REDUCED L-GLUTATHIONE
INVESTIGATOR'S BROCHURE
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1. **A Brief Description of the Drug Substance and the Formulation**

1.1 Drug Substance, Including the Structural Formula

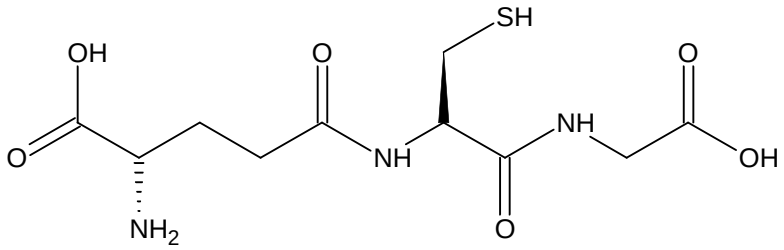
Reduced L-glutathione is a well-known tripeptide, γ-glutamyl-cysteinyl-glycine, abbreviated GSH. It is the most abundant low-molecular-weight thiol, and GSH/glutathione disulfide is the major redox couple in animal cells. "Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including kwashiorkor, seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes)."(1)

1. GSH is obtained commercially by yeast fermentation and is purified by the manufacturing procedures which give substantially pure product free from endotoxins (1571 Section 12 Part 7). See attached documentation from Kohjin Company. GSH is widely sold both as a drug and as a dietary supplement.

Full formula name: N-(N-L-γ-Glutamyl-L-cysteinyl)glycine

Abbreviated name: GSH

Structural formula:



Formula: $C_{10}H_{17}N_3O_6S$

Molecular Weight: 307.3

1.2 Drug Formulation per Capsule

GSH powder is encapsulated, 500 mg per capsule, under sanitary conditions without the addition of excipients or flow agents. See attached documentation from Complete Packaging & Manufacturing LLC.

1.3 Overview and Scientific Rationale for the Proposed Preliminary Effects Size Study of Orally Administered Glutathione to Augment Weight Gain in Children with Cystic Fibrosis

The Sponsor-Investigator, Clark T. Bishop, M.D., intends to augment weight gain in children with cystic fibrosis (CF) by oral administration of reduced L-glutathione. Difficulty in gaining weight is characteristic of many pancreatic insufficient CF children, and is associated with steeper declines in lung function and decreased longevity (2-6).

New research findings establish that the CFTR mutations responsible for CF with pancreatic insufficiency also cause diminished efflux of GSH from epithelial cells, resulting in a deficiency of GSH in epithelial lining fluids (7-13).

The importance of GSH in the epithelial lining fluid of the gut is also well established in the scientific literature. GSH helps to keep intestinal mucus thin, serving to defend the intestinal system against reactive oxygen species (ROS), and keeping inflammation in check under normal circumstances (14-18). GSH is also important to ancillary systems, such as the liver and pancreas, and further serves to conjugate potentially toxic substances (19-24). GSH has long been used as an adjuvant therapy in diabetes and irritable bowel syndrome (25-28), has been used to treat cachexia (29), and has been used to normalize digestive disorders in livestock by inclusion in animal feeds (30-32). Fasting, malnutrition, and starvation exist in a negative spiral with diminishing GSH levels (33-34). Lower levels of GSH in mice correlated with slower growth and decreased weight (35). Other experiments on the supplementation of GSH have noted attenuation of reperfusion injury of the gut (36), enhanced bile flow (37), amelioration of colitis (38) and reversal of abnormally high levels of lipid peroxidation (39). Specific transporters also permit supplemented GSH to increase GSH levels in the jejunum and liver, especially (40).

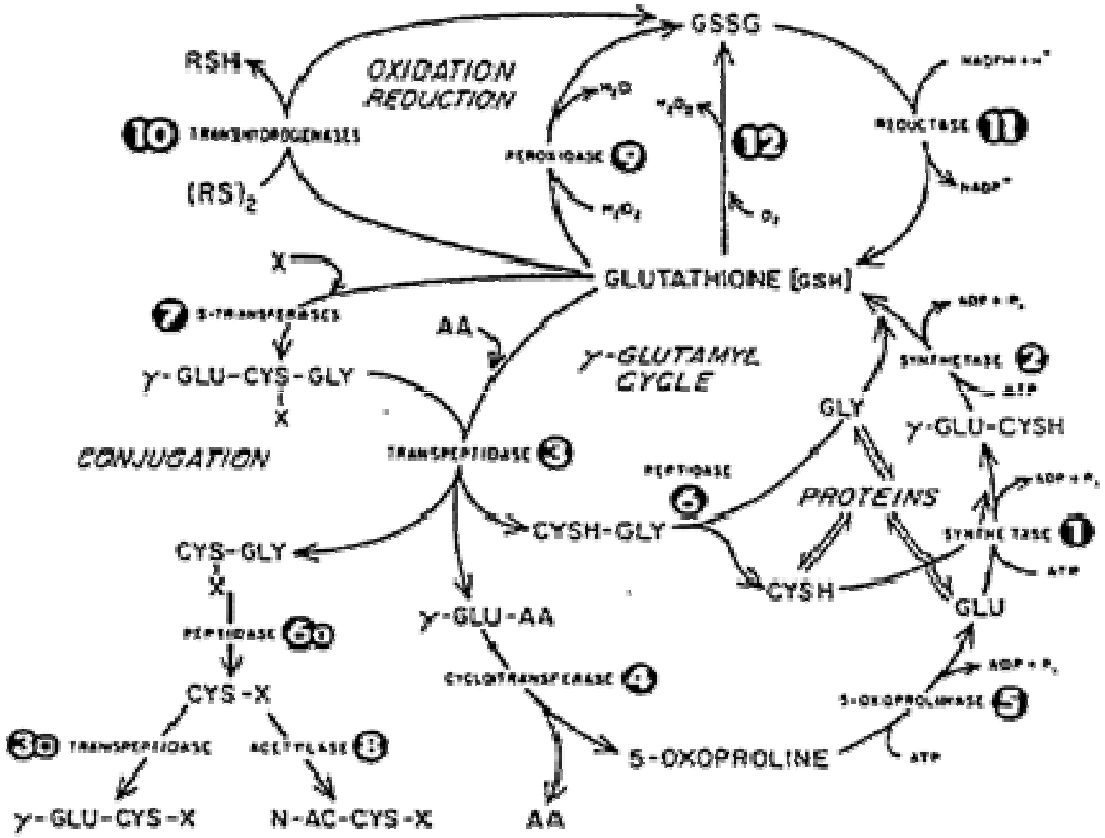
Since hallmarks of CF-related gastrointestinal pathology include increased intestinal mucus viscosity, increased inflammation, and increased oxidative stress (among many other pathological phenomena in a complex web of interaction; see research plan in 1571 Section 12 Part 6a for more detail), increasing levels of GSH in the lumen of the gut can be hypothesized to aid in the amelioration of these symptoms. Oral administration of GSH is the method by which we will attempt to increase lumenal levels of GSH in CF children with growth failure. Primary outcome measures include weight and height percentiles, body mass index, growth velocity, and arm circumference. Secondary indicators related to digestive function include steatocrit, fecal fat levels, and several others.

2. A Summary of the Pharmacological and Toxicological Effects of the Drug in Humans and Animals

2.1 Glutathione Metabolism Summary

To elucidate the complex and numerous actions and interactions of GSH and its oxidation product, GSSG, observe Figure 2, taken from a review published in 1983 (41).

Figure 2



Professor Alton Meister's research elucidated the **γ-glutamyl cycle** and many of the following processes (4):

❶	Initial step in the intracellular synthesis of glutathione.
❷	Completion of the intracellular synthesis of glutathione.
❸	Reaction involving γ-glutamyltranspeptidase.
❸a	Reaction involving γ-glutamyltranspeptidase.
❹	Intracellular γ-glutamyl amino acids are substrates of γ-glutamyltranspeptidase.
❺	Conversion of 5-L-oxoproline to L-glutamate.
❻	Cysteinylglycine is split by dipeptidase.
❻a	Substituted cysteinylglycine is split by dipeptidase.
❼	Electrophilic addition via S-transferases.
❽	N-acetylation of substituted cysteines.
❾	Peroxidase reaction forming disulfide linked molecule.
❿	Transhydrogenase reaction forming glutathione from dimer.
⓫	Conversion of dimer to glutathione via GSSG reductase.
⓬	Conversion of glutathione to dimer via oxygen molecule.

Glutathione (L-gamma-glutamyl-L-cysteinylglycine) is a ubiquitous tripeptide, found in all eukaryotic cells, including all mammalian tissue. Reduced L-glutathione (GSH) is available to the mammalian organism through cellular synthesis from constituent amino acids, and also through dietary intake. Glutathione is present in nearly all foods, and a normal diet would provide approximately 100-250 mg per day (42).

The metabolism of glutathione in mammalian animals is virtually identical to glutathione's metabolism in humans, and therefore the above Figure 2 applies both to mammalian animal models as well as human beings. Glutathione serves as the body's primary water-soluble antioxidant and conjugator of toxins and xenobiotics. It also maintains proper mucus viscosity, and its redox state regulates many important functions, such as inflammation.

The glutathione system, with its accompanying enzymes, has been comprehensively analyzed since 1921, when glutathione was first identified. Excellent reviews of basic glutathione biochemistry in animals and in humans include books written or edited by Vina (43), Sakamoto et al. (44), Taniguchi, et al. (45), Sies and Wendel (46), Sies (47) and Pressman and Buff (48).

Because we are interested solely in the oral administration of glutathione, and therefore the fate of glutathione ingested by humans, the remainder of this IB addresses primarily those issues, rather than a review of the entire glutathione system as represented in the above Figure 2.

2.2 Pharmacology Data

Uptake from the digestive system of intact GSH has been shown in pig and rabbit intestinal border membrane vesicles (49-50). Oral GSH has been reported to increase significantly the plasma levels in rats (51-52). There is a report that this increase of plasma levels of GSH does not take place in humans under certain conditions (53) but the generality of this conclusion is questioned by other researchers (54). Our trial will not settle the issue of whether GSH is taken up intact by the jejunum in humans. That contested issue is not relevant to our trial, for we are examining only the increased levels of GSH in the epithelial lining fluid of the gut through oral administration of GSH.

It is known that intestinal epithelial cells (55-58) and cells of the buccal cavity in humans (59) can take up dietary glutathione and can use it for protection against oxidative injury. Therefore, high lumenal concentrations of GSH can easily be attained via GSH supplementation, and this is our intent in this clinical trial.

2.3 Toxicology Data

When GSH was repeatedly administered to humans in doses up to 5 grams per day, both orally and intravenously, no toxicity was observed (60-61). There are no reports in the scientific literature indicating any toxicity from use of glutathione, whether in oral, aerosol, or IV form and whether in animals or in humans. Established pharmacopeias, such as the European Pharmacopeia (62), the Merck Index (63), the Physicians' Desk Reference (64), Thomson Micromedex (65), and others, concur that

no toxicity has ever been manifested from oral use of glutathione in animals or in humans:

1) Drugdex: "No serious adverse effect attributable to glutathione has been reported."
2) Martindale: "A mild zinc deficiency was found with chronic administration." Other than that, no adverse effects have been noted. It should be kept in mind that the daily regimen of all cystic fibrosis patients includes zinc supplementation in their ADEKs vitamins.
3) The PDR: The PDR summary of glutathione shows no reports of overdosage of glutathione, and no reports of adverse reactions to glutathione in the many research reports of use of glutathione in humans (http://www.pdrhealth.com/drug_info/nmdrugprofiles/nutsupdrugs/glu_0126.shtml).
4) The European Pharmacopeia: No adverse effects reported in the scientific literature.
5) The Merck Index: No adverse effects have been reported.

Glutathione is given in cases of drug or radiation poisoning, and is considered a systemic antitoxin (67). Furthermore, GSH is considered the "non-toxic" means by which the body can store cysteine and NO (1). Furukawa et al note, "Oral administration even of relatively high levels of GSH has been demonstrated to be safe and without adverse effects" (68). Ascorbate spares GSH, and is often used in oral administration of GSH to maintain its reduced form (68).

We have the results of toxicology tests on *four* pediatric cystic fibrosis patients who have used high dose glutathione (same dose (66 mg/kg/day), same material, same trial population as in the proposed clinical trial) for *years*—the longest for *over seven years.* We reproduce the results of those toxicology reports below:

I. Patient A, Male, DOB 11/13/96, delF508/delF508 mutations, oral glutathione at 66/mg/kg/day since 10/28/98. He was above the 90th percentile for weight at birth, but though he grew, it was slow. His growth curve was very flat from age 19-23 months, and his weight percentile fell to the 50th. He started on oral GSH at age 23 months and immediately began to grow again. Now, at age 9, he weighs 94 lbs which is at the 95th percentile for weight.

Laboratory Tests performed 12/22/05 (within normal range unless otherwise noted):

Protein	7.3		
Albumin	4.2		
Bilirubin, Total	0.3		
Bilirubin, Conjugated	0.0		
Bilirubin, Unconjugated	0.4		
Alkaline Phosphatase	296		
ALT	65	Hi	(normal range 10-35)
AST	38		
WBC	8.6		
RBC	5.23	Hi	(normal range 4.00-5.23)
Hemoglobin	14.9		

Hct	44.2		
MCV	84.5		
MCH	28.5		
MCHC	33.7		
RDW	11.9		
PLTS	510	Hi	(normal range 150-400)
MPV	8.3		
Neut, Abs	3.9		
Granulocytes, Auto	45.9		
Lymphocytes, Auto	46.2		
Monocytes, Auto	7.9		
Lymphocytes, Abs	4.0		
Monocytes, Abs	0.7		

Comments: ALT is slightly elevated which is typical for male delF508 homozygotes. Conclusion is that over 7 ½ years of glutathione treatment has not produced toxicity. His growth is exceptional for a child with cystic fibrosis.

II. Patient B, Male, DOB 6/14/99, delF508/delF508 mutations, oral glutathione at 66 mg/kg/day since 6/28/99. This child has been on oral GSH since he was an infant. He weighs 47 lbs which is the 50th percentile for weight.

Laboratory Tests performed 12/22/05 (within normal range unless otherwise noted):

Protein	7.8		
Albumin	4.4		
Bilirubin, Total	0.8		
Bilirubin, Conjugated	0.0		
Bilirubin, Unconjugated	0.6		
Alkaline Phosphatase	200		
ALT	51	Hi	(normal range 10-25)
AST	59	Hi	(normal range 15-50)
WBC	10.0		
RBC	5.39	Hi	(normal range 4.00-5.20)
Hemoglobin	15.0		
Hct	44.8		
MCV	83.1		
MCH	27.8		
MCHC	33.4		
RDW	12.9		
PLTS	489	Hi	(normal range 150-400)
MPV	8.0		
Neut, Abs	4.8		
Granulocytes, Auto	47.8		
Lymphocytes, Auto	45.1		
Monocytes, Auto	7.1		
Lymphocytes, Abs	4.5		
Monocytes, Abs	0.7		

Comments: ALT and AST are slightly elevated, which is typical for male delF508 homozygotes. Conclusion is that over 6 years' worth of glutathione treatment has not produced toxicity.

III. Patient C, Male, DOB 5/28/02, delF508/delF508 mutations, oral glutathione at 66 mg/kg/day since 6/12/02. He has been on oral GSH since an infant. He weighs 39 lbs which is the 85th percentile for weight.

Laboratory tests performed 12/22/05 (within normal range unless otherwise noted):

Protein	7.0		
Albumin	4.1	Hi	(normal range 3.1-3.9)
Bilirubin, Total	0.4		
Bilirubin, Conjugated	0.0		
Bilirubin, Unconjugated	0.4		
Alkaline Phosphatase	290		
ALT	47	Hi	(normal range 5-45)
AST	48		
WBC	17.9	Hi	(normal range 6.0-17.0)
RBC	5.12		
Hemoglobin	14.4		
Hct	43.5	Hi	(normal range 34.0-40.0)
MCV	84.9		
MCH	28.1		
MCHC	33.1		
RDW	12.9		
PLTS	456	Hi	(normal range 150-400)
MPV	8.0		
Neut, Abs	11.4	Hi	(normal range 1.5-8.5)
Granulocytes, Auto	63.6	Hi	(normal range 15-36)
Lymphocytes, Auto	31.1	Lo	(normal range 44.0-74.0)
Monocytes, Auto	5.3		
Lymphocytes, Abs	5.6		
Monocytes, Abs	0.9		

Comments: Patient had an upper respiratory infection at time of laboratory testing. ALT is slightly elevated, which is typical for male delF508 homozygotes. Results on WBC neutrophils, granulocytes, and lymphocytes affected by viral cold and are not of concern. Conclusion is that over 3 years worth of glutathione treatment has not produced toxicity. As with patient A, this child's growth is exceptional.

IV. Patient D, Male, DOB 10/15/1999, del F508/ G542X, oral glutathione at 66 mg/kg/day since April 2004. This child had abdominal pain and cramps prior to starting oral GSH. He noticed improved appetite, decreased abdominal cramping, decreased requirement for pancreatic enzymes, and improved stool characteristics. Selected labs are shown below. Weight has not been a problem for this child. At 6 yrs old, he weighs 50 lbs which is the 75th percentile.

Test	10-9-2003	5-19-2005
AST	102	44
ALT	119	45
LDH	739	617
Bili	0.1	0.8
BUN	20	20
Cr	0.5	0.4

Comments: This child has been on high dose oral GSH for 21 months with improved symptoms and no evidence of toxicity. ALT, AST, and LDH fell after he had been on GSH for 13 months.

2.4 Pharmacology and Toxicology Data from Animal and In Vitro Models

2.4.A: Genotoxicity and Mutagenicity

There are numerous studies showing that there is "an inverse relationship between the level of GSH and the occurrence of DNA base pair modifications" (Beddowes et al., 2003, 110 [125]). That is, the lower the level of cellular GSH, the greater the number of chromosomal aberrations found. It should be noted that eukaryotic cells buffer from the more general cytosolic pool of GSH, a reduced glutathione pool specifically for protection of nucleic structures. That buffered pool is extremely resistant to depletion. When depleted, however, apoptosis occurs. Reduced glutathione is one of the key elements in cellular protection from chromosomal aberrations. Cell lines studied to reach this conclusion include HepG2 cells (Wang and Hu, 2000 [126]; Beddowes et al., 2003 [125]), AS52 cells (Will et al., 1999 [127]), human fibroblasts from patients suffering from 5-oxoprolinuria (which are unable to synthesize GSH, Edgren et al., 1981 [128]), and in native Chinese hamster V79 cells genetically engineered to express rat CYP1A1, CYP1A2, and CYP2B1 (Parry et al., 1996 [129]). In fact, Parry et al (1996 [129]) suggest using GSH depletion as a marker indicating aneugenicity has taken place.

2.4.A.1: The Ames Test

On this topic, we contacted the world's foremost expert on the genotoxicity of glutathione, Dr. Silvio De Flora of the University of Genoa. We reproduce his email response of 24 April 2006 here (the original email is available upon request):

Dear Dr. Hudson,

Thank you for your clarifications explaining the rationale for the use of GSH in cystic fibrosis.

I confirm that GSH is not mutagenic in the Ames test, which renders further testing unnecessary. For instance, in the paper by S. De

Flora et al. (Carcinogenesis 5, 505-510, 1984) we reported that NAC, GSH and GSSG, tested up to 10 mg/plate (a huge dose!), were devoid of toxic and mutagenic activity to any of the 7 Salmonella tester strains (TA1535, TA1537, TA1538, TA97, TA98, TA100 and TA102), with or without liver S9 fractions. In contrast, cysteine (from two different manufacturers) was clearly mutagenic at high dose (mg/plate), especially in the presence of S9 mix. This effect was borderline in TA98, rather weak in TA100 and more pronounced in TA97 and TA102. In previous studies, cysteine had been found to be nonmutagenic in the Ames test (M.P. Rosin and H.F. Stich, Mutat. Res. 70, 269-278, 1978) but had thereafter found to revert TA100 in the presence of rat liver or Kidney S9 fractions (H. Glatt et al., Science 220, 961-963, 1983). In the last study, even GSH was found to be mutagenic in the presence of rat Kidney S9 fractions. This finding was questioned and ascribed to cleavage of GSH by GGT, which is unlikely to occur in vivo, since GGT is localized in the outer surface of the Kidney tubule cells, which is exposed to low GSH concentrations (D. Ross et al., Mutat. Res. 175, 127-131, 1986). Therefore, it is my opinion that GSH is not mutagenic and that further analyses are not needed, since this issue has already been sufficiently explored. This conclusion differentiates GSH from other antioxidants, such as ascorbic acid, which often become prooxidants. On the contrary, there is a rationale of combining GSH (or NAC) with ascorbic acid. GSH has been shown, both in vitro and in vivo, to maintain a reducing milion in the cell, which can reduce dehydroascorbic acid (reviewed in F. D'Agostini et al., Int. J. Cancer 88, 702-707, 2000). In fact, we found that NAC prevents the adverse effects of ascorbic acid (ibidem; F. D'Agostini et al., Carcinogenesis 26, 657-664, 2005).

Yours sincerely,

Silvio De Flora

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Dr. De Flora's email address is sdf@unige.it, if it is necessary to confirm the accuracy of this statement.

In addition to Dr. De Flora's research and the research by others he cites in his email, we would also like to draw your attention to Abu-Shakra, 2003 (124), which also attests that GSH, up to very high doses of 50 mmol per plate, is not mutagenic in the Ames test with Salmonella strain TA1535.

2.4.A.2: SCE Testing

MacRae/Stich (Mutat Res 68: 351-365, 1979) was not the final word on SCE testing of glutathione. Testing which is of more recent vintage than 1979 shows no formation of SCEs from GSH. Speit, Wolf, and Vogel (1980, [130]), found that "GSH . . . essentially did not induce SCEs" (p. 268) in very large doses up to 50 μmol per plate (please see Table 1, p. 268 for raw figures). The authors go on to say that, "Glutathione in either form [reduced or oxidized] did not lead to an increase of SCE frequency" (p. 270), and "The peptide GSH . . . did not lead to an increase of the SCE rate" (p. 271), and "GSH is more stable, and it may be considered **certain** that neither the reduced nor the oxidized form induces SCEs" (p. 271, emphasis added). Speit and Vogel (1982 [131]) actually explain MacRae and Stich's findings by pointing out that an examination of those authors' methods shows what those authors actually found was not that GSH induced SCEs, but that H2O2 induces SCEs—because MacRae and Stich used a cell line abnormally sensitive to H2O2 which compromises the interpretation of their results (p. 176, p. 181). Speit, Wolf, and Vogel (1982 [131]) further compared SCE-inducing capacity of GSH and Vitamin C. They discover that in the bone marrow of Chinese hamsters, a huge dose of up to 100 μmol of GSH did not induce SCEs (please see Table 2 and 3, p. 275 in [135] for raw figures). They state, "Glutathione [itself] did not increase the SCE rate" (p. 274 [135]), while Vitamin C did increase the SCE rate! They also state, "SCE induction is reduced . . . by glutathione in our experiments. Sulfhydryl compounds such as . . . glutathione are known for their antimutagenic and anticlastogenic effects" (p. 277 [135]). The levels of GSH used in these experiments greatly exceed the amounts we could ever hope to achieve with oral administration of GSH at 66 mg/kg/day.

2.4.A.3: Micronucleus Assays

The scientific literature affirms that glutathione does not cause micronuclei, but rather inhibits their formation.

S. Deb and A. Chatterjee (1998 [132]) report on the effect of arecoline on mutagenesis via the depletion of cellular reduced glutathione levels. Male Swiss albino mice were studied, and their bone marrow cells examined after sacrifice. Chromosomal aberrations "were scored as isochromatid breaks (both terminal and interstitial) and chromatid breaks" and scored from "first cycle metaphases" (p. 244); thus scoring was of micronuclei. Mice were administered arecoline, arecoline and BSO (a depletion agent for GSH), and GSH itself. They found that GSH depletion increased the mutagenic effects of arecoline, and the authors found that "GSH alone failed to induce any aberrations" (p. 244). The dose used for GSH in the mice was 400 mg/kg, far exceeding the 66 mg/kg as the proposed dose for this IND. Raw figures upon which the authors base their conclusion regarding exogenous GSH are found in Table 1, p. 245.

P. Rita et al (1991 [133]) were interested in the effect of glutathione on the formation of mitomycin-C induced micronuclei in bone marrow erythrocytes, in this case using Swiss albino mice. In addition to showing that glutathione decreased the

number of micronuclei formed when mitomycin-C was administered, this research team also looked at whether GSH alone induced micronuclei formation. The dose of oral GSH given to the mice was up to 160 mg/kg of body weight, an extremely high dose far exceeding our proposed dose. Their conclusion was, "Table 1 shows the incidence of micronuclei in young bone marrow erythrocytes in mice treated with GSH alone. There was no statistically significant increase in the frequency of micronuclei compared to control" (p. 132). Raw figures are provided in the article in Table 1 on page 132.

K. Sai et al (1992 [134]) studied micronucleus formation as the result of administration of potassium bromate, which is known to cause such aberrations. As in the previously cited articles, they were interested in whether exogenous GSH could attenuate the formation of micronuclei in reticulocytes in peripheral blood of male F344 rats injected with potassium bromate. They did find this attenuation effect. As part of the study, some rats were given only GSH. The dose of GSH injected was 800 mg/kg, an extremely high dose far exceeding our proposed dose of 66 mg/kg. The authors found that "GSH . . . alone did not induce MNRETs [micronucleated reticulocytes]" (p. 115). Table 1 of their results (p. 116) displays the raw data on which they base this conclusion.

All three of the studies of exogenous glutathione cited above used doses higher than what we propose in our study protocol, and all three studies concur that exogenous GSH does not cause micronucleus formation in vivo in rodents.

2.4.B: Carcinogenicity

1. Exogenous reduced glutathione has been shown to directly inhibit carcinogenesis in a rodent model (mouse skin tumors and hamster buccal pouch cancer), and this finding has been successfully replicated (Schwartz and Sklar, 1996 [138]; Rotstein and Slaga, 1988 [136]; Trickler et al., 1993 [137]; Sklar et al., 1993 [139]; Perchellet et al., 1985 [140]).

Rotstein and Slaga (1988 [136]) used female SENCAR mice, shaved their backs, and applied DMBA, a known cancer-causing agent, for 16 weeks to induce skin tumors. Then DMBA was ceased for 4 weeks, after which topical applications of 5 and 25 μmol GSH were applied for 30 weeks. "The GSH-treated group has a cancer incidence of 50%, which was significantly lower than the control group ($p<0.05$) [70%]" (p. 1548). In a second experiment with a lower dose of DMBA, "the application of GSH significantly inhibited cancer incidence . . . the carcinoma incidences in the GSH group treated with 25 μmol (30%) and in the group treated with 5 μmol (40%) were significantly less than the cancer occurrence ($p<0.05$) in the [control] group (60%)" (p. 1549). The authors conclude, "This paper presents data that show that GSH is capable of inhibiting tumor progression in the murine skin multistage carcinogenesis model" (p. 1550).

Trickler et al (1993 [137]) painted the buccal pouches of young adult Syrian hamsters (average weight 100 grams) with DMBA in an effort to create oral carcinomas. For 14 weeks, ten hamsters received only DMBA (Group 1), ten received DMBA plus 1 mg GSH orally on alternating days (Group 2), ten received only 1 mg GSH every other day (Group 3), and ten served as controls (Group 4). The animals were then sacrificed and their buccal pouches fixed and examined. No animal in Groups 3 or 4 developed carcinomas. Furthermore, animals receiving DMBA plus GSH had significantly reduced numbers of tumors, and significantly

reduced tumor volume than Group 1 (mean number of tumors for Group 1 was 81 at 14 weeks, for Group 2 the number was 35; tumor volume in Group 1 at 14 weeks was 380 cubic millimeters, versus 90 cubic millimeters for Group 2). Thus, oral GSH effectively inhibited carcinogenesis in this study.

Schwartz and Sklar (1996 [138]) replicate Trickler et al's (1993 [137]) study. Using forty Syrian Golden young adult male hamsters, one group has its buccal pouch painted with DMBA, a second group has the DMBA painting but is also given 1 mg of GSH orally, a third group are untreated controls, and a fourth group is given 1 mg of GSH orally without any DMBA. After 14 weeks, the animals were euthanized and buccal pouches were examined. The GSH-only group did not develop any carcinomas. The DMBA-only group did develop carcinomas. Interestingly, the DMBA+GSH group did develop some tumors, but according to the authors, "There was a significant inhibition of tumor development in the [DMBA-treated] animals receiving GSH. After 14 weeks, there were fewer tumors and the overall tumor burden was notably smaller (315 vs. 3,040 cubic millimeters). This difference is statistically significant ($p<0.001$ by Student's t-test). . . . In addition, the level of dysplasia observed in the DMBA group was also higher than in the GAH and DMBA group (2.97 and 1.84, respectively)" (p. 231). Furthermore, "In the animals that had received systematically administered GSH in addition to the DMBA applications to the cheek pouch, the leukoplakic lesions as well as the carcinomas were not only smaller, but they demonstrated significantly increased staining for the wild-type p53 protein, particularly in the cytoplasm of the invasive foci of malignant epithelial cells. The level of relative p53 expression was higher in the buccal pouch treated with GSH and DMBA than in the DMBA tumor control (3.20 and 2.64 respectively) . . . Large numbers of proliferating endothelial cells and small capillaries were observed within the stroma of the epidermoid carcinomas in the tumor control animals. The comparable carcinomas in the DMBA-GSH animals exhibited scattered capillaries and no clusters of proliferating endothelial cells. The increased development of squamous cell carcinoma was associated with a marked increase in endothelium-lined vascular spaces, as defined by Factor VIII immunohistochemical staining. Inhibition of carcinogenesis by GSH was correlated with an absence of these immunohistochemical patterns. The relative level for angiogenesis was increased in the DMBA treatment group (3.10 and 2.30 for the DMBA and the DMBA+GSH groups, respectively). GSH treatment during oral carcinogenesis reduced the number of carcinomas observed, dysplastic sites per tissue, and the level of angiogenesis in these tissues. In contrast, the level of tumor suppressor protein expression for p53 was increased" (p. 232).

Shklar et al (1993 [139]) also used young adult male Syrian hamsters, and again their cheeks were painted with DMBA. Group 4 received 50 □g of reduced glutathione orally by pipette in a manner so that on alternate days they received either DMBA painting or oral reduced glutathione. The hamsters weighed about 100 g each. Animals were sacrificed 12-14 weeks later and their buccal pouches examined. The GSH group experienced statistically significant reductions ($p<0.001$) in areas of leukoplasia, number of tumors, mean tumor volume, and tumor burden.

Perchellet et al (1985 [140]) looked at the induction in mouse skin tumors of epidermal ornithine decarboxylase (ODC) by 12-O-tetradecanoylphorbol-13-acetate (TPA), with ODC being one of "the essential components of mouse skin tumor promotion" (p. 567). The backs of CF-1 mice were shaved and painted with TPA.

GSH was injected into some of the mice at the dose of 250 mM prior to painting. In vivo and in vitro ODC activity was observed. In vitro reduction of ODC activity by GSH was 63% and in vivo the reduction was 53%. While 100% of the TPA-only treated mice developed papillomas, only 89% of the GSH-treated mice developed them. The number of papillomas per mouse at 22 weeks was 13.2 for the TPA-only group and only 5.7 for the GSH-treated mice. All of these differences in indices were statistically significant.

2. Furthermore, reduced glutathione has been shown to inhibit human ovarian cancer cell lines (A2780 and IGROV-1) in in vitro concentrations ranging from 10-300 □g/ml (Perego et al., 1997 [141]), as well as inhibition of human R3230AC mammary adenocarcinoma cells (Karmali, 1984 [142]) and human hepatocarcinoma cells (Novi, 1980 [143]).

3. Reduced glutathione is officially approved for use in cancer patients in the European Union. Prima facie, this implies that reduced glutathione is non-carcinogenic, as this would have to have been proven before such approval could have been granted. TAD (Italy), Gluthion (Italy), Ipatox (Italy), Maglut (Italy), Ridutox (Italy), Rition (Italy), Scavenger (Italy) are among the approved formulations for use in cancer patients in Europe. Clinical trial results support this use. For example, Dalhoff et al. (1992 [60]) administered 5 grams of oral glutathione per day to patients with hepatocellular carcinoma, with duration of 119-820 days. Patients in general survived longer and experienced regression of their tumors. The clinical data on intravenous/intramuscular injection of GSH in cancer patients are so numerous that it cannot be efficiently reproduced here: please see section 4.2 of the IND. Clinical trials included patients with gastric cancer, colorectal cancer, ovarian cancer, bladder cancer, non-small-cell lung cancer, head cancer, neck cancer, and endometrial cancer. In no case did exogenous GSH adversely affect the clinical outcomes of these patients; in contrast, the patients experienced improvement in clinical outcomes.

2.4.C: Toxicity in Animals

Since the mammalian glutathione system is virtually identical in mammalian animals and in humans, and since the clinical trial subjects are human, we have elected to concentrate in this Investigator's Brochure on what is known in the scientific literature about human use of glutathione (see Section 2.3).

3. **A Summary of the Pharmacokinetics and Biological Disposition of reduced L-glutathione in Human Models Comparing the Glutathione Systems of Humans and Animal Mammals**

In this trial, as noted above, we focus on the specific pharmacokinetics of oral administration of glutathione, rather than the entirety of the well-understood mammalian glutathione system. It is to the topic of the pharmacokinetics and biological disposition of orally ingested GSH that we will now turn.

As noted in Section 2.2, experts differ as to whether dietary glutathione is taken up intact from the human jejunum or not (53, 54). (It is taken up intact in the rat, mouse, and pig jejunum (49, 50, 51, 52, 70) One study demonstrates that oral glutathione does not increase blood levels of glutathione in CFTR-knock-out mice [122], but at

the same time, the U.S. Food and Drug Association asserts, “Literature reports clearly describe that orally administered glutathione is well absorbed” [123]. Because it is irrelevant to our trial whether GSH is taken up intact from the human jejunum or not, we will lay out the pharmacokinetics of both possibilities without prejudice.

3.2. Pharmacokinetics in the Event that GSH Is Not Taken Up Intact in the Human Intestine

If GSH is not taken up intact in the human jejunum, its primary activities as GSH would take place in the intestinal lumen, before being hydrolyzed by gamma-glutamyl transferase in the intestine. Lumenal GSH comes from bile (which contains high levels of GSH, probably in GSSG form (71), 1-6 mM (57), which levels are an indicator of hydrogen peroxide metabolism in the liver (71), diet, export from intestinal epithelial cells, and by breakdown of desquamated cells. If cleaved, (assuming it is not taken up intact), the constituent amino acids would then be sent by the bloodstream to the cells, especially hepatocytes, for retrieval. The constituent amino acids would then be used for hepatic protein synthesis, for synthesis of GSH itself, or in the case of excess glutathione disulfide such would be sent for biliary excretion. Some glutathione conjugates would also be excreted in bile, though much would be sent through venous secretion to the kidneys for further metabolism before urinary excretion. Interestingly, bile glutathione conjugates are metabolized in the intestinal lumen to cysteinyl-conjugates, which are then reabsorbed into the bloodstream, where they are picked up by the kidneys and acetylated before excreted in urine (72).

Urinary excretion, then, is the final exit route of whatever is left from the operation of the glutathione system. The liver effluxes GSH itself uni-directionally, with efflux across the sinusoidal plasma membrane into the caval perfusate (71). In the rat liver, the rate of such efflux is 0.3% per minute (71). There appear to be two hepatic pools of GSH, one with a half life of approximately 2 hours, and one with a half-life of approximately 30 hours; the former is used for detoxification, antioxidant, and protein synthesis functions; the latter is used for mitochondrial protection. Circulating conjugated GSH (including conjugated GSH from the liver through venous secretion) (73), or reduced GSH is freely filtered through the renal system, with gamma-glutamyl transferase (and two other peptidases) again playing the key role in hydrolysis (with 90% extraction from glomerular filtrate in rat experiments) (71). The enzymatic action occurs at the brush border of the proximal tubular epithelium (73). Urinary excretion of any unnecessary amino acids occurs, though amino acids would also be returned to the liver. Before final excretion from the body through urine, glutathione conjugates have been metabolized to mercapturates, which is how the excretion of toxins neutralized by glutathione takes place (73, p 111).

But the kidney itself needs GSH to function. “About 80 percent of the GSH synthesized in the liver is exported from the hepatocytes, and most of this is utilized by the kidneys, which also carry a major toxic burden” (67). Thus there is significant interorgan transport of GSH between the liver and the kidneys. The kidneys accept GSH from the liver; the liver accepts the products of the hydrolysis of GSH from the kidneys. Normal levels of GSH in rat liver, small intestine, and kidney cells are given

by Ormstad and Orrenius (73): GSH, nmol/10^6 cells): Liver, about 50; Small Intestine; about 10; Kidneys, about 30 (73, p 110).

The activities of dietary glutathione per se (in this model of non-uptake of intact GSH from the human jejunum) would take place in the intestinal lumen. Its role there has been summarized in the scientific literature in this fashion: "The primary role of glutathione is to protect cells from oxidative stress. It is abundantly distributed in the mucosal cells of gastrointestinal tract both in animals and man. The highest concentration is found in the duodenum. The amount of glutathione ingested with foods, age and drug or ethanol consumption affect glutathione concentration. The detoxifying capability of glutathione is directly related to its thiol group and to its function as a substrate for enzymatic activity; in fact, glutathione regulates the action of glutathione-peroxidases and glutathione-transferases. It has been documented that a direct relation between glutathione concentration and mucosal damage or between glutathione-related enzymes and cancer occurrence is present in various pathological conditions of the gastrointestinal tract (from oesophagus to rectum). The present review underlines: a) the role of oxidative stress in numerous physiological and pathological conditions in experimental animals and man; b) the need to maintain a normal antioxidant potential in the mucosal cells of the gastrointestinal tract; and c) the possibility to evaluate, through clinical studies, how glutathione concentration, food intake, and gastrointestinal diseases are associated." (74)

Martensson et al. (57) find that oral administration of GSH to mice whose GSH is depleted by BSO increases the GSH in jejunal, gastric, and colonic mucosa as well as in the pancreas as shown in Table 1.

Table 1
GSH Levels After Treatment with BSO, GSH Ester, and GSH

	Jujunal Mucosa Micromol/g	Jujunal mucosa mitochondria, nmol per mg protein	Gastric mucosa micromol/g	Colon mucosa miccromol/g	Pancreas micromol/g	Plasma micromol/g
Controls	0.27 ± 0.03	1.1 ± 0.03	0.32 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.90 ± 0.20
GSH(p.o.)	0.94+0.1	4.8+0.30	0.60+0.20	0.34+0.15	0.12+0.04	
Not treated With BSO	2.29+0.35	8.7+0.71	1.73+0.29	1.70-+0.13	1.71+0.18	58.3+6.0

Mice were given BSO (L-buthione-SR-sulfoximine, a transition-state inhibitor of gamma-glutamyl cysteine synthesase) for 7 days together with other compounds. Groups of four to six mice were given GSH ester (0.9 ml of 160 mM) by gastric lavage (p.o.), or by i.p. injection. GSH (+ ethanol and Na2SO4; see Materials) and a mixture of L glutamate, L-Cysteine, glycine, ethanol, and Na2SO4 were given in the same way as indicated in doses of 4 mmol/kg (except cysteine was at 2.5 mmol/kg) twice daily (at 11 a.m. and 6 p.m.) for 7 days.

The oral administration of glutathione to the epithelial surfaces of the GI tract thus has at least four effects:

1) increasing levels of glutathione in GI mucosa, enterocytes, and the pancreas;

2) direct reduction of oxidants with or without enzymatic assistance;

3) conjugation of toxins and xenobiotics through gamma-glutamyl transferase in the plasma membrane of the intestinal epithelium;

4) downregulation of inflammation in the gastro-intestinal region through redox status alteration.

3.3 Pharmacokinetics in the Case That GSH Is Taken Up Intact by the Human Intestine

There are specific GSH transporters in the human jejunum (54). If GSH is taken up intact in the human jejunum, this would not prevent GSH from performing all the other functions and roles it would play in the intestinal lumen as described in Section 3.2. However, in this model of intact uptake, some proportion of oral glutathione would enter circulation via these jejunal transporters. Plasma concentrations of GSH are fairly low (and have a rapid turnover with a half-life of 1.6 minutes:) (73), but erythrocyte concentrations of GSH can reach high levels several orders of magnitude higher than in plasma. In circulation, GSH would enter into redox reactions directly with such radicals as hydrogen peroxide. Some circulating GSH would be taken up by cells intact or cleaved through transpeptidase activity allowing for cellular resynthesis of GSH (radio-labelled GSH in most cells, including kidney cells, has a half-life of 1 hour; (75) in erythrocytes, lung, spleen, and nervous system the turnover rate may be several days (73). Some would also be filtered by the renal system using gamma-glutamyl transferase as described in the previous section. Constituents of GSH will be taken up by the hepatic system, with storage, cleavage, resynthesis, and biliary excretion as possible outcomes, as noted in the previous section.

One argument that GSH is taken up intact is presented here: “Glutathione given orally does raise GSH in vivo. This has been demonstrated both in animals and in humans.(67) In one study, an oral bolus of 15 mg/kg to the human appears to raise plasma GSH two-to five-fold, (77) with great variability in effect between the five subjects tested. Equivalent amounts of individual amino acid precursors of GSH failed to raise plasma GSH above baseline. In another study that used healthy, fasted subjects, plasma GSH did not rise following oral administration of GSH (78). Perhaps plasma GSH is so well buffered in healthy subjects that it is difficult to influence by oral dosing. The enterocyte cells that line the intestinal lumen absorb GSH via non-energy-requiring, carrier-mediated diffusion, and later export it into the blood.(67)

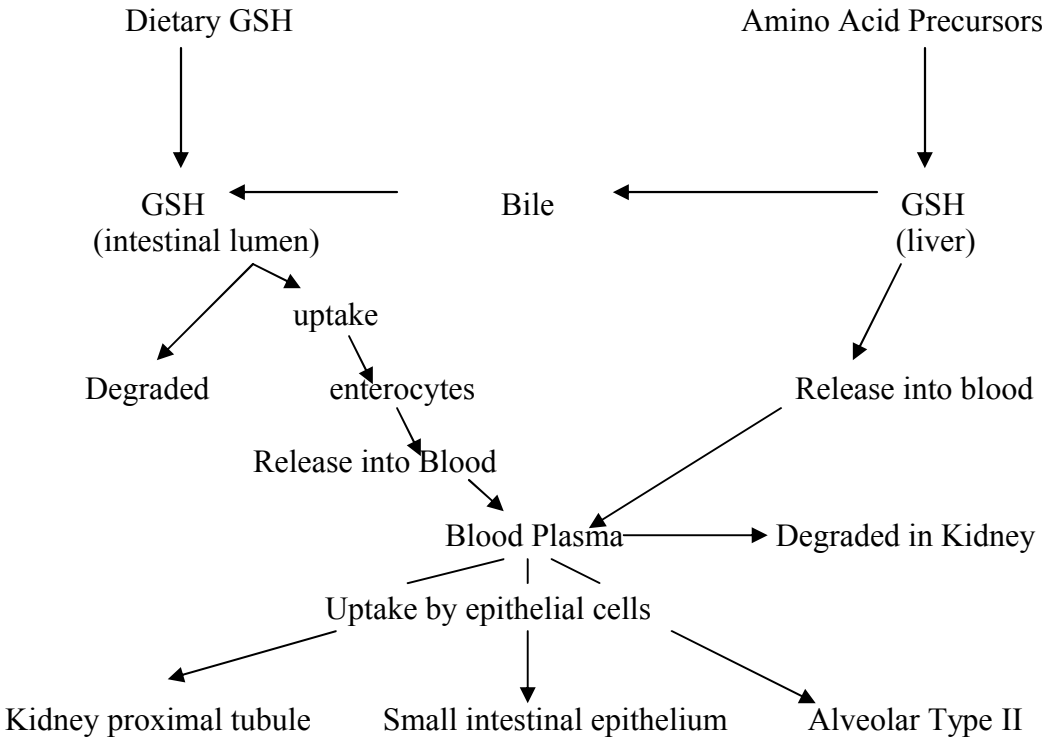
GSH also can be absorbed intact by epithelial cells other than the enterocytes, such as lung alveolar cells, vessel endothelial cells, retinal pigmented epithelial cells, and cells of the kidney's proximal tubule; it seems also to cross the blood-brain barrier (67). Intact GSH also can be delivered directly into the lungs as an aerosol (77). Other cells - brain endothelial and nerve cells, red blood cells, lymphocytes - appear

incapable of absorbing GSH as the intact tripeptide; rather they must synthesize GSH anew from cysteine (or cystine) that they transport inward from the outside (67) Here transpeptidase enzymes on the outside surface of the cell assist by removing single amino acids from circulating GSH, some of which are then subsequently absorbed (See Figure in Section 1.2). "Thus, administering GSH as the whole molecule may be worthwhile as a means to directly replete GSH in the intestinal lining cells or other epithelia in vivo; otherwise, it is not a particularly cost-effective way to accomplish GSH repletion." (67)

In this trial we are interested in such direct repletion of the intestinal epithelium; however, it should be noted that cost-effectiveness criteria for normal persons—as alluded to in the above quotation--may not hold in the case of CF patients, whose genetic mutation prevents efflux of synthesized GSH from many epithelia in the body.

3.4 Summary of Pharmacokinetics

We can characterize the fate of orally administered glutathione in humans (and animal mammals) by reference to this diagram, originally produced by Hagen and Jones (78)



After oral administration of GSH, the model of non-intact uptake by the human jejunum holds that GSH will act as a mucolytic and antioxidant in the intestinal lumen, then be degraded or conjugated. Whether degraded or conjugated, the resultant will be absorbed into the bloodstream: constitutent amino acids will be used by cells for synthesis of GSH, conjugates will be filtered by the renal system and

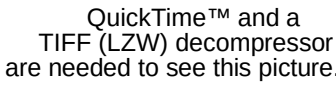
excreted in urine. GSSG would be excreted in bile. Some GSH conjugates may also be excreted in bile, then metabolized in the intestines and reabsorbed to be filtered by the kidneys.

If one holds to the theory that GSH is absorbed intact into the bloodstream from the intestine via specific transporters in the jejunum, GSH would still fulfill the same mucolytic and antoxidant roles in the intestines as described above after oral administration, but some GSH would also be transported intact into the bloodstream. Intact absorbed GSH in the bloodstream would be part of direct antioxidant and conjugation processes and could also be cleaved by cells by transpeptidases to be used in resynthesis of cellular GSH or even absorbed intact by cells. After such use, oxidized and conjugated GSH would meet the same fate in the liver and kidneys as described previously.

Biological disposition and excretion of glutathione has been measured in sheep, with data indicating the following after 600 mg of intravenous glutathione being administered as shown in the Figures 2A, 2B, 2C, and 2D from a U. S. Patent (see next page) (79).


QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Biological disposition of oral glutathione fed to normal and CFTR-knockout mice has also been measured (300 mg/kg) as shown in figures labeled, “Effect of GSH Oral treatment (300 mg/kg) in ELF at 60 min,” and “Effect of GSH Oral treatment (300 mg/kg) in Serum at 60 min” (80).



4. A Summary of Information Relating to Safety and Effectiveness in Humans

4.1 Summary of Oral Use of Reduced L-Glutathione in Humans; Published Clinical Data

a. Cachexon

Cachexon, made by Telluride Pharmaceutical Corporation of Hillsborough, New Jersey, is formulated as a capsule in which the only active ingredient is 500 mg of reduced l-glutathione. Cachexon is an FDA-approved orphan drug, whose indication for use is AIDS-related cachexia. There are no reports of any adverse events by physicians and/or patients using Cachexon in the 6 years that it has been marketed as an orally administered drug. (81)

b. Foreign Formulations

There are several foreign drug formulations of reduced glutathione for use in humans. The only active ingredient in each of these formulations is reduced l-glutathione. These formulations include: TAD (Italy and Hong Kong), Gluthion (Italy), Ipatox (Italy), Maglut (Italy), Ridutox (Italy), Rition (Italy), Scavenger (Italy), Glutathin (Japan), and Glutoxim (Russia). No reports of adverse effects from the use of any of these drugs has been noted in the scientific or regulatory literature, and none of these drugs has been withdrawn from any market because of adverse events related to their use. Indications for use include alcohol and drug poisoning, radiation trauma, and chemotherapy-induced neuropathy (82)

c. High Dose Oral Use in Hepatocellular Carcinoma

In a study by Dalhoff, et al. (60) eight patients with this cancer were administered 5 grams of glutathione (dissolved in orange juice) daily. Two of the patients, both female, discontinued the treatment due to increased odorous flatulence and gastrointestinal irritation. Three of the remaining six patients (the six were aged 27-63) survived longer than one year and exhibited regression of their tumors. The daily dose of 5 grams of oral glutathione was continued in these six patients from 119-820 days.

d. Low Dose Oral Use in Meniere's Disease

Twenty-five patients with Meniere's disease were given oral glutathione (300 mg/day) along with Vitamin C (600 mg/day) and rebamipide (300 mg/day). Twenty-one showed marked improvement of vertigo, and no toxicity was noted (83)

e. Low Dose Long Duration Use of Oral GSH in Alcoholic Hepatopathy

Eighty patients with alcoholic hepatopathy were divided into two groups. The first group received 300 mg GSH daily, and their hepatic function scores improved significantly over 30 days of use. No toxicity of oral GSH was noted.(84)

4.2 Summary of Other Forms of Administration: Intravenous/ Intramuscular Injection, and Aerosol/Intertracheal/Intranasal Administration; Published Clinical Data

a. Intravenous/Intramuscular Injection

1. Fifty patients with gastric cancer were enrolled in a randomized double-blinded placebo-controlled trial to determine whether the addition of glutathione to the IV administration of cisplatin reduced neurotoxicity (85). The dose was 1.5 g/meters-squared in 100 mL of normal saline over a 15 minutes period prior to cisplastin administration. On days 2 and 5, patients also received 600 mg of GSH in an intramuscular injection. At week 9, no patients in the glutathione arm showed neuropathy, while 16/25 in the control arm did. Glutathione did not reduce the efficacy of the chemotherapy. No adverse events from the use of glutathione were noted.

2. Fifty patients with colorectal cancer were enrolled in a randomized, double-blinded, placebo-controlled trial to determine whether addition of glutathione to the IV administration of oxaliplatin reduced neurotoxicity (86). Protocol was precisely the same as in 4.2.a.1. A statistically significant reduction of neuropathy in the GSH arm was noted ($p=0.003$). No adverse events from the use of glutathione were noted, and glutathione did not diminish the efficacy of the chemotherapy.

3. Bohm, et al.(87) studied 50 patients with advanced ovarian cancer. Before chemotherapy administration with cisplatin and carboplatin, patients received 2500 mg GSH intravenously for two cycles. No toxicity from the use of glutathione was noted, and glutathione was effective in reducing neurotoxicity, and survival was also enhanced.

4. In another study (88), 79 patients with ovarian cancer being treated with cisplastin and cyclophosphamide were pretreated before each chemotherapy session with 2500 mg of GSH intravenously over 345 total courses. No toxicity from the use of glutathione was noted, and glutathione was effective in reducing neurotoxicity.

5. This same team studied 32 patients with ovarian cancer over 5 cycles with the same protocol as #4, and with the same results. (89)

6. This same team studied 35 patients with ovarian cancer, with the same protocol and same results as #4. (90)

7. The same team studied 40 patients with ovarian cancer over five cycles of chemotherapy with the same protocol and the same results as #4. (91)

8. The pharmacokinetics of high dose IV glutathione was determined by Aebi et al.) (92). They found no toxicity from the use of IV glutathione, and their abstract is worth quoting in full:

“Parenteral glutathione has therapeutic potential for targeted delivery of cysteine equivalents. Thus, high doses of reduced glutathione (GSH) protect from the nephrotoxic and urotoxic effects of cisplatinum and oxazaphosphorines. In order to elucidate the underlying mechanisms the kinetics and the effect of glutathione on plasma and urine sulphydryls were studied in 10 healthy volunteers. Following the intravenous infusion of 2 g m-2 of glutathione the concentration of total glutathione in plasma increased from 17.5 +/- 13.4 mumol l-1 (mean +/- SD) to 823 +/- 326 mumol l-1. The volume of distribution of exogenous glutathione was 176 +/- 107 ml kg-1 and the elimination rate constant was 0.063 +/- 0.027 min-1 corresponding to a half-life of 14.1 +/- 9.2 min. Cysteine in plasma increased from 8.9 +/- 3.5 mumol l-1 to 114 +/- 45 mumol l-1 after the infusion. In spite of the increase in cysteine, the plasma concentration of total cyst(e)ine (i.e. cysteine, cystine, and mixed disulphides) decreased, suggesting an increased uptake of cysteine from plasma into cells. “Urinary excretion of glutathione and of cyst(e)ine was increased 300-fold and 10-fold, respectively, in the 90 min following the infusion. The present data suggest that the concentration of sulphydryls in the urinary tract and, more importantly, the intracellular availability of cysteine increase markedly following parenteral glutathione. The high intracellular concentration of cysteine may protect against cisplatinum and oxazaphosphorine toxicity either directly or indirectly by supporting the synthesis of glutathione.”

9. A Phase I trial of using glutathione as a pretreatment for cisplatin chemotherapy was performed Sixteen patients over 44 total cycles were evaluated. (93) Protocol and results were the same as #4.

10. Nine patients with advanced bladder cancer were studied with the same protocol as #4 (94). No toxicity from the use of glutathione was noted, and neurotoxicity was significantly reduced.

11. One hundred fifty-one patients with ovarian cancer were pretreated with GSH before chemotherapy, using the same protocol as in #4 over 6 cycles. (95) Use of the GSH pretreatment improved quality of life for these women significantly: there was a “statistically significant improvement in depression, emesis, peripheral neurotoxicity, hair loss, shortness of breath and difficulty concentrating.”

12. Twenty patients with non-small-cell lung cancer or head cancer or neck cancer were pretreated with 5000 mg of IV GSH prior to chemotherapy (96). No toxicity from the use of glutathione was noted, and hemotologic toxicity from use of the chemotherapy drugs was significantly reduced.

13. Thirty-three patients with ovarian cancer were pre-treated prior to chemotherapy with IV GSH according to protocol as in #4. (97) Neuroprotection was offered by GSH for these patients, and no toxicity of glutathione itself was noted.

14. Forty-two patients with uremic anemia were given 1200 mg of IV GSH twice a week for 12 weeks.(98) No toxicity from the glutathione was noted, and the treatment increased measures of hemoglobin, red blood cells, and hematocrit.

15. Forty patients with peripheral artery disease were enrolled in a randomized, double-blinded placebo-controlled trial of IV GSH twice a day for five days (99) No toxicity was noted, and significant improvement in walking distance and macrocirculatory and microcirculatory parameters was noted.

16. Thirty-two patients with amyotrophic lateral sclerosis were enrolled in an open, crossover, randomized clinical trial. On treatment, patients received a 600 mg intramuscular injection of GSH daily for 12 weeks (100) A slight slowing of the disease was shown, and no toxicity was noted.

17. IV GSH was given to 10 normal and 10 diabetic patients (101) Total glucose uptake improved significantly in both groups, and no toxicity was reported.

18. Twenty-eight hemodialyzed patients were given IV GSH at the end of their dialysis sessions for at least nine months (102). Dose was 1200 mg IV GSH. Hemoglobin and hematocrit improved in 60% of the patients.

19. Nine patients with early, untreated Parkinson's Disease were treated with IV GSH, 600 mg twice daily for 30 days in open label use (103), A 42% decline in disability was noted, with effects lasting two to four months. No toxicity was noted.

20. Four hemodialyzed patients were given the same protocol as #18 for 90 days (104) The same results were noted as for #18, again with no toxicity noted.

21. Infertile male patients with dyspermia were given every-other-day intramuscular injection of 600 mg GSH in a placebo-controlled double-blinded crossover trial (105). No toxicity was noted, and there was a significant positive effect on sperm motility and morphology.

22. Patients receiving radiation treatment for endometrial cancer were given 1200 mg IV GSH as a pretreatment (106). No toxicity of the GSH therapy was noted, and incidence of diarrhea was significantly reduced compared to controls

23. Forty patients in intensive care were given a continuous infusion of 70mg/kg/day of GSH (107). No toxicity was noted, and indicators of free radical production were significantly reduced.

24. A double-blind placebo-controlled crossover study of twenty atherosclerotic patients was performed, with treatment being 600 mg of IV GSH every day for seven days (108). No toxicity was noted, and GSH infusion significantly decreased blood viscosity and increased blood filtrations. Thromboplastin time was lengthened as well.

25. Twenty-six of 98 patients about to undergo thrombolysis were treated with 3 grams of IV GSH before the procedure (109) Relevant paramteres improved and no toxicity was noted.

b. Aerosol/Intratracheal/Intranasal Administration of Glutathione; Published Clinical Trials

1. Fourteen ventilated preterm infants received 1 mg/kg or 10 mg/kg of intratracheally administered liposomal GSH in one dose (110). Pulmonary glutathione levels were increased, malondialdehyde levels were decreased, and no toxicity was noted.

2. Seven patients with cystic fibrosis were given 600 mg of unbuffered aerosolized glutathione, doses given every 12 hours for 3 days (111). Superoxide release diminished, and no toxicity was noted.

3. Twenty-three patients with cystic fibrosis were given thrice-daily doses of 300 mg or 450 mg of buffered aerosol GSH for 14 days (112). Lung glutathione levels significantly improved, PGE(2) levels were reduced, CD4(+) and CD8(+) lymphocyte levels increased, and no toxicity was noted.

4. Nineteen children with cystic fibrosis were enrolled in randomized, placebo-controlled, double-blinded trial of aerosolized buffered GSH (113). Peak flow significantly increased in the treatment group, and improvement in FEF25-75 approached statistical significance. No adverse effects were noted.

5. Sixty children with chronic otitis media were enrolled in a placebo-controlled randomized double-blinded study. The thirty treatment patients received 600 mg GSH in saline given intranasally every 3-4 waking hours for two weeks (114). In the treatment group, 66.6% of the patients improved compared with 8% of the controls. No toxicity was noted.

6. Thirteen normal subjects and thirteen chronic rhinitis sufferers (ages 4-15) were given 600 mg unbuffered GSH aerosol once a day for 10 days (115). No side effects were observed and there was a significant improvement in nasal obstruction, rhinorrhea, and ear fullness.

7. Ten adult patients with idiopathic pulmonary fibrosis were given 600 mg aerosolized unbuffered glutathione every 12 hours for 3 days (116). No adverse events occurred, and glutathione concentrations in the lung epithelial fluid increased, with release os superoxide anions decreased.

8. Eighteen asthmatic adults, half assigned to placebo, were given 2400 mg of aerosolized unbuffered GSH once a day for 6 days (117). No adverse events were reported and asthma symptoms were ameliorated.

9. Eight adult asthma patients were treated with 600 mg aerosolized unbuffered GSH three times each (118). Bronchoconstriction was noted in some patients.

10. Fourteen adult HIV+ patients were treated with 600 mg aerosolized unbuffered GSH every 12 hours for 3 days (119) No adverse events were noted, and levels of glutathione in the lung epithelial lining fluid normalized.

11. Seven adult emphysema patients were treated with 120 mg of aerosolized unbuffered GSH twice daily for years (120). No adverse events were reported and there was a marked improvement in the course of the disease for all patients.

12. Twelve asthma patients were given one 600 mg dose of aerosolized unbuffered GSH (121). No adverse events were reported, and treatment significantly improved response to "fog" challenge.

5. A Description of Risks and Side Effects

5.1 Possible Risks and Side Effects

According to the scientific literature, there are no risks or side effects of oral administration of reduced l-glutathione. However, there have been anecdotal reports of transitory increase in flatulence, which diminishes after several days of consistent use. Also, Dalhoff et al (60) report two of their patients experienced gastrointestinal irritation. There have also been anecdotal reports of decreased need for use of pancreatic enzymes based on changes in stool characteristics in CF patients.

5.2 Precautions and Monitoring in the Clinical Trial Context

We will advise patients on the risk of transitory increased flatulence.

If gastrointestinal irritation results, the Principal Investigator will adjust the dosage of oral glutathione in an attempt to minimize that symptom; if symptom persists, patient will be instructed to cease treatment material. Gastrointestinal irritation will be reported as an adverse event.

Should stool characteristics change significantly with use of oral glutathione, the pediatric gastroenterologist on the research team will determine if a change in pancreatic enzyme therapy is warranted, and if so, what the new dosage of enzymes should be.

Because the results of any investigation of a new drug cannot be predicted beforehand, we will exclude patients who are in a very fragile nutritional condition, as manifested by tube-dependency or actively losing weight. We will also exclude patients who are in overall fragile health, operationalized as FEV1<50% predicted, presence of ABPA, previous culture of Burkholderia cepacia, or bowel surgery/hospitalization for bowel obstruction in the last six months.

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