.6 mM have been observed. Transient increases in intracellular cysteine and glutathione in HIV-infected patients after single oral doses of NAC (30 mg/kg) have been observed by some (Kinschere et al, 1994; de Quay et al, 1992). Roederer in a study of 7 HIV patients who received NAC 1000-2000 mg daily was able to demonstrate a return of the relative intracellular GSH levels to a normal range within one week (Roederer, 1993). In contrast, one report has suggested that NAC administration in HIV, although increasing plasma cysteine, may not increase GSH concentrations in vivo, possibly due to decreased levels of the enzyme gamma-glutamyl cysteine synthetase (GGCS) necessary for the synthesis of GSH from CYS (Witschi et al, 1995).

Even if NAC does not increase glutathione levels, it still may decrease the incidence of sulphonamide hypersensitivity reactions. The chemical group most essential to the function of glutathione is the thiol or sulfhydryl group. Sulfhydryl groups are reactive nucleophiles which can detoxify electrophilic metabolites and they also play a role in maintaining critical protein sulfhydryl groups in the reduced form rather than the disulfide form. Since NAC also contains a sulfhydryl group, it can fulfill some of the functions of glutathione. Therefore, NAC might decrease the incidence of T/S reactions in HIV patients and could even improve immune function even if it did not increase glutathione levels.

**NAC and HIV Infection**

Many of the pathophysiological events in the course of HIV infection are not due directly to viral replication, but are secondary to the host immune response to infection and the subsequent dysregulation of the immune system (Figure 3). Cytokines, such as tumor necrosis factor (TNF), interleukin-2 (IL-2), IL-6 and interferons alpha and gamma play a major role in HIV pathogenesis and can stimulate HIV replication through the activation of the transcription factor NF-KB (Fauci et al, 1996). Stimulation of cells of the immune system by the cytokines leads to increased intracellular production of reactive oxygen intermediates (oxidative stress). This could also serve to further deplete glutathione.
In cell line models of acute and chronic HIV infection, NAC has been shown in submillimolar concentrations to inhibit HIV viral transcription induced by TNF and IL-1 by 10-30 fold (Roederer et al, 1990; Simon et al, 1994; Roberts et al, 1995; Roederer et al, 1993; Droge, 1993; Kalebic et al, 1991; Malorni et al, 1993). NAC could, therefore, play a role in maintaining viral latency by inhibiting cytokine stimulated expression of integrated virions and decreasing oxidative stress. Therefore, if HIV infection itself is a stimulator of drug hypersensitivity reactions, NAC may be protective by an alternate mechanism.

Further, during opportunistic infections (including PCP) and immunization, various researchers have shown an increase in HIV plasma viral loads (Stanley et al, 1996; Brichacek et al, 1996). This increased viral replication is associated with increased oxidative stress. GSH is the main host defense against oxidative stress via several chemical and enzymatic reactions, reduces the generated reactive oxygen species to non-toxic species. One group has demonstrated that HIV infected patients who have adverse progression to T/S have more rapid rates of progression to AIDS and death. Increased oxidative stress could be important (Veenstra et al, 1997). The combination of increased oxidative stress resulting from 1) acute PCP; 2) high dose of T/S and increased production of reactive metabolite and; 3) increased HIV viral replication could combine to further deplete glutathione and explain why T/S reactions are observed more commonly during treatment for PCP than for prophylaxis.

Side Effects of NAC

NAC is used as a mucolytic agent for the treatment of chronic bronchitis and other pulmonary diseases complicated by the production of viscous mucous such as cystic fibrosis. It is also used as an antidote to acetaminophen poisoning. It has also been used for the prevention of cardiotoxicity by doxorubicin. Stomatitis, vomiting and diarrhea are the most common side effects experienced with the oral use of NAC and appears to be dose related (Holdiness, 1991). A few susceptible patients especially asthmatics may experience bronchospasm associated
with the administration of nebulized NAC. Hypersensitivity reactions following intravenous administration of NAC have been reported and can include facial edema, urticaria, hypotension and bradycardia. Other adverse symptoms that have been noted in less than 5% of treated patients include hypertension, chest pain, headache, lethargy and fever. Therefore, the compound appeared to be safe for oral use in the context of this study.

II RATIONALE FOR STUDY

PCP continues to be the most common AIDS-defining opportunistic infection. Sixty percent of all new AIDS cases in Canada present with PCP (Wilk et al, 1997). Many more patients develop PCP later in their illness. PCP is a significant cause of morbidity and mortality and contributes significantly to the overall cost of caring for patients with HIV. T/S is the preferred agent in terms of ease, cost and efficacy for both treatment and prophylaxis of PCP. Despite this superior efficacy, its use is frequently limited by adverse hypersensitivity reactions. Although desensitization programs can decrease the incidence of hypersensitivity reactions in the short term, evidence for long term efficacy of these programs is limited and life-threatening reactions have been occasionally observed during the de-sensitization procedures (Caumes et al, 1996). If another strategy could be developed to decrease the risk of T/S hypersensitivity reactions in HIV, then more patients could continue this drug for PCP treatment and prophylaxis. This should translate into fewer PCP episodes and increased response rates to treatment, thereby improving the overall health of these patients and decreasing health care costs.

This study would also provide a better understanding of the features, frequency and risk factors for T/S hypersensitivity reactions in HIV and may provide a better understanding of drug metabolism.

If NAC were found to decrease the incidence of T/S reactions in this population, other studies could be performed to determine the minimally effective NAC dose, thereby decreasing cost,
inconvenience and possible intolerances.

a) Study Design

A multi-centered, randomized trial. Although the optimal design of this trial would have been double blind and placebo controlled, it was not felt to be feasible. NAC is available by prescription only as a liquid (as a 20% solution). It has a very distinctive odor and taste which cannot be masked. This meant that the patients could not be blinded. Additionally, the study nurse administering the medication could also not be blinded. In order to minimize bias, the investigator(s) who was following the patients and would be making the clinical decisions about outcomes would be blinded where possible. We recognized that on occasion, patients would inadvertently unblind their treating physicians. It was not thought that the decision to discontinue treatment would be affected by this knowledge. To ensure any bias would be minimized, the following was requested. When a decision was made to discontinue T/S, the patient was to be seen by a second physician in the study centre. This physician had to agree that the adverse reaction was likely due to T/S and that the patient should have the T/S discontinued for this reason. The case would then be counted as a primary endpoint. If the second assessment disagreed, the patient was judged withdrawn from the study.

b) Inclusion Criteria

- Patients known to be HIV-infected.

- CD$_4$ count $\leq 200 \times 10^6$/L or CD$_4$ percentage $< 20\%$ or previous AIDS-defining illness.

- Patient consents to study and is willing and able to complete follow-up.
c) Exclusion Criteria

- Previous allergic reaction to sulphonamides or trimethoprim.
- Treatment with T/S since HIV diagnosis including treatment for acute PCP episode.
- Significant neutropenia (neutrophils < 1000 x 10⁶/L).
- The patient prefers another form of prophylaxis.
- The patient is taking a study drug other than investigational anti-retroviral agents.
- The patient is taking NAC from other sources and not willing to discontinue it during study period.

At the time this trial was developed, it was thought to be unethical and hazardous to rechallenge patients who had previously experienced T/S reactions, therefore, this study was restricted to primary PCP prophylaxis. The inclusion criteria reflected the population for whom this is indicated. Any patient with a previous T/S reaction was excluded. Including patients who had received T/S since their HIV diagnosis without a reaction could bias the study towards a low risk group for hypersensitivity. As the incidence of reaction appears to increase following HIV infection, those who had received T/S prior to their diagnosis were included. We recognized that this might include some patients who had received T/S at a time they were unaware of their HIV status. As hematologic toxicity is common, patients with neutropenia were excluded. At the time this trial was developed, aerosol pentamidine was often a preferred form of prophylaxis. Patients choosing this agent were excluded. As hypersensitivity reactions to the anti-retroviral agents in use at the time were uncommon, we allowed patients to co-enrol in studies of anti-retroviral therapy in attempts to increase recruitment. There was no reason to believe that NAC would interfere with their anti-retroviral activity.
d) Intervention

When a potential study patient was identified by an investigator, the study nurse at that centre confirmed eligibility and consent was obtained. The study nurse then called the study coordinator at the Canadian HIV Trials Network (CTN) in Vancouver who reviewed the eligibility. If acceptable, the study number and drug regime was assigned according to the next allocation on the randomization list generated by the CTN data centre. The site pharmacist was informed of patient assignment. Randomization was stratified by study site using variable, permuted blocks of two and four. The random allocation sequence was computer generated at the CTN.

All patients received T/S one single strength tablet twice a day orally. Patients were randomized to receive or not receive NAC orally one hour prior to each dose of septra at a dose of 15 cc of 20% solution (3 g) for a total daily dose of 6 g. NAC was given in cola or orange juice to make it more palatable. NAC was administered for the first two months and patients were followed for three additional months on T/S alone.

e) Justification of Dose Regimen

The optimal dose of NAC (if any) to decrease sulphonamide hypersensitivity reactions is unknown. Therefore, the doses to treat acetaminophen overdose were used as a guide. In this setting, a loading dose of 140 mg/kg is followed by q4h doses of 70 mg/kg. Unlike reactive metabolites of acetaminophen, sulphonamides in themselves do not cause a significant depletion of glutathione. Therefore, it was felt unlikely that as high a daily dose of NAC would be required. Nonetheless, because of the poor oral bioavailability, we did not want to use an insufficient dose to show efficacy. The final dose chosen (6 gm/day) was based on what was expected to be safe and yet sufficient to be effective. In a pilot study, 16/17 HIV positive patients were able to tolerate this dose for several months but did complain of an unpleasant taste and did not feel that they would be able to tolerate higher doses. One patient (being
treated for PCP) and therefore, receiving twice this dose of NAC was unable to tolerate the NAC because of severe nausea. In order to minimize the unpleasant taste, NAC was given in orange juice or cola. If NAC was found to be effective in this trial, then optimal doses, routines and schedules for administration could be examined in future studies. Although there is a commercial non-prescription tablet form of the drug available in health food stores, nutritional supplement companies and buyers clubs, it was not felt to be optimal for this study. NAC and other thiols are readily and quickly oxidized to non-functional products called disulfides. Non-prescription products are not subjected to routine NAC analysis by regulatory agencies, nor are there guidelines for the appropriate storage of such compounds and, therefore, it is difficult to be certain of the label claims for NAC content of these products.

The optimal dose of T/S for PCP prophylaxis is clear. Based on the evidence at the time of trial development, the US Public Health Service Force recommended one double strength tablet (800 mg sulfamethoxazole, 160 mg trimethoprim) daily.

To optimize serum levels of NAC, we felt that it should be taken bid. For greatest effect, we felt that it should be tied to the dose of T/S. Therefore, the latter was given as one single strength tablet twice daily. There was no evidence to suggest that this would be associated with less efficacy than taken as a once daily dose. It would also allow us to determine whether or not this dose schedule (control arm) was associated with fewer sulphonamide reactions than reported with standardized dosing with daily double strength tablets.

In the literature, the vast majority of T/S reactions occur within the first two weeks of initiation of treatment. It is possible that NAC could delay rather than completely prevent these reactions. We, therefore, wished to ensure it was given during the highest risk period. The initial patients on study received the drug for 3 months of T/S therapy. After 46 patients had been enrolled, we determined that there did not appear to be any significant delays in T/S reactions. Therefore, for reasons of cost and convenience, subsequent patients received NAC for only the initial two months of the study. The original group of patients were included in the final analysis. Prolonged follow-up after NAC was discontinued then allowed an
evaluation of the treated patients who did not develop a reaction while on NAC to serve as their own controls.

f) Study Development (Appendix I)

A pilot study was begun at the Sunnybrook Health Science Centres in January 1991 to evaluate the feasibility of the protocol. Ten patients were enrolled in the pilot study over a six month period. Subsequently, a full protocol was developed and the IND (investigational new drug) application was filed. University of Toronto ethics approval was obtained and initial funding from the Physicians' Services Incorporated was granted. Bristol Myers Squibb agreed to provide the NAC free of charge for the study in April 1992. Based upon the initial sample size estimate, three centres in Toronto (Sunnybrook, St. Michael's Hospital and The Toronto Hospital) began recruiting patients in 1992. Initially, there were two strata to the study - patients being treated with T/S for an acute episode of PCP and patients on primary prophylactic therapy. Over one year, 59 patients were enrolled into the trial, including 46 into the prophylaxis arm and 12 in the treatment arm. At the time, given the widespread use of prophylactic treatment for PCP and the changing nature of HIV, it became apparent that we would never be able to recruit sufficient numbers of patients to the treatment arm and that arm was eliminated for feasibility reasons. The sample size was recalculated. In order to recruit the required number of patients, it was realized that further centres would be needed. In addition, there was a manufacturing problem and a short supply of the drug N-acetylcysteine during April to October 1992 when recruitment was very poor.

In November 1993, a protocol summary was sent to the investigators of the Canadian HIV Trials Network (CTN). Investigators were asked about their desire to participate in the trial and to estimate the number of patients they expected they could enroll over the next year. The study was revised and submitted to the Scientific Review Committee, the Community Advisory Board and the Steering Committee of the Canadian HIV Trials Network. New case report forms were developed and submitted to the CTN statistical centre for computerized data entry. The study was accepted in January 1994 and additional funding secured from the CTN
to enable completion of the trial. A study coordinator to be centered in Vancouver was hired in May 1994 and the CTN centres submitted the protocol to the local institutional ethics review boards. The pharmaceutical company, Bristol Myers Squibb, who initially agreed to supply the N-acetylcysteine for the study was re-approached and agreed to supply NAC to complete the new and revised trial. In March 1994, the rights of the produced N-acetylcysteine were sold from Bristol Myers Squibb to Roberts Pharmaceutical. After several meetings, they agreed to fulfill the commitments to supply mucomyst for the study. Patients were enrolled through the CTN starting in January 1994. It was estimated that each participating centre could recruit one patient per month and that recruitment could be completed in 15 months and follow-up of enrolled patients completed in 20 months.

**g) Recruitment**

Initiating and maintaining enthusiasm for recruitment in a multi-centred clinical trial is a complex task. This is particularly difficult in HIV research where many of the centres are participating in multiple and often conflicting trials and because of the ever changing nature of the disease and its therapy. The significance of the clinical question being addressed, the perceived importance by the investigators of rapid recruitment and completion of the trial and their sense of ownership is crucial. Another factor important to recruitment is to ensure that the workload associated with the study is kept to a minimum and that excessive data that will not be utilized will not be collected. It is important that a sufficient number of centres enroll in the study to enable recruitment in a reasonable time period and that the centres chosen are treating the patient population who fit the entry criteria for the trial. Recruitment must be monitored on an ongoing basis and if recruitment falls below the expected rate, alternative strategies need to be considered.

**Recruitment Strategies (Appendix II)**

Actual recruitment rates lagged behind that expected with 2-10 patients (average 4) enrolled in the study each month.
A number of reasons were identified, including:

- With the changing nature of HIV care and management, more patients were initiated on primary PCP prophylaxis by their primary care physician rather than the hospital based clinics, where the trial was conducted.

- Difficulty of clinic physicians to remember the trial when initiating T/S for PCP prophylaxis.

- Greater enthusiasm on behalf of both the patients and investigators to enroll in competing anti-retroviral trials. These trials often required the same patient population and pharmaceutical-based trials were usually more financially lucrative for the centres. For patients receipt of new, unlicensed HIV therapies was more desirable. As the NAC was given for only 2 month and patients could obtain it from health food stores, they perceived less direct personal benefit from study participation.

Steps that were taken to address these issues were as follows:

- An investigators' meeting was held at the Canadian Association for HIV Research (CAHR) conference in May 1994 describing the theory of the study and the importance of the clinical question. We reinforced the importance of clinician-initiated trials in the visibility of the CTN. Another investigators' meeting was held at the CAHR conference in May 1995 to review progress and discuss recruitment strategies. The study remained on the agenda of the Ontario Region of the CTN biannual meetings.

- Regular phone conferences were held between the principal investigator, the CTN research coordinator and the study site nurses. The purposes of these phone conferences were to discuss problems with the study and to share ways to increase recruitment, (i.e. post-it note reminders in patient charts), to enhance enthusiasm, and congratulate efforts.
• One on one phone conversations between the principal investigator and site investigators of the CTN and the research coordinator with the site study nurses for those sites lagging in their recruitment to discuss issues and to offer assistance.

• The "Think NAC" campaign. Engraved pens were made available to the study coordinators and investigators. Brightly-coloured posters were prepared to hang in offices and waiting rooms to remind physicians and patients about the study. Advertisements were put into gay magazines in Toronto and Vancouver to enable patients to self-refer.

• The "NAC Newsletter" was developed to explain the theory of the trial, inclusion/exclusion criteria, the location of the participating sites, recruitment, etc. All participating centres identified their referring primary care physicians and a copy of the newsletter was sent to them as well as AIDS organizations and treatment information centres (i.e. Community AIDS Treatment Information Exchange - CATIE) in the area. This was intended to increase referral from outside the study centres.

• Presentation of trial updates to the primary care physician rounds in Toronto, Noon HIV Rounds at Toronto General Hospital and the primary care physician rounds in Vancouver to further remind investigators and update status.

• There was recruitment of additional sites, especially those in more isolated areas who did not have the same number of competing trials (i.e. Windsor, Sudbury) and who were more likely to initiate PCP prophylaxis.

• Regular mail-outs to participating centres of trial recruitment in total by region and by site and indication of the site expectations and actual recruitment rates.

• The study allowed for co-enrollment of patients participating in trials of anti-retroviral agents. The reciprocal was not always true.
h) Follow-up

The patients continued T/S, one single strength twice daily for 5 months. At that time, investigators were permitted to choose any T/S dosing schedule. NAC was maintained for the initial 2 months or until T/S was discontinued whichever was first.

Patients were seen in follow-up at weeks 2, 4, 6, 8, 10, 12, 16 and 20 after randomization. Patients were seen biweekly during the first 2 months to ensure all hypersensitivity reactions were identified. Follow-up also occurred at 2 week intervals for 1 month after NAC was discontinued to ensure no early reactions developed and then continued monthly until the study was complete.

If a reaction occurred, the patient was seen as soon as possible and then at 2 days and 4 days for clinical and laboratory evaluation to evaluate the severity and extent of the reaction and to ensure the reaction resolved. The patient was assigned an alternate form of prophylaxis if a reaction occurred or the treating physician could attempt T/S desensitization.

i) Data Collection (Appendix III)

At baseline, information was collected on:

- patient demographics
- status of HIV disease and previous opportunistic infections or malignancies
- HIV associated signs/symptoms
- concurrent medications
- baseline laboratory studies, including chest x-ray, EEG, hematology and
- biochemistry
- vital signs
At follow-up visits, information was collected on:

- intercurrent HIV associated events
- changes in medications
- compliance to assigned treatment
- signs and symptoms of adverse events
- intolerance to treatment
- laboratory studies to evaluate for toxicity

If a reaction occurred, information was collected on:

- features, extent and timing of reaction
- use of symptom-relieving medications
- laboratory assessment of the reaction and extent of organ involvement
- date drugs discontinued

If the patient discontinued medications for any other reason, data was collected on:

- reason for drug discontinuation
- date of discontinuation
- presence or absence of reaction

Data was collected by a nurse at the study centre and forwarded to CTN study coordinator in Vancouver. The data forms were checked for completeness. Any missing data or questions were referred back to the study nurse for clarification. Once completed, data was entered in computer in duplicate at the CTN data centre.
j) Monitoring and Source Documentation

There were insufficient funds available to allow for full data monitoring. Partial monitoring was performed for 117 cases as outlined in Appendix IV.

The most intensive monitoring was performed on inclusion/exclusion criteria, certain baseline clinical and laboratory parameters and concurrent therapy, details of drug discontinuation and hypersensitivity reactions. These were thought to be the most important data to enable trial analysis with respect to the primary outcome. Monitoring was most extensive in the centers enrolling the greatest number of patients. Where possible, centers close together were monitored at the same time. The monitoring was last done in April 1996, when 229 patients were randomized. No patient enrolled prior to CTN involvement were monitored. No monitoring was done after April while waiting for further recruitment. When the study was closed due to lack of efficacy, it was determined that no further monitoring would be performed for economic reasons.

k) Outcome Measures

Primary Outcome

The primary outcome measure initially determined for this trial was the need to discontinue T/S for any two of a) rash; b) severe pruritus; c) fever. Rash was defined as a diffuse erythematous eruption involving more than 50% of the body surface area. Fever was defined as a documented fever $> 38.5^\circ C$ on at least 2 occasions at least 2 hours apart and within 48 hours. Severe pruritus was that which was intolerable to the patient. For patients on T/S, the drug is the most common cause of this combination of side effects. It was felt initially that this "major" adverse reaction was what we hoped to prevent by the use of NAC. At the CTN investigators meeting, it was determined that most physicians manage T/S hypersensitivity in a similar way - i.e. if a "major" reaction such as that defined above would occur, that T/S would be discontinued and an alternative agent chosen for prophylaxis. In contrast, if a milder
reaction occurred - most physicians would try to treat “through it” with or without the use of Tylenol/ASA/Atarax or anti-histamines for symptomatic control. Thus, the clinically important effect of NAC would be its ability to allow patients who would otherwise develop rash, fever and/or pruritus to continue to take T/S. After the first interim analysis and with the recommendation of the Safety and Efficacy Review Committee (Appendix V), the primary outcome measure was changed to “the need to discontinue T/S for any extent of rash, fever or pruritus”. There were a number of factors which led to this change:

1) The primary outcome as previously described was poorly documented:
   - in the earlier cases (before CTN involvement) the presence/absence of pruritus was not documented
   - the fever criterion was not well documented - many patients developed their reactions at night or on weekends and often elected to discontinue T/S and then saw the study nurse the following day when fever had resolved
   - many patients did not record their temperature or if so, only on one occasion when they saw the study nurse the next morning. At that point, they were frequently afebrile

2) It was felt that the major concern was the need to discontinue T/S and not necessarily the extent of the reaction. If some patients had only extensive rash and not pruritus or fever and the drug was discontinued, by the original criteria they would not be counted as a primary endpoint. In practice, they did, however, have a significant reaction to T/S and if the drug use continued, it is likely that fever +/- pruritus would follow. Therefore, a failure may inadvertently be called a success.

3) In a true intention to treat analysis, the endpoint would be the need to discontinue T/S for any reason. We did not use the approach as there is no biologic plausibility as to why NAC would prevent some of the common adverse reactions to T/S such as nausea, neutropenia, altered taste, etc. In fact, nausea or malaise could be caused by NAC or
T/S and differentiation could be difficult. If these symptoms were a major problem and more frequent than hypersensitivity, even if NAC prevented hypersensitivity reactions, this observation might not be detected in the analysis, i.e. if more patients in the T/S arm had rashes and more patients in the NAC arm had nausea, the two groups might be statistically equivalent yet NAC may be very effective in preventing hypersensitivity. Also, if NAC was later found effective at a lower dose, nausea may not be a confounder. The intention to treat analysis was maintained as a secondary outcome.

Secondary Outcomes

1) The need to discontinue T/S because of a “severe” hypersensitivity reaction as defined above.

2) Any rash/fever/pruritus. NAC rather than preventing hypersensitivity reaction may modify the severity of the reaction and enable patient to continue T/S.

3) The need to discontinue T/S for any reason.

4) Description of the extent, severity, duration of all T/S hypersensitivity reactions.

5) For the patient assigned to NAC who did not develop a reaction during the first two months, the incidence of T/S hypersensitivity reaction during the subsequent 3 months (off NAC).

6) Time to discontinuation of T/S due to allergic reaction.
1) Statistical Considerations

1. Sample Size Justification

In patients with HIV receiving T/S, the reported rates for the development of rash/fever/pruritus ranges from 5-68%. It appears that this is somewhat dose related and that for treatment the rates are 5-68% and for prophylaxis 5-40% (see Tables 1,2). A midrange baseline value of 30% was chosen for sample size calculation.

In order for NAC to be cost effective (alternative forms of prophylaxis available) and acceptable (unpleasant taste) it would need to be very effective in reducing the incidence of hypersensitivity. When the trial was initiated in Toronto, we estimated a 70% efficacy. When the trial was revised and submitted to the CTN, it was determined that this was overly optimistic and that a clinically significant difference could be missed. The sample size was therefore, based on the assumption that NAC could decrease the rate of hypersensitivity reaction requiring discontinuation of treatment by 50%, i.e. from 30% to 15%.

It was estimated that 10% of patients would be lost to follow-up before completion of the study secondary to nausea, death, entering other trials, etc. Using a 2-sided alpha of 0.05 and a power of .8, 266 patients would need to complete the study (293 enrolled in the trial) using Fischer’s exact or corrected Chi square and 240 to complete trial (264 enrolled) using the uncorrected Chi square. Expecting 15-20 patients to be enrolled per month, it was estimated that enrollment could be completed in 15 months.

2. Final Analysis

The primary analysis examined differences in the incidence of the primary outcome (need to discontinue T/S because of any extent of fever or pruritus or rash) during the first 2 months on study.
Patients receiving less than 4 weeks of T/S or NAC therapy for reasons other than an allergic reaction to T/S were omitted. Patients who receive T/S tend to be at relatively high risk for a reaction up to 4 weeks from the initiation of therapy. To treat them as success if they had less than 4 week exposure would be inappropriate.

3. Statistical Analysis

The uncorrected Chi square test was used for comparisons of categorical variables between the treatment groups. Continuous variables were compared using the t-test. The time to the T/S reaction was compared between the treatment groups with a log rank test. The logistic regression was used to determine the independent and significant association of the primary outcome, discontinuation of T/S due to hypersensitivity reaction and the following variables: age, sex, race (black), heterosexual partner as risk factor, AIDS at baseline, baseline CD4 counts, baseline TSH, use of fluconazole and treatment group. The adequacy of the models was tested using Hosmer and Lemeshow goodness-of-fit tests (Hosmer et al, 1989). A two-tailed p value of less than 0.048 (O'Brien Fleming adjustment) was considered to indicate statistical significance for all tests. Analyses were performed using SAS statistical software (version 6.11).

4. Interim Analysis (Appendix V)

The interim analysis were conducted by the Data and Methodology Centre at the CTN. Results were reported to the Safety and Efficacy Review Committee (SERC), who were responsible for making recommendations to continue or terminate the enrollment.

The original plan was to conduct a single interim analysis after half the target number of patients had completed two weeks treatment. Since the majority of adverse reactions occur
quickly after onset, it was reasonable to expect that the classification of patients as success or failure would not change after 2 weeks. During the course of the study, it became apparent that adverse reactions occurred after 2 weeks (evaluation made blind to treatment allocation) and the classification was reset to 4 weeks. The analytic strategy incorporated the efficacy approach - whereby all patients lost to follow-up or discontinuing their assigned treatment prior to the first 4 weeks were excluded. The O'Brien Fleming approach was incorporated in the analysis. The criteria for statistical significance at the interim analysis was a nominal $p$-value of .005.

The SERC met in April 1995 and after review of the first interim analysis recommended continuation of the trial. All personnel connected with the study remained blinded to the results. The SERC were concerned, however, about the enrollment rate and recommended a second interim analysis. This was conducted in August 1996 and followed the same criteria as the first interim analysis.

At that point, 234 patients were enrolled. Forty-six patients were not included in the analysis for the following reasons:

- 8 patients had recently been enrolled and did not have sufficient follow-up
- 11 patients never started study medications after randomization
- 13 patients did not have a follow-up after baseline
- 13 patients received less than 4 wks of study medication

Consequently, 188 patients were included in the primary analysis, 96 in T/S alone arm and 92 in NAC and T/S arm. In total 43 patients had to discontinue T/S because of fever/rash/pruritus: 23 patients (24%) receiving T/S alone and 20 patients (22%) receiving T/S and NAC ($p=.85$) with a 95% confidence interval - 11%, 15%.

A futility analysis was performed (Lan et al, 1984). The outcomes for evaluable patients yet to be randomized ($n=52$) were simulated on the basis of the hypothesized outcome rates (NAC
decreased hypersensitivity treatment reaction from 30% to 15%) and combined with observed results of the 188 evaluable patients to date. One thousand simulations were run and combined with the observed results to generate 1000 2 x 2 contingency tables of the primary outcome by treatment. Chi Squares were computed for each table. The proportion which resulted in a significant p-value (p<.05) was 10/1000. By employing the strategy of adjusting for multiple analyses as stipulated in the original analysis plan, then the chances of reaching statistical significance was 7/1000 or .007. Therefore, it was concluded that it was highly unlikely that the study, if continued, would result in a statistically significant difference.

This information was presented to the SERC on October 7, 1996 at the CTN biannual meetings. They recommended that the trial be terminated. These recommendations were accepted by Martin Schecter, the Director of the CTN and Sharon Walmsley, the Principal Investigator of the study. The investigators were notified and the trial closed on October 31, 1996. Patients who were receiving NAC at the time of study closure were permitted to complete their course if desired. An abstract of the interim analysis was prepared and presented at the 4th Conference on Retroviruses and Opportunistic Infections in Washington in January 1997.

IV RESULTS

As of October 2, 1996, 238 patients were enrolled in the clinical trial at 15 participating CTN sites across Canada. Figure 4 and Table 3 outline the enrollment over time by site and region. Of the 238 patients randomized, 40 patients were not included in the analysis for the following reasons: 12 patients were never started on study medications after the randomization; 14 patients were lost to follow-up after the baseline visit; and 14 patients received less than 4 weeks of T/S or NAC for reasons other than fever, rash or pruritus (Table 4).

More patients in the T/S and NAC arms were unevaluable than in the T/S alone arm (23 vs 17 respectively) but this difference was not statistically significant (p=.39). Of the 13 patients
randomized to NAC and T/S who did not receive four weeks of treatment, four discontinued NAC because of subjective side effects (3 nausea, 1 unpleasant taste) but were able to continue T/S. Of the other nine patients to receive less than 4 weeks of T/S and NAC, 2 discontinued because of unpleasant taste, 1 because of fatigue, 3 because of nausea and vomiting, 1 because of arm pain and the other 2 were lost to follow-up. The proportion of un evaluable patients (4/238 = 17%) exceeds that of the 10% estimate used for the sample size calculation. Therefore, 198 patients were included in the final analysis, including 102 randomized to T/S and 96 randomized to NAC and T/S.

The baseline characteristics of patients randomized to the two study groups are outlined in Table 5. The mean CD4 count of all the randomized patients was 154/mm³ and only 12% of patients had a previously documented AIDS-defining illness. The only significant differences between the treatment groups with respect to baseline characteristics were the percentage of men (86% vs 98%), proportion of patients reporting heterosexual contact as their primary risk transmission category (29% vs 16%), and the proportion of black patients (19% vs 7%) for patients randomized to T/S and NAC and T/S respectively. There were similar differences in the distribution when all randomized study patients were considered.

Primary Outcome (Tables 6, 7)

There were 45 patients (23% of total) who had to discontinue T/S because of any extent of fever, rash or pruritus including 25/102 (25%) of patients on T/S alone and 20/96 (21%) of patients randomized to T/S and NAC (p=.65) (Table 6). The difference in the incidence of allergic reactions requiring T/S discontinuation between treatment groups is 4% (95% CI -16%, +9%). There were no statistically significant differences in the specific symptoms leading to drug discontinuation between the two groups.

Using the uncorrected Chi Square test for categorical variables, and the t test for continuous variables, there was no statistically significant difference (p<.048) in the incidence of T/S
hypersensitivity reaction with regard to potential prognostic variables including gender, race, previous AIDS diagnosis, CD4, or concurrent use of fluconazole (Table 7a). The only factor independently associated with a statistically significant increase in T/S hypersensitivity reactions was an increased baseline serum TSH, \( p = .04 \). Following a logistic regression analysis, incorporating the above variables again baseline serum TSH was the only factor predictive of a hypersensitivity reaction, O.R. 1.284, 95% confidence interval 1.028-1.603, \( p = .0275 \) (Table 7b). The adequacy of the model was confirmed by the goodness of fit test of Hosmer and Lemeshow, \( p = .97 \).

**Secondary Outcomes (Tables 6, 8, 9, 10)**

The incidence of severe hypersensitivity reactions characterized by any two of a) fever > 38.5°C on two occasions; b) intolerable pruritus; c) rash > 50% of body surface area was not statistically significantly different between the two treatment arms. This severe reaction occurred in 10 (10%) of patients randomized to T/S alone and 6 (6%) of patients randomized to T/S and NAC (\( p = .51 \)). In total 34 (33%) of patients randomized to T/S alone and 33 (36%) of patients randomized to T/S and NAC had to discontinue T/S for any reason (\( p = .88 \)). There were 4 minor allergic reactions in the T/S alone arm and 1 in the T/S and NAC arm that resolved and did not result in the discontinuation of study medications. Therefore, overall any hypersensitivity symptoms occurred in 21 (23%) of T/S and NAC patients and 29 (28%) in the T/S group alone (\( p = .29 \)). Using the log rank test, there was no statistically significant difference between the treatment groups (\( p = .52 \)) in the time to discontinuation of T/S because of a hypersensitivity reaction (Table 8). The median number of days to drug discontinuation was 13 days and 14 days in the T/S alone and the T/S and NAC treatment groups respectively.

Of the patients randomized to T/S and NAC, no patients developed an allergic reaction after two months (i.e. after the NAC was discontinued) while they remained on T/S. Similarly, of the 4 patients who continued T/S after discontinuing NAC prior to 4 weeks of treatment, none had a subsequent allergic reaction.
There were more patients (24%) in the T/S and NAC arm who discontinued therapy after baseline for reasons other than fever/rash/pruritus than in the patients randomized to T/S alone (10%) \( p=0.01 \). The largest proportion of the discontinuation were in the first 4 weeks of therapy and were related to intolerance (taste, nausea or vomiting) to NAC (Table 9). When analyzed by year of enrollment, there was no significant difference in the frequency of T/S reactions \( p=0.86 \) (Table 10).

Characteristics of the T/S Hypersensitivity Reaction (Table 11)

Overall 50/198 patients in this study developed fever, rash or pruritus that was thought to be related to the use of T/S and in 45/50 patients the drug was discontinued because of these symptoms. In a multivariate analysis using a logistic regression, there was no correlation with the development of any of these symptoms and age, gender, race, heterosexual intercourse as a risk factor, prior AIDS, concurrent use of fluconazole or baseline CD4 (Table 7). The only factor found to be associated was increased baseline thyroid stimulating hormone (TSH) \( OR=1.284, 95\% \text{ CI} 1.028-1.603, p=0.0275 \). The skin reaction, occurring in 40 patients, was described as erythematous-flat in 47.5%, erythematous-raised in 45% and involving the mucosa in 7.5%. The reaction involved more than 50% body surface area in 70%. In all cases, the allergic symptoms resolved with the discontinuation of T/S. The median duration of the rash was 5 days range 1-45 days. For those who experienced pruritus, it was described as mild in 25%, moderate in 15% and intolerable in 60%. Antihistamines, non-steroidal anti-inflammatory agents, acetaminophen, acetyl salicylic acid or steroids were taken by 100% of patients with a reaction. There were no cases of exfoliative dermatitis or Stevens-Johnston reaction. There were no cases of associated encephalitis, pneumonitis, epididymitis, myocarditis, or thyroiditis. Two patients reported arthralgias and one myalgias. During the reaction, 11% of patients had neutropenia \(< 1000/\text{mm}^3 \) and 19% eosinophilia \( > 400 \times 10^9/\text{L} \). The Coomb's test became positive in 2/19 (11%). Increases in liver transaminases greater than 5 times above baseline was noted in 3%. Increases in amylase greater than twice the
baseline was not noted in any patient. Renal insufficiency (creatinine increased twice baseline) was not noted in any patient. Increases in TSH were not noted in any patient during the reaction. New abnormalities on chest x-ray or electrocardiogram were not detected in any patient during the reaction.

V LIMITATIONS OF THE STUDY

a) The Single Blind Design

Blinding techniques and placebo provide the means for eliminating factors unintentionally associated with treatment and to protect against the pre-determined biases and prejudices of the patients and physicians participating in the trial. As there was some subjective interpretation of the primary outcome of this study, i.e. the need to discontinue T/S, the physician might be more or less likely to determine the patient has reached a primary endpoint if he or she believes the patient to be receiving NAC, especially if the patient may have inadvertently unblinded him/her to randomization. Similarly, a patient or study nurse may be more or less likely to report adverse symptoms and insist upon being discontinued from the trial based upon knowledge of the randomization arm. The knowledge of whether or not a patient was assigned to NAC may have influenced their decision to start assigned treatment or withdraw from the study. Although the lack of blinding may have introduced biases in either direction, it was not felt that the magnitude of the potential effect of the lack of unblinding on outcome was significant.

In retrospect, most patients would be unfamiliar with the taste of liquid NAC. It may have been possible to identify a substance with an unpleasant taste which could have been used as a placebo. Perhaps even 1 ml of NAC could have changed the taste of cola or orange juice sufficiently to allow for blinding without influencing efficacy.
b) **NAC Dose and Absorption and Timing**

The dose and timing of NAC needed to prevent T/S hypersensitivity reactions (if possible) is unclear. It is possible that higher or more frequent doses than utilized in this study may have been protective. There were more patients who discontinued the trial or were lost to follow-up who were assigned to NAC and T/S. Given the unpleasant taste, compliance (despite recording to the contrary) may have been poor. Therefore, patients may not have received enough drug to demonstrate an effect. It was unclear whether or not the patient should have received a “loading dose” of NAC prior to challenge with T/S. As the reactions typically occurred 1-2 weeks into therapy, this was not felt to be important. A study of 50 patients did not find that use of NAC 800 mg/d for the two weeks prior to initiation of T/S had any effect on the frequency of hypersensitivity reactions to T/S relative to placebo (Akerlund et al, 1995).

We also recommended that the NAC be given one hour prior to the dose of T/S. It is unclear how compliant patients were with this schedule and whether or not it was important. It was also unclear whether or not the NAC was absorbed from the gastrointestinal tract and to what extent and if so, whether or not it was converted to glutathione. Although we had originally planned to measure thiol levels in patients pre- and post- treatment, this was not feasible. Measurement of glutathione is technically difficult and our previous work has demonstrated that samples need to be processed within five minutes of blood letting. This was not possible in the context of a multi-centred clinical trial and even not feasible for a single centre. These evaluations will need to be done outside the study context to better understand the observed lack of efficacy.

c) **Subjectivity and Documentation of Primary Endpoint**

There is variability on the part of physicians as to their “threshold” of tolerance of the signs and symptoms of T/S hypersensitivity and the need to discontinue treatment. This diagnostic suspicion bias can also vary in a physician from one patient to another. Although some physicians might try to push through a reaction with graval, aspirin or tylenol, other physicians may discontinue treatment immediately based on their past experience. As outlined
previously, despite attempts to make the primary endpoint as objective as possible, details of the T/S reaction were often poorly documented. This could lead to misclassification of patients. Although all patients were instructed on what to do in the event of a reaction, they occasionally developed the endpoint at night or on a weekend and presented themselves to on-call physicians or to Emergency Room doctors who were unfamiliar with the protocol. Although we attempted to have two physicians assess the primary endpoint independently, this frequently was not possible. The need for reclassification of some endpoints by the principal investigator who remained blinded to the randomization arm for some patients supports the notion that there was some error involved. None of these events occurred with sufficient frequency to have significantly influenced the outcome of the trial.

**d) Lost To Follow-up**

Twelve patients, although randomized, never started study medications and 14 were lost to follow-up after baseline. In both cases this occurred more frequently in the T/S alone arm. There could be some systematic biases introduced consequent to this high rate of drop off. Patients have selected to or not to participate depending on whether or not they received NAC. Patients may have not tolerated the NAC and elected not to follow-up subsequently. Patients may have developed an allergic reaction and elected not to return for follow-up.

**e) Duration of the Trial**

Recruitment for this trial was much slower than anticipated. HIV disease is rapidly evolving and through the duration of the trial, new treatments of the underlying diseases were introduced. These treatments could have had impact upon the primary outcome. For example, ritonavir, one of the new protease inhibitors which was introduced in 1996, is able to inhibit cytochrome p450, an enzyme important in T/S metabolism. Inhibition of this enzyme could theoretically result in decrease of hydroxylamine metabolites and therefore, decrease the risk of T/S hypersensitivity. We did not find that the rate of T/S varied by years with the percentage of reactions not significantly different for patients enrolled in 1991 through 1996. Although
recruitment was slow we do not feel that this affects the generalizability of the results. Slow recruitment was more frequently because the doctor did not remember or recommend the patient to the study rather than a selection for the "right" patient or refusal by the latter.

f) Power

This study was designed to detect a 50% decrease in the primary endpoint. It was felt that given the poor taste and additional cost associated with the use of NAC in its current formulation that it needed to be fairly effective to be used routinely. It is possible that NAC had a clinical benefit of 20-30% that could not be detected in this trial. If a cheaper, more tolerable, more efficacious formulation could be developed, a lesser decrease in reaction rate could be clinically relevant.

There were more patients unevaluable than predicted prior to study onset. The study was terminated prior to achieving the 240 evaluable patients estimated to be necessary to demonstrate effect. The overall incidence of T/S hypersensitivity reactions of 23% was lower than the 30% used to calculate the sample size. These three factors combined served to decrease the power of the trial. With the number of patients that were available for evaluation at study termination, the power to detect the hypothesized rate decrease was 0.72 with a significance of 0.048. The futility calculation determined that there was only a .007 chance of the trial reaching statistical significance if all remaining patients were enrolled, so we believe that we have confirmed our observation. The confidence intervals around the primary outcome are narrow (-16%, +9%), further enforcing the confidence in the conclusions.

A different study design in which NAC was used to rechallenge patients with previous history of T/S hypersensitivity reactions could have decreased the number of patients required for study. Although considered initially, at the time the trial was designed it felt it was unethical to rechallenge patients with a history of adverse reaction because of concerns about serious and life-threatening events. Further, the decreased efficacy of aerosolized pentamidine as primary and secondary PCP prophylaxis had not been realized at that time, and this form of
prophylaxis was felt to be a safer alternative than rechallenge with T/S.

g) Imbalances in Baseline Demographics

There were more men randomized to the T/S alone arm. Consistent with this, more patients in the T/S and NAC arm reported heterosexual transmission as their primary HIV risk group. There is no biological reason to suspect that the incidence of T/S reactions would vary with gender and in fact, in this trial, hypersensitivity reactions were observed in similar proportions of women and of men (p=NS). Similarly, there was no reason to suspect that NAC would have differential beneficial effects between the genders. There were more black patients randomized to the T/S and NAC group. This may have had an overall effect on outcome, however, similar proportions of black patients had T/S reactions. Acetylator phenotype has been associated with race with a lower rate of slow acetylator phenotype in oriental populations and an increased incidence in Arabic populations. No differences have been described between black and Caucasian races. There is no published data on different rates of hypersensitivity reaction by race. The randomization scheme should have allocated slow acetylators equally between the arms. The differences in the baseline characteristics was felt to have occurred by chance alone given the randomization scheme that was used. Similar differences in characteristics were seen when all enrolled patients were considered reinforcing that there was no evidence of selective drop out by any one group. Also in the analysis none of the baseline characteristics were shown to be independent risk factors for the primary outcome.

VI CONCLUSION

_Pneumocystis carinii_ pneumonia remains the commonest opportunistic infection complicating HIV (Wilk et al, 1997). Despite the effectiveness of T/S for treatment and for primary and secondary prevention of this infection, hypersensitivity reactions to T/S are often dose-limiting. The reason for the increased incidence of T/S hypersensitivity reactions for patients living with HIV remains unknown. Our study failed to show a protective effect of NAC in
preventing T/S hypersensitivity reactions when used together for primary PCP prevention. In this study, we could not demonstrate that NAC at a dose of 3 gm bid (in the 20% solution) could prevent, delay or modify the features of the T/S reaction.

Overall, 23% of patients initiating primary PCP prophylaxis with one single strength tablet of T/S twice daily developed a hypersensitivity reaction requiring the drug to be discontinued. This value is consistent with the literature where the incidence of reactions varies from 5-40%. The use of T/S as one single strength tablet twice daily did not appear to be associated with a lower rate of reaction than the standard dosing schedule of one double strength daily or three times weekly.

The commonest symptom resulting in drug discontinuation was rash with or without fever or pruritus (n=41). Fever (n=2) or pruritus (n=2) alone were much less common. The reaction occurred within 14 days for the majority of patients (62%) although another 24% developed a reaction between week 2 and week 4. This indicates the high risk period over which patients must be observed following the initiation of therapy. Although 8% of patients overall had a more severe reaction which we defined as any two of rash > 50% body surface area, intolerable pruritus or fever > 38.5°C on two occasions, no cases of Steven-Johnson or exfoliative dermatitis were seen. Organ involvement as part of the hypersensitivity reaction was uncommon. We could not identify any patient demographics associated with increased risk of reaction, such as gender, race or previous AIDS diagnosis. Unlike previous data, CD₄ level did not appear to affect the rate of reactions. Despite the changing nature of HIV care, the incidence of reactions did not change over time. Although fluconazole has been shown to reduce the formation of the sulfamethoxazole hydroxylamine metabolite in healthy volunteers and in vitro in human liver microsomes (Mitra et al, 1996) its use did not appear to be protective in this study, however, the number of patients on this antifungal agent was small. The only factor found to be statistically associated with the hypersensitivity reaction was baseline TSH value. This difference does not make biologic sense and average values in patient groups who did and did not have a reaction remain within the normal range of laboratory values. Therefore, although statistically significant, this was not felt to be clinically
NAC was not well-tolerated in the present formulation. Even if it had been shown to be efficacious in this study, it is unlikely that many patients would be able to tolerate the dose used for prolonged periods of time. Overall, 12/109 (11%) of patients had to discontinue NAC because of nausea, vomiting or unpleasant taste as opposed to only 1/103 (1%) of patients discontinuing drug for these reasons in the T/S only group. A percentage of the withdrawal and lost to follow-up which was higher in the NAC and T/S group may have been attributed to these side effects as well.

The results of this trial do not support the hypothesis that glutathione deficiency increases the risk of T/S hypersensitivity reactions in HIV (van der Ven et al, 1991). However, we can also not conclude that the hypothesis is incorrect. We cannot be certain that sufficient NAC was taken or absorbed to increase glutathione levels sufficiently to detoxify reactive T/S metabolites. Detailed pharmacokinetic and pharmacodynamic studies including measurements of intra-cellular plasma thiol of patients with HIV and AIDS using different doses, timing and formulations of NAC and other compounds could help to clarify this issue. This study was designed to detect a 50% decrease in the primary endpoint. It was felt that given the poor taste and additional cost associated with the use of NAC in its current formulation that it needed to be fairly effective to be used routinely. It is possible that NAC had a clinical benefit of 20-30% that could not be detected in this trial. If a cheaper, more tolerable, more efficacious formation could be developed, a lesser decrease in reaction rate could be clinically relevant. Perhaps the use of other compounds for generating glutathione, such as procysteine, glutathione esters or liposomal glutathione would be more effective given their increased absorption (Kalayjian et al, 1994; Porchaska et al, 1993; Giorgi et al, 1992).

Even if glutathione deficiency is responsible for increasing T/S reactions we are not certain of the mechanism of this deficiency. Increasing serum levels of glutathione might be effective if the mechanism of the deficiency were increased catabolism or increased conjugation, elimination or oxidation. If the problem were one of decreased synthesis, use of NAC may not
correct this deficiency. One group has demonstrated that NAC may not increase glutathione concentrations in vivo possibly due to decreased level of the enzyme gamma glutamyl cysteine synthetase necessary for the synthesis of GSH from CYS (Witschi et al, 1995). The amount of NAC used may have been insufficient especially if other sources of oxidative stress, such as HIV replication itself, acute infections or other medications contributed to the glutathione deficiency. There may be other confounding factors which may have unbalanced the two groups in regards to the risk of T/S hypersensitivity reactions such as acetylator phenotype (Carr et al, 1994) or medications that could interfere with T/S metabolism and influence the risk of T/S hypersensitivity. However, we would have expected the randomization process to have minimized this risk. We did not measure acetylation phenotype status in this trial. Measurement of the phenotype involves use of caffeine as a metabolic probe. Urine must be collected for an eight hour period after the ingestion of a standard dose of caffeine, stored at -20°C and then analyzed by high pressure liquid chromatography. This was not practical for a multicentered trial. Further, patients must discontinue all medications which could alter cytochrome p450 function for at least four days prior to the assay. As many HIV associated medications use this metabolic pathway their discontinuation would not have proven ethical. To fully evaluate acetylator phenotype, genotyping must also be performed. Intercurrent illness can change phenotype but not genotypic acetylation status.

Despite the rationale behind the hypothesis of glutathione deficiency contributing to the increased rate of T/S reactions, this theory could be incorrect. In parallel work in which we employed a highly constrained sample collection and preparation protocol with both plasma and lymphocytes, we could not observe any differences in the concentrations of thiols or disulfides or in thio disulfide ratios in patients who did not report a history of T/S hypersensitivity reaction (Walmsley et al, 1997). This suggests that the mechanism of this predisposition involves factors other than thiol/disulfide status.

In summary, in this study the use of NAC could not be shown to decrease the incidence of T/S hypersensitivity reactions when used for the primary prevention of PCP in HIV. The reason for the increased incidence of reactions relative to the general population remains unknown.
REFERENCES


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.


Mallolas J, Zamora L, Gatell JM et al. Primary prophylaxis for *Pneumocystis carinii* pneumonia: a randomized trial comparing cotrimoxazole, aerosolized pentamidine and dapsone plus pyrimethamine. AIDS. 1993; 7:59-64.


Walker RE, Lane HC, Boenning CM et al. The safety, pharmacokinetics and antiviral activity of N-acetylcysteine in HIV-infected individuals. 8th International Conference on AIDS. Amsterdam, 1992. Abstract # MoB 0022.


Table 1: Reported Incidence of Sulphonamide Hypersensitivity in HIV When Used For *Pneumocystis carinii* Pneumonia Prophylaxis

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal/Year</th>
<th>T/S Dose</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fischl</td>
<td>JAMA 1988</td>
<td>DS bid</td>
<td>30%</td>
</tr>
<tr>
<td>Ruskin</td>
<td>Lancet 1991</td>
<td>DS MWF</td>
<td>25%</td>
</tr>
<tr>
<td>Stein</td>
<td>AAC 1991</td>
<td>DS MWF</td>
<td>7-13%</td>
</tr>
<tr>
<td>Blum</td>
<td>J AIDS 1992</td>
<td>DS od</td>
<td>40%</td>
</tr>
<tr>
<td>Schneider</td>
<td>NEJM 1992</td>
<td>SS - DS od</td>
<td>21-26%</td>
</tr>
<tr>
<td>Hardy</td>
<td>NEJM 1992</td>
<td>DS</td>
<td>10%</td>
</tr>
<tr>
<td>Martin</td>
<td>Arch Int Med 1992</td>
<td>DS bid MWF</td>
<td>23%</td>
</tr>
<tr>
<td>Podzamczer</td>
<td>AIDS 1993</td>
<td>DS bid MWF</td>
<td>6%</td>
</tr>
<tr>
<td>Mallolas</td>
<td>AIDS 1993</td>
<td>DS MWF</td>
<td>10%</td>
</tr>
<tr>
<td>Neilsen</td>
<td>Dan Med Bull 1993</td>
<td>SS od</td>
<td>5%</td>
</tr>
<tr>
<td>Schneider</td>
<td>JID 1995</td>
<td>SS-DS od</td>
<td>30-40%</td>
</tr>
<tr>
<td>Bozette</td>
<td>NEJM 1995</td>
<td>DS bid</td>
<td>20%</td>
</tr>
</tbody>
</table>

DS double strength (160 mg trimethoprim, 800mg sulfamethoxazole)
SS single strength (80 mg trimethoprim, 400 mg sulfamethoxazole)
OD once daily
BID twice daily
MWF every Monday, Wednesday and Friday
T/S trimethoprim-sulfamethoxazole
Table 2: Reported Incidence of Sulphonamide Hypersensitivity in HIV When Used For *Pneumocystis carinii* Pneumonia Treatment

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal/Year</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gordin</td>
<td>Ann Intern Med 1984</td>
<td>53%</td>
</tr>
<tr>
<td>Small</td>
<td>Arch Int Med 1985</td>
<td>41%</td>
</tr>
<tr>
<td>Wharton</td>
<td>Ann Intern Med 1986</td>
<td>67%</td>
</tr>
<tr>
<td>Smith</td>
<td>AIDS 1992</td>
<td>13%</td>
</tr>
<tr>
<td>Klein</td>
<td>AIDS 1992</td>
<td>12%</td>
</tr>
<tr>
<td>Medina</td>
<td>NEJM 1990</td>
<td>37%</td>
</tr>
<tr>
<td>Hughes</td>
<td>NEJM 1993</td>
<td>17%</td>
</tr>
<tr>
<td>Toma</td>
<td>CID 1993</td>
<td>28%</td>
</tr>
<tr>
<td>Hughes</td>
<td>JID 1995</td>
<td>23%</td>
</tr>
<tr>
<td>Walmsley</td>
<td>J AIDS 1995</td>
<td>29%</td>
</tr>
<tr>
<td>Sattler</td>
<td>JID 1994</td>
<td>5%</td>
</tr>
<tr>
<td>Shafer</td>
<td>J AIDS 1989</td>
<td>68%</td>
</tr>
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</table>
Table 3: Enrollment by Investigator and Center

<table>
<thead>
<tr>
<th>Site</th>
<th>Investigator</th>
<th>Pre-CTN</th>
<th>Post-CTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic</td>
<td>Thompson</td>
<td>---</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Schlech</td>
<td>---</td>
<td>11</td>
</tr>
<tr>
<td>Quebec</td>
<td>Duperval</td>
<td>---</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Senay</td>
<td>---</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Toma</td>
<td>---</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Tsoukas</td>
<td>---</td>
<td>5</td>
</tr>
<tr>
<td>Ontario</td>
<td>Cohen</td>
<td>---</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Fong</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Ford</td>
<td>---</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Mackie/Gilmore</td>
<td>---</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Rachlis</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Smaill</td>
<td>---</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Walmsley</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>Prairies</td>
<td>Williams</td>
<td>---</td>
<td>6</td>
</tr>
<tr>
<td>Pacific</td>
<td>Montaner</td>
<td>---</td>
<td>15</td>
</tr>
<tr>
<td>Subtotal</td>
<td></td>
<td>60</td>
<td>178</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>238</td>
<td></td>
</tr>
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</table>

CTN = Canadian HIV Trials Network
Table 4: Enrollment Summary

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>T/S and NAC</th>
<th>T/S alone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized</td>
<td>119</td>
<td>119</td>
<td>238</td>
</tr>
<tr>
<td>Who never started septra</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>No follow-up after baseline</td>
<td>6</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Less than 4 weeks of septra</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Less than 4 weeks of NAC</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Included in the primary analysis</td>
<td>96</td>
<td>102</td>
<td>198</td>
</tr>
</tbody>
</table>

T/S = trimethoprim-sulfamethoxazole
NAC = N’acetylcysteine
<table>
<thead>
<tr>
<th></th>
<th>T/S and NAC (n=96)</th>
<th>T/S alone (n=102)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% Male)</td>
<td>83 (86%)</td>
<td>100 (98%)</td>
<td>.005</td>
</tr>
<tr>
<td>Race/Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>69 (72%)</td>
<td>89 (87%)</td>
<td>.052</td>
</tr>
<tr>
<td>Black</td>
<td>18 (19%)</td>
<td>7 (7%)</td>
<td></td>
</tr>
<tr>
<td>Oriental</td>
<td>1 (1%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>2 (2%)</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>6 (6%)</td>
<td>2 (2%)</td>
<td></td>
</tr>
<tr>
<td>HIV Risk Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual</td>
<td>58 (61%)</td>
<td>60 (59%)</td>
<td>.95</td>
</tr>
<tr>
<td>Bisexual</td>
<td>11 (12%)</td>
<td>13 (13%)</td>
<td>.91</td>
</tr>
<tr>
<td>IV Drug Use</td>
<td>9 (9%)</td>
<td>12 (12%)</td>
<td>.72</td>
</tr>
<tr>
<td>Heterosexual Contact</td>
<td>28 (29%)</td>
<td>16 (16%)</td>
<td>.041</td>
</tr>
<tr>
<td>Transfusion</td>
<td>3 (3%)</td>
<td>2 (2%)</td>
<td>.96</td>
</tr>
<tr>
<td>Other</td>
<td>9 (9%)</td>
<td>20 (20%)</td>
<td>.06</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1%)</td>
<td>3 (3%)</td>
<td>.66</td>
</tr>
<tr>
<td>Age (mean, std dev)</td>
<td>38.1 (11.2)</td>
<td>38.6 (9.3)</td>
<td>.76</td>
</tr>
<tr>
<td>Disease Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>48(50%)</td>
<td>52 (51%)</td>
<td>.85</td>
</tr>
<tr>
<td>ARC</td>
<td>33(34%)</td>
<td>37 (36%)</td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>13(14%)</td>
<td>10 (10%)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (2%)</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>Thyroid Stimulating</td>
<td>1.92 (1.40)</td>
<td>2.18 (1.53)</td>
<td>.24</td>
</tr>
<tr>
<td>Hormone (mean, std dev)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD, count (mean, std dev)</td>
<td>148 (92)</td>
<td>160 (106)</td>
<td>.40</td>
</tr>
</tbody>
</table>

categorical variable compared by uncorrected Chi Square test, continuous variables by the t-test
ARC - AIDS related complex
Table 6:  Reason For Discontinuation of Trimethoprim-Sulfamethoxazole (T/S) by Treatment Arm

<table>
<thead>
<tr>
<th>Reason for Discontinuation</th>
<th>T/S and NAC (n=96)</th>
<th>T/S alone (n=102)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>6</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Rash and fever</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Rash and pruritus</td>
<td>1</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Rash, pruritus and fever</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total hypersensitivity related*</td>
<td>20</td>
<td>25</td>
<td>45</td>
</tr>
<tr>
<td>Lost to follow-up/compliance</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>34</td>
<td>67</td>
</tr>
</tbody>
</table>

*Chi Square Test p=.65

Difference in the incidence of hypersensitivity reactions between treatment groups is 4% (95% confidence interval -16%, +9%)
Table 7A: Primary Outcome and Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>No Primary Outcome n=153</th>
<th>Primary Outcome n=45</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>38.3 (± 10.1)</td>
<td>38.6 (±10.9)</td>
<td>.8754*</td>
</tr>
<tr>
<td>Sex</td>
<td>140 (92%)</td>
<td>43 (96%)</td>
<td>.366**</td>
</tr>
<tr>
<td>Race</td>
<td>23 (15%)</td>
<td>2 (4%)</td>
<td>.060**</td>
</tr>
<tr>
<td>Heterosexual</td>
<td>31 (20%)</td>
<td>13 (29%)</td>
<td>.216**</td>
</tr>
<tr>
<td>Diagnosis of AIDS</td>
<td>17 (11%)</td>
<td>6 (13%)</td>
<td>.683**</td>
</tr>
<tr>
<td>CD₄ (cells/mm³)</td>
<td>158.6 (±103.4)</td>
<td>140 (±83.9)</td>
<td>.2809*</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>1.92 (±1.32)</td>
<td>2.46 (±1.84)</td>
<td>.0357*</td>
</tr>
</tbody>
</table>

* t-test
** Chi Square
Table 7B: Logistic Regression - Primary Outcome and Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.013</td>
<td>0.972 - 1.056</td>
<td>.5445</td>
</tr>
<tr>
<td>Sex (Male)</td>
<td>3.054</td>
<td>0.514 - 18.135</td>
<td>.2193</td>
</tr>
<tr>
<td>Race (Black)</td>
<td>0.330</td>
<td>0.068 - 1.603</td>
<td>.1692</td>
</tr>
<tr>
<td>Heterosexual partner</td>
<td>2.095</td>
<td>0.828 - 5.302</td>
<td>.1183</td>
</tr>
<tr>
<td>Diagnosis of AIDS</td>
<td>0.832</td>
<td>0.259 - 2.667</td>
<td>.7564</td>
</tr>
<tr>
<td>CD$_4$ (100 cells/mm$^3$)</td>
<td>0.800</td>
<td>0.521 - 1.228</td>
<td>.3066</td>
</tr>
<tr>
<td>Use of fluconazole</td>
<td>2.435</td>
<td>0.658 - 9.013</td>
<td>.1826</td>
</tr>
<tr>
<td>Treatment arm</td>
<td>0.842</td>
<td>0.391 - 1.812</td>
<td>.6601</td>
</tr>
<tr>
<td>TSH (mU/L)</td>
<td>1.284</td>
<td>1.028 - 1.603</td>
<td>.0275</td>
</tr>
</tbody>
</table>

Hosmer and Lemeshow goodness of fit p=.97
Table 8: Time of Discontinuation of Trimethoprim-Sulfamethoxazole (T/S) Due To Allergic Reaction

<table>
<thead>
<tr>
<th>Time Period</th>
<th>T/S and NAC</th>
<th>T/S alone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 week</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1-2 weeks</td>
<td>11</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>2-4 weeks</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 4 weeks</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>25</td>
<td>45</td>
</tr>
</tbody>
</table>

log rank test p=.52
Table 9: Patients Who Discontinued Therapy for Reasons Other Than Fever/Rash/Pruritus Following the Baseline Evaluation

<table>
<thead>
<tr>
<th></th>
<th>T/S and NAC (n=109)</th>
<th>T/S (n=103)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 weeks</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 4 weeks</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td><strong>26 (24%)</strong></td>
<td><strong>10 (10%)</strong></td>
</tr>
</tbody>
</table>

*uncorrected Chi Square test
Table 10: Frequency of Trimethoprim-Sulfamethoxazole Hypersensitivity Reactions by Year of Enrollment

<table>
<thead>
<tr>
<th>Study Year</th>
<th>Number Enrolled</th>
<th>Number Included</th>
<th>Number Discontinued Due to T/S Reaction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>12</td>
<td>8</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>1992</td>
<td>18</td>
<td>16</td>
<td>5 (31%)</td>
</tr>
<tr>
<td>1993</td>
<td>30</td>
<td>23</td>
<td>4 (17%)</td>
</tr>
<tr>
<td>1994</td>
<td>51</td>
<td>44</td>
<td>12 (27%)</td>
</tr>
<tr>
<td>1995</td>
<td>83</td>
<td>71</td>
<td>15 (21%)</td>
</tr>
<tr>
<td>1996</td>
<td>44</td>
<td>36</td>
<td>7 (19%)</td>
</tr>
</tbody>
</table>

The incidence of reactions between treatment groups over time were compared with a log rank test, p=0.86.
Table 11: Symptoms Among Patients Who Discontinued Trimethoprim-Sulfamethoxazole (T/S) Because Of Hypersensitivity

<table>
<thead>
<tr>
<th>Symptom</th>
<th>T/S and NAC (n=20)</th>
<th>T/S alone (n=25)</th>
<th>Total (n=45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash &gt; 50% BSA</td>
<td>12</td>
<td>16</td>
<td>28</td>
</tr>
<tr>
<td>Rash</td>
<td>7</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>Erythematous/macular</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Erythematous/raised</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mucosal</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Intolerable pruritus</td>
<td>9</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Temperature &gt; 38° C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BSA = body surface area
Figure 1. Glutathione Metabolism

cysteine precursors
  - N-acetylcysteine
  - procysteine
deeacylation

cysteine (CYS)
  δ-glutamylcysteine synthetase
  glutathione synthetase

glutathione

  glutathione reductase
glutathione disulfide (GSSH)

  toxic reactive intermediates (oxidative stress)
Figure 2. Metabolism of Sulfamethoxazole

- Sulfamethoxazole
  - N-acetyltransferase: 50-70%
    - N-acetylsulfamethoxazole
      - Cytochrome p450: 10-15%
        - Hydroxylamine metabolite
          - Reactive metabolite
            - Scavenged by glutathione
              - Glutathione conjugate
                - Hypersensitivity reaction
  - Covalent binding to cellular proteins
  - Variable %
Figure 3. Potential Effects of N-acetylcysteine and Anti-retroviral Drugs on HIV Expression

HIV (RNA virus)

\[ \text{RT inhibitors} \quad \text{inhibit} \quad \text{reverse transcriptase} \]

Proivirus (dsDNA)

\[ \text{integration} \]

Latency

\[ \text{NAC} \quad \text{inhibits} \quad \text{NFKB activation} \]

Expression
HIV transcription & replication

\[ \text{cytokines} \quad \text{TNF, IL-1, IL-6} \quad \text{interferons} \]

reactive oxygen species (oxidative stress)

\[ \text{RT} = \text{reverse transcriptase} \quad \text{ddC} = \text{dideoxycytidine} \quad \text{NFKB} = \text{nuclear factor kappa B} \]
\[ \text{AZT} = \text{zidovudine} \quad \text{3TC} = \text{lamivudine} \quad \text{TNF} = \text{tumor necrosis factor} \]
\[ \text{DDI} = \text{didanosine, videx} \quad \text{d4T} = \text{stavudine, zerit} \quad \text{IL} = \text{interleukin} \]
Patient Enrollment = Actual Enrollment
# of Sites with Ethics Approval = Expected Enrollment

Month

NAC Enrollment

Jan-Dec 1991
Jan-Dec 1992
Jan-Dec 1993
Jan 1994
Feb 1994
Mar 1994
April 1994
May 1994
June 1994
July 1994
Aug 1994
Sept 1994
Oct 1994
Nov 1994
Dec 1994
Jan 1995
Feb 1995
Mar 1995
Apr 1995
May 1995
June 1995
July 1995
Aug 1995
Sept 1995
Oct 1995
Nov 1995
Dec 1995
Jan 1996
Feb 1996
Mar 1996
Apr 1996
May 1996
June 1996
July 1996
Aug 1996
Sept 1996
Oct 1996
APPENDICES
Appendix I

Documentation of Study Development

- investigational IND
- ethics approval and consent
- funding PSI
- agreement to supply drug
  (Bristol Myers Squibb)
- letters to CTN investigators
- approval from CTN
- agreement for continued drug supply
  (Roberts Pharmaceutical)
- standard operating procedures
April 23, 1992

Director,
Bureau of Human Prescription Drugs
Place Vanier
Tower B
355 River Road,
Vanier, Ontario
K1A 1B8

Dear Sirs:

We submit the following protocol entitled "A randomized trial of the use of N-acetylcysteine for the prevention of sulfonamide hypersensitivity reactions when used for the treatment and prevention of Pneumocystis carinii pneumonia in patients with Human Immunodeficiency Virus Infections" for an investigational new drug IND.

As you know this drug has been on the Canadian market as adjunctive therapy for patients with abnormal or viscid mucous secretions, and also as an antidote to prevent or lessen hepatic injury which may occur following the ingestion of a potentially hepatotoxic quantity of acetaminophen.

The study will be primarily supported by a grant from the Physicians Services Incorporated (PSI) Foundation. The manufacturer of the agent will be supplying the drug, thus the reason for this IND submission.

If you require further information, please feel free to contact me.

Sincerely,

Sharon Walmesley M.D. F.R.C.P.(C)
Dr. Sharon Walmsley  
Sunnybrook Health Science Centre 
Dept. of Medicine/Medical Microbiology 
Infectious Diseases  
2075 Bayview Ave., Suite A-226  
Toronto, Ont. M4N 3M5

Dear Dr. Walmsley:

This will confirm the receipt of your preclinical New Drug Submission for N-ACETYLCYSTEINE, control number 1HP921398 and your letter dated April 23, 1992, which was received in this office on May 1, 1992. This material has been forwarded to the Bureau of Human Prescription Drugs. You are requested to refer to the file number and control number in any communication relating to this preclinical submission.

You are reminded that under paragraph C.08.005 (1) (b) of the Food and Drug Regulations, the sale of a new drug for clinical testing is prohibited if, within 60 days after the date of receipt of the preclinical submission, the Director has sent a notice by registered mail that the preclinical submission is not satisfactory.

You are further reminded of the requirements respecting labelling and qualified investigators contained in paragraphs C.08.005 (1) (c), (d), and (e) of these Regulations.

Yours sincerely,

Doris Cook (Mrs.)  
Submission and Notification Administration Division

Canada
May 24, 1994

Dr. Claire A. Franklin
Director
Bureau of Human Prescription Drugs
Place Vanier
Tower "B"
355 River Road
Vanier, Ontario K1A 1B8

Dear Dr. Franklin:

Re: A Randomized Trial of the Use of N'Acetylcysteine for the Prevention of Sulfonamide Hypersensitivity Reactions When Used for the Prevention of Pneumocystis Carinii Pneumonia in Patients with HIV Infections Control No. 1HP921398

With regards to the above protocol, we would like to inform you that Canadian HIV Trials Network is going to act on behalf of the Principal Investigator, Dr. Sharon Walmsley, to recruit patients. Dr. Walmsley has been recruiting a number of patients from the Toronto General Hospital site. However, Dr. Walmsley has applied to Canadian HIV Trials Network to facilitate the recruitment of patients. Thus, additional Canadian Investigators will be involved in the above study. Please find enclosed the documentation of the Statement of Investigators for the following sites (Dr. Schlec, Dr. Senay, and Dr. Williams) who have also provided us with a copy of their ethics approval. I will forward the remaining Statement of investigators as soon as they are received.

In addition, please find enclosed a copy of the recently revised pages of the protocol named above. You will find that specific revisions (marked in bold) were made to:

a) p. 8 - indicating that randomization will now take place at the Canadian HIV Trials Network
b) p.9 - Follow-up will be at weeks 2, 4, 6, 8, 10, 12, 16, and 20 after randomization (the patient will be on Mucomyst (or no drug) for the first 8 weeks and trimethoprim-sulfamethoxazole for the full 20 weeks.

c) p.12 - Interim Analysis is added to the protocol prior to the sample size section.

d) Frequency of the blood work is decreased - The CBC and differential as well as biochemistry are no longer required on week 6 and 10. Please refer to the flow chart (attached).

e) The CD4 count will take place on week 8 and 20 of the follow up. Please refer to the flow chart (attached).

Please feel free to call if you have questions or require more information.

Sincerely,

Shideh Khorasheh
Shideh Khorasheh
National Clinical Trials Coordinator
November 29, 1991

Dr. J. Uetrecht
Faculty of Pharmacy
19 Russell Street, Rm. 517
University of Toronto

Dear Dr. Uetrecht:

Re: Your research study entitled, "Prevention of Sulfonamide Hypersensitivity Reactions with N-Acetylcysteine"

I have asked Dr. Bernard Dickens to review your protocol and the approval extended by Sunnybrook Health Science Centre.

Dr. Dickens has advised that he does not feel further review will be necessary and recommends expansion of the study to St. Michael's Hospital and the Toronto General Hospital.

During the course of the research, any significant deviations from the approved protocol and/or any unanticipated developments within the research should be brought to the attention of the Office of Research Services.

Best wishes for the successful completion of the project.

Yours sincerely,

Susan Pilon
Executive Officer
Human Subjects Review Committee

cc: Dr. D. Dickens, Chair,
    Human Subjects Review Committee, University of Toronto
    Dr. E. Blandin, Chair
    Research Ethics Committee, Sunnybrook Health Science Centre
March 23, 1992

Dr. I W. Fong
Director, Div of Infectious Diseases and
HIV Clinic
St. Michael's Hospital
M5B 1W8

Dear Bill,

Re: REB 80U — Randomized Trial of the Use of N-acetylcysteine in
Prevention of Sulfonamide Hypersensitivity Reactions in patients with...
Infection

Thank you for your letter of March 9, 1992.

I am writing to issue final approval for this study. This approval is valid for a period of 18 months from the date of this letter. Continued beyond that date will require a further review.

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated development should be brought to the attention of the Human Subjects Committee.

While this letter serves as approval by the St. Michael's Ethics Committee for the Use of Human Subjects in Research, additional approval may be needed from this institution. This can be coordinated temporarily through the Vice President of Medical Affairs.

Sincerely,

Jeff Nelson MD FRCP
Chairman
Research Ethics Board

Joel/dp
January 22, 1992

Dr. I. Salit
EN G-216

Dear Dr. Salit:

I am pleased to inform you that as of January 20, 1992 the Toronto Hospital Committee for Research on Human Subjects has tabled and approved the following research project which received approval from The University of Toronto Human Subjects Review Committee on November 29, 1991:

"Prevention of Sulfonamide Hypersensitivity Reactions with N-Acetylcysteine"

Best wishes for the successful completion of your project.

Yours sincerely,

(Mrs.) M. Raza
Administrative Assistant
Research Directorate

cc: Dr. A. Aberman
Pharmacy
Nursing
Medical Records-NOH
MEMORANDUM

TO: Dr. S. Walmsley et al.
    Department of Medicine
    A-226

FROM: Eric M. Meslin, Ph.D.

DATE: January 16, 1992

SUBJECT: Prevention of sulfonamide hypersensitivity reactions with N-Acetylcysteine

The Committee has reviewed the changes to the above study and has approved the amendment. The study may proceed at Sunnybrook Health Science Centre.

We would ask that the Committee be notified if there are any deviations in the protocol in the future or if it is terminated prematurely.

Eric M. Meslin, Ph.D.
Chair, Research Ethics Committee
MEMORANDUM

TO: Dr. J. Uetrecht  
Faculty of Pharmacy  
University of Toronto  
19 Russell St, Room 517  
Toronto M5S 2S2

FROM: Eric M. Meslin, Ph.D.  
Chair, Research Ethics Committee

DATE: August 16, 1990

SUBJECT: Prevention of sulfonamide hypersensitivity reactions with N-Acetylcysteine.

Thank you for your response to the concerns of the Research Ethics Committee with respect to the above study. This study is now ethically acceptable for performance at Sunnybrook Health Science Centre.

Signature

Eric M. Meslin, Ph.D.  
Chair, Research Ethics Committee

2075 Bayview Avenue  
North York, Ontario  
Canada, M4N 3M5  
University of Toronto
Approval by Review Committee on the Use of Human Subjects

Principal Investigator : Dr. J. Uetrecht, Pharmacy

Title : A Randomized Trial of the Use of N-Acetylcysteine for the Prevention of Trimethoprim Sulfamethoxazole Hypersensitivity Reactions When used for the Prevention of Pneumocystis Carinii Pneumonia in Patients with the Human Immunodeficiency Infection (Revised protocol)

Review Committee : Professor J. Boyle and the Ethics Committee at Sunnybrook Hospital

Documents Submitted to Review Committee : A protocol, changes to the protocol and a consent form.

Subjects : Patients of the HIV clinics of investigators of the Canadian Clinical Trials Network.

Procedures : As described in the attached consent form.

Method for Obtaining Consent : Consent form, as attached. Patients are to be given a copy of the form to keep.

Remarks : 

Date of Approval : November 28, 1994.

*During the course of the research, any significant deviations from the approved protocol and/or any unanticipated developments within the research should be brought to the attention of the Office of Research Services.

*A copy of this approval form is available to Review Committee members upon request.

SP/tp

cc: Ms. A. Ratchford
Dr. A. McGeer
Dr. A. Rachlis
Sunnybrook Hospital
Dr. I. Fong
St. Michael's Hospital
Dr. S. Walmley
Dr. I. Sait
Toronto Hospital
Wellesley Hospital
Dr. N. Shear
Dr. P. Wells
Professor B. Dickens

Susan Pilon, Executive Officer
Human Subjects Review Committee
CONSENT FORM

A RANDOMIZED TRIAL OF THE USE OF N-ACETYLCYSTEINE FOR THE PREVENTION OF SULPHONAMIDE HYPERSENSITIVITY REACTIONS WHEN USED FOR THE PREVENTION OF PNEUMOCYSTIS CARINI PNEUMONIA IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS SYNDROME

INVESTIGATORS: DR. SHARON WALMSLEY

DR. IRVING SALT

Purpose of the Study

Your physician has decided to treat you with a drug called Bactrim or Septra (these are the trade names for a combination of trimethoprim and sulfamethoxazole). Although this medication can be life-saving, and may prevent pneumonia, it is associated with a high incidence of serious side effects in patients who are HIV positive. These side effects usually include a skin rash but can include fever and inflammation of organs such as the thyroid gland, heart or liver (hepatitis). There are studies that suggest that this high incidence of side effects is due to an abnormally low level of a substance in the blood called glutathione. This study is designed to see if giving an amino acid called N-acetylcysteine (NAC) will decrease these side effects. N-acetylcysteine can be used by the body to make glutathione.

Procedure

In order to determine if the treatment works, it is important to give N-acetylcysteine to only about 50% of the patients in the study. If you agree to participate in this study, the determination of whether you receive N-acetylcysteine will be made randomly by a computer. If you are randomized to the group that receives N-acetylcysteine, it will be added to a cola drink or orange juice and given to you 1 hour before each dose of Bactrim/Septra (twice per day) for the first two months you are on the Bactrim/Septra.

You will be followed by the study nurse on weeks 2, 4, 6, 8, 10, 12, 16 and 20 after starting the study. At each visit, he/she will ask you questions about your health in general, whether you have missed any doses in your medication and for symptoms of allergy to Septra/Bactrim. At these
visits, you will have 3 teaspoons of blood drawn to ensure you do not develop side effects to the medication. At the initial visit, you will also be asked to have a chest x-ray and ECG (electrocardiogram) if they have not been done in the previous six months.

The only other procedure involved in the study is that 2 small (1 teaspoon) blood samples will be drawn from a vein in your arm, one sample will be taken before the first dose of medication and another one week later. The samples will be used to measure glutathione levels. This will be done whether or not you receive N-acetylcysteine.

If you develop symptoms of an allergic reaction, you will be seen as soon as possible by the study nurse and blood (approximately 5 teaspoons), urine tests, a chest x-ray and ECG will be performed. You will be assessed again 2 and 4 days later to ensure your allergy resolves.

**Risks and Discomforts**

N-acetylcysteine is used to treat patients who have taken an overdose of acetaminophen (Tylenol) and it is not associated with dangerous side effects. It does have an unpleasant smell and can cause nausea, but its taste will be masked to a large degree by giving it in a cola drink or orange juice.

The needle stick required to draw blood causes some discomfort, but it may be possible to take this sample when other samples are being taken so that no extra needle stick will be required.

**Benefits**

If N-acetylcysteine works to decrease serious side effects of Bactrim, it could be a great benefit to you and other patients. Even if you do not receive N-acetylcysteine during this study, if the study is successful, it could be used if you ever required Bactrim on another occasion.

It has been suggested in laboratory studies that N-acetylcysteine may improve immune function in patients who are HIV positive, but there has been no reported study in patients to determine if this is true.

**Confidentiality**

All information concerning individual patients will be kept confidential and only the principal investigators and study nurse will have access to the information. Each patient will be given a number in the initial randomization and other records and samples such as the blood samples will be identified by this number.

**Rights**

Your participation in this study is voluntary and will not affect any other part of your care. If you decide not to participate, you can be assured that you will receive the best possible care. Even if you decide to participate you can change your mind at any time.
No special insurance protection is provided for compensation for any injury that you might experience as a result of participation in this study.

You are encouraged to ask any questions you might have about this study now or at any time in the future.

By signing this consent form, you acknowledge that all questions have been answered to your satisfaction and that you voluntarily choose to participate in this study. You understand that you can withdraw from the study at any time and it will not affect any other aspect of your care.

______________________________  ______________________________

(date)  (signature of volunteer)

______________________________  ______________________________

(date)  (signature of witness)

If you have any questions at some later time, you can call any one of the following numbers.

Dr. S. Walmsley  340-3871

Dr. L. Salfit  340-3697
January 14, 1992

Ms. Judy Tseung
Grants Coordinator, Research
Sunnybrook Health Science Centre
Reichmann Research Building
1st Floor, S-Wing, Room 104
2075 Bayview Avenue
Toronto, Ontario
M4N 3M5

Dear Ms. Tseung:

Ref. No. 91-36

I am pleased to enclose our cheque for $30,000 representing the first of four installments on a grant of $95,000 awarded to Dr. Sharon Walmsley, for her research entitled, "Randomized Trial of the Use of N-Acetylcysteine in the Prevention of Sulfonamide Hypersensitivity Reactions in Patients with HIV Infection."

The grant was awarded to cover the expenses of this study for a two year period commencing January 1st, 1992. Future installments will be forwarded in the following amounts: June 1992 - $30,000; December 1992 - $20,000; and June 1993 - $15,000.

We will look forward to receiving an accounting of the disbursements made under this grant at the end of each twelve month period.

Yours sincerely,

K. McGuire (Miss) - Administrative Assistant

Encl.

cc Dr. S. Walmsley
April 3, 1992

Dr. Sharon Walsley
A226 Sunnybrook Health
Science Centre
2075 Bayview Avenue
Toronto, Ontario
M4N 3M5

Dear Dr. Walsley:

We are pleased to inform you that your request for Mucomyst has been approved by our Research Committee. Consequently, Bristol-Myers Squibb accepts to provide you with a total of 4,104 vials of 30ml Mucomyst, free of charge, to enable your research group to complete the project entitled:

"Randomized Trial of the Use of N-acetylcysteine in the Prevention of Sulfonamide Hypersensitivity Reactions in Patients with HIV infection"

As indicated in our conversation, you should provide us with a tentative schedule of the shipments as well as the quantity needed for each shipment. As agreed, Mucomyst shipments will be sent upon request from you accompanied by a study progress report. It is also understood, that if a manuscript is prepared, a copy should be sent to us at least 30 days prior to its submission to the Editor and would expect that BMS support be acknowledged. This is essentially what is indicated in the Clinical Supplies Agreement that is included. If you agree with the CSA, please sign one of the originals and return it to me with the product shipment schedule and all appropriate informations for shipments.

I trust you will find all of the above to your entire satisfaction.

Yours sincerely,

Claude Auclair, Ph.D.
Director, Clinical Research
Oncology, CNS and Anti-infective Products
CA/hk

cc: Dr. I. Fong - Dr. A. Rachlis - Dr. I. Salit - Dr. N. Shear - Dr. J. Ulrecht - Mr. P. Wells
CLINICAL SUPPLIES AGREEMENT

Dr. Sharon Walmsley  
A226 Sunnybrook Health Science Centre  
2075 Bayview Avenue  
Toronto, Ontario  
M4N 3M5

Dear Dr. Walmsley:

Re: "Randomized Trial of the Use of N-acetylcysteine in the Prevention of Sulfonamide Hypersensitivity Reactions in Patients with HIV infection"

The Bristol-Myers Squibb Pharmaceutical Group agrees to provide you with Mucomyst to enable your research group to the conduct of your clinical research study. This letter of agreement sets forth the terms and conditions we would like to offer for your consideration:

1. The Bristol-Myers Squibb Pharmaceutical Group recognizes your authorship for the protocol entitled: "Randomized Trial of the Use of N-acetylcysteine in the Prevention of Sulfonamide Hypersensitivity Reactions in Patients with HIV infection".

2. In order for you to proceed with your study, the Bristol-Myers Squibb Pharmaceutical Group agrees to provide you with a total of 4,104 vials of 30ml Mucomyst free of charge.

Mucomyst product will be shipped to the following location:


3. Every shipment will be made on your request and upon receipt of a study progress report. The quantity of vials to be sent at each shipment will also be determined by you. However, in order to planify anticipated shipments, you will provide us with a list of shipment dates and quantity of product per shipment. We need to receive these forecast before the first shipment is made.

4. For the purpose of this agreement, you will be deemed an independent contractor and not an employee of the Bristol-Myers Squibb Pharmaceutical Group.
5. Publication of study results in the scientific literature is encouraged, but the Bristol-Myers Squibb Pharmaceutical Group reserves the right to review any paper written utilizing data generated from this study before such paper is presented or submitted for publication. Any publication or abstract must be submitted to the Bristol-Myers Squibb Pharmaceutical Group at least 30 days before sending it to the editors.

6. The terms of this Agreement shall be from the date of this Agreement is accepted by you until the study is either completed or terminated.

7. The Bristol-Myers Squibb Pharmaceutical Group retains the right to terminate its support for good and sufficient reasons at any time.

8. In performing this study, you agree to follow and comply with all applicable terms, rules and regulations relating to the conduct of such study and, in particular, the laws, rules and regulations which have been promulgated by the HPB.

9. It is the responsibility of the investigator to file an IND with the HPB if one is required and to inform HPB and BMSPG of any Serious Adverse Events that may occur during the course of the study.

If the foregoing terms meet with your approval, kindly confirm your acceptance thereof by dating, signing and returning to us the copy of this letter (enclosed for that purpose).

Very truly yours,

THE BRISTOL-MYERS SQUIBB PHARMACEUTICAL GROUP

BY:

Claude Auclair, Ph.D.
Director, Clinical Research
Oncology, CNS & Antinfective Products

Patrick Mérat, M.D.
Vice-president
Scientific Affairs

Agreed to and accepted:

Sharon Walmsley, M.D.

Date

Date

Date
SEPTMBER 7, 1993

Dr. Walter Schiech
ACC 5014 Victoria General Hospital
1278 Tower Road
HALIFAX, NS B3H 2Y9

FAX# (902) 428-2023

Dear Dr. Schiech:

I have developed a clinical study entitled:

"A randomized trial of the use of N-acetylcysteine for the prevention of sulphonamide hypersensitivity reactions when used for the prevention of Pneumocystis carinii pneumonia in patients with HIV infection."

We have been enrolling patients in this study in Toronto for the past year but have had some difficulty in recruiting adequate numbers. To achieve reasonable power approximately 250 patients will need to complete the study. We originally had a treatment arm to the study as well but because of the declining number of cases of acute infection we felt this arm should be discontinued.

In order to complete the study in timely fashion, I will be submitting this study to the Canadian HIV Clinical Trials Network for consideration for support. If accepted by the network I am hopeful that your centre will be interested in enrolling patients.

Please find on the next page a brief synopsis and rationale for the study. Obviously, a letter of support from your centre would help to strengthen the application to the network.

The deadline I hope to reach for submission is Friday September 10/93. I realize that this is short notice, but if you feel you would be interested in participating could you please FAX back to me the following letter of intention as soon as possible to (416) 595-5826. The full protocol is available on request.

Thanks for your support. Hope we have the opportunity to work together on this project.

Best Regards,

Sharon Walmsley, M.D. F.R.C.P.(C).
LETTER OF INTENTION TO PARTICIPATE

"A randomized trial of the use of N-acetylcysteine for the prevention of sulphonamide hypersensitivity reactions when used for the prevention of *Pneumocystis carinii* pneumonia in patients with HIV infection."

If the above study is supported by the Clinical Trials Network I will be interested in participating as a co-investigator, and will supervise the recruitment, enrollment and follow-up of suitable patients at my center.

Name ____________________________________________

Signature ____________________________________________

Centre ____________________________________________

Over the next 12 months I expect that I will be able to enrol ________ patients from my center.
Randomized trial of the use of N-acetyl-cysteine in the prevention of sulfonamide hypersensitivity reactions in patients with HIV infection

OBJECTIVE

The objective of this proposal is to test the hypothesis that giving N-acetylcysteine (NAC) to patients with Human Immunodeficiency Virus Infection (HIV) will decrease the extraordinary incidence of serious adverse reactions to trimethoprim-sulfamethoxazole (T/S) observed in this population.

RESEARCH DESIGN

A randomized single blind, single cross-over trial. Patients who are to receive trimethoprim/sulfamethoxazole (T/S) for the prevention of PCP in HIV infection and who give consent will be randomized to receive or not receive N-acetylcysteine (NAC). The patient and study nurse will not be blinded as to study assignment (NAC has a very distinctive taste which cannot be blinded). The study site investigator assessing outcome will be blinded.

Patient Selection

The study population will consist of patients who are to receive T/S for prophylaxis for PCP. The following criteria will be used:

Inclusion Criteria
   (i)  patients known to be HIV positive
   (ii) CD4 lymphocyte count less than 200 x 10^6/L or CD4% < 20% or an AIDS diagnosis
   (iii) consent to study and able to come for follow up

Exclusion Criteria:
   (i)  previous allergic reaction to sulfonamides or trimethoprim
   (ii) treatment with T/S since diagnosis of HIV infection, including treatment for an acute episode of PCP
   (iii) neutropenia (PMN <1000 x 10^6/L)
   (iv) patient prefers another form of prophylaxis
   (v)  taking a study drug other than AZT, DDI or dDC.
   (vi) patients taking NAC obtained from other sources

All HIV positive patients whose CD4 count drop to these levels should receive prophylaxis for PCP. T/S is considered the treatment of choice, and is recommended where not contraindicated. Exclusion of patients who have received treatment with T/S elsewhere and have not had an allergic reaction avoids selecting patients with a lower risk of allergy.

Standard doses of T/S for prophylaxis will be used for all patients. The U.S. public health service task force recommends a dose of one double-strength tablet (800 mg sulfamethoxazole, 160 mg trimethoprim) daily for PCP prophylaxis. Although many physicians administer the drug only 3 days a week, the Task Force did not feel the data was sufficient to recommend using T/S for less than 7 days per week. To optimize serum levels of NAC, we felt it should be given at least twice per day, and tied to the T/S administration. Therefore for purposes of this study the T/S will be given as one single strength tablet (400mg sulfamethoxazole, 80 mg trimethoprim) orally twice daily. The T/S will be continued indefinitely if no adverse reactions occur. After completion of the study protocol, physicians may change the T/S dosage schedule if desired.

Intervention

When an eligible patient is identified by an investigator, the study nurse at that center will be notified. Eligibility will be confirmed, patient consent obtained, and the patient will be randomized.

The intervention is NAC taken orally one hour prior to each dose of T/S at a dose
of 15 cc of a 20% solution (3 grams). The dose is given 1 hour prior to T/S to ensure absorption prior to the T/S. The total daily dose of NAC is 6 grams. NAC is available only as a liquid (20% solution), and will be given in cola or orange juice to make it more palatable.

NAC will be maintained for 2 months or until T/S is discontinued. Prophylaxis will be given indefinitely, but adverse reactions to T/S usually occur in the first few weeks. The NAC will be given for the first two months to ensure that its beneficial effects relate to prevention and not just a delay in the development of hypersensitivity reactions. Patients will continue to be followed after the NAC is discontinued to determine if adverse reactions develop.

Patients will be seen by the study nurse one week after starting therapy, then every two weeks for the first 2 months. Followup will be maintained after the NAC is discontinued to assess T/S reactions, biweekly for 1 month then monthly for 2 months. Laboratory tests to assess for toxicity will be performed once monthly during the study period.

If a reaction occurs, the patient will be seen immediately, and followed at day 3 and 5 following the reaction. Laboratory tests will be performed to assess the extent and the severity of the reaction. The patients will also be followed by their own physician over this time.

Patient compliance will be assessed at each visit by pill counts for T/S and volume measurements for NAC. The only other clinical use of NAC is for acetaminophen overdose, and it is unlikely that patients in non-drug arm will be able to acquire the liquid form of this drug by other means. However, patients who use small quantities of NAC obtained from health food stores in tablet form will be ineligible.

Outcome measure

The primary outcome measure for this trial is the need to discontinue T/S because of any two of rash, diffuse severe pruritus, and fever. Rash is defined as a diffuse erythematous eruption involving at least 50% of body surface area. Severe pruritus is defined as that which is intolerable to the patient. Fever is defined as documented oral temperature >38.5°C on at least two occasions within 48 hours at least two hours apart. If the reaction is mild and the patients and treating physician agree to continuing treatment together with the use of antihistamines, nonsteroidal antiinflammatory agents or steroids in attempts to diminish the hypersensitivity reaction, the patients will not be counted as reaching the primary outcome. The use of these agents will be recorded and the patient will continue in the study and be observed.

Analysis

The primary analysis will be an intention to treat analysis to examine differences in the rates of the primary outcome (discontinuation of T/S secondary to rash/fever/pruritus) during the first 3 months of prophylaxis, between the two groups by the Chi-square test.

Three secondary analysis will be done. First an efficacy analysis to compare the difference in the rates of the primary outcome in patients assigned to NAC who take at least 80% of both medications compared to patients not assigned to NAC who take at least 80% of their doses of T/S. Secondly, the incidence of any rash between the two treatment groups will be compared. Thirdly, an analysis will also be performed to compare the differences in the incidence of fever and rash in patients assigned to NAC during the time on study drug and during the period of follow-up while off NAC.

The remainder of the analysis will be descriptive. The data will be examined to determine if certain patient characteristics are associated with T/S hypersensitivity reactions. Also the degree, incidence, and severity of these reactions will be determined.
Application to Conduct a Clinical Trial in HIV and Related Diseases with the Canadian HIV Trials Network

Project Title: A RANDOMIZED TRIAL OF THE USE OF N-ACETYLCysteine FOR THE PREVENTION OF TRIMETHOPRIM-SULFAMETHOXAZOLE HYPERSENSITIVITY REACTIONS WHEN USED FOR THE PREVENTION OF PNEUMOCYSTIS CARINII PNEUMONIA IN PATIENTS WITH HIV INFECTION.

Principal Investigator:
(Name, address and affiliated institution)
DR. SHARON WALLSLEY
TORONTO GENERAL HOSPITAL, EN G-219
200 ELIZABETH STREET
TORONTO, ON M5G 2C4

Type of Submission:

☐ Preliminary Proposal
☒ Full Protocol

Please note: We would greatly appreciate receiving your thoughts and suggestions regarding the format and the content of this application form.
A Randomised Trial of the use of N-acetylcysteine (NAC) for the prevention of sulphonamide hypersensitivity reactions when used for the prevention of Pneumocystis carinii pneumonia in patients with the human immunodeficiency virus infection, submitted by Dr. Sharon Weinsley.

Type of Application: This is a complete application

Objective

To test the hypothesis that giving N-acetylcysteine (NAC) to patients with HIV will decrease the extra-ordinary incidence of serious adverse reactions to Trimethoprim–Sulfamethoxazole observed in this population.

Background

Trimethoprim–Sulfamethoxazole (TMP–SMX) is the drug of choice for the prevention of Pneumocystis carinii pneumonia in HIV patients. For unknown reasons HIV patients have an unusually high incidence of hypersensitivity reactions to this antibacterial combination. The hypersensitivity reactions are usually fever with rash. The incidence of such reactions ranges from 15 to 30% in HIV infected patients compared to less than 1% in non-HIV infected patients who are treated with this combination.

It is known that glutathione deficient lymphocytes from patients with a genetic defect in glutathione synthesis have an increased susceptibility to sulphonamide metabolite toxicity in vitro. Sensitive lymphocytes from patients who have had hypersensitive reactions to sulphonamides can be protected in vitro by the addition of glutathione. Patients with HIV have been found to have abnormally low levels of cysteine and glutathione in their blood and low glutathione levels in peripheral blood mononuclear cells. NAC has been used clinically to regenerate glutathione in individuals who have ingested an overdose of acetaminophen. In such individuals, glutathione prevents the hepatic toxicity due to acetaminophen, presumably by repleting glutathione in the hepatocytes and preventing the hepatotoxic effect of the reactive metabolite generated by acetaminophen metabolism.
At this time no strategies have been developed to prevent the hypersensitivity reactions that commonly limit the usefulness of TMP-SMX for the prevention of PCP in HIV patients. This study aims to undertake such a first evaluation.

HIV patients with <200 CD4 cells receiving TMP-SMX for PCP prophylaxis, will ingest it as one adult strength tablet BID. One-half will be randomized to ingest NAC as 15ml of a 20% solution (3g) one hour prior to each TMP-SMX tablet, for the 2 months study period.

The primary endpoint will be a hypersensitivity reaction defined as 2 or more of fever, diffuse rash or intense pruritis. It is estimated that the incidence of same without NAC will be 30%. A beneficial effort of NAC in reducing this by 50% will require 293 patients using alpha 0.05 (2-sided) and a power of 80%.

This study is already underway in Toronto. In Toronto patients experiencing hypersensitivity reactions and controls, glutathione concentrations in plasma and monocytes, will be measured.

**Strengths of the Proposal**

1. This an innovative idea addressing a clinically important issue in HIV infected patient management.

2. The Principal Investigator is working with a group of colleagues who are experts in the area of sulfonamide induced hypersensitivity reactions.

**Weaknesses of the Proposal**

1. Concern was expressed that some studies have shown that the bioviability of NAC is very poor in doses as high as 9 g. In this regard, procysteine has been found to be a much better absorbed drug for cysteine and glutathione generation than NAC.
2. A prophylactic regimen in which TMP/SMZ is given as one adult tablet twice a day rather than as one double strength tablet once a day assumes that the two dosage regimens are equivalent. Members of the committee were unclear that there were data that this was so.

3. The plan to undertake an intention to treat analysis needs to be reconsidered because, as described, it will not be that.

4. No provision is made to record use of other anti-oxidants.

**Evaluation**

The proposal is a well developed study based on considerable local experience with the problem of sulfonamide hypersensitivity reactions. The proposal was seen as an interesting first step into an important area of HIV care.

The Community Advisory Committee evaluation was that the CAC support the trial as submitted. CAC members based in Toronto mentioned that they had not heard of it even though it is currently underway. They suggested that this lack of communication may account for recruitment problems and were also concerned that the availability of over the counter NAC may pose problems for recruitment as well.

**Recommendation**

The Scientific Review Committee recommended that this study be accepted as submitted with provision that the Principal Investigator address 1) the issue of the intention to treat analysis design. 2) that an interim analysis by an independent group be performed. 3) that patients use of other OTC oxygen scavengers (vitamin C, etc) be recorded. 4) the applicant consider rolling over this study into one in which other cysteine sources such as procysteine might be used more effectively.

November 18, 1993.

[Signature]

Chairman SRC
JANUARY 4, 1994

Dr. Donald P. Zarowny
Program Head, Scientific Industrial Liaison
The Canadian HIV Trials Network
200-1033 Davey Street
VANCOUVER, BC V6E 1M7

Dear Don:

RE: A RANDOMIZED TRIAL OF THE USE OF N’ACETYLCYSTEINE FOR THE PREVENTION OF TRIMETHOPRIM SULFAMETHOXAZOLE HYPERSENSITIVITY REACTIONS WHEN USED FOR THE PREVENTION OF PNEUMOCYSTIS CARINII PNEUMONIA IN PATIENTS WITH HIV INFECTION

Thank you for your letter concerning the above trial and the comments of the Scientific Review Committee.

I enclose the responses of myself and my co-investigators to the points that have been raised. I am sorry for the delay, but the holiday season interrupted my ability to reach necessary contacts to appropriately address the questions.

I am trying to trimline the budget as we discussed, but I am still concerned as to how we will deal with the March 31, 1994 deadline.

To clarify one point, there has been a typographical error in the body of the grant. Patients will be randomized to receive or not to receive N’acetylcysteine for the first two months (not three months) and be followed for an additional three months.

If you require further information at this time, feel free to contact me.

Sincerely,

Sharon L. Walmsley, MD, FRCP(C)
Division of Infectious Diseases

SLW/ast
A Randomized Trial of the use of N-acetylcysteine for the prevention of sulphonamide hypersensitivity reactions when used for the prevention of Pneumocystis carini pneumonia in patients with HIV infection. Submitted by Dr. Sharon Walmsley.

The presentation is relatively straightforward and I have few concerns which are:

1. The budget of $500,000 seems high to me and if the trial needed to secure funding from the CTN, justification would have to be more compelling.

2. The study will attempt to enroll nearly 300 patients but this may be problematic as prevention of PCP improves each year.

3. Is the laboratory determination of the glutathione necessary in all the patients. Perhaps this determination could be completed in a subset of the population. Since an early phase of the study has been completed perhaps the data should now be analyzed rather than continuing to make the same observations in a bigger group.
Title:
A Randomized Trial of the use of N-Acetylcysteine for the Prevention of Trimethoprim-Sulfamethoxazole Hypersensitivity Reactions when used for the Prevention of Pneumocystis Carinii Pneumonia in Patients with HIV Infection.

Submitted by: Sharon Walmsley

Type of submission: Full protocol

Type of trial: Phase III (comparative)

Objective:
The objective of this proposal is to test the hypothesis that giving N-acetylcysteine (NAC) to patients with Human Immunodeficiency Virus Infection (HIV) will decrease the extraordinary incidence of serious adverse reactions to trimethoprim-sulfamethoxazole (T/S) when used for the prevention of pneumocystis carinii pneumonia (PCP).

Background:
An extensive background is given, outlining the importance of PCP prophylaxis in HIV+ patients and the extent of the problem with adverse reactions to T/S in this population. As many as 50% of HIV+ patients need to discontinue T/S for adverse reaction compared to 1% in the general population. Additionally, the limitations of alternatives to T/S are given. A good theoretical justification for the use of NAC for the prevention of adverse reactions is provided.

Strengths of Proposal:
The proposal's strength is that it is a single-blind, multi-centre, parallel-groups, community based randomized trial addressing an important clinical question with a modest amount of follow-up and intervention. It is well designed, and care is taken to provide proper randomization and blinded assessment of outcome. Sample size is adequate to detect a reduction in adverse reactions from 30% to 15%.

Weaknesses of Proposal:
The major weakness of the proposal is the patient accounting for the outcome measure. The investigators state their wish to preform an intent-to-treat analysis, but then outline a number of randomized patients who will be ineligible or invaluable for analysis. A true intent-to-treat analysis (which is easily justified in for this trial) should include all randomized patients where the outcome is simply the discontinuation of T/S for whatever reason during the first 2 months of use. The
justification for this approach is justified further by the investigators intentions to do secondary efficacy analyses.

The investigators propose to count discontinuation of T/S during the third month of its use, even though NAC will be given for 2 months only. There does not seem to be a good reason for this.

Evaluation:

This is an clinically relevant, well designed trial.

Recommendation:

Approval pending stated improvements to the intent-to-treat analysis.
Canadian HIV Trials Network
Scientific Review Committee Evaluation

17 November, 1993

Title
A randomized trial of the use of N-acetylcysteine for the prevention of sulphonamide hypersensitivity reactions when used in the prevention of Pneumocystis carinii pneumonia in patients with human immunodeficiency virus infection.

Submitted by
S Wlamsley (P.I.)
J Uetrecht
A Rachlis
I Salti
B Fong
N Shear
A Meegeet
P Wells

Type of submission
Full protocol

Type of protocol
Phase II

Objective
To test the hypothesis that giving N-acetylcysteine (NAC) to patients with Human Immunodeficiency Virus (HIV) infection will decrease the extraordinary incidence of serious adverse reactions to trimethoprim-sulfamethoxazole (T/S) observed in this population.

Background
The incidence of treatment-terminating adverse reactions due to trimethoprim-sulfamethoxazole (T/S) in HIV infected adults has been observed to be between 30% and 50%, whereas the incidence in similarly treated uninfected adults has been estimated to be approximately 1%. These reactions include morbilliform rash, generalized pruritis and drug fever.

The etiology of these reactions, particularly in this population, is unknown but there are relevant observations include: (1) immune dysregulation present in HIV infected adults; (2) impaired acetylation of sulfonamide arylamine in this population, possibly aggravated by concurrent infection; (3) genetically determined differences in acetylation rates; and (4) reduced concentrations of glutathione in this population when compared with uninfected adults. These observations suggest that pre-treatment of HIV infected adults with N-acetylcysteine, in order to increase glutathione synthesis as well as increase available thiols, could decrease the incidence of adverse reactions to T/S in this population. Consequently, a trial is proposed in which HIV infected adults will be prospectively treated with N-acetylcysteine when being receiving T/S prophylaxis against Pneumocystis carinii pneumonia. N-acetylcysteine is a relatively harmless, although unpleasant, treatment so that its use would not be expected to involve excess risk to this population. Preliminary research in this population, as described in the protocol, supports this conclusion.

The proposed study will be a randomized, single-blind cross-over trial design in which one group of subjects will drink a 3 g N-acetylcysteine solution one hour before oral ingestion of single-strength cotrimoxazole (80 mg trimethoprim + 400 mg sulfamethoxazole) twice daily for three months or until they cease using T/S. They will continue T/S prophylaxis and be followed thereafter. A comparison group will receive only T/S.
The primary outcome measure is discontinuation of T/S prophylaxis due to drug-induced rash, pruritus and/or fever during the first three months of prophylaxis. Analysis will be intention to treat. Efficacy analyses will include difference in this outcome among subjects receiving 80% of the prescribed doses of each drug, the incidence of any rash (regardless of T/S discontinuation), and the incidence of rash and fever among subjects receiving N-acetylcysteine during and following cessation of this treatment. It is estimated that 293 subjects will be studied on a 1:1 randomization basis and with a 50% decrease in adverse events attributable to N-acetylcysteine treatment, over a total study time of 36 months.

Strength of Proposal
This is a very strong proposal. The protocol is clear, concise and clear with regard to the information provided provided in it, the design issues and logistical concerns it addresses. All appropriate documentation is provided (CRFs, ethics approval, HPB correspondence, funding reports, etc.).

The research design is relatively straightforward. Sufficient numbers of subjects will be studied so that types I and II errors will be avoided, based on the estimated incidence of adverse events in the study population. Observation time is relatively short but it should provide useful information on the incidence of adverse events attributable to cotrimoxazole prophylaxis against Pneumocystis carinii pneumonia, and the potential benefit of N-acetylcysteine to reduce this incidence.

Partial funding of this study has been received from the Physicians's Services Incorporated Foundation. In addition, in vivo studies will be carried out using blood specimens from subjects enrolled in the Toronto region. These studies have received peer-reviewed support (CANSFAR). The budget and its justification are clearly set out in the proposal, and do not raise any unusual concerns.

Weaknesses of proposal
There are several minor issues that should be addressed but these should not delay starting the study.

First, the prophylaxis dose of cotrimoxazole is a single-strength cotrimoxazole taken twice daily. There is no apparent reason to consider this dosage should be less efficacious than a single daily double-strength cotrimoxazole dose, nevertheless, this is not discussed in the protocol, in particular in the informed consent information provided to potential research subjects. As it is presently designed, this study cannot assess this treatment modification (and probably should not be modified to do so). However, providing appropriate information to research subjects can easily be done.

Second, the cost and potential unpalatability of N-acetylcysteine may limit its future utility, even if it is found to be efficacious. This problem is alluded to in the protocol where it is stated that its effects have to be "substantial in order to be clinically important". This raises the concern about the opportunity costs of such a study. This does not detract from the scientific merit of the proposed study but it does raise concern about the clinical salience of the study. Balancing this concern is that the study has been found to be ethically acceptable. Emphasis on the scientific merits of the study (that is as a step on the way to developing safer treatments) rather than on its potential to identify a novel therapeutic intervention, would be more appropriate in this context.
Third, the potential impact of N-acetylcysteine on HIV disease progression or manifestations is discussed only minimally in the protocol and in the informed consent information provided to potential research subjects. There is no reference to this issue in the analysis of data that will be collected in this study.

**Evaluation**

This is a very well prepared protocol, and other than the weaknesses discussed above, the study is acceptable, and should be recommended, as a Network trial. The concerns, discussed above, should not delay the initiation of the study.

**Recommendation**

Approval of this protocol, as submitted, as a Network trial, pending clarification of concerns discussed above.
JANUARY 4, 1994

Dr. F. Aoki
Chairman, Scientific Review Committee
The Canadian HIV Trials Network
200-1033 Davey Street
VANCOUVER, BC V6E 1M7

Dear Dr. Aoki:

RE:  A RANDOMIZED TRIAL OF THE USE OF N'ACETYLCYSTEINE FOR THE PREVENTION OF TRIMETHOPRIM SULFAMETHOXAZOLE HYPERSENSITIVITY REACTIONS WHEN USED FOR THE PREVENTION OF PNEUMOCYSTIS CARINII PNEUMONIA IN PATIENTS WITH HIV INFECTION

Thank you for your Committee's comments concerning our protocol. Enclosed are the responses of our group in regards to the questions that have been raised.

1) Bioavailability of NAC

There is minimal published data on this matter in HIV patients. De Quay et al (article enclosed) were able to demonstrate moderate elevations in plasma glutathione and cysteine in patients with HIV after the oral administration of 30 mg/kg of NAC. There is data on the absorption of procysteine in abstract form (enclosed - from the AIDS conference) in a rat model. This abstract would suggest that procysteine is more readily absorbed in this model. Giogi et al (enclosed) achieved raised glutathione levels in patients treated with IM glutathione or po procysteine that were not statistically different. Dr. Clifford Lane (in unpublished studies) found it difficult to achieve adequate serum levels of glutathione when using up to 9 g/day of NAC.

This data is difficult to interpret for our study. Our laboratory work as well as that of Dr. Chris Tscoukas (oral communication) has shown that the measurement of glutathione in the laboratory is very difficult and affected by many variables. The previous methods that have been described do not give specific information about changes in individual thiol and disulfide pools which may be clinically very important. Further, even if NAC does not increase serum glutathione levels, it may decrease the incidence of sulphonamide toxicity. The sulphydryl groups of the compound are reactive nucleophiles which may directly detoxify electrophilic metabolites. It is unclear whether procysteine is better absorbed in HIV patients but even if so may not offer advantages over NAC.

The mechanism of glutathione deficiency in HIV is unclear. If the deficiency is nutritional then supplementation with either NAC or procysteine would be useful. If procysteine were better absorbed in humans, theoretically it could be advantageous.
if, however, the mechanism of glutathione deficiency is an enzymatic problem with glutathione synthesis, then neither of these precursor compounds will be able to overcome the enzymatic block. In this regard, my pharmacology co-investigators are attempting to synthesize glutathione esters. These compounds could detoxify directly and not depend upon the synthesis of glutathione. Theoretically, they would be advantageous in that they could overcome glutathione deficiency by either mechanism. Once synthesized, we will have this compound approved by the HPB and we would then like to proceed with the studies to determine their efficacy in preventing sulphonamide hypersensitivity reactions in a parallel or roll over study to this one.

Now, on a more practical level, we feel that we should continue our studies with NAC for the following reasons:

1) Sixty patients have already been enrolled and the data would be lost if we were to change compounds at this time.

2) Roberts Pharmaceutical (who have taken over the distribution of NAC from Bristol Myers Squibb) have agreed to supply free of charge all of the NAC required to complete this study. This amounts to a considerable dollar value contribution to the study.

3) Procysteine is only available through Pre-Radical Science Incorporated of the United States. It would have to be approved by the HPB in Canada which would significantly delay our study. Further, according to Dr. Schnittman, NIH (oral communication), the company has had considerable financial difficulties and may not be able to continue to support the ACTG trials ongoing with procysteine. Dr. D. Goldberg, Vice-President of this company, has not been available by phone for the past two weeks for me to consult re: possible collaboration. It would be unfortunate if the trial could be completed because of drug availability.

4) There is little published literature to support the hypothesis that procysteine would work when NAC will not.

5) If found to be beneficial, procysteine is not available in Canada where NAC is.

6) We plan to develop the methyl ester of glutathione and study it. Theoretically, this will be a better compound than procysteine for the study in addition to NAC.

2) **Dose of Trimethoprim Sulphamethoxazole for Prophylaxis**

The exact dose of NAC required to decrease the incidence of hypersensitivity reactions to sulphonamides is unknown and was derived empirically. The dose of NAC that was chosen was based on what was expected to be safe and tolerable (to increase compliance) yet sufficient. We though there was an increased likelihood of demonstrating response if the daily dose of Trimethoprim Sulphamethoxazole was tied to NAC. However, we also did not want to overwhelm the drug detoxification system with too much Septra at any one time. Therefore, we decided to give the Trimethoprim Sulphamethoxazole as one single strength tablet bid (800 mg/400 mg) for a total daily dose equivalent to that which is routinely recommended.
There is no literature to suggest this schedule would not be as efficacious to prevent PCP. In fact, the minimally effective dose of Trimethoprim Sulfamethoxazole for PCP prophylaxis is currently unknown but under study. Nielsen et al. (paper enclosed) used this dose of Trimethoprim Sulfamethoxazole in 166 AIDS patients for secondary prophylaxis against PCP and were able to demonstrate a relapse rate of 5.1% (confidence interval 0-11%) which is consistent with that observed in studies of higher doses.

The period of this trial is short (5 months) and after this period, the investigators are free to use whatever dose of Trimethoprim Sulfamethoxazole they prefer.

A statement regarding the dose of Trimethoprim Sulfamethoxazole will be added to the Consent Form.

We do recognize that by decreasing the per dose amount of Trimethoprim Sulfamethoxazole, that this manoeuvre alone may decrease the number of toxic metabolites and decrease the incidence of hypersensitivity reactions to sulfonamides. This will be valuable information even if NAC is not found to be effective.

3) Analysis

The hypothesis in this study is that NAC will decrease the incidence of hypersensitivity reactions requiring the discontinuation of Trimethoprim Sulfamethoxazole. This is the primary outcome we will be measuring in an intention to treat fashion. We felt it necessary to define the hypersensitivity reaction for consistency.

NAC would not be expected to decrease the incidence of GI intolerance or hematological toxicity. Therefore, we did not use the discontinuation of Trimethoprim Sulfonamides as any reason as the primary outcome measure. These events and the reason for discontinuation of Septra will be recorded in the secondary analysis. If, however, the committee feels strongly about using discontinuing of Trimethoprim Sulfamethoxazole for any reason as the primary endpoint we would change. The rate of hypersensitivity reaction will be counted during the two months on NAC (the three months was a typographical error).

4) Other Anti-Oxidants

All other medications taken during the study period will be recorded in the case report form. We will make a separate category for anti-oxidants and name a few to ensure that they are not overlooked.

5) Interim Analysis

We agree that an interim analysis be performed by an independent group. This will increase the sample size minimally.
5) **Recruitment**

This study will enrol patients who are about to initiate PCP prophylaxis. Therefore, the improvement in PCP prophylaxis will not interfere with recruitment.

7) **Glutathione Levels**

The laboratory glutathione levels will not be determined in all patients, but only in the Toronto subset. The preliminary laboratory work has been on the refinement of the assay. There is not data for patients involved in the study so far.

8) **Justification**

We agree that the cost and potential unpalatability of NAC may limit its future use in this setting. This, however, a preliminary study. If NAC is found to be effective then the minimally effective dose or other potentially useful compounds, such as the methyl esters of glutathione could be studied.

We felt it necessary to use a modest dose for this study to ensure we could demonstrate efficacy if present. This, however, doses increase the cost and potential intolerability of the drug.

We also feel strongly that the laboratory component of this study is crucial to our understanding of the potential benefits of NAC (or related compounds) in this setting.

9) **Effects of NAC on HIV**

Although this is an interesting hypothesis, we would not expect a two month course of treatment with NAC to significantly impact on the course of the HIV infection. Therefore, this point has been de-emphasized in the protocol. CD4 counts are being recorded to ensure there is no relationship between hypersensitivity reaction and CD4.

10) **Acetylator Phenotype**

The role of acetylator phenotype is an interesting but complex question. One of our co-investigators (Dr. Neil Shear) previously showed that there may be an increase in the rate of hypersensitivity reactions in patients of the slow acetylator phenotype. However, more recently it has been demonstrated that Trimethoprim Sulfamethoxazole is primarily metabolized by the unimodal acetyl transferase pathway. Therefore, in theory, acetyl phenotype should not matter. As the ratio of fast to slow acetylator phenotypes is approximately 50/50 in the population, this should balance out in the study. The techniques for the determination of acetylator phenotype and genotype requires the use of white blood cells and are technically demanding. There has been some hesitation by our laboratory in performing these studies in patients infected with HIV. What we could do, however, is to collect a sample of blood on each patient and freeze it and the genotypes could be determined retrospectically if NAC was found to be efficacious. At that time, we could apply for further funding to complete this aspect of the study.
I hope that we have been able to answer these questions to the satisfaction of the committee. I anxiously await your response as we are anxious to proceed with this study in a multicentre fashion.

Sincerely,

Sharon Walmsley, MD, FRCP(C)
Division of Infectious Diseases

SLW/ast
November 24, 1993

Dr. Sharon Walmsley
Toronto General Hospital, EN G-219
200 Elizabeth Street
Toronto, ON
M5G 2C4

Dear Dr. Walmsley:

Re: A Randomized Trial of the Use of N-Acetylcysteine for the prevention of Trimethoprim-Sulfamethoxazole Hypersensitivity reactions when used for the prevention of Pneumocystis Carinii Pneumonia in patients with HIV Infection.

On November 21, 1993 I faxed to you the recommendations from the Scientific Review Committee with respect to your study. I promised at that time to provide you with further comments.

The first thing is that the four points in the recommendations should be resolved as quickly as possible. I will help you in any way I can and I am sure that Dr. Joel Singer will also provide some advice if you wish.

We have to discuss the budget immediately. You did a great job of using the "Zarowny" prototype budget. Of course the resulting magnitude did raise some questions from some people sitting around the meetings as to the amount of support required from the Network. Consequently, we now have to revisit the budget and evaluate what resources the Network can put to your project.

I am also enclosing copies of the reviewers' comments, some of which are quite interesting and worthy of thought.

In one of the discussion periods an interesting question was raised regarding the phenotype of the patients who would be studied as indicated by
determining whether they were slow or fast acetylators. The question was asked does this makes a difference with respect to the development or production of toxic metabolites. I know you have access to world authorities at "sick kids" on this and will be able to provide us with some comments.

Sharon, if you have any questions that I can help you with in addition to the budgetary comments please do not hesitate to contact me.

Yours very truly,

Donald P. Zarowny, M.D., M.Sc.
Programme Head
Scientific Industrial Liaison

DPZ/jsz

cc  Dr. J. Singer
    Dr. M. Schechter
    Dr. F.Aoki
**Trial Name:** A randomized trial of the use of N-acetylcysteine for the prevention of sulphonamide hypersensitivity reactions when used for the prevention of Pneumocystis carinii pneumonia in patients with HIV infection

**Sponsor:** Physician Services Inc., CanFAR, Bristol Myers Squibb

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>Known Sites</th>
<th>Investigator(s)</th>
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<tbody>
<tr>
<td>Walmsley, Dr. Sharon</td>
<td>St. Paul's, Vancouver</td>
<td>Peter Phillips</td>
<td>604-631-5317</td>
</tr>
<tr>
<td>Div. of Infectious Diseases</td>
<td>Southern Alberta HIV Clinic, Calgary</td>
<td>John Gill</td>
<td>403-670-2481</td>
</tr>
<tr>
<td>Toronto Hospital</td>
<td>Royal U. Hospital, Saskatoon</td>
<td>Kurt Williams</td>
<td>306-966-1660</td>
</tr>
<tr>
<td>200 Elizabeth Street, ENG-219</td>
<td>U. of Manitoba, Winnipeg</td>
<td>Fred Aoki</td>
<td>204-788-6625</td>
</tr>
<tr>
<td>Toronto, Ontario</td>
<td>St. Joseph's, London</td>
<td>Janet Gillmore</td>
<td></td>
</tr>
<tr>
<td>MSG 2C4</td>
<td>McMaster University, Hamilton</td>
<td>Fiona Small</td>
<td>416-521-9800</td>
</tr>
<tr>
<td>Telephone: 416-340-3871 Fax: 595-5826</td>
<td>St. Michael's, Toronto</td>
<td>Bill Fong</td>
<td>416-864-5746</td>
</tr>
<tr>
<td>Therapy: Prophylaxis for ADI</td>
<td>Toronto Hospital (General)</td>
<td>Sharon Walmsley</td>
<td>416-340-3871</td>
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<td>Drug(s) Tested: N-Acetylcysteine (NAC)</td>
<td>Sunnybrook, Toronto</td>
<td>Anita Rachlis</td>
<td>416-480-4689</td>
</tr>
<tr>
<td>AIDS Defining Illness: PCP</td>
<td>Ottawa General</td>
<td>Bill Cameron</td>
<td>613-737-8169</td>
</tr>
<tr>
<td>Inclusion Criteria: HIV Positive</td>
<td>Montreal General</td>
<td>Emil Toma</td>
<td>514-843-2611</td>
</tr>
<tr>
<td>Exclusion Criteria: previous allergic reaction to sulphonamides or trimethoprim</td>
<td>Victoria General, Halifax</td>
<td>Wally Schlech</td>
<td>902-428-3742</td>
</tr>
</tbody>
</table>

CD4 Count Range: 0 to 200
- treatment with TMP-SMX since diagnosis of HIV
- a study drug other than AZT, ddI or ddC
- NAC from other sources

**Trial Type:** Pilot Study

**Target:** 200

**Enrolment Status:** Enrolled

**Telephone:**

**End Date:**

**Purpose:** The objective of this study is to test the hypothesis that giving N-acetylcysteine (NAC) to patients with HIV infection will decrease the extraordinary incidence of serious adverse reactions to trimethoprim-sulfamethoxazole (sefpral) observed in this population. This study is a PCP prophylaxis study. NAC will be maintained for two months or until sefpral is discontinued.
March 9, 1994

Sharon L. Walmsley, MD., FRCP(C)
The Toronto Hospital
Division of Infectious Diseases
200 Elizabeth St., ENG 219
Toronto, Ontario M5G 2C4

RE: Mucomyst Study

Dear Dr. Walmsley:

It was a pleasure speaking to you on the telephone the other day. I look forward to meeting you on March 17th.

We have discussed the options of your group paying for medication vs Roberts providing funds for a field monitor.

We have made a decision to provide the medication for the entire study to you free of charge (7,000 vials). Thus, you will take care of funding a field monitor from your study budget.

Since this was the agreement which you had originally made with BMS, I am certain this is acceptable to you.

With Best Regards,

[Vice President's Signature]

FSC/cc

bcc: R. Loy
     A. Rivest
     A. Woodruff
     T. Griffin
December 22, 1995

Dr. Sharon L. Walmsley
The Toronto Hospital
Division of Infectious Diseases
200 Elizabeth Street, ENG-219,
Toronto, Ontario
M5G 2C4

Dear Dr. Walmsley:

As you are aware, Roberts Pharmaceutical Canada Inc. made a commitment to you to supply 7000 x 30mL vials of Mucomyst for the H.I.V. NAC trial. As of November 30, 1995, we had supplied 6,600 vials or 94.3% of the total agreed to on March 9, 1994.

Colleen Tooke, our Sales & Marketing Assistant, has brought a few issues to my attention that requires some discussion with you.

- On December 19, 1995, Shideh Khorasheh indicated to Colleen that three (3) sites have been shipped Mucomyst that have been not used.

The sites and investigators with unused stock are:

Dr. Fred Aoki
Health Sciences Centre, Manitoba
(240 vials - 02/94, 240 vials - 07/94)

Dr. Bill Cameron
Ottawa General Hospital
(Shipped - 09/94)

Dr. Bayoumi
Wellesley Hospital, Toronto
(Shipped - 04/95)

*Dr. Bayoumi replaces Dr. Fanning at Wellesley

We are concerned that the investigators have ordered Mucomyst and then let the supplies lie dormant in excess of one (1) year.

...
Apparently it was suggested by Shidch that Roberts move the Mucomyst around preferably by having the product returned to us for redistribution.

- Shidch also suggested that you may require an additional 3000 vials. As this was not part of our initial agreement and since we have not budgeted for these additional vials, I am not sure how we can honour this new request.

Please call me regarding these concerns.

Yours truly,

ROBERTS PHARMACEUTICAL CANADA INC.

[Signature]

A. E. Woodruff
General Manager

AEW: 1105/jt
Canadian HIV Trials Network

Standard Operating Procedures

Randomization Procedures

Purpose
This procedure describes the process by which a patient is screened by the HIV trial centre before being accepted into the trial.

Procedures for randomization
1. In response to a phone call from the site coordinator, the national coordinator reviews the eligibility of the patient with the site coordinator by completing the randomization form containing the inclusion/exclusion criteria.

2. If the patient meets all the inclusion/exclusion criteria, the national coordinator assigns the patient a study number and drug regimen according to the randomization list provided by the National Data Centre.

3. The national coordinator signs and dates the randomization form and FAXes the form to the site coordinator and the site pharmacist for their records.

4. The national coordinator will then record the patient’s randomization and the date and other relevant information on a master list specific to the trial.
Canadian HIV Trials Network

Standard Operating Procedures

Drug Ordering Procedure

**Purpose**

This procedure describes the steps to be followed when ordering shipment of drugs from Roberts Pharmaceutical Canada Inc.

**Procedures**

1. When the patient is randomized to the NAC Trial, the national coordinator will notify the Roberts Pharmaceutical Canada Inc. in order to send the mucomyst to the site pharmacist.

2. In addition, when your drug supply is low, the pharmacist should notify the national coordinator two weeks in advance. The national coordinator will then notify the Roberts Pharmaceutical Inc. and the mucomyst is then send out to the site pharmacist.
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Canadian HIV Trials Network

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Serious Adverse Event

A. Notification of the Canadian HIV Trials Network by the Sites

Purpose
This procedure describes the process and obligations of the trial sites in the reporting of the serious adverse event (SAE) to Canadian HIV Trials Network.

Definition
Discuss with Joel.
An SAE is reported if the patient is reported to have any of the two following symptoms:

1. Rash - Diffuse erythematous eruption involving at least 50% of body surface area.
2. Fever - Oral temperature >38.5 C on at least two occasions within 48 hours at least two hours apart.

Please note that when the decision is made to discontinue Septra (Trimethoprim-sulfamethoxazole), the patient will be seen by a second physician at the study centre. Two approaches will be used to ensure any bias is minimized because in some cases the patient will unblind their treating physicians.

Procedure
1. All SAE must be reported by the site to the national coordinator at the Canadian HIV Trials Network by Phone within 24 hours of the investigator having learnt of it.

2. The site then completes the SAE forms found in the case report folders (copy attached).

3. The site then FAXes the SAE form to the Canadian HIV Trials Centre immediately.

4. The national coordinator will then check the form for eligibility of an SAE and for completeness (errors, omissions, inconsistencies, signature of the investigator and date).

5. The national coordinator calls or faxes the site and requests sites to fax back corrections immediately.
Canadian HIV Trials Network

Standard Operating Procedures

Reporting of Septra Reaction

A. Notification of the Canadian HIV Trials Network by the Sites

Purpose
This procedure describes the process and obligations of the trial sites in the reporting of the Septra reaction (orange form) to Canadian HIV Trials Network.

Definition
A septra reaction is reported if the patient is reported to have any of the two following symptoms:

1. Rash - Diffuse erythematous eruption involving at least 50% of body surface area.
2. Fever - Oral temperature >38.5°C on at least two occasions within 48 hours at least two hours apart.
3. Pruritus - Severe pruritus is defined as that which is intolerable to the patient.

Please note that when the decision is made to discontinue Septra (Trimethoprim-sulfamethoxazole), the patient will be seen by a second physician or a study nurse who is unaware of randomization at the study centre. This approach will be used to ensure any biases are minimized because in some cases the patient inadvertently will unblind their treating physicians.

Procedure
1. The septra reaction must be reported by the site to the national coordinator at the Canadian HIV Trials Network by phone within 24 hours of the investigator having learnt of it.

2. The site then completes the septra reaction form (orange form) and the discontinuation form found in the case report folders (copy attached).

3. The site then FAXes the septra reaction form along with the discontinuation form to the Canadian HIV Trials Network immediately.

4. The national coordinator will then check the form for eligibility of a septra reaction and for completeness (errors, omissions, inconsistencies, signature of the investigator and date).
Canadian HIV Trials Network

Standard Operating Procedures

Blinding Procedure for Investigators

**Purpose**

This procedure describes the steps to be followed in order to keep the investigators blinded to the study regimen of the patient.

**Procedures**

Please note: All the NAC trial investigators are blinded to the study drugs. Therefore, when a patient enters the NAC study, the study coordinator will ask the investigator to sign a prescription for both the NAC (mucomyst) and the trimethoprim-sulfamethoxazole (septra). The study nurse takes both of the prescriptions back to the site pharmacist. In a case when the patient is randomized to the placebo arm (i.e. septra only), the nurse coordinator and the pharmacist will destroy the prescription for the NAC (mucomyst). This way, the investigator will remain blinded to the drug regimen for the patient.

Please note: NAC can be dispensed one month at a time.
Canadian HIV Trials Network

Standard Operating Procedures

Protocol Exemptions

Purpose
This procedure describes the steps to be followed when a patient does not meet the inclusion/exclusion criteria which is considered for randomization to a trial.

Definition
A site may wish to randomize a patient for whom, during the randomization process, it is discovered that he/she meets all but a single inclusion or exclusion criterion. A protocol exemption refers to permission given by the principal investigator to allow a patient in this circumstance to be randomized to the trial.

Procedures
1. The coordinator, during discussion with the site regarding the patient's eligibility, informs the site of the patient's ineligibility regarding the particular inclusion/exclusion criteria.

2. If the site wishes to appeal to the principal investigator for a protocol exemption, the coordinator records the specific conditions of the exemptions on behalf of the site.

3. If the principal investigator agrees to grant permission to allow the patient a protocol exemption, the coordinator records the specific conditions of the exemption on the randomization sheet.

4. The coordinator then writes the site a letter of exemption for that patient for the site's files.
Appendix II

Recruitment Strategies

- notice of investigators meetings
- gay newspaper advertisement
- NAC campaign
- NAC newsletter
- example of trial update
MEMO

TO: NAC TRIAL INVESTIGATORS AND COORDINATORS

FROM: Dr. DON ZAROWNY, PROGRAMME HEAD, SCIENTIFIC AND INDUSTRIAL LIAISON, CTN
DR. SHARON WALMSLEY, PRINCIPAL INVESTIGATOR

DATE: MAY 5, 1994

SUBJECT: NAC TRIAL BREAKFAST MEETING

We would like to invite you to an investigators and coordinators (NAC trial) breakfast meeting on Thursday June 2, 1994. The breakfast meeting is tentatively scheduled to take place from 7:00 am to 8:30 am at Chelsea Delta Hotel in Toronto. You will be notified of the location of the room in the Delta Hotel.

In order to start making the necessary arrangements, please inform Shideh Khorasheh (at the above fax number) whether or not you are planning to attend this meeting prior to May 13, 1994.

YOUR PROMPT REPLY WILL BE GREATLY APPRECIATED!
PCP Prevention Study Seeks Volunteers

If you are:
- HIV positive
- have a CD4 count of less than 200
- interested in contributing to knowledge of AIDS

We are:
- conducting a study to explore the theory that N-acetylcycteine (NAC) may decrease the incidence of allergic reactions to Septra in HIV positive people.

If you are interested in participating, please contact the Study Coordinator, Mr. Brown, 631-5441 at St. Paul's Hospital. To be eligible for the study, please contact us BEFORE you start Septra.
MEMO

TO: DR. TSOUKAS AND TONI DI GIROLAMA

FROM: DR. SHARON WALMSLEY, PRINCIPAL INVESTIGATOR
      DR. DON ZAROWNY, PROGRAM HEAD
      SHIDEH KHORASHEH, CLINICAL TRIAL COORDINATOR

DATE: JANUARY 20, 1995

SUBJECT: NAC RECRUITMENT

We hope that all is well with you. We wanted to take this opportunity to wish everyone a happy new year. Please find enclosed several pens that we are providing for you and your study coordinator in order to express our appreciation for your involvement in the NAC study. There are also a few extra pens included which can be given to the family physicians at your site so that they also remember the NAC study when they start their patients on septrra.

In addition, I would like to provide everyone with an update of enrollment at their sites.

Ethics Approval Date: November 1994
# of patients to date: 2
Future Expectation: 1 per month

In addition, please find enclosed a graph that shows the date when the patients were enrolled to the NAC study at your site.

There are a total of 18 sites involved in the NAC study and if you could kindly enroll one patient per month at your site, we can finish the study in 9 months.
Thanking you for your continued cooperation.

Sincerely,

Shideh Khorasheh
Clinical Trials Coordinator
A RANDOMIZED TRIAL TO DETERMINE THE EFFECTIVENESS OF N'ACETYLCYSTEINE (MUCOMYST) TO PREVENT TRIMETHOPRIM-SULFAMETHOXAZOLE (SEPTRA) HYPERSENSITIVITY REACTIONS IN HIV

FOR MORE INFORMATION CONTACT:
## UPDATE

**The Study Continues**

This newsletter is to update you on the progress of the NAC trial. The NAC study (N-acetylcysteine for prevention of sulphonamide hypersensitivity) was initiated in late 1991 with its objective to determine if NAC (mucomyst) could prevent Sulpha hypersensitivity reactions. This could allow more people with HIV to use Septra which remains the most valuable therapy for the prevention and treatment of PCP. Fever and rash are among the symptoms associated with this hypersensitivity which occurs in approximately 30% of persons living with HIV. In 1991, the study developed by principal investigator Dr. Sharon Walmsey was initiated in Toronto centers only. In January of 1994, this study was redesigned and was approved by the Canadian HIV Trials Network with a multicenter focus.

### Study Design

Patients are entered into the study when the CD4 count is <200x10^6/L or 20% and Septra is to be started for PCP prophylaxis. For the first two months they receive Septra they are randomized to receive or not to receive NAC. They are followed for a total of 5 months.

### Current Enrollment

At the present time, there are a total of 18 sites involved across Canada with total of 144 patients enrolled in the study.

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<th># of Sites</th>
<th>Enrollment</th>
</tr>
</thead>
<tbody>
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<td><strong>Total</strong></td>
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## Data Collection

Data collection is rolling along smoothly and an interim analysis was recently performed. We are pleased to inform you that at the meeting of April 24, 1995, the Safety and Efficacy Review Committee of the Canadian HIV Trials Network reviewed the Interim Analysis for the NAC trial and recommended that the trial be continued.

### We need your help

The estimated sample size for the trial is approximately 300. However, enrollment has been much slower than expected and this was the major concern of the Safety and Efficacy Review committee. At the current rate of enrollment, more than 20 months will be required to complete the study. Thus, your help is essential to keep the study going. If we would all redouble our efforts and enroll one patient per month, this study could be completed in eight months. If you have an eligible patient who is interested in the study, please contact the study coordinator in your area.

Dr. Sharon Walmsey and the team of investigators as well as the Canadian HIV Trials Network would like to thank you for your interest in the NAC study.
**RECRUITMENT**

**Update**

This newsletter is to update you on the progress of the NAC trial. The study still continues and through everyone’s help and enthusiasm, the recruitment rate has increased a great deal during the last six months. The average patient enrollment has been nine patients per month. So congratulations to all of you for all your effort. We hope to be able to increase or continue recruitment at current rate. A total of 196 patients have been enrolled to date. Our target is 296 patients with 250 evaluable. We hope to complete the study and be able to present the preliminary analysis at the International Conference in Vancouver this summer. So please contact Shideh at 1-800-661-4664 if you have an eligible patient.

**Welcome on Board**

Recently two new sites joined the team of the NAC investigators. We would like to welcome Dr. Roger Sandre from Sudbury, Ontario and his study coordinator, Ms. Sue Jefferson. We would also like to extend our welcome to Dr. David Burdge from Vancouver, BC and his study coordinator Ms. Elizabeth

---

**HAPPY NEW YEAR**

**NAC Housekeeping**

Data collection is rolling along. However, I would like to remind everyone to forward the case report forms on a regular basis so that I can deal with the queries and problems right away.

In addition, please remember to inform Shideh right away if a patient experiences a septa reaction or a serious adverse event. Serious adverse events are described as: death, life-threatening, disabling or incapacitating, requires or prolongs hospitalization, a congenital anomaly or cancer.

I also would like to remind the pharmacists to notify Shideh ahead of time when their supplies of mucomyst is low. A period of one to two weeks is required in order for the Roberts Pharmaceutical Canada Inc. to ship out the drug supplies.

---

**Current Enrollment**

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<thead>
<tr>
<th>Regions</th>
<th># of Sites</th>
<th>Enrollment</th>
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</thead>
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<td>Ontario</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
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---

**Merry Christmas**

Dr. Sharon Walsmley, Shideh and the team of the Canadian HIV Trials Network would like to wish you a Merry Christmas and a Happy New Year. Cheers to another successful working year.
TO: ALL NAC TRIAL INVESTIGATORS

FROM: DR. SHARON WALMSLEY, PRINCIPAL INVESTIGATOR
       SHIDHEH KORASHEH, NATIONAL TRIAL COORDINATOR

DATE: SEPTEMBER 7, 1995

SUBJECT: NAC RECRUITMENT

We hope that all is well with you. We would like to take this opportunity to thank everyone for their interest and participation in the NAC study. In the past, we have encouraged everyone to redouble their efforts in order to increase recruitment into the NAC study. We know that at times, we appeared to be a nuisance to everyone. However, we are delighted to inform everyone that all your efforts have been worth while. The recruitment has picked up over the summer time. The total number of patients in the NAC study is now 175. During the summer time, the number of patients enrolled into the study were:

<table>
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<th>Months</th>
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<td>9</td>
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<td>August / 1995</td>
<td>11</td>
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<tr>
<td>Total</td>
<td>28</td>
</tr>
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The following graph shows the breakdown of the numbers over the summer time by region.

Finally, I am also providing you with a graph which shows the number of actual patients' enrollment (Total = 109) in the NAC study since January 1994 which is the official date when CTN took on the study. In the graph, I have also presented you with the expected number of enrollment. The expected patient's enrollment equals the number of sites with their ethics approval. For example, in October 1994, when 15 active sites were involved in the study, the expected enrollment is 15 (1 patient/active site) but the actual enrollment in October was only 7 patients.
So congratulations to all of you for all your effort. Thank you for your continued cooperation.

Sincerely,

Shideh Khorasheh
National Trials Coordinator
April 19, 1995

Dr. Sharon Walmsley
Toronto Hospital
Division of Infectious Diseases
200 Elizabeth Street, ENG-219
Toronto, Ontario
M5G 2C4

Dear Sharon:

As we discussed at the last open meeting of the Ontario Region of the CTN two weeks ago, I will continue to do everything I can to support the success and conduct of the NAC trial of cotrimoxazole tolerance. In addition to having the National Study Coordinator fax out a monthly pie chart of accruals and evaluable patients in the study, I would suggest that you write a personal letter to sites, satellites and potential investigators concerning the rationale for your study, and promote the fact that the study has every opportunity given accrual to answer the important question at hand. This enclosed editorial review on hypersensitivity reactions to drugs in patients with HIV (AIDS 1995, 9:217-222 by Coopmans et al) might be a useful enclosure with such a letter. This well written review addresses your hypothesis and should increase the resolve in the CTN to bring your study to successful completion.

So far, the Ontario Region has with administrative funds supported hiring of a National Coordinator, and provided additional support to new satellites participating in your study in Ontario. I trust that funds, from the National Centre for support of the local Coordinator have arrived via Dr. Rachlis at Sunnybrook.

Please let me know if there’s anything else I can do to facilitate this from an administrative point of view.
As a centre, I will redouble my efforts to have patients in primary care settings local to Ottawa informed through their physicians at their opportunity to contribute.

Best personal regards.

Sincerely,

Bill Cameron MD,FRCP
Assistant Professor of Medicine
Division of Infectious Diseases
Ontario Ministry of Health Career Scientist
Director, Ontario Region/Canadian HIV Trials Network

BC:lw

cc: Bob O’Neill
MAY 4, 1995

Ms. Shideh Khorasheh  
Clinical Trials Coordinator  
Canadian HIV Trials Network  
200-1033 Davie Street  
VANCOUVER, BC V6E 1M7

Dear Shideh:

Thank you for preparing the memo to the Site Investigators and to Coordinators. I think this time we should send a copy to each of the Investigators and Co-ordinators to make sure they receive it. I would like to reword it as follows:

Dear NAC Investigators: (but direct them personally)

We hope that all is well with you. We would like to take this opportunity to remind everybody about the importance of the NAC Trial. As you know, the purpose of this study is to determine whether or not glutathione, when supplied as N'acetylcysteine or Mucomyst, can prevent the high incidence of sulfonamide hypersensitivity reactions in patients living with HIV. Although the dose and the formulation of NAC that we are using in this study may not be optimal in terms of the appropriate compound for long term use, the dose was deliberately selected to be high to determine whether or not there is effect and can be later refined. We enclose for you a recent paper published in the AIDS Journal which re-emphasizes the importance of this question.

At the meeting of April 24, 1995, the Safety and Efficacy Review Committee of the Canadian HIV Trials Network reviewed the interim analysis for the NAC trial and recommended that the trial be continued. However, enrolment of this trial has been much slower than expected. This was a major concern of the Committee and your help is essential to keep the study going. In this regard, we provide you with an update of enrolment at your site.

Ethics Approval Date:  
# of Patients Enrolled to Date:  
# of Patients Completing the Study:  
# of Patients Expected to Date:  
Future Expectations: 1 patient per month

In addition, please find enclosed a graph which shows the dates on which patients were enrolled into the NAC Study at your site. We also provide you with a graph which shows the total number of actual patient enrolment across the country (total = 77) since January 1994, which is the official date when the CTN took on this study. In the graph, we have presented you with the expected number of patients we hoped would be enrolled. The expected number of patient enrolment has been calculated by determining the number of sites who have their Ethics Approval at the time and anticipating one patient per centre enrolment per month. For example, in October 1994,
when 15 active sites were involved in the study, the expected enrolment was 15 patients (1 patient per active site), but the actual enrolment in October was only 7 patients. At the current rate of enrolment, more than 20 months will be required to complete this study. If we could all redouble our efforts and enrol one patient per month, this study could be completed in eight months. An important enrolment strategy that a number of centres have found useful is to ensure that people are thinking about the trial. In this regard, we enclose a poster that you might place in the examining rooms of your physicians to remind them of the trial and perhaps even distribute to your family doctors, as they may be an important source of patient referral.

Thank you for your continued co-operation and if there is anything any of us can do to help you improve the enrolment at your site, please feel free to contact us.

Sincerely,


Sincerely,

Sharon L. Wairmsley, MD, FRCP(C)
Division of Infectious Diseases

SLW/ast

encl. poster paper
Appendix III

Case Report Forms

- baseline

- follow-up

- discontinuation of study medications

- septra hypersensitivity

- compliance
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</table>
N-ACETYLCYSTEINE

INCLUSION/EXCLUSION

INCLUSION CRITERIA

Codes: 1 = Yes, 2 = No

1. IS THE PATIENT KNOWN HIV POSITIVE? □

2. DOES THE PATIENT HAVE CD4 COUNT < 200 X 10^6/L. CD4% < 20% OR AN AIDS DIAGNOSIS? □

3. DOES THE PATIENT CONSENT TO STUDY AND ABLE TO COME FOR FOLLOW UP? □

EXCLUSION CRITERIA

1. HAS THE PATIENT HAD A PREVIOUS ALLERGIC REACTION TO SULPHONAMIDE OR TRIMETHOPRIM-SULFAMETHOXAZOLE? □

2. HAS THE PATIENT BEEN TREATED WITH SULFA SINCE BEING DIAGNOSED WITH HIV? □

3. IS THE PATIENT RECEIVING ANY STUDY DRUGS OTHER THAN AZT, DDI OR DDC? □

4. DOES THE PATIENT HAVE A PMN < 1000 X 10^6 PRIOR TO DOSING? □

5. DOES THE PATIENT PREFER ANOTHER FORM OF PROPHYLAXIS? □

6. IS THE PATIENT TAKING NAC FROM ANOTHER SOURCE? □
N-ACETYLCYSTEINE

PATIENT NUMBER [ ] [ ]

DATE OF ASSESSMENT [ ] [ ] [ ]

PATIENT INITIALS [ ] [ ] [ ]

DAY MONTH YEAR

HIV RELATED ILLNESS

Codes: 1 = Yes, 2 = No

1. IS THE PATIENT SYMPTOMATIC? [ ]

If No skip to next section, if Yes.

   a. ORAL CANDIDIASIS [ ]
   b. WEIGHT LOSS > 10 LBS. SINCE HIV DIAGNOSIS [ ]
   c. CHRONIC DIARRHEA [ ]
   d. NIGHT SWEATS [ ]
   e. SALMONELLA BACTEREMIA [ ]
   f. Nocardia [ ]
   g. ORAL HAIRY LEUKOPLAKIA [ ]
   h. HERPES ZOSTER [ ]
   i. LYMPHADENOPATHY [ ]
   j. IMMUNE THROMBOCYTOPENIA [ ]
   k. OTHER (SPECIFY) [ ]
   l. OTHER (SPECIFY) [ ]
### N-ACETYL-CYSTEINE

#### BASELINE EVALUATION

**PATIENT NUMBER**

**PATIENT INITIALS**

**DATE OF ASSESSMENT**

**OPPORTUNISTIC INFECTIONS/MALIGNANCIES**

1. **HAS THE PATIENT HAD ANY OPPORTUNISTIC INFECTIONS OR MALIGNANCIES?**
   - [ ]
   - **If No skip to next section, if Yes.**
   - **Codes:** 1 = Yes, 2 = No
   - **Status Codes:** 1 = Active, 2 = Stable, 3 = Resolved

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<td>b. KAPOST'S SARCOMA</td>
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<td></td>
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<tr>
<td>c. EXTRA PULMONARY TB</td>
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<td></td>
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<tr>
<td>d. MAC</td>
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<tr>
<td>e. CYTOMEGALOVIRUS</td>
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<tr>
<td>f. TOXOPLASMSISMOSIS</td>
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<td></td>
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<tr>
<td>g. CRYPTOSPORIDUM</td>
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<td></td>
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<tr>
<td>h. ISOPORA</td>
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<tr>
<td>i. PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY</td>
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<tr>
<td>j. NON-HODGKINS LYMPHOMA</td>
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<td>k. DISSEMINATED STRONGLOIDS</td>
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<td>l. HISTOPLASMSISMOSIS</td>
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<td>m. CHRONIC HERPES SIMPLEX</td>
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<td>n. 1° CNS LYMPHOMA</td>
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<tr>
<td>o. OTHER (SPECIFY)</td>
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**DATE OF ONSET**

**Status**

- [ ]
- [ ]
N-ACETYLCYSTEINE

DATE OF ASSESSMENT

1. FIRST DOSE OF SEPPTRA RECEIVED AT [ ] HRS.
   Day  Month  Year

2. DATE OF FIRST DOSE OF SEPPTRA
   Day  Month  Year

SEPPTRA TO BE GIVEN AS ONE SINGLE STRENGTH TABLET BID

3. FIRST DOSE OF MUCCOMYST RECEIVED AT [ ] HRS.
   Day  Month  Year

4. DATE OF FIRST DOSE OF MUCCOMYST
   Day  Month  Year

5. WAS A GLUTATHIONE LEVEL DONE?  Codes: 1 = Yes, 2 = No
   Day  Month  Year
   a. DATE GLUTATHIONE LEVEL DONE
   Day  Month  Year
   b. TIME GLUTATHIONE LEVEL DONE [ ] HRS.

6. PATIENT WEIGHT (KG)

VITAL SIGNS

1. TEMPERATURE
   [ ] °C

2. BLOOD PRESSURE
   [ ] / [ ]

3. PULSE

4. RESPIRATION

HEMATOLOGY

1. DATE OF HEMATOLOGY
   Day  Month  Year

2. DATE OF THYROID
   Day  Month  Year

3. WBC (x 10^9/L)

4. HGB (g/L)

5. PLT

6. GRAN%  9. TSH (mU/L)

7. LYMPH %

8. EOS X 10^9  or %

10. COOMBS TEST  Codes: 1 = Positive, 2 = Negative
**N-ACETYLCYSTEINE**

**BASELINE LAB DATA**

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<thead>
<tr>
<th><strong>PATIENT NUMBER</strong></th>
<th><strong>PATIENT INITIALS</strong></th>
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**DATE OF ASSESSMENT**

**BIOCHEMISTRY**

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<th>2. DATE OF IMMUNOLOGY</th>
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<tr>
<th>3. BUN (mmol/L)</th>
<th>12. CD4 (x 10^6/L)</th>
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<th>4. CR (u mol/L)</th>
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<th>5. GLU (mmol/L)</th>
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<th>2. pH</th>
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<th>7. S.G.</th>
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<tr>
<td>Codes: 1 = Negative, 2 = Positive, 3 = Trace, -1 = Not done</td>
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<th>3. PROTEIN</th>
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**CXR**

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**COMMENTS:**

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**EKG**

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N-ACETYLCYSTEINE

PATIENT NUMBER [ ] [ ]

CONCURRENT THERAPY

PATIENT INITIALS [ ] [ ] [ ]

DATE OF ASSESSMENT

1. IS THE PATIENT ON ANY CONCURRENT THERAPY? Codes: 1 = Yes. 2 = No

   If No. skip to next section. If yes,

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<td>c. ddC</td>
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<td>d. ddT</td>
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<td>e. 3TC</td>
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<td>- Specify:</td>
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PLEASE PROCEED TO PAGE 2
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<td>cc. prednisone</td>
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<tr>
<td>ee. vitamin E</td>
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<td>gg. zinc</td>
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<td>hh. selenium</td>
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<td>ii. chromium</td>
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<td>jj. co-enzyme Q</td>
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<td>kk. quercetin</td>
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<td>ll. lipoic acid</td>
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<td>oo.</td>
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<tr>
<td>pp.</td>
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</tbody>
</table>

2. HAS THE PATIENT USED RECREATIONAL DRUGS? Codes: 1 = Yes, 2 = No [ ]

COMMENTS: _______________________________________________________
_________________________________________________________________
_________________________________________________________________

3. AVERAGE ETOH INTAKE PER WEEK ___________________________
N-ACETYLCYSTEINE

PATIENT NUMBER

PATIENT INITIALS

DATE OF ASSESSMENT

WEEK NUMBER

VITAL SIGNS

1. TEMPERATURE

2. BLOOD PRESSURE

3. PULSE

4. RESPIRATION

SEPTRA DOSE AND FREQUENCY

1. SINCE LAST REPORT DID PATIENT TAKE ALL DOSES OF SEPTRA?
   Codes: 1 = Yes, 2 = No

2. NUMBER OF DOSES OF SEPTRA MISSED

3. REASON FOR MISSING DOSES

4. DID PATIENT HAVE ANY ADVERSE EFFECTS?
   Codes: 1 = Yes, 2 = No
   IF YES:

5. DESCRIBE:

N-ACETYLCYSTEINE DOSE (g/Dose)

1. SINCE LAST REPORT DID PATIENT TAKE ALL DOSES OF MUCOMYST?
   Codes: 1 = Yes, 2 = No

2. NUMBER OF DOSES OF MUCOMYST MISSED

3. REASON FOR MISSING DOSES

3b. IS MUCOMYST TAKEN 1 HR. PRIOR TO SEPTRA

4. DID PATIENT HAVE ANY ADVERSE EFFECTS?
   Codes: 1 = Yes, 2 = No
   IF YES:

5. DESCRIBE:

6. HAS THERE BEEN ANY CHANGE IN THE PATIENT'S MEDICATIONS?
   IF YES PLEASE RECORD ON CONCOMITANT MEDICATIONS SHEET
### Hematology

1. **Date of Hematology**: [ ] [ ] [ ]

2. **WBC ($\times 10^9$)**: [ ] [ ] [ ]
3. **HGB (g/L)**: [ ] [ ] [ ]
4. **PLT**: [ ] [ ] [ ]
5. **GRAN%**: [ ] [ ] [ ]
6. **LYMPH %**: [ ] [ ] [ ]
7. **EOS X $10^9$**: [ ] [ ] [ ] or [%] [ ] [ ]

### Biochemistry

1. **Date of Biochemistry**: [ ] [ ] [ ]

2. **BUN (mmol/L)**: [ ] [ ] [ ]
3. **CR (umol/L)**: [ ] [ ] [ ]
4. **GLU (mmol/L)**: [ ] [ ] [ ]
5. **CPK (U/L)**: [ ] [ ] [ ]
6. **AMYL (U/L)**: [ ] [ ] [ ]
7. **AST (U/L)**: [ ] [ ] [ ]
8. **ALT (U/L)**: [ ] [ ] [ ]
9. **ALP**: [ ] [ ] [ ]
10. **GGT**: [ ] [ ] [ ]

### Immunology (At 8 Weeks and Last Followup Only)

1. **CD4 ($\times 10^5$/L)**: [ ] [ ] [ ]
## Date of Assessment

1. IS THE PATIENT ON ANY CONCURRENT THERAPY? Codes: 1 = Yes, 2 = No
   If No, skip to next section. If yes, fill in the table with the details of the concurrent therapy.

### Daily Dose

<table>
<thead>
<tr>
<th>Dose</th>
<th>Start Date</th>
<th>Stop Date</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ddI</td>
<td></td>
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<tr>
<td>ddC</td>
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<td>ddT</td>
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<td></td>
</tr>
<tr>
<td>3TC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other antiretrovirals</td>
<td></td>
<td></td>
<td>Specify:</td>
</tr>
<tr>
<td>ganciclovir</td>
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</tr>
<tr>
<td>foscarnet</td>
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<tr>
<td>acyclovir</td>
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<td></td>
<td></td>
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<tr>
<td>ciprofloxacin</td>
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<td></td>
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<tr>
<td>clofazimine</td>
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<td>edambutol</td>
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<td>clarithromycin</td>
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<tr>
<td>a interferon</td>
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<tr>
<td>amphotericin</td>
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<td>clindamycin</td>
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PLEASE PROCEED TO PAGE 2
<table>
<thead>
<tr>
<th>DAILY DOSE</th>
<th>START DATE</th>
<th>STOP DATE</th>
<th>INDICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>aa. itraconazole</td>
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<td></td>
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<tr>
<td>bb. nystatin/clotrimazole</td>
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<tr>
<td>cc. prednisone</td>
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<tr>
<td>dd. vitamin C</td>
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<td></td>
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<tr>
<td>ee. vitamin E</td>
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<td></td>
<td></td>
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<tr>
<td>ff. beta carotene</td>
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<tr>
<td>gg. zinc</td>
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<td>hh. selenium</td>
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<tr>
<td>ii. chronix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>jj. co-enzyme Q</td>
<td></td>
<td></td>
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<tr>
<td>kk. quercetin</td>
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<tr>
<td>ll. lipoic acid</td>
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<td>mm. caffeic acid</td>
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<td>nn.</td>
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<td>pp.</td>
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</table>

2. HAS THE PATIENT USED RECREATIONAL DRUGS? Codes: 1 = Yes, 2 = No

COMMENTS:

________________________________________________________________________

________________________________________________________________________

3. AVERAGE ETHOH INTAKE PER WEEK

________________________________________________________________________
N-ACETYLCYSTEINE

PATIENT NUMBER [__]  [__]
PATIENT INITIALS [__]  [__]

Briefly describe the events leading to discontinuation of either or both of the study medications.
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Codes: 1 = Yes, 2 = No

WAS SEPTRA DISCONTINUED ____________________________ [__]

DATE THAT SEPTRA WAS DISCONTINUED [__] [__] [__]

WAS THE DRUG DISCONTINUED BECAUSE OF:

1. ALLERGY REACTION TO SEPTRA [__]

2. INTOLERANCE TO SEPTRA [__]

   Describe: __________________________

3 OTHER ADVERSE EVENT [__]

   Describe: __________________________

4. SWITCH TO ANOTHER AGENT [__]

5. DEATH [__]

6. OTHER (SPECIFY) __________________________ [__]

WAS MUCOMYST DISCONTINUED? ____________________________ [__]

DATE THAT MUCOMYST WAS DISCONTINUED [__] [__] [__]

WAS THE DRUG DISCONTINUED BECAUSE OF:

1. ALLERGY REACTION TO MUCOMYST [__]

2. INTOLERANCE TO MUCOMYST [__]

3 OTHER ADVERSE EVENT [__]

4. ENTRY INTO ANOTHER TRIAL [__]

PLEASE PROCEED TO PAGE 2
N-ACETYLCYSTEINE

DISCONTINUATION OF STUDY MEDICATIONS

PATIENT NUMBER

PATIENT INITIALS

5. DEATH

6. UNABLE TO TAKE P.O. MEDS

7. OTHER (SPECIFY)

HAS THE PATIENT REQUIRED THE USE OF ANY OF THE FOLLOWING MEDICATIONS?:

1. ANTIHISTAMINES

DOSE AND FREQUENCY:

DATE

2. NSAID

DOSE AND FREQUENCY:

DATE

3. TYLENOL/ASA

DOSE AND FREQUENCY:

DATE

4. STEROIDS

DOSE AND FREQUENCY:

DATE

COMMENTS:
REQUIRED INVESTIGATIONS:
Day 5: CBC, differential, eosinophil count, AST, ALT, ALP, CPK, GGT, amylase, BUN, creatinine, uric acid, fasting glucose, lipids, calcium, phosphorus, sodium, potassium, chloride, bicarbonate, i.v. fluids, stat glucose, electrolyte levels

IN THE EVENT OF SEPTIC INFECTION / TOXIC REACTION
PLEASE COMPLETE THE FOLLOWING INFORMATION:

DATE OF INITIATION OF SEPTRA

DATE OF ONSET OF ADVERSE REACTION

WAS SEPTRA DISCONTINUED? (1 = Yes, 2 = No)

DATE THAT SEPTRA WAS DISCONTINUED

DATE OF SEPTRA AT TIME OF REACTION

DATE OF FOLLOW-UP BST STUDY / CLOTS

Was there any other medication at the time of the reaction?

DAY 1 OF REACTION

DAY 3 OF REACTION

DAY 5 OF REACTION

FEATURES OF REACTION
Codes 1 = Yes, 2 = No

SYMPTOMS:

1. DID THE PATIENT HAVE A FEVER?

MAXIMUM TEMPERATURE

2. DOES PATIENT HAVE A RASH?
   IF YES:
   a. RED, FLAT
   b. RED, RAISED
   c. RED, NUCLEUS / BLISTERS
   d. MUCOSAL

PLEASE PROCEED TO PAGE 2 OF SEPTRA REACTION FORM
Patient Number

Date: Yyy.2 = 90

Investigations

Patient initials

Day 1

(Day 1, follow-up)

Day 2

(Day 2, follow-up day 1-3)

Day 3

(Day 3, follow-up day 5-9)

1. Estimate of BSA involved (G) [ ] [ ] [ ]

2. Does the patient have pruritus? [ ] [ ] [ ]

3. Estimate what was the severity:
   Code: 1 = Mild, 2 = Moderate, 3 = Severe

4. Did investigator 42 think reaction likely due to SEPTRA? [ ]

5. Did investigator 42 think reaction likely due to SEPTRA? [ ]

6. Duration of temperature greater than 38°C? [ ] [ ] [ ]

7. Duration of rash (Days) [ ] [ ] [ ]

8. Did patient have two temp greater than 38°C at least two hours apart? [ ]

9. Did Patient have rash greater than 50% BSA? [ ]

10. Did Patient have intolerable pruritus? [ ]

Please proceed to page 3 of SEPTRA reaction form.
## E. Drug Reaction Form

**PATIENT NUMBER**

**PATIENT INITIALS**

**Code:** 1 = Yes, 2 = No

**L. Did Patient Take the Following Medication During the Reaction?**

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Date Drug Initiated</th>
<th>Date Drug Discontinued</th>
<th>Number of Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihistamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSAID</td>
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<tr>
<td>Tylenol/ASA</td>
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<td></td>
<td></td>
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<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please proceed to page 4 of SEPTA Reaction Form.
<table>
<thead>
<tr>
<th>NO.</th>
<th>Fragestellung</th>
<th>Ja</th>
<th>Nein</th>
<th>Zweifel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pankreatitis</td>
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</tr>
<tr>
<td>3</td>
<td>Ulcus duodeni</td>
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<tr>
<td>4</td>
<td>Arthritis</td>
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<tr>
<td>5</td>
<td>Dyspepsia</td>
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<tr>
<td>6</td>
<td>Megakaryosarcoma</td>
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<tr>
<td>7</td>
<td>Ankylose</td>
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</tr>
</tbody>
</table>

14. Are there any other features of the Septra reaction that you want to indicate? (1 = Yes, 2 = No)

If yes, specify:

If patient continued on Septra despite minor hypersensitivity reactions, please complete the following questions and return card. Continue to provide all follow-up information.

Codes: 1 = Yes, 2 = No

15. Did the symptoms resolve? [ ]
16. Did the symptoms decrease to an acceptable level? [ ]
17. Was Septra eventually discontinued? [ ]
   If yes:
   a) Date Septra was discontinued
18. Reason for discontinuation (specify):

________________________________________

________________________________________
# N-ACETYLCYSTEINE

**SEPTRA REACTION LAB DATA**

**DAY 1**

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Patient Initials</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Date of Assessment</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

## HEMATOLOGY

<table>
<thead>
<tr>
<th>Date of Hematology</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

<table>
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<tr>
<th>Date of Thyroid</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>WBC (× 10⁹/L)</th>
<th></th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>HGB (g/L)</th>
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<table>
<thead>
<tr>
<th>PLT</th>
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<table>
<thead>
<tr>
<th>GRAN%</th>
<th></th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>LYMPH %</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>EOS X 10⁹</th>
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<table>
<thead>
<tr>
<th>TSH (mU/L)</th>
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</tr>
</thead>
</table>

| Coombs Test Codes: 1 = Positive, 2 = Negative |     |       |      |

## BIOCHEMISTRY

<table>
<thead>
<tr>
<th>Date of Biochemistry</th>
<th>Day</th>
<th>Month</th>
<th>Year</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>BUN (nmol/L)</th>
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<table>
<thead>
<tr>
<th>CR (µmol/L)</th>
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<th>GLU (nmol/L)</th>
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<th>CPK (U/L)</th>
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<table>
<thead>
<tr>
<th>GGT</th>
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</thead>
</table>
URINALYSIS

1. DATE OF URINALYSIS: _________________________
   Day: _______  Month: _____  Year: _______

2. pH: _______  _______

3. PROTEIN: _______

4. BLOOD: _______

5. LEUK: _______

6. EOS: _______

7. S.G.: _______

   Codes: 1 = Negative, 2 = Positive, 3 = Trace, -1 = Not done

8. KETONES: _______

9. BILIRUBIN: _______

10. GLUCOSE: _______

CXR

1. RESULTS OF CXR: _______
   Codes: 1 = Normal, 2 = Abnormal, -1 = Not Done

COMMENTS: __________________________________________

EKG

1. RESULTS OF EKG: _______
   Codes: 1 = Normal, 2 = Abnormal, -1 = Not Done

COMMENTS: __________________________________________
## Hematology

1. **Date of Hematology**

2. **WBC (x 10^9/L)**
3. **HGB (g/L)**
4. **PLT**
5. **GRAN%**
6. **LYMPH %**
7. **EOS X 10^9**

## Biochemistry

1. **Date of Biochemistry**

2. **BUN (mmol/L)**
3. **CR (umol/L)**
4. **GLU (mmol/L)**
5. **CPK (U/L)**
6. **AMYL (U/L)**
7. **AST (U/L)**
8. **ALT (U/L)**
9. **ALP**
10. **GGT**
**N-ACETYLCYSTEINE**

**SEPTRA REACTION LAB DATA**

**DAY 5**

<table>
<thead>
<tr>
<th><strong>PATIENT NUMBER</strong></th>
<th><strong>PATIENT INITIALS</strong></th>
</tr>
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</table>

**DATE OF ASSESSMENT** ..............................................................

<table>
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<tr>
<th>Day</th>
<th>Month</th>
<th>Year</th>
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</table>

**HEMATOLOGY**

1. **DATE OF HEMATOLOGY**

2. **DATE OF THYROID**

3. **WBC (x 10^9/L)**

4. **HGB (g/L)**

5. **PLT**

6. **GRAN%**

7. **LYMPH %**

8. **EOS X 10^9** or % ............................................

9. **TSH (mU/L)**

---

**BIOCHEMISTRY**

1. **DATE OF BIOCHEMISTRY**

2. **BUN (mmo/L)**

3. **CR (umol/L)**

4. **GLU (mmo/L)**

5. **CPK (U/L)**

6. **AMYL (U/L)**

7. **AST (U/L)**

8. **ALT (U/L)**

9. **ALP**

10. **GGT**
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</tr>
<tr>
<td>3</td>
<td>Trace</td>
</tr>
<tr>
<td>-1</td>
<td>Not done</td>
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</table>

**URINALYSIS**

1. DATE OF URINALYSIS
   - Day [ ]
   - Month [ ]
   - Year [ ]

2. pH
   - [ ]

3. PROTEIN
   - [ ]

4. BLOOD
   - [ ]

5. LEUK
   - [ ]

6. EOS
   - [ ]

7. S.G.
   - [ ]

8. KETONES
   - [ ]

9. BILIRUBIN
   - [ ]

10. GLUCOSE
   - [ ]

Codes: 1 = Negative, 2 = Positive, 3 = Trace, -1 = Not done
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<thead>
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<th>No. MLS Returned</th>
<th>Reason Returned</th>
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N-ACETYLCYSTEINE
FOLLOWUP: SERIOUS ADVERSE EVENT

PATIENT IDENTIFICATION

PATIENT NUMBER _______ __________ PATIENT INITIALS _______ __________

DATE OF REPORT __________ __________ __________

EVENTS WHICH ARE FATAL, LIFE-THREATENING, DISABLING OR INCAPACITATING, OR REQUIRE OR PROLONG HOSPITALIZATION, AND WHICH ARE UNRELATED TO HIV PROGRESSION SHOULD BE RECORDED ON THE SERIOUS ADVERSE EVENT FORM AS SHOULD UNEXPECTED EVENTS, CANCERS AND CONGENITAL ABNORMALITIES

Codes: 1 = None; 2 = Yes

1. SPECIFY SERIOUS ADVERSE EVENT:

IF NONE ABOVE, END OF FORM.

2. TYPE OF REPORT

Codes: 1 = Initial; 2 = Follow-up; 3 = Final

3. DATE OF ONSET OF EVENT

4. DURATION

(Number of min., hours, days, weeks, etc.)

5. UNITS

Codes: 1 = sec; 2 = min; 3 = hr; 4 = day; 5 = wk; 6 = month

6. PATTERN

Codes: 1 = Intermittent; 2 = Continuous -1 = Unknown

7. SERIOUS ADVERSE EVENT CLASSIFICATION: Codes: 1 = Yes, 2 = No (You may indicate “Yes” to more than one item)

a. Death (Complete Death Form)

b. Life threatening

c. Hospitalization or prolongation of hospitalization

d. Severe or permanent disability

e. Cancer

f. Unexpected adverse event

g. Other

3. INDICATE STUDY DRUG AT ONSET OF EVENT

Codes: 1 = mucomys, 2 = Not on mucomys

9. RELATIONSHIP OF EVENT TO MUCOMYST

Codes: 1 = Unrelated, 2 = Unlikely, 3 = Possible, 4 = Probable, 5 = Definite

10. ACTION TAKEN WITH STUDY DRUG

Codes: 1 = None, 2 = Drug interrupted, 3 = Drug discontinued

PLEASE PROCEED TO PAGE 2 OF SERIOUS ADVERSE EVENT FORM
N-ACETYLCYSTEINE

ASSOCIATED SYMPTOMS AND/OR SIGNS

SYMPTOM/SIGN

DATE
Day Month Year

DURATION CODES TOX. GRADE

Duration codes: 1 = Sec; 2 = Min; 3 = Hr; 4 = Day; 5 = Wk; 6 = Month
Toxicity codes: 1 = Mild; 2 = Moderate; 3 = Severe; 4 = Serious

CONFIRMATORY DATA: LABORATORY, TEST, X-RAYS, ETC

TEST

DATE
Day Month Year

RESULT (specify)

19. WAS TREATMENT GIVEN FOR ADVERSE EVENT? [ ]
Codes: 1 = Yes; 2 = No

20. IF YES (describe)

21. RECHALLENGE [ ]
Codes: 1 = Yes; 2 = No

22. INDICATE DOSE(S) AND DATES OF RECHALLENGE(S):

23. CURRENT STATUS [ ]
Codes: 1 = Recovered
2 = Reaction still active
3 = Convalescent
4 = Other

PLEASE PROCEED PAGE 3 OF SERIOUS ADVERSE EVENT FORM
N-ACETYLCYSTEINE
SERIOUS ADVERSE EVENTS PAGE 3

PATIENT NUMBER

PATIENT INITIALS

24. WAS THERE AN ALTERNATIVE CAUSE FOR THE SERIOUS ADVERSE EVENT? □
Codes: 1 = Yes  2 = None  -1 = unknown

25. COMMENTS: (Specify relevant intercurrent or concomitant illness, possible drug interactions, etc.)

(PLEASE PRINT):

DEMOGRAPHIC DATA

26. DATE OF BIRTH

Day  Month  Year

27. SEX

Codes: 1 = Male  2 = Female

28. RACE

Codes: 1 = Caucasian  2 = Black  3 = Oriental  4 = Hispanic  5 = Other

29. HEIGHT (cm)

30. WEIGHT AT TIME OF EVENT (kg)

PLEASE PROCEED PAGE 4 OF SERIOUS ADVERSE EVENT FORM
CONCOMITANT MEDICATION

- Record any non-study medications (including vitamins) which have been stopped, started or had a change of dose since the last visit. Be sure to include any medications that were started after the last assessment and stopped before the current one.
- Start dates are required for medications which have been started since the last follow-up.
- Stop dates are required only for medications which have been terminated.
- If you do not know the exact date that a medication was started or stopped, please estimate it to the best of your ability.

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</tbody>
</table>

DEATH

1. DATE OF DEATH ..................................................................................
   Day | Month | Year

2. CAUSE OF DEATH: ...........................................................................

3. HAS AN AUTOPSY BEEN PERFORMED? .................................................
   Codes: 1 = Yes, 2 = No

OTHER (SPECIFY) ..................................................................................

PLEASE PROCEED PAGE 5 OF SERIOUS ADVERSE EVENT FORM
Appendix IV

Monitoring

- standard operating procedures
- descriptions of monitoring
- listing of number patients monitored by centre
- list of patients monitored
Canadian HIV Trials Network

Standard Operating Procedures

Site Monitoring for the NAC study

Purpose

This procedure describes the process and the requirements for conducting data verification during a site visit by a monitor.

Procedure

1. The National Centre in consultation with the Regional Directors identifies satellites that require site visits and monitoring of the data forms (case report forms).

2. The Regional Director is responsible for identifying an independent monitor and contracting for their services. The National Center will offer advice if required.

3. The detailed procedures for the NAC Study are described in the attached six page document.

4. The monitor will liaise with the National Centre during the monitoring process. The contact is with the Scientific and Industrial Liaison Program. The contact names are Shideh Khorasheh or Dr. Zarowny and they can be reached by phone at 1-800-661-4664 or by fax at 1-604-631-5210.
Information on the NAC Study for the Monitors

NAC Case Report Forms

The duration of the study is 5 months (20 weeks). The patients are either randomized to take Septra or Septra+Mucomyst. The NAC case report forms can be divided into the following sections:

A. Entry/Baseline Forms

This section consists of the following forms:

1. Randomization/Identification page
2. Inclusion/Exclusion page
3. Baseline Evaluation (2 pages)
4. Baseline Data (2 pages of laboratory data and 2 pages of concurrent therapy)

B. Follow-up Forms

There are a total of 8 follow-up visits in the NAC study. The follow-up visits take place at weeks 2, 4, 6, 8, 10, 12, 16, and 20. Each follow-up contains 4 pages of information:

1. Laboratory Data (2 pages)
2. Concurrent Therapy (2 pages)

C. Other Forms

1. Discontinuation Form (2 pages)
2. 'Septra' Reaction Form (4 orange pages)
3. 'Septra' Reaction Laboratory Data for Day 1, 3, and 5 (5 pages)
4. Patient Compliance Record for 'Mucomyst' (green page)
5. Patient Compliance Record for 'Septra' (purple page)
6. Serious Adverse Event Form (5 pages)
Classification of Outcomes of Patients in the NAC study

The patients on the NAC Study can be divided into 3 categories re their outcome:

1) Complete

These are the patients that complete the whole 20 weeks of the study. These patients must then have:

a) All of Entry/Baseline forms
b) 8 sets of Follow-up Forms
c) Compliance Record Forms

2) Discontinuation

Patients are lost to follow-up from the study for different reasons: dislike of the taste of mucomyst, adverse events, or other reasons.

In this case, for example if a patient is lost at week 4, then the case report form for this patient must have:

a) All of Entry/Baseline forms
b) Follow-up Forms up to week 4
c) Discontinuation Form
d) Patient Compliance Record Form

3) Septra Reaction

This is our primary endpoint for the NAC Study. In the instances, when a patient has a Septra Hypersensitivity to septra, then the case report form for this patient must have the following forms:

a) All of Entry/Baseline forms
b) Follow-up Forms up to week when the septra reaction happened
c) Discontinuation Form
d) 'Septra' Reaction Form (4 orange pages)
e) 'Septra' Reaction Laboratory Data for Day 1, 3, and 5 (5 pages)
f) Patient Compliance Record
Information on the NAC Study for the Monitors

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**Trial Monitoring for the NAC Study**

For all the patients, the following forms must be verified by the monitor by using the original records available at the site. The data recorded on the case report forms must be validated against the clinical notes (patient's chart) and the laboratory reports. Where there are inconsistencies, a single line should be drawn through the incorrect data and the correct value written clearly beside it. The correction must be initialed by the investigator or the study nurse.

A) **Entry/Baseline Forms**

In the forms called Randomization/Identification and the Inclusion/Exclusion Criteria, the following important information must be monitored.

1. The patient's hospital file number, date of birth, sex, initials and the risk factors for HIV should be checked against the patient's hospital or clinic chart.

2. The patient's eligibility for the trial must be checked. The important points are:
   a) **Informed consent** - Check that the center has a copy of the consent form which both the patient and the investigator have signed. The patient must be over 16 years old.

   b) **HIV infected** - The evidence of infection for HIV should be validated and the CD4 for that patient should be below 200 or less than 20% or an AIDS diagnosis.

   c) Check for evidence that the patient has never been treated with 'Septra' before since being diagnosed with HIV. If the clinical notes indicate that the patient has previously been treated with sulfa since HIV diagnosis, then the patient should not be included in the study.

In addition, during entry into the study, certain data is collected during the baseline visit. The following **Baseline Data** should be checked against the patient's hospital or clinic chart.
Information on the NAC Study for the Monitors

1. **Vital Signs** - Temperature, Blood Pressure, Pulse, and Respiration
2. **Hematology** - the required test are WBC, HGB, PLT, GRAN, LYMPH, EOS
3. **Thyroid** - TSH
4. **Coombs Test**
5. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
6. **Immunology - CD4**
7. **Urinalysis** - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE
8. **Chest x-ray**
9. **EKG**
10. The patient's **concurrent therapy** should be checked. All the medications that the patient is currently receiving and the date that the patient was started on the medication must be recorded. In addition, one must indicate the reason for taking that specific medication.

**B) Follow-up Forms**

We do not require that the data in all 8 follow-up visits has 100% source documentation.

The monitor is **only** required to check the date of each follow-up visit with the patient's medical chart in order to ensure that the follow-up visit actually did take place.

**C) Other Forms**

**Discontinuation Form**

In an event when a patient has discontinued from the study due to 'Septra' reaction, other adverse event, death, or withdrawal from the study for any other reason, the discontinuation form must get completed and the information on the form must be checked against the patient's hospital chart.

'Septra' Hypersensitivity Reaction (orange pages)

This is the most important form in this case report because it involves the primary outcome of the study. Check all the information on this form to ensure that everything is recorded correctly.
Information on the NAC Study for the Monitors

1. Whether the patient had a fever, the maximum temperature and duration of the fever.

2. Whether the patient had a rash and the duration of the rash. It is important to check that the nurse has recorded whether the rash was (a) Red/flat, (b) Red/raised, (c) red/necrosis/blisters, or (d) mucosal.

3. Estimate of the body surface area involved by rash. The nurses are provided with a burn chart in which they are required to shade in the body areas where the rash has taken place. This chart must be in the patient's case report form in an event where a 'Septra' reaction has taken place.

4. It is also important to record whether the patient had pruritis and the severity of the pruritis.

5. Check the patient's records for any treatment such as antihistamines, Tylenol, steroids or other medication that could have been given for the reaction.

6. Note if the patient's sepsa was discontinued. If it was discontinued, indicate the reasons for discontinuation.

7. The patient is seen on Days 1, 3, and 5 after the 'Septra' reaction takes place and certain laboratory and other tests are performed during this time. All of the laboratory values in the case report form must be checked against the patient's clinical records.

Septra Reaction Lab data for Day 1

The following lab data are required for Day 1 of the 'Septra' reaction:

1. Hematology - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. Thyroid - TSH
3. Combs test
4. Biochemistry - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
5. Urinalysis - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE
6. Chest x-ray
7. EKG
Information on the NAC Study for the Monitors

Septra Reaction Lab Data for Day 3

The following lab data are required for Day 3 of the 'Septra' reaction:

1. Hematology - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. Biochemistry - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT

Septra Reaction Lab Data for Day 5

The following lab data are required for Day 5 of the 'Septra' reaction:

1. Hematology - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. Thyroid - TSH
3. Biochemistry - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
4. Urinalysis - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE

Serious Adverse Event Form

In the case where an adverse event has taken place, check all of the information on this form against the patient's medical chart.

Patient Compliance Records for Mucomyst

In the NAC study, only the mucomyst is dispensed by the site pharmacist. The pharmacist's records should be checked for mucomyst dispensal.
Trial Monitoring for NAC

General Points

Entry criteria and all baseline data as well as all the follow-up data must be validated against the clinical notes (patient's chart) and the laboratory reports. Where there are inconsistencies, a single line should be drawn through the incorrect data and the correct value written clearly beside it. The correction must be initialed by the investigator or the study nurse.

The case report form for the study is simple and can be divided into the following sections in an orderly fashion.

1. Randomization/Identification page
2. Inclusion/Exclusion page
3. 2 pages of baseline evaluation
4. 4 pages of baseline information which consists of 2 pages of laboratory data and 2 pages of concurrent therapy
5. 4 pages of follow-up information which consists of 2 pages of laboratory data and 2 pages of concurrent therapy (please note that there are 8 sets of pages for these follow-up visits)
6. Discontinuation Form (2 pages)
7. 'Septra' Reaction Form (4 orange pages)
8. 'Septra' reaction laboratory data for Day 1, 3, and 5 (5 pages)
9. Patient compliance record for 'Mucomyst' (green page)
10. Patient compliance record for 'Septra' (purple page)
11. Serious Adverse Event Form (5 pages)

Entry Criteria:

The following information are found in the first two pages of the case report form which are the Randomization/Identification and the Inclusion/Exclusion Criteria pages.

1. The patient's hospital file number, date of birth, sex, initials and the risk factors for HIV should be checked against the patient's hospital chart.

2. The patient's eligibility for the trial must be checked. The important points are:
   a. Informed consent - Check that the center has a copy of the consent form which both the patient and the investigator have signed. The patient must be over 16 years old.
   b. HIV infected - The evidence of infection for HIV should be validated and the CD4 for that patient should be below 200 or less than 20% or an AIDS diagnosis.
Check for evidence that the patient has never been treated with 'Septra' before since being diagnosed with HIV. If the clinical notes indicate that the patient has previously been treated with sulfa since HIV diagnosis, then the patient should not be included in the study.

**Baseline Data**

The following data which are required during the baseline visit should be checked against the patient's hospital chart:

1. **Vital Signs** - Temperature, Blood Pressure, Pulse, and Respiration
2. **Hematology** - the required test are WBC, HGB, PLT, GRAN, LYMPH, EOS
3. **Thyroid** - TSH
4. **Coombs test**
5. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
6. **Immunology** - CD4
7. **Urinalysis** - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE
8. **Chest x-ray**
9. **EKG**
10. The patient's concurrent therapy should be checked. All the medications that the patient is currently receiving and the date that the patient was started on the medication must be recorded. In addition, one must indicate the reason for taking that specific medication.

**Follow-up data**

The following data which are required during the follow-up visits should be checked against the patient's hospital chart. There are a total of 8 follow-up visits required in this study. The follow-up visits take place at the following weeks: Week 2, 4, 6, 8, 10, 12, 16, and 20. Please note that the hematology and the biochemistry tests are not required in weeks 6 and 10 of the study.

1. **Vital Signs** - Temperature, Blood Pressure, Pulse, and Respiration
2. **Compliance Record** - the patient's compliance with both the mucomyst and the septra should be checked
3. **Adverse Effects** - any suspected adverse reaction due to 'Septra' or 'Mucomyst' should be checked
4. **Hematology** - the required tests are WBC, HGB, PLT, GRAN, LYMPH, EOS
5. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
6. The patient's concurrent therapy should be checked. All the medications that the patient is currently receiving and the date that the patient was started on the medication must be recorded. In addition, one must indicate the reason for taking that specific medication. It is important to ensure that all of the changes in the patient's medication have been recorded.
7. Immunology - Please note that the CD4 counts are required during week 8 and 20 of the study.

**Discontinuation Form**

In an event when a patient has discontinued from the study due to 'Septra' reaction, other adverse event, death, or withdrawal from the study for any other reason, the discontinuation form must get completed and the information on the form should be checked against the patient's hospital chart.

**'Septra' Hypersensitivity Reaction**

This is the most important form in this case report because it involves the primary outcome of the study. Check all the information on this form to ensure that everything is recorded correctly.

1. Whether the patient had a fever, the maximum temperature and duration of the fever.

2. Whether the patient had a rash and the duration of the rash. It is important to check that the nurse has recorded whether the rash was (a) Red/flat, (b) Red/raised, (c) red/necrosis/blisters, or (d) mucosal.

3. Estimate of the body surface area involved by rash. The nurses are provided with a burn chart in which they are required to shade in the body areas where the rash has taken place. This chart must be in the patient's case report form in an event where a 'Septra' reaction has taken place.

4. It is also important to record whether the patient had pruritis and the severity of the pruritis.

5. Check the patient's records for any treatment such as antihistamines, tylenol, steroids or other medication that could have been given for the reaction.

6. Note if the patient's septra was discontinued. If it was discontinued, indicate the reasons for discontinuation.

7. The patient is seen on Days 1, 3, and 5 after the 'Septra' reaction takes place and certain laboratory and other tests are performed during this time. All of the laboratory values in the case report form must be checked against the patient's clinical records.

**Day 1 data**

The following lab data are required for Day 1 of the 'Septra' reaction:
The following lab data are required for Day 3 of the 'Septra' reaction:

1. **Hematology** - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. **Thyroid** - TSH
3. **Coombs test**
4. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
5. **Urinalysis** - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE
6. **Chest x-ray**
7. **EKG**

**Day 3 data**

The following lab data are required for Day 3 of the 'Septra' reaction:

1. **Hematology** - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT

**Day 5 data**

The following lab data are required for Day 5 of the 'Septra' reaction:

1. **Hematology** - WBC, HGB, PLT, GRAN, LYMPH, EOS
2. **Thyroid** - TSH
3. **Biochemistry** - BUN, CR, GLU, CPK, AMYL, AST, ALT, ALP, GGT
4. **Urinalysis** - PH, S.G., PROTEIN, BLOOD, LEUK, EOS, KETONES, BILIRUBIN, GLUCOSE

**Serious Adverse Event Form**

In the case where an adverse event has taken place, check all of the information on this form against the patient's medical chart.
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**Note:**
- 30 N02: Location code for St. Paul's vendor.
- 29 W09: Location code for Cochin vendor.
- 27 W09: Location code for Metallic and Cochin vendors.
- 26 W02: Location code for 220 Metropolitan vendor.
- 26 W07: Location code for 220 Metropolitan vendor.
- 26 W02: Location code for 220 Metropolitan vendor.
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Appendix V

- first and second interim analysis

- recommendations of SERC and Dr. Schecter

- letter to investigators terminating study
NOVEMBER 23, 1994

Dr. Joel Singer
Canadian HIV Trials Network
200-1033 Davie Street
VANCOUVER, BC V6E 1M7

Dear Joel:

Thank you for your letter of November 8, 1994, where you outlined some of your concerns about the statistical analysis of the NAC trial and the concerns of the Safety and Efficacy Committee.

As we discussed on the phone, I think a lot of these problems date back before the Canadian HIV Trials Network involvement in this study. My own study nurse admits that when she first began entering patients into the trial, she would take all comers, and now recognizes that a number of patients could be recognized as being non-compliant and these were the cause of some of the lost to early follow-up. Similarly, without a study coordinator, there was a tendency to be less rigorous in some of my study sites in terms of the follow-up and recording of data. I know that Shideh is doing her best to try and get the data that is available retrospectively and my study nurse is also going through the charts to see if we can better clarify this information.

In terms of your concerns about the primary outcome, given what has happened that patients are being removed from the study because of both major and minor rash, I agree and think for the primary outcome, that we are going to have to lump all rashes together. I think, however, that we should leave the protocol as it is and not allow openly for all patients to be removed for any rash, but rather for one that the clinician feels is significant. I agree that if NAC is effective, that it should decrease the frequency of the less severe rashes as well. As we have discussed by phone, I believe all of these rashes are due to allergy or hypersensitivity and they cannot be distinguished from "toxicity rashes".

In terms of the patient that were lost to follow-up, I think as I outlined, this is largely due to poor selection and lack of study coordination and hopefully this problems will decrease in time. We will try to determine whether or not these patients have continued on Septra which may be useful. In terms of those who have been excluded because of adverse reaction, we did include a 10% loss for these reasons in our initial sample size and I think the numbers are within that range. I think that it would be reasonable to include those patients in the analysis who have been followed for at least four weeks, as we would expect most of the hypersensitivity reactions to occur within the first two weeks. I do not, however, feel that we should include in analysis those patients who are lost or who discontinue the trial within the first month. In the big sense, one could look at the fact that if you are not able to tolerate the Mucomyst, this would not be an appropriate therapy. However, as I have discussed with you, we have chosen a large dose to determine whether or not this treatment works and if so, we can later clarify the dose. Therefore, the ultimate dose that patients may be required to take may be considerably lower or a different formulation which would be better tolerated. Therefore, I think that we may lose seeing an effect if we counted these patients as failures.
As I see it, the major problems with this protocol at present is recruitment. I will try to address this in a number of ways. First of all, on November 24, 1994, I am having a meeting in Toronto with our primary care physicians to explain the study once again our problems with recruitment and try to encourage them to randomize their patients. I will provide them with some hand outs and some signs to post in their office to try and capture more patients. I also have Shideh setting up a phone conference with the study coordinators across the country to try and determine what the barriers are to recruitment and see if we can improve this. Following that meeting, I would like to watch recruitment over the next three months. Given the enrolment as we previously depicted in the study, I would expect to see approximately 15 patients per month and at the end of three months, if this is not realized, then I think we have to reconsider the trial.

Finally, as one further option, if we continue to have problems with recruitment, would be to add another arm. This would include patients who had a previous hypersensitivity reaction to Septra. There was no reason to believe that if Mucomyst is effective, that it would not also work in this group. Obviously, this muddles the waters and it makes it difficult to make certain whether or not the primary prophylaxis group in patients who previous had a reaction are truly equivalent. However, I would rather add this arm than lose the study entirely and I think to start from scratch with a new protocol in that regard would waste a lot of valuable time.

I look forward to your comments on these thoughts and I will talk to you soon.

Sincerely,

Sharon L. Wainsley, MD, FRCP(C)
Division of Infectious Diseases

SLW/ast
November 8, 1994

Dr. Sharon Walmsley,
Toronto Hospital

Dear Sharon,

Shideh told me that she spoke to you to relay some of the concerns about the NAC study. I asked Shideh to summarize the status of patients who have already been randomized. These are attached for your perusal.

There are a number of things that are important to note. First, there have been more discontinuations (12) for minor rash than for major rash (10). The members of the Safety Committee feel that these ought to be included as primary outcomes. At least one of the clinicians indicated that physicians tend to stop septran for minor rash to avoid more major problems. I know that from your perspective this may not be appropriate. From a practical point of view, excluding these patients from the analysis poses a problem because of their large number, the possibility that they may have evolved into major rashes, and more importantly the fact that if NAC is effective, one might think that the frequency of less severe rashes ought to decrease as well. If this is true (ie that the risk reduction for minor rashes is equivalent to the risk reduction for major rashes) then the sample size requirements for the study would actually decrease if these events were included as part of the primary analysis. With regard to these events, one of the clinicians wondered whether one could distinguish between rashes that would be due to allergy rather than to toxicity. The question was whether NAC was supposed to alleviate both types.

The second issue concerns the evaluation of patients lost for other reasons. As you will note, some patients discontinued mucomyst because of the taste, and some patients discontinued at least some of their study medications because of nausea or other medical reasons. Finally, 15 patients were "lost to follow-up". There seem to be two ways one could approach these problems. One way is just to exclude all of these patients from the primary analysis. This will present a problem because of the number being excluded. You would have to recruit 500 to get the evaluable 300 that you want. On the other hand, if the vast majority of the adverse effects of septran occur in the first month, then one could consider patients lost beyond week 4 without an adverse reaction to be successes. As far as other medical problems go, if patients are able to continue on septran (at least some of them were) they should be followed for adverse reactions and
counted as failures if such failures occur. If patients have to stop septra for other medical reasons, it is difficult to know what to do with them. It really comes down to what question the study is attempting to answer, and the most unbiased way of answering it.

The goal is to limit the adverse events which prevent patients from discontinuing septra, but NAC is aimed particularly at the rash/fever that people develop. The most specific question (the one that I think you are trying to address) concerns whether NAC can help to limit these particular reactions. The major methodological problem, particularly if there is differential withdrawal in the two groups, is whether it is fair to only consider those patients who remain on all their study medications.

In any case, these are the issues that we must discuss. I hope to speak to you tomorrow along with Shideh.

Sincerely,

Joel Singer
November 8, 1995

Sharon Walmsley, MD
Toronto Hospital
Division of Infectious Diseases
200 Elizabeth St, ENG-219
Toronto, Ontario
M5G 2C4

Dear Dr. Walmsley,

Re: CTN057: A Randomized Trial of the use of N-Acetylcysteine for the Prevention of TMP-SMX Hypersensitivity Reactions

The Safety and Efficacy Review Committee reviewed the enrollment into the above mentioned trial at their meeting on November 1st 1995. SERC was very pleased to hear that enrollment into this trial has increased to a rate of 10 patients per month through the efforts of yourself and Shideh Khorasheh. We recognize that is quite an accomplishment since enrollment is usually low in the summertime. We would like to congratulate you and Shideh on your creative efforts to increase enrollment. We are sure that we share your hopes that you will be able to maintain this high rate of enrollment to the end of the study.

Sincerely,

[Signature]

Dr. David Roy
Chair
Safety and Efficacy
Review Committee
Canadian HIV Trials Network

cc. Dr. Martin Schechter
Shideh Khorasheh
Dr. Don Zarowny
Second Interim Analysis of
A Randomized Trial of the use of N-Acetylcysteine for the Prevention
of TMP-SMX Hypersensitivity Reactions

Presented to the Safety and Efficacy Review Committee October 7, 1996

1. Trial Summary

This is a randomized open-label trial of N-Acetylcysteine (NAC) vs placebo for the prevention of TMP-SMX (Septra) hypersensitivity reactions in patients receiving TMP-SMX as primary prophylaxis for PCP. The incidence of serious reactions to Septra among HIV infected individuals is approximately 30% to 50%, as compared to less than 1% in HIV negative patients. It was hypothesized that the increased incidence of adverse reactions was due to low glutathione levels and that therapy with NAC would increase the glutathione levels and thus reduce the incidence of adverse reactions. According to the protocol, patients on Septra and NAC were to take NAC for 2 months or until Septra was discontinued. Prophylaxis with Septra was to continue indefinitely. Patients were to return for follow-up visits at weeks 2, 4, 6, 8, 10, 12, 16 and 20 after randomization. The trial is necessarily unblinded because the strong unpleasant taste of NAC is difficult to mask.

This trial started enrolling patients in 1991 in three sites in Toronto as a non-Network trial. In January 1994, with 46 patients enrolled, the trial was expanded to multiple sites and was accepted as a Network trial. As of August 31, 1996, 234 patients had been enrolled out of the targeted 266. Note that the goal of 266 was set on the assumption that 240 (90%) of those patients would be evaluable. The primary outcome of this trial is discontinuation of Septra due to a rash of any severity. Patients with less than 4 weeks of Septra or NAC therapy for reasons other than an allergic reaction to Septra were omitted from the analysis because patients who receive Septra tend to be at relatively high risk for rash up to 4 weeks, and to treat them as successes if they had less than 4 weeks exposure would be inappropriate.

2. Summary of Results

- Of the 234 patients randomized to date, 46 were not included in primary analysis due to less than 4 weeks of study medication.

Eight recently randomized patients did not have follow up, 11 patients, although randomized, never started study medication, 13 were lost to follow-up after baseline. 13 additional patients received less than 4 weeks of Septra or NAC.

Thus, 188 patients were included in the primary analysis, 96 in the Septra alone arm and 92 in the NAC and Septra arm.

- 43 rashes/pruritus/fever that resulted in discontinuation of the medication occurred during the trial: 23 (24%) among patients receiving Septra alone and 20 (22%) among patients receiving Septra and NAC (p = .85).
- 27 of all allergic reactions to septran occurred within 2 weeks and 38 occurred within 4 weeks of randomization. The median time to rash was 2 weeks. There was no difference in time to rash between the two treatment arms.
- The severity of symptoms was similar between treatment groups.
- There was only one patient who discontinued NAC prior to experiencing a rash. For this patient, the time between discontinuation of NAC and the rash was 7 days. The patient refused to take NAC after the first dose because of taste.

3. Enrolment Summary

The enrolment statistics are summarized in Table 1.

Table 1: Enrolment Summary

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<td>8</td>
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<td>Who never started sepran</td>
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<td>7</td>
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<td>No follow-up after baseline</td>
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<td>13</td>
</tr>
<tr>
<td>Less than 4 weeks of sepran</td>
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<td>2</td>
<td>10</td>
</tr>
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<td>Less than 4 weeks of NAC</td>
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<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Included in the primary analysis</td>
<td>92</td>
<td>96</td>
<td>188</td>
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Of the 226 patients enrolled so far, excluding 8 recently randomized patients, 38 are unevaluable because of no follow-up after baseline (n=24) or less than 4 weeks of therapy (n=14). More patients in the sepran and NAC arm were unevaluable than in the sepran alone arm (21 vs 17), but the difference was not statistically significant (p=0.07). Of the 12 patients on sepran and NAC with less than four weeks of therapy, four discontinued NAC because of nausea (3) and taste(1) but stayed on sepran for more than one month. Of the remaining eight patients in this arm, two discontinued NAC because of taste, one because of fatigue, one because of nausea, one because of pain in the arm and three were lost to follow-up. One patient on sepran alone with less than four weeks of follow-up was lost to follow up and the other stopped his medications because of complaints of shakiness and feeling warm. No fever, pruritus or rash was registered for this patient.

The proportion of unevaluable patients (38/226 = 17%) is greater than the 10% that was expected and was accounted for in the sample size calculation and similar to one (16%) reported in the first interim analysis. The average rate of enrolment after April 1995 was 6 patients per month.
4. Baseline Characteristics

The only significant differences between the treatment groups with respect to their baseline characteristics were differences between the numbers of men and women in the two treatment arms (87% vs 98%) and between the numbers of patients whose risk factor was heterosexual contact (29% vs 15%) (Table 2). There was a similar difference in the distribution of men and women between the two arms when all randomized patients were considered: 100 of the 114 patients (88%) randomized to septra and NAC were male, as compared to 109 of the 113 patients (96%) randomized to septra alone.

Note that the risk factor categories are not mutually exclusive.

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<td>36 (90%)</td>
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<td>16 (17%)</td>
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<td>Homosexual</td>
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<td>57 (61%)</td>
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<td>2 (2%)</td>
<td>2 (2%)</td>
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<td>Other</td>
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<td>38.6 (10.7)</td>
<td>39.0 (8.6)</td>
<td>.75</td>
</tr>
<tr>
<td>Patients with Previous OI's</td>
<td>17 (82%)</td>
<td>14 (15%)</td>
<td>.47</td>
</tr>
<tr>
<td>Current Diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>48 (53%)</td>
<td>47 (51%)</td>
<td>.44</td>
</tr>
<tr>
<td>ARC</td>
<td>29 (32%)</td>
<td>37 (40%)</td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>13 (14%)</td>
<td>9 (10%)</td>
<td></td>
</tr>
<tr>
<td>TSH (mean, std dev)</td>
<td>1.98 (1.41)</td>
<td>2.14 (1.49)</td>
<td>.36</td>
</tr>
<tr>
<td>CD4 count (mean, std dev)</td>
<td>147 (92)</td>
<td>164 (108)</td>
<td>.24</td>
</tr>
</tbody>
</table>
5. Discontinuations of Septra

The numbers of patients on each treatment arm who discontinued septra for various reasons are shown in Table 3. The primary comparison of interest is the number of patients who discontinued septra because of a rash, pruritus or fever of any severity. Twenty-three of 96 (24%) patients on septra alone and 20 of 92 (22%) patients on septra and NAC discontinued septra because of an allergic reaction of any severity (p=.85). The difference in the incidence of allergic reactions between treatment groups is 2% (95% CI: -15%, 11%).

We performed a secondary analysis adjusting for factors which were maldistributed between groups at baseline (gender, heterosexual contact as risk factor, and race). The odds ratio on the treatment effect for this analysis was even closer to 1.0 than the unadjusted analysis.

Of the five patients on NAC and septra who discontinued septra for other reasons, one discontinued because of severe dysphagia, one because of diarrhea, one due to change in the dose and two patients withdrew from the study at their own request. Of the four patients on septra alone who discontinued septra for other reasons, one discontinued because of neutropenia and three patients withdrew from the study at their own request. The symptoms among patients who discontinued are described in Table 4.

Table 3: Reason for Discontinuation of Septra

<table>
<thead>
<tr>
<th>Reason for Discontinuation</th>
<th>Septra and NAC (n=92)</th>
<th>Septra alone (n=96)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash alone</td>
<td>5</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Rash and fever</td>
<td>6</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Rash and pruritus</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Rash, pruritus, and fever</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Fever</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pruritus</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total rash related</td>
<td>20</td>
<td>23</td>
<td>43</td>
</tr>
<tr>
<td>Lost to follow up/compliance</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>32</td>
<td>65</td>
</tr>
</tbody>
</table>
Table 4: Symptoms among Patients who Discontinued

<table>
<thead>
<tr>
<th></th>
<th>Septra and NAC (n=20)</th>
<th>Septra alone (n=23)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash &gt; 30% BSA</td>
<td>12</td>
<td>15</td>
<td>27</td>
</tr>
<tr>
<td>Rash</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red/flat</td>
<td>7</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>Red/raised</td>
<td>9</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Mucosal</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Intolerable pruritus</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Temperature &gt; 38.5°C</td>
<td>9</td>
<td>6</td>
<td>15</td>
</tr>
</tbody>
</table>

There was no significant difference in the time to discontinuation of septra because of rash between the treatment groups (Table 5). The median times to discontinuation of septra were 13 days and 14 days in the septra alone and septra and NAC treatment groups respectively (p=.65).

Table 5: Time of Discontinuation of Septra due to Allergic Reaction

<table>
<thead>
<tr>
<th></th>
<th>Septra and NAC</th>
<th>Septra alone</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 week</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1-2 weeks</td>
<td>11</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>2-4 weeks</td>
<td>5</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 4 weeks</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>23</td>
<td>43</td>
</tr>
</tbody>
</table>

6. Futility Analysis

We simulated the outcomes for the evaluable patients yet to be randomized (N=53) on the basis of the hypothesized outcome rates at the beginning of the trial and combined these with the observed results of the 183 evaluable patients already seen. It was hypothesized that NAC would reduce the incidence of septra associated allergic reactions sufficient to cause drug discontinuation from 30% to 15%. One thousand simulations were run. The results of the 1000 simulations for the remaining patients were combined with the observed results to generate 1000 2x2 contingency tables of the primary outcome by treatment. Chi-squares were computed for each table. The proportion of the chi-squares which resulted in a significant p-value (p<.05) was 10/1000.

If one employs the more appropriate strategy of adjusting for multiple analyses as was stipulated in the original analysis plan, then the chances of reaching statistical
significance is 7/1000 or .007. Thus, it is highly unlikely that this study, if continued, would result in a difference that is statistically significant.
Dear Dr. Schechter,

I am writing to you on behalf of the Safety and Efficacy Review Committee concerning the recommendations forthcoming from our review of the second interim analysis of the randomized open-label trial of N-acetyl cysteine for the prevention of allergic reaction to TMP SMX. The meeting took place in Montreal on October 7, 1996.

SERC reviewed the first interim analysis of this protocol in April 1995. At that time, the committee recommended that the trial continue but that a second interim analysis be conducted at a later point in time to determine whether continuation of the trial was warranted based on the data.

We now recommend that the trial be terminated because the observed difference in outcome between treatment groups is small and not statistically significant, and even under very optimistic assumptions, it is highly unlikely that the outcomes between groups would become significantly different if the investigators continued to accrue patients to reach the sample size indicated in the protocol. Estimates of the size of the treatment difference would be somewhat more precise if the trial continued but we do not believe that this is reason enough to warrant continuation of the trial. There would be little additional knowledge gained at the cost of considerable time and resources. The report upon which this recommendation is based is attached.

We have shared the report and the recommendation with Dr. Walmsley, and she concurs with the recommendation.

We would also recommend that Dr. Walmsley communicate the recommendation to site investigators and that recruitment stop immediately. Patients currently on study should be notified as soon as possible. Since the experimental drug does not seem to cause any toxicities, patients who have been randomized to receive N-acetyl cysteine may continue its use if they so choose.

Finally, we wish to commend Dr. Walmsley on her superb efforts in her conduct of the trial. Early on, there were serious difficulties in recruitment, and she expended considerable effort to recruit new trial sites and to stimulate existing sites to increase enrolment.

We would appreciate it if you could formally inform Dr. Walmsley of these recommendations.

Sincerely,

Joel Singer for David Roy,
Chair, Safety and Efficacy Review Committee
Oct. 16, 1996

Dr. Sharon Walmsley,
Toronto Hospital

Dear Sharon,

It would be helpful to us if you would indicate your plans with respect to further data collection and data analysis. Since the results are not going to change, I would suggest that the next visit for each patient be the last in terms of data collection and for data analysis. If there are any patients on mucomyst who wish to continue mucomyst, we would have to collect safety data on these patients.

I think it would be worth having a telephone discussion involving you, Shideh, me and Ognjenka Djurdjev (who will be working on the data analysis) just so we can review the data analysis plans. Since we went through this exercise for the first interim analysis, I doubt things will change much, although some of the secondary analyses which are necessary for the paper probably require additional discussion.

I look forward to hearing from you.

Sincerely,

Joel Singer, PhD
Programme Head, Data and Methodology
October 28, '96

1024/96

Dr. Sharon Walsmsley
Toronto Hospital
Division of Infectious Diseases

Dear Sharon:

re: NAC study

As you are aware, SERC has recently reviewed the NAC study and has sent the attached correspondence to me. The letter indicates that you are aware of SERC's recommendation and that you concur with it. Members of the CTN Staff will be in touch with you to assist you in carrying out the necessary steps.

I wish to note the following quote in the letter from Dr. Roy: "Finally, we wish to commend Dr. Walsmsley on her superb efforts in her conduct of the trial. Early on, there were serious difficulties in recruitment, and she expended considerable effort to recruit new trial sites and to stimulate existing sites to increase enrolment." On behalf of the CTN, I would like to thank you for your efforts and I share in SERC's recognition of your contribution.

While one trial may be ending, it appears that another very exciting opportunity for the CTN is just beginning in collaboration the CPCRA. I understand that you will be acting as the Canadian Principal Investigator for this new trial and I know that you will bring the same energy and commitment to this new endeavor. With best wishes.

Yours sincerely,

Martin

Martin Sbechert OBC, MD, PhD, FRCPC
Canadian HIV Trials Network
200 - 1033 Davie Street
Vancouver, BC V6E 1M7
Telephone: 800-661-4664
Fax: 604-631-5005

FAX TRANSMISSION

TO: See Below
FROM: Dr. Sharon Walmsley, Principal Investigator
Shideh Khorasheh, National Clinical Trials Coordinator
DATE: October 31, 1996
PAGES: 4

Dr. Fred Aoki/George Esteves (204) 783-5255
Dr. David Burdige (604) 875-3063
Dr. Bill Cameron/Nanci Hawley-Foss (613) 737-8682
Dr. Jeffrey Cohen/Nancy McFarland (519) 254-0883
Dr. Duperval/Mimose Dambreville (819) 820-6444
Dr. Bayourni (416) 926-5024
Dr. Bill Fong/Manuel Laural (416) 864-5870
Dr. Peter Fu/Tracy Stevenson (613) 548-6080
Dr. Ian Mackie/Fran Clarke (519) 438-7602
Dr. Julio Montane/Leila Sour (5527 and 5412
Dr. Anita Rachlis/Miriam Bast/Salma Esmail (416) 480-5808
Dr. Roger Sandre/Sue Jefferson (705) 523-7077
Dr. Walter Schlech/Susan Hyndman (902) 428-4032
Dr. Helene Senay/Lynn Lapointe (418) 654-2786
Dr. Fiona Smal/lyn Kelleher (905) 521-8675
Dr. Thompson/Rachel Roy (506) 857-5597
Dr. Emil Toma/Lise Cyr (514) 849-2140
Dr. Chris Tsoukas/Chantal Perpete (514) 937-1424
Dr. Sharon Walmsley/Jennifer Clarke (416) 595-5826
Dr. Kurt Williams/Nona Ford (306) 975-0383
observed. Shideh will be collecting the case report forms for these patients up to the end of their last visit so that their results can be included in the final analysis.

In addition, please find enclosed a copy of the abstract of the study results which has been submitted to the 4th Conference on Retroviruses and Opportunistic Infections which will take place in Washington in January 1996. Your site will be included as a member of the NAC study group. A manuscript will be started soon and after all data is collected, the final analysis will be completed. We will ask for your comments at that time.

Although we are disappointed that the trial did not show a protective effect of NAC, we have learned important information about PCP prophylaxis and septa hypersensitivity reactions. We would like to take this opportunity to personally thank you for all the hard work and effort you have provided towards the trial. I think we as a group have done well to recruit so well to this study. We congratulate the coordinators and investigators in meeting our objectives.

Sincerely,

Sharon L. Waalmsley, MD, FRCP(C)
Division of Infectious Diseases

cc: Dr. Don Zarowny
Dr. Dr. Joel Singer
Dr. Michael O'Shaughnessy
Dr. Martin Schechter

Shideh Khorasheh
National Clinical Trials Coordinator
4th Conference on Retroviruses and Opportunistic Infections
Official Abstract Form

Category: G
Subject category: From the list of subjects below, choose the most appropriate description of the paper's content and enter the letter on the line above.
A. Virology
B. Immunology and Vaccines
C. Pathogenesis
D. Epidemiology and Infection Control
E. Antiretroviral Therapy
F. Retroviral Diagnostics
G. Opportunistic or HIV-Associated Diseases

Please consider this abstract for:
☐ Regular abstract deadline, October 16, 1996. (If accepted, paper will be scheduled in poster or slide sessions.)
☐ Late breaker abstract deadline, December 20, 1996. (Abstract must reflect new data. If accepted, Late Breakers will be scheduled in slide sessions on Sunday, January 26, 1997.)
☐ Student/fellow travel grant consideration. (see page 2 for details)

Instructions: Complete this form and submit it for receipt by October 16 for the regular abstract deadline or December 20 for the Late Breaker abstract deadline. Only this original form is acceptable (no photocopies). Refer to page 2 of this packet for further instructions. Type the title (initial capital only) first, then list all authors (all capital letters), with an underscore for the person presenting the paper; finally list institutions and short addresses (do not give departments, divisions, building, zip code, etc.).

ONLY A FONT OF 10 POINT OR LARGER IS ACCEPTABLE.

The full name and institutional mailing address of the author who will present the paper must be typed in the space to the right. This is the address to which your abstract notification will be sent. The signature of the author on the back of this form implies that all coauthors are aware their names appear on this abstract and that this abstract conforms to all particulars pertaining to the regulations governing the consideration of abstracts.

KEY WORDS should be listed in high-priority first, using words similar to those in Medline and Index Medicus.

A Randomized, Multicenter, Controlled Trial Of The Use Of N'-acetylcysteine (NAC) For The Prevention Of Trimethoprim- Sulfamethoxazole (TMP-SMX) Hypersensitivity Reactions In HIV.


Background:
The incidence of serious adverse reactions to TMP-SMX is increased in HIV. This could be related to low plasma and intracellular glutathione leading to a decreased ability to detoxify reactive drug metabolites.

Objective:
To test the hypothesis that giving NAC to patients with HIV to regenerate glutathione could decrease the incidence of adverse reactions to TMP-SMX when given as primary PCP prophylaxis.

Methods:
Two hundred and thirty-four patients were randomized to receive TMP-SMX (one single strength bid) alone or with NAC 3 gm orally twice per day, one hour prior to each dose of TMP-SMX. NAC was continued for 2 months and patients followed for 5 months.

Results:
One hundred and eighty-eight patients were included in the primary analysis, 46 patients were not included due to < 4 weeks of study medication. The primary endpoint measure was the need to discontinue TMP-SMX because of any two of rash, diffuse severe pruritis and fever and was observed in 23(24%) of patients receiving TMP-SMX alone and 20 (22%) receiving TMP-SMX and NAC (p=0.85; 95% CI: -11%, 15%). The mean time to rash was 2 weeks and did not differ between the two treatment arms. Equal numbers of patients in each group discontinued TMP-SMX for any reason. The trial was terminated by the Safety & Efficacy Review Committee at the interim analysis on the basis of lack of efficacy.

Conclusions:
The use of oral NAC (6 gm/day) did not decrease the incidence of hypersensitivity reactions to TMP-SMX in patients with HIV.

PLEASE TYPE THE FOLLOWING INFORMATION BELOW ON THE EXACT LINE SPECIFIED--DO NOT COMBINE INFORMATION ON LINES.

PLEASE DO NOT USE POST OFFICE BOX NUMBERS.

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Last Name: WALMSLEY
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Key word 1: PCP
Key word 2: allergic reaction
Key word 3: N'-acetylcysteine