NCI, DCPC
Chemoprevention Branch and Agent Development Committee
CLINICAL DEVELOPMENT PLAN:
l-SELENOMETHIONINE

DRUG IDENTIFICATION

**CAS Registry No.:** 3211-76-5

CAS Name (9CI): (S)-2-Amino-4-(methylseleno)butanoic Acid
Synonyms: l-Selenomethionine-^{75}Se (CAS No. 1187-56-0)
Sethotope (Squibb)
Selenomethionine Se 75 Injection Diagnostic
(CIS Radiopharmaceuticals)

Related Compounds:
Selenium (Se)
d-Selenomethionine
d,l-Selenomethionine
Selenomethionine (NOS)

Molecular Wt.: 196.1

Structure:

$$CH_3-Se-CH_2-CH_2-\underset{\underset{NH_2}{|}}{\overset{\overset{H}{|}}{C}}-\overset{\overset{O}{\|}}{C}-OH$$
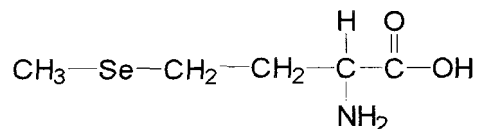
EXECUTIVE SUMMARY

Selenium (Se) is an essential nonmetallic trace element with a nutritional intake of 50–350 μg/day [1] and an estimated dietary MTD of 819 μg Se/day (0.15 μmol/kg-bw/day) [23]. Organic Se compounds, primarily selenomethionine and selenocysteine, ingested in grains, meat, yeast, and certain vegetables are the major source for humans [4]. Low Se status has been linked epidemiologically to increased incidence of cardiovascular disease [4] and increased incidence or mortality from cancers in various organs, such as bladder, breast, colon and rectum, lung,

and prostate [*e.g.*, 12], although these relationships are very inconsistent. For example, seven of 14 retrospective breast cancer studies found significantly lower serum Se in cases compared with controls [5,13]; however, this may result from the fact that certain tumors concentrate Se at the expense of the host [14]. Out of 12 prospective studies using serum or toenail measurements to determine Se status, the only one showing an inverse correlation between serum Se and breast cancer risk lacked statistical significance (trend p=0.45) [5,15,16]. This study, and several others associating increased risk for cancer of

the stomach, esophagus, lung, and all sites with low serum Se levels, were conducted in Finland, where subjects had substantially lower Se status than other populations before the addition of Se to fertilizers began in 1985 [17]. The association between cancer risk and tissue Se levels, especially in the breast, may be nonlinear, with the chemopreventive effect of supplementation limited to deficient or marginal populations. In contrast, the most consistent inverse associations between cancer risk and Se in prospective studies have been observed in populations with concomitant low levels of serum α-tocopherol, retinol and β-carotene, and in males (*e.g.*, stomach, pancreas, all sites) [5].

In animal studies, pharmacological levels of inorganic or organic Se compounds have inhibited chemically and virally induced tumors in mammary glands, colon, skin, lung, trachea, liver, stomach and pancreas, as well as the development of transplanted tumors [18,19]. Published animal chemoprevention studies with all Se compounds indicate that Se inhibits the postinitiation phase of mammary carcinogenesis and the initiation phase of colon carcinogenesis, the inhibitory effect is reversible, and chemopreventive doses approach toxic levels [18]. *l*-Selenomethionine, specifically, was ineffective in mouse colon and lung carcinogenesis models; however, racemic or unspecified enantiomers inhibited mammary tumor development in several rat models. The combination of α-tocopherol acetate with *d,l*-selenomethionine slightly improved the efficacy of the latter in rat mammary glands. Two on-going NCI, Chemoprevention Branch-sponsored preclinical studies with *l*-selenomethionine are evaluating modulation of biomarkers of prostate cancer in the estrogen/testosterone-exposed Noble rat and the effect on prostate cancer in CA/TP/MNU/TP-exposed Wistar rats in combination with α-tocopherol acetate.

The only well-defined function for Se in animals is as a constituent of the Se-dependent form of glutathione peroxidase (GSH-Px) [20], where it is incorporated into the active site of the enzyme as the substituted amino acid, selenocysteine. GSH-Px is a cytosolic enzyme that reduces both hydrogen peroxide and organic hydroperoxides, providing potential antioxidant activity; however, other chemopreventive mechanisms may exist, since GSH-Px activity plateaus in blood and plasma and does not always correlate to tissue Se levels at cancer inhibitory doses. Besides the antioxidant activity of GSH-Px, Se com-

pounds have other antiinitiation effects through altered carcinogen metabolism (by affecting heme metabolism), as well as antiproliferative effects resulting from inhibition of DNA [21] and protein synthesis [22,23], and altered immune function [24,25].

Some evidence suggests that the chemopreventive activity of both inorganic and organic Se compounds may depend on conversion to selenide though different pathways and on the subsequent generation of monomethylselenonium metabolites (as shown in Figure 1). However, other mammalian Se-containing proteins may contribute to these activities. For example, selenoprotein P (found in plasma, kidney, and lung) is hypothesized to have antioxidant activity [21].

In contrast to inorganic Se, *l*-selenomethionine can be converted directly to selenocysteine for incorporation into GSH-Px [27], iodothyronine 5′-deiodinase [23] and other selenoproteins, or used to synthesize Se-adenosyl-*l*-methionine [22,26,28]. Another important difference is that it can be incorporated nonspecifically into proteins in place of *l*-methionine when levels of dietary methionine are low [4]. Because of this, selenomethionine is sequestered and accumulates in tissues to a greater extent than inorganic forms of Se, especially in pancreas, kidneys, liver, gastrointestinal (GI) tract, muscle and mammary glands of rats and mice; therefore, the elimination phase is extended [26,28,29]. Macaque monkeys chronically treated with *l*-selenomethionine showed a similar pattern of distribution with a total tissue Se increase of 13–28-fold. These characteristics suggest that the potency of *l*-selenomethionine as a chemopreventive agent depends on the level of dietary methionine, a concept which was confirmed in a rat chemoprevention experiment where suboptimal levels of dietary methionine significantly reduced the protective effect of *d,l*-selenomethionine [30].

Most animal studies specifically evaluating the anticarcinogenic or toxic effects of selenomethionine have used racemic mixtures of *d*- and *l*-enantiomers, both of which can be metabolized by rodents [*e.g.*, 31–33]. The *d*-enantiomer is not normally found in nature except in certain bacteria and is not metabolized or incorporated into proteins by humans. Since it may have different biological/toxicological properties than the *l*-enantiomer [34], the latter was chosen for potential development. Some clinical trials have administered Se-enriched brewer's yeast, in which
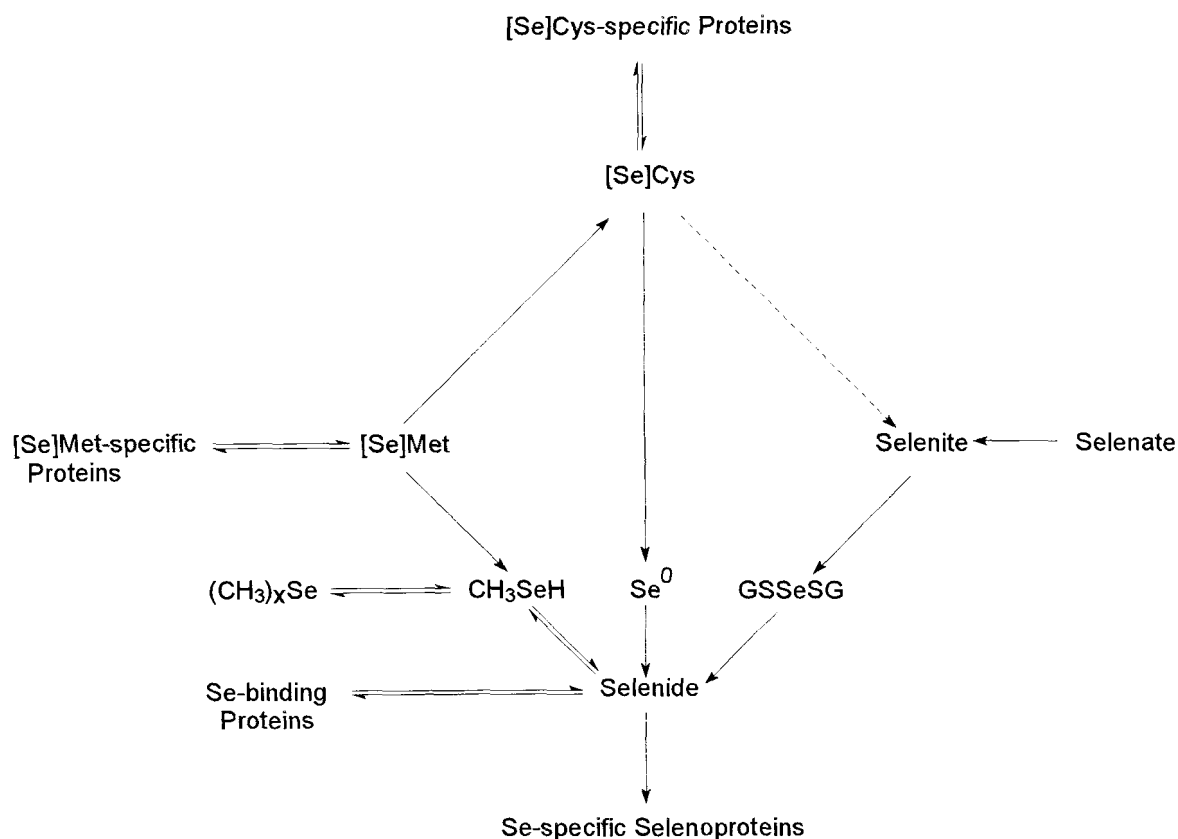
Figure 1. Pathways of Se metabolism leading to the synthesis of selenoproteins in animals [22,26]

approximately 50% of the Se is in the form of *l*-selenomethionine.

Chronic exposure of experimental animals to inorganic or organic Se has resulted in growth failure and weight loss, as well as liver damage, splenomegaly, pancreatic enlargement, anemia, dermatitis, skeletal muscle atrophy, hair loss, and abnormal nails. In NCI, Chemoprevention Branch-sponsored preclinical studies, *l*-selenomethionine produced decreased weight gain, elevated liver enzymes, and liver pathology, as well as altered hematopoiesis in rats and dogs. Non-human primates appear to be more sensitive to *l*-selenomethionine toxicity than rodents or dogs. Any difference in toxicity between inorganic and organic Se compounds due to the difference in fate is unclear, although rats consuming organic Se compounds showed a trend toward less growth inhibition compared with inorganic Se compounds [33]. Although inorganic Se has been shown to impair reproduction in birds and fish, *l*-selenomethionine was not teratogenic in non-human primates except at doses associated with significant maternal toxicity.

In humans, Se appears to have only a 10-fold range of safety between physiological and toxic levels [35]. Since the $t_{1/2}$ of *l*-selenomethionine in humans has been estimated at 200–300 days, there is a potential for accumulation. No specific reports on human toxicity of selenomethionine (NOS) were found in the literature. Based on a Chinese survey, a conservative RDA of 70 and 50 µg Se (NOS) (*ca.* 0.013 and 0.01 µmol/kg-bw/day) was estimated for American men and women, respectively [36]. In another Chinese study, adverse effects following ingestion of *ca.* 5,000 µg Se/day (*ca.* 0.9 µmol/kg-bw/day) as organoselenium compounds in local produce were described as garlic breath, nausea, fatigue, irritability, dermatitis, hair loss, and nail tenderness or loss [37]. Recently, an oral Reference Dose (RfP) of 350 µg Se (NOS)/day (*ca.* 0.06 µmol/kg-bw/day) was published, which represents a safe intake meant to protect populations at highest risk, and could be loosely interpreted as the NOEL [1]. The human MTD of dietary Se is estimated to be 819 µg Se/day (*ca.* 0.15 µmol/kg-bw/day) [3], or about 15 times the RDA for women.

Although no trials with *l*-selenomethionine specifically were identified, three Phase III trials with Se-enriched yeast or Se (NOS) have been initiated or completed. The NCI-funded Nutritional Prevention of Cancer Project (Dr. Larry Clark, University of Arizona) is assessing the chemopreventive effect of supplementation with 200 µg or 400 µg Se/day (*ca.* 0.04 or 0.07 µmol/kg-bw/day) as Se-enriched brewer's yeast (*ca.* 50% of the Se as *l*-selenomethionine) for at least two years on patients at high risk for skin cancer living in relatively low Se areas of the U.S. (selenomethionine (NOS) was provided as an alternative for those intolerant to brewer's yeast). Additional endpoints include total mortality, cancer mortality, incidence of lung, prostate, and colorectal cancer, incidence of colorectal polyps, and incidence of elevated PSA. Long-term safety is also being assessed by plasma Se levels, clinical chemistry, and hair and nail changes.

In collaboration with the Cancer Institute of the Chinese Academy of Medical Sciences, the NCI, Cancer Prevention Studies Branch (Dr. Philip Taylor, CPSB) evaluated the effect of supplementation with Se in combination with other agents in a completed Phase III trial in Linxian, China, the area with the world's highest mortality rate from esophageal/gastric cardia cancer. Following daily treatment with 50 µg Se (*ca.* 9 nmol/kg-bw/day) as Se-enriched brewer's yeast plus 15 mg β-carotene and 30 mg vitamin E (α-tocopherol) or placebo for 5¼ years, total mortality rate was significantly decreased (9%), primarily the result of lower total and stomach cancer rates. A 42% reduction in esophageal cancer risk in a subset of subjects was not statistically significant.

Finally, an independent Phase III trial in Norway (Norwegian Cancer Society) is evaluating the effect of a daily combination dose of 101 µg Se (NOS) (0.02 µmol/kg-bw/day), 15 mg β-carotene, 1,600 mg Ca^{2+} (NOS), 75 mg vitamin E, and 150 mg vitamin C for three years on incidence and size of colon polyps and incidence of colon cancer. No results have been published to date.

l-Selenomethionine is under clinical development by the NCI, Chemoprevention Branch based on the inverse epidemiological association between breast and prostate cancer risk and Se status (primarily from ingestion of organoselenium compounds in food) and the suggested reduction in esophageal cancer risk seen in the Chinese Phase III trial (Dr. P. Taylor). In support of these studies, selenomethionine (NOS) has

shown chemopreventive efficacy in animals and is credited with at least five chemopreventive mechanisms. Of equal importance is the brief report from the NCI-funded Phase III clinical trial (Dr. L. Clark) that daily ingestion of 200 or 400 µg Se (0.04 or 0.07 µmol/kg-bw) as Se-enriched brewer's yeast (*ca.* 50% of Se as *l*-selenomethionine) for two years has not caused changes in hair and nails or clinical chemistry associated with Se toxicity. The NCI, Chemoprevention Branch is planning a short-term, double-blind Phase I/IIa trial to study the safety and pharmacodynamics of *l*-selenomethionine in combination with *d*-α-tocopherol acetate. The agents will be administered to patients with breast DCIS for a period of 1–4 weeks between biopsy and surgery to evaluate modulation of intermediate biomarkers such as proliferation, DNA ploidy, and nuclear pleomorphism. A second Phase II trial is being considered to evaluate the effect of the *l*-selenomethionine/*d*-α-tocopherol acetate combination on intermediate biomarkers in esophageal dysplasia.

l-Selenomethionine was chosen in preference to Se-enriched yeast for these trials because purity and stability of the single-agent formulation can be better controlled; the amount of *l*-selenomethionine and the composition of Se-enriched yeast may vary from lot to lot. Clinical supplies of the agent and one-year stability test data are available.

PRECLINICAL EFFICACY STUDIES

Epidemiological studies have shown inconsistent relationships between Se levels in serum, plasma, or tissue and cancer incidence or risk for specific targets such as breast, lung, colorectal, hematological, bladder, and prostate [7]. Preclinical studies of both inorganic and organic Se have shown efficacy in some of the same organs, including mammary glands, colon, skin, and lung. However, the major human dietary source of Se is from organic compounds (primarily selenomethionine and selenocysteine), and preclinical studies with selenomethionine have demonstrated efficacy only in mammary models.

NCI, Chemoprevention Branch-funded preclinical efficacy studies of *l*-selenomethionine and *d,l*-selenomethionine have shown a lack of effect in mouse colon and lung carcinogenesis models, respectively. Dietary *l*-selenomethionine was not effective in the MAM acetate-induced mouse colon at 2 ppm Se (*ca.* 3.3 µmol/kg-bw/day) and *d,l*-selenomethionine was ineffective in MCA-induced mouse lung at 4 ppm Se

(*ca*. 6.5 μmol/kg-bw/day); however, inorganic forms of Se were also inactive in these models (sodium selenite and selenate). Inappropriate doses or models may have been the cause, or, as suggested previously, effects may only be seen in Se-deficient or marginally adequate populations. An ongoing Chemoprevention Branch-sponsored study is evaluating the effect of *l*-selenomethionine on prostate cancer in the CA/TP/MNU/TP-exposed Wistar rat model.

In contrast, more than 60 published animal studies have shown inhibition of tumorigenesis in ten organ systems by various forms of Se [18]. Inhibition of mammary gland tumors in rodents was achieved by various forms of Se at nontoxic doses in 20 out of the 24 studies reviewed. For selenomethionine specifically, the racemic mixture inhibited DMBA-induced rat mammary tumor multiplicity, incidence, and number [31,32,34,38,39], in addition to increasing latency in the MNU-induced model [39]. However, it should be noted that the mammary cancer chemo-preventive efficacy of the inorganic forms, selenate and selenite, appeared to be somewhat greater than *d,l*-selenomethionine administered to rats at comparable doses of 3 ppm Se in the diet (*ca*. 1.9 μmol/kg-bw/day) during the promotional phase [32]. Finally, an unspecified enantiomer of selenomethionine was effective against both DMBA-induced rat mammary carcinogenesis *in vivo* [30,38] and DMBA-induced mutagenesis in the standard Ames *Salmonella* assay *in vitro* [40,41]. Chemopreventive efficacy was slightly improved *in vivo* by combining 3 ppm Se as *d,l*-selenomethionine (*ca*. 1.9 μmol/kg-bw/day) with 500 ppm α-tocopherol acetate. In this study, DMBA-induced tumor incidence in rats was significantly inhibited by 32%, compared with 22% for *d,l*-selenomethionine alone [32]. In the same study, selenite plus α-tocopherol acetate significantly inhibited tumor incidence by 55%.

A significant effort in the NCI, Chemoprevention Branch program is focused on the identification and validation of intermediate biomarkers of cancer and the evaluation of the potential for chemopreventive agents to modulate these markers. Such studies in animals contribute to the development of more efficient screens for identifying new chemopreventive agents, as well as identifying biomarkers to be used as surrogate endpoints in clinical trials [42]. Currently, modulation of biomarkers of prostate cancer by *l*-selenomethionine, α-tocopherol acetate and the combination is being evaluated in the estrogen/testos-terone-induced Noble rat model. Endpoints include histological biomarkers such as number and grade of dysplastic lesions, nuclear and nucleolar morphometry (measured by computer-assisted image analysis), DNA ploidy and proliferative biomarkers (PCNA and BrdU labelling).

PRECLINICAL SAFETY STUDIES

Chronic exposure of experimental animals to high doses of organic or inorganic Se has resulted in growth failure and weight loss, as well as liver damage, splenomegaly, pancreatic enlargement, anemia, dermatitis, hair loss, and abnormal nails [43]. In general, it appears that males are more sensitive than females and non-human primates are more sensitive than rodents or dogs. Selenate, selenite and selenomethionine were toxic at similar Se levels in the diet [33,44]. In an NCI, Chemoprevention Branch-sponsored acute toxicity study, the LD_{50} for *l*-selenomethionine in rats was estimated to be 26 mg/kg-bw (133 μmol/kg-bw). In a 90-day study, chronic administration resulted in decreased weight gain, elevated liver enzymes, liver pathology, and altered hematopoiesis in rats and dogs. Se (both inorganic and organic) is recognized as a teratogen in birds, fish, and crustaceans; however, *l*-selenomethionine was only teratogenic in non-human primates and hamsters at doses associated with significant maternal toxicity [45,46]. ADME studies in rats show that selenomethionine administration results in greater tissue accumulation in rats than inorganic forms of Se, especially in kidney, liver, and muscle; a similar response was seen in non-human primates.

Safety: The NCI, Chemoprevention Branch has completed an acute toxicity study of *l*-selenomethionine in Fischer 344 rats, and 28- and 90-day oral toxicity studies of *l*-selenomethionine in Fischer 344 rats and Beagle dogs. In the acute toxicity study, doses ranged from 10–80 mg/kg-bw (51–408 μmol/kg-bw); signs were decreased activity, ataxia, abnormal defecation, and body weight loss, with observations of clear fluid in the thoracic cavity and pulmonary congestion in the higher dose groups at necropsy. In the 90-day rat study of 0.5–4.5 mg *l*-selenomethionine/kg-bw/day (2.5–22.9 μmol/kg-bw/day) ig, sex differences were observed even though plasma Se levels were equivalent. The NOELs were 1.0 and 0.5 mg *l*-selenomethionine/kg-bw/day (5.1 and 2.5 mmol/kg-bw/day) for males and

females, respectively. At higher doses, reduced body weight gain, elevated liver enzymes, toxic hepatitis, pancreatic inflammation/degradation, and extramedullary hematopoiesis in the spleen were observed, with 100% mortality at the highest dose in females. In the 28-day toxicity study, ig doses ranged from 0.75–6.0 mg *l*-selenomethionine/kg-bw/day (3.8–30.6 µmol/kg-bw/day). Observations were similar to those seen in the 90-day study; doses of 0.75 and 1.5 mg *l*-selenomethionine/kg-bw/day (3.8 and 7.6 µmol/kg-bw/day) had no effect except for an increase in liver weight.

When Beagle dogs were exposed to oral *l*-selenomethionine doses of 0.1–3.0 mg/kg-bw/day (0.5–15.3 µmol/kg-bw/day) for 28 days, there were no survivors in the high-dose group and no deaths in the low-dose group. Males were more sensitive than females. Hematology and clinical chemistry tests showed reduced platelets and leukocytes and elevated liver enzymes. Microscopic pathological changes indicated toxic hepatitis and hypocellular bone marrow. After 90-day exposure of Beagle dogs to doses of 0.1, 0.3, and 1.0 mg *l*-selenomethionine/kg-bw/day (0.5, 1.5, and 5.1 µmol/kg-bw/day) by capsule, males were again more sensitive than females; only 1/3 survived in the high-dose group. Clinical signs of reduced body weight gain, emesis, elevated hepatic enzymes, hemorrhage, and lymphocyte depletion were supported by microscopic observations of inflammation, telangiectasis, hemorrhage, vacuolar changes and brown pigment in liver hepatocytes, thymic atrophy, and GI hemorrhages. The NOEL was 0.3 mg *l*-selenomethionine/kg-bw/day (1.5 µmol/kg-bw/day) in this study.

In published studies, male rats administered 10 ppm Se (*ca.* 6.3 µmol/kg-bw/day) in a semipurified diet for six weeks as sodium selenite, sodium selenate, *d*- or *l*-selenomethionine experienced severe growth depression and total mortality [33]. The NOEL for all compounds appeared to be 2.5 ppm Se (*ca.* 1.6 µmol/kg-bw/day). In contrast, a second study comparing selenite with both selenomethionine enantiomers found no toxic effects in rats given 10 ppm Se in a commercial diet for 12 weeks. However, *l*-selenomethionine produced a greater weight reduction at 16 ppm Se (10.1 µmol/kg-bw/day) than the *d*-enantiomer or sodium selenite [47]. The lack of agreement between the two studies may have resulted from differences in the basal diet. The commercial chow used in the second study appears to have attenu-

ated the toxicity of Se; adequate methionine content is believed to be the protective factor.

Non-human primates appear to be more sensitive to *l*-selenomethionine toxicity than rodents or dogs. In female macaque monkeys given time-weighted average doses of up to 300 µg *l*-selenomethionine/kg-bw/day (1.5 µmol/kg-bw/day) ig for 30 days, a dose-related decrease in body weight was observed [34]. Based on these data and the occurrence of hypothermia, dermatitis, xerosis, cheilitis, menstrual disturbances and GI distress, the MTD was estimated at 150 µg/kg-bw/day (0.8 µmol/kg-bw/day) in this species. One female macaque treated with 600 µg *l*-selenomethionine/kg-bw/day (3.0 µmol/kg-bw/day) ig for 15 days developed severe body weight loss and a seven-fold increase in polychromatic erythrocyte micronucleus frequency over control values [48].

Embryolethality and teratogenicity have been reported primarily in birds and fish following inorganic or organic Se exposure. However, total doses of 75 and 100 µmol *l*-selenomethionine/kg-bw/day ig administered to hamsters over gestation days 5–8 did not produce teratogenic effects [46]. The resorption rate increased at both doses, but it was associated with severe maternal body weight loss. When given as a single dose of 75, 88 or 100 µmol *l*-selenomethionine/kg-bw ig on gestation day eight, the incidence of abnormal litters significantly increased in the lower dose group; however, all dose regimens resulted in severe maternal toxicity.

No teratogenic effects from *l*-selenomethionine were found in female macaque monkeys administered 25, 150 or 300 µg/kg-bw/day ig (0.13–1.5 µmol/kg-bw/day) during organogenesis (gestation days 20–50) [45]. One embryonic and two fetal deaths in the high-dose group were not significantly different from concurrent or historical controls. No negative effects were found in the neonates, despite increased Se levels in fetal tissues [49].

ADME: In published studies, the plasma Se concentration in male rats given 2.5 ppm Se as *l*-selenomethionine in the diet (*ca.* 1.6 µmol/kg-bw/day) for six weeks was 0.56 µg/ml (7 µM) [33]. It is important to note that the increased body burden of Se resulting from feeding 5.0 ppm Se as *l*-selenomethionine (*ca.* 3.2 µmol/kg-bw/day) did not correlate to increased plasma Se levels [33]. In rats, plasma levels plateaued at 7.3 µM Se from dietary doses of *l*-selenomethionine greater than 2.5 ppm Se, while the skeletal muscle burden doubled between

2.5 and 5.0 ppm Se [33]. In the same study, erythrocyte Se levels did reflect a proportionate increase between doses of 2.5 and 5.0 ppm *l*-selenomethionine.

In rats fed 3 ppm Se as selenomethionine (NOS) (*ca.* 1.9 μmol/kg-bw/day) in AIN-76A diet for up to 30 weeks [38], tissue concentrations of Se (μg/g) after eight weeks were as follows: kidney, 29.3±2.5 (0.37 μmol/g); liver, 17.0±0.61 (0.22 μmol/g); muscle, 4.1±0.24 (0.052 μmol/g); plasma, 0.93±0.04 (12 μM); and mammary glands, 0.57±0.05 (0.007 μmol/g). The estimated body burden and the mammary gland levels of Se were higher than those from selenite and three high-Se food sources; however, this high level of Se did not correlate to increased chemopreventive efficacy. GSH levels in liver were not affected by selenomethionine, although they were raised significantly by selenite, which is a more potent chemopreventive agent in rat mammary glands [38]. In this study, the carcinogen was DMBA, which must be activated by the liver before reaching its target; the outcome might have been different with MNU, a direct-acting carcinogen.

The distribution of 4 μCi [75]Se-*l*-selenomethionine was studied in mice after a single iv administration [29]. The elimination $t_{1/2}$ was seven days; the agent was incorporated rapidly into pancreas, but the highest levels of ^{75}Se throughout the study were in liver, kidneys, and GI tract. The levels remained fairly constant in liver for the seven days of the experiment, but decreased in most other tissues.

The pharmacokinetics of *l*-selenomethionine in adult female macaque monkeys was investigated in a published study. At 25, 150, 300 or 600 μg Se as *l*-selenomethionine (*ca.* 0.3–7.6 μmol/kg-bw/day) for 30 days, plasma, hair and urinary Se levels were dependent on the dose [50]. Erythrocyte Se was slower to respond and often continued to increase after dose interruption. Two monkeys died after 10–15 days on the high dose; total tissue Se increased 13–28-fold, with the liver and kidneys showing the highest levels.

CLINICAL SAFETY: PHASE I STUDIES

No Phase I clinical trials of *l*-selenomethionine have been conducted to date. The NCI, Chemoprevention Branch is planning a short-term multidose double-blinded Phase I/IIa trial to study the safety and pharmacodynamics of *l*-selenomethionine in combination with *d*-α-tocopherol acetate in patients

with DCIS. *l*-Selenomethionine (400 μg Se/day or *ca.* 0.07 μmol/kg-bw/day) and *d*-α-tocopherol acetate will be administered for of one week or more in the period between biopsy and surgery. Modulation of intermediate biomarkers such as proliferative index, DNA ploidy, and nuclear pleomorphism index will be evaluated.

An ongoing NCI-funded Phase III clinical trial (Dr. L. Clark, University of Arizona) is assessing both efficacy and long-term safety of Se-enriched brewer's yeast (50% *l*-selenomethionine) in humans at 200 and 400 μg Se/day (*ca.* 0.04 and 0.07 μmol/kg-bw/day). It should be noted that Se-enriched brewer's yeast has not been analytically characterized except for Se content.

Drug Effect Measurement: The most commonly used index of Se status is the plasma or serum concentration [51]. In humans, as in rats, these measures respond rapidly to depletion or supplementation; however, increased body burden of Se caused by high intake of *l*-selenomethionine may not correlate to a proportionate increase in plasma Se. In subjects ingesting 200 μg Se/day as *l*-selenomethionine (*ca.* 0.04 μmol/kg-bw/day), plasma levels plateaued between 11 and 16 weeks at 2.4 μM Se, and erythrocyte levels plateaued at 0.4 ng Se/g Hb (0.005 nmol/g Hb) by 28 weeks [17,52]. These parameters are apparently not useful for measuring increases in body burden over long periods of time, but can register intake changes over several weeks [5,33]. Toenail Se content may be a more reliable indicator of body burden for long-term chronic studies, although the correlation coefficient is only 0.48 [5,53]; hair can also reflect Se intake but it is subject to superficial contamination by Se-containing dandruff shampoos and may not be reliable.

For assessment of functional Se status, GSH-Px activity has been evaluated in whole blood, plasma, erythrocytes, and platelets. Interestingly, a saturation point (plateau) for GSH-Px activity was reached at the same concentration of Se for all blood components [52]. In women treated with 200 μg Se/day (*ca.* 0.04 μmol/kg-bw/day) as *l*-selenomethionine, GSH-Px activity plateaued at plasma concentrations of 98.7–114.5 μg Se/l (1.25–1.46 μM) after *ca.* 14 days [52]. The rate of GSH-Px activity response to increased serum levels of Se depends on the chemical form of Se [17]; dietary inorganic Se intake shows a rapid response while organic forms, including selenomethionine, are incorporated into non-GSH-Px

protein to a greater extent [54] by direct replacement of methionine in the polypeptide chain, resulting in a slower GSH-Px response.

Safety: A nutritionally adequate daily intake ranging up to 350 µg Se (*ca.* 0.06 µmol/kg-bw) has been proposed for humans [3], although the RDA for North American males and females is 70 and 50 µg Se (*ca.* 0.013 and 0.011 µmol/kg-bw), respectively [36]. The recently published oral RfP of 350 µg Se (NOS)/day (0.06 µmol/kg-bw) represents a safe intake meant to protect populations at highest risk [1]. No specific reports of human toxicity from *l*-selenomethionine were found in the literature. In Enshi County, China, adverse effects following daily ingestion of 5,000 µg Se (*ca.* 0.9 µmol/kg-bw/day) as nonspecific organoselenium compounds in local produce included garlic breath, nausea, fatigue, irritability, dermatitis, hair loss, and nail changes and loss [37]. A low level of adverse effects is observed at an estimated dietary intake of 1,540 µg Se/day (*ca.* 0.28 µmol/kg-bw/day) [3]. In the Phase III clinical trial studying the effects of 200 and 400 µg Se/day (*ca.* 0.04 and 0.07 µmol/kg-bw/day) as Se-enriched yeast (*ca.* 50% *l*-selenomethionine), changes in hair, nails and liver and kidney function tests did not suggest clinical differences between the placebo and high-dose groups [55].

ADME: A model to describe the kinetics of sodium selenite in humans has been developed based on a single oral dose of 200 µg Se (2.5 µmol) enriched with ^{75}Se [56]. Approximately 84% of the dose was absorbed, with 65% remaining in the body after 12 days. The model consists of four plasma components, a tissue component representing the liver and pancreas, and a longer residence tissue component. The ADME of *l*-selenomethionine may differ somewhat. Selenite is absorbed passively, and metabolized into selenodiglutathione (GSSeSG) before incorporation into proteins or methylation for excretion. In contrast, about 75% of a selenomethionine dose is actively transported from the intestine by the methionine carrier in humans [4]. It may then be incorporated directly into proteins in place of *l*-methionine when levels of dietary methionine are low [4], catabolized to selenocysteine for incorporation into GSH-Px [27] and other selenoproteins [21,28], or released into the Se pool by further catabolism or transsulfuration to be metabolized to trimethylselenonium and excreted in the urine, but this is a minor pathway in rats at nontoxic doses (3–4% of total) [22,57] (see Fig. 1).

Organic Se compounds such as selenomethionine are sequestered and accumulate in tissues [26] to a greater extent than inorganic forms of Se, especially in kidney, liver, and muscle [29] of rats, mice, and monkeys. For example, 211 µCi ^{75}Se-*l*-selenomethionine iv is rapidly incorporated into pancreatic proteins, which is the basis of its use as a radiological diagnostic [58]. The elimination t½ for this agent has been reported to be 70 days in humans [cited in 59]. In another pharmacokinetic study, a single 200 µg (2.5 µmol) dose of ^{75}Se as *l*-selenomethionine administered to healthy males had a whole-body residence time approximately five times longer than the turnover time in the slowest tissue pool (61–86 days), suggesting considerable recycling of Se [60]. This time frame was confirmed in two different reports, which estimated a t½ of 200–300 days [58, 61].

The kinetics of a single higher oral dose of 540 µg Se (*ca.* 0.1 µmol/kg-bw) as *l*-selenomethionine has been evaluated in healthy male volunteers. The rate of urinary Se excretion reached a maximum within only four hours [62], with approximately 26% of the dose recovered in the urine within 24 hours. In two chronic (16 and 32 weeks) human studies, subjects received daily doses of 200 µg Se (*ca.* 0.04 µmol/kg-bw/day) as *l*-selenomethionine; plasma Se levels plateaued at week 11 and 16 at 2.1 and 2.4 µM, respectively. In humans, GSH-Px accounts for only 10% of erythrocyte Se, indicating that GSH-Px activity may not be a reliable measure of *l*-selenomethionine burden. In the 32-week study, GSH-Px activity plateaued in blood after *ca.* 12 weeks of treatment [17,52]; erythrocyte levels of Se appeared to plateau later at *ca.* 28 weeks and continued to rise after dosing was discontinued.

CLINICAL EFFICACY: PHASE II/III STUDIES

Epidemiological data showing an inverse association between Se status and cancer are inconsistent for several reasons. In retrospective studies, the presence of cancer may decrease plasma Se; in prospective studies, the chemopreventive effect may be limited to marginally deficient populations, or the measurements of Se status may be inaccurate, especially in dietary studies [5,10,53]. The most consistent associations are in males and in populations with concomitantly low vitamin E, retinol and β-carotene intake [5,7,12]. Randomized, placebo-controlled trials of Se-enriched brewer's yeast alone and in com-

bination with other nutrients have been undertaken to clarify the cancer chemopreventive effects. However, Se-enriched brewer's yeast contains organic compounds in which Se has been substituted for sulfur during growth in Se-enriched media; the result is that *ca.* 50% of the Se is in the form of *l*-selenomethionine in addition to unknown amounts of selenoglutathione, selenodiglutathione, selenocysteine, unidentified Se-containing compounds, and analytically undefined non-selenium products [63]. One NCI-funded Phase III trial (Dr. L. Clark, University of Arizona) of Se-enriched brewer's yeast is in progress; a second completed trial, funded by the NCI, CPSB (Dr. P. Taylor) in collaboration with the Chinese Academy of Medical Sciences, evaluated the effect of Se-enriched brewer's yeast in combination with other nutrients on esophageal/gastric cardia cancer incidence and mortality. Finally, the effect of a combination of Se (NOS) with other nutrients on colon polyps has been assessed in an independent trial in Norway. No Phase II Se trials have been funded by the Chemoprevention Branch, but a Phase II trial is being considered to evaluate the effect of the *l*-selenomethionine/*d*-α-tocopherol acetate combination on intermediate biomarkers in esophageal dysplasia.

The NCI, CPSB (Dr. P. Taylor) collaborated with the Cancer Institute of the Chinese Academy of Medical Sciences to evaluate the effect of Se in combination with other agents in a completed Phase III trial in Linxian, China, the area with the world's highest mortality rate from esophageal/gastric cardia cancer [61,64]. Participants (n=29,584) from the general population were treated daily with 50 μg Se (ca. 9 nmol/kg-bw/day) as Se-enriched brewer's yeast plus 15 mg β-carotene and 30 mg vitamin E (α-tocopherol) or placebo for 5¼ years. Endoscopy performed on a sample of subjects found a 42% reduction in esophageal cancer risk compared with the placebo group, but the difference was not statistically significant. However, total mortality was significantly decreased 9% in this group (p=0.03), and was attributed to lower total and stomach cancer rates. It should be noted that this population had subclinical deficiencies in several nutrients, including vitamin E, although levels of Se were near normal (according to Western standards).

The ongoing NCI-funded placebo-controlled Phase III Nutritional Prevention of Cancer Project (Dr. L. Clark, University of Arizona) is assessing the

effect of 200 and 400 μg Se/day (*ca.* 0.04 and 0.07 μmol/kg-bw/day) as Se-enriched brewer's yeast on multiple forms of cancer. Since the study was originally intended to evaluate the effect of Se on non-melanoma skin cancer, participant selection was based on high risk for this condition, but the objectives were expanded in later years to include multiple retrospective endpoints such as mortality, cancer mortality, incidence of lung, prostate, colorectal, and skin cancer, incidence of colorectal polyps, and effect on PSA. Long-term safety is also being assessed by plasma Se levels, clinical chemistry, and hair and nail changes. The project was initiated in 1983, but no interim data are available.

An independent double-blind, placebo-controlled colon trial in Norway (Norwegian Cancer Society) of a daily combination of 101 μg Se (NOS) (1.3 μmol), 15 mg β-carotene, 1,600 mg Ca^{2+} (NOS), 75 mg vitamin E, and 150 mg vitamin C administered for three years began in July 1989 [65]. Over a period of 18 months, a total of 116 patients with colon polyps were accrued and allocated to separate groups based on the size of the largest polyp (<5 mm, 5–9 mm, >9 mm); each group was then randomized to the placebo or treatment arm. The endpoints were modulation of histological biomarkers (*e.g.* changes in polyp diameter, number of polyps reaching 5 or 10 mm, new polyp incidence) and colon cancer incidence. No results have been published to date.

PHARMACODYNAMICS

In animal models, pharmacological doses of Se appear to be necessary to inhibit carcinogenesis. In rats, the effective oral dose of *d,l*-selenomethionine against mammary cancer (3 ppm Se, or *ca.* 1.9 μmol/kg-bw/day) [32] is 15–30-fold higher than the nutritional requirement for Se in this species (0.1–0.2 ppm Se, or *ca.* 0.06–0.13 μmol/kg-bw/day) [reviewed in 37]. The chemopreventive dose is also within the range (2.5–5 ppm Se or *ca.* 1.6–3.2 μmol/kg/day) at which rat plasma Se and GSH-Px activity plateau and skeletal muscle burden doubles [33]. Using the MTD from the NCI-funded 90-day toxicity study in rats (2.5 μmol Se/kg-bw/day), the calculated chemopreventive index (MTD divided by effective dose) is only 1.32.

In humans, the dose calculated directly from the rat mammary study would be 10,500 μg Se, or 26,078 μg *l*-selenomethionine/day (*ca.* 1.9 μmol/kg-bw/day). However, if the estimated human MTD of

819 µg Se/day (*ca.* 0.15 µmol/kg-bw/day) and rat chemopreventive index (1.32) are used to calculate the approximate effective human dose of *l*-selenomethionine, the result is *ca.* 620 µg/day (*ca.* 0.1 µmol/kg-bw Se/day). Although the pharmacokinetics/dynamics of *l*-selenomethionine appear to be similar in humans and animals—plasma Se and GSH-Px activity plateau while tissue levels increase—the plateau occurs in humans at a dose (200 µg Se/day or *ca.* 0.04 µmol/kg-bw/day) [17,52] which is within the nutritional range for daily intake (50–350 µg Se, or *ca.* 0.01–0.06 µmol/kg-bw [1,36]). This suggests that chemopreventive efficacy may be obtained at lower doses in clinical trials if the mechanism is antioxidation and/or a specific selenoprotein is present in the target tissue. The safety of chronic doses up to 400 µg/day Se (*ca.* 0.07 µmol/kg-bw/day) as *l*-selenomethionine has been suggested by preliminary results from the ongoing NCI-funded Phase III trial (Dr. L. Clark, University of Arizona) of Se-enriched brewer's yeast. This dose is well below the estimated human MTD of 819 µg Se/day (0.15 µmol/kg-bw/day), a value which is ~16-fold greater than the lowest nutritional dose (50 µg Se/day, or *ca.* 0.02 µmol/kg-bw/day) [36], ~6-fold lower than the toxic levels observed in China (5,000 µg Se/day or *ca.* 0.9 µmol/kg-bw/day) [37], and ~2-fold lower than the estimated LOAEL (1,540 µg Se/day or *ca.* 0.28 µmol/kg-bw/day) [3].

PROPOSED STRATEGY FOR CLINICAL DEVELOPMENT

Drug Effect Measurement

Se availability from *l*-selenomethionine may be lower than for other forms of the element because of its ability to replace methionine in proteins; protein-bound Se is unavailable to undergo the transsulfuration necessary for release into the main Se pool [52]. For this reason, specific drug effect measurements for selenomethionine intake should be given careful consideration, since it appears that misclassification of Se status may result from reliance on a single measurement [53]. For short-term Phase II trials, urinary and plasma levels of Se may be useful; however, these measures are indicative of only recent *l*-selenomethionine intake and should be compared with tissue Se levels.

Plasma levels in long-term selenomethionine trials may be inaccurate measures of Se status. Based on

two human studies of chronic *l*-selenomethionine administration at 200 µg Se (in the form of Se-enriched yeast), plasma Se levels plateaued at *ca.* 2.2 µM between weeks 11 and 16 [17,52]. Thus, plasma levels might be an appropriate measure of human intake for short-term studies (up to 16 weeks) at that dose; however, the plateau may occur earlier at a higher dose. Erythrocyte Se appeared to plateau later (*ca.* 28 weeks), but continued to rise after dosing was discontinued. In epidemiological studies, the Se content of hair and nails has been used as an indicator of body burden [55]. Hair levels of Se are subject to superficial contamination, but nail levels can register Se intake over the previous several months [5]. For long-term studies at doses above 200 µg Se/day (*ca.* 0.04 µmol/kg-bw/day) as *l*-selenomethionine, toenail Se may be the most accurate measurement of status; however, this only has a correlation coefficient of 0.48 with intake [53]. Since none of these parameters appear to provide accurate information which could reflect chemopreventive activity, it might be useful to investigate blood levels of monomethylselenonium, which may be an active chemopreventive metabolite [66].

Safety Issues

At chemopreventive dietary *l*-selenomethionine doses (3 ppm Se or *ca.* 1.9 µmol/kg-bw/day) in rats, tissue Se concentrations were highest in the kidney, liver, and muscle. Since *l*-selenomethionine can be nonspecifically incorporated into proteins in place of methionine, chronic or high doses could potentially produce tissue accumulation, inhibition of protein and DNA synthesis and toxicity, especially in these organs. For example, adverse hepatic effects were observed in both rats and dogs in the Chemoprevention Branch-funded 90-day toxicity studies. In rats, doses of 2–4.5 mg/kg-bw/day (10–23 µmol/kg-bw/day) decreased body weight, elevated liver enzymes and caused toxic hepatitis, pancreatic inflammation, and extramedullary hematopoiesis. Similar responses occurring in dogs at a lower dose (5.1 µmol/kg-bw/day) included decreased weight gain, elevated liver enzymes, hemorrhage, lymphocyte depletion, and liver lesions. Since non-human primates also accumulate Se in the liver [50], liver enzymes may be appropriate indicators of toxicity in future clinical trials.

The estimated whole body residence time of 430 days for *l*-selenomethionine in humans [58] suggests

that chronic dosing will result in tissue accumulation, presumably in protein. A brief report on the Phase III cancer prevention trial with Se-enriched brewer's yeast (*ca.* 50% *l*-selenomethionine) (Dr. L. Clark, University of Arizona) indicates that cases of hair, nail, or liver and kidney enzyme changes indicative of Se toxicity have not been identified in cohorts receiving either 200 or 400 μg Se/day (*ca.* 0.04 or 0.07 μmol/kg-bw/day) for up to two years [55]; however, the data are not available from the blinded study. It is not known if these are appropriate indicators of *l*-selenomethionine toxicity at these dose levels; serum hepatic and kidney enzyme concentrations in the plasma may be the most sensitive.

The proposed Phase II trial of *l*-selenomethionine plus *d*-α-tocopherol acetate has a duration of 1–4 weeks which should not impose any risk of toxicity. For longer term studies, the tendency of *l*-selenomethionine to accumulate nonspecifically in body proteins could be hazardous, particularly in subjects with a dietary deficiency of *l*-methionine. In order to minimize nonspecific protein deposition and the potential for accumulation, a dietary supplement of methionine should be considered. The estimated daily requirement of methionine plus cysteine (cysteine can replace methionine) for an adult is 13 mg/kg-bw/day (910 mg/day) [67].

Pharmacodynamics Issues

As noted above, determining appropriate biological indicators of effective doses may require significant developmental effort, since neither body burden of Se, GSH-Px activity nor GSH levels appear to correlate to the chemopreventive activity of selenomethionine in rat mammary glands. Epidemiological studies have relied on plasma or serum levels of Se as indicators of cancer protection; however, studies in rats show that plasma Se levels plateau at doses below effective chemopreventive levels following dietary exposure to selenate or *l*-selenomethionine [33].

Regulatory Issues

The dose of 400 μg Se/day as *l*-selenomethionine (*ca.* 0.7 μmol/kg-bw/day) proposed in the NCI, Chemoprevention Branch planned Phase I/IIa trial is less than half of the estimated MTD for Se (819 μg/day, or *ca.* 0.15 μmol/kg-bw/day) and should therefore not be a safety risk in view of the short duration of exposure (1–4 weeks).

Intermediate Biomarker Issues

An NCI, Chemoprevention Branch short-term Phase I/IIa clinical trial is planned to study the effect of *l*-selenomethione in combination with *d*-α-tocopherol acetate in presurgical breast cancer patients. Suggested intermediate biomarker endpoints are DNA ploidy, nuclear and nucleolar polymorphism, and proliferation.

Supply and Formulation Issues

Clinical supplies for the planned Phase II studies and one-year stability data are available. Capsules containing 25, 50, 100, 200, and 400 μg Se as *l*-selenomethionine have been formulated. *l*-Selenomethionine was chosen in preference to Se-enriched yeast because of the difficulty in establishing *l*-selenomethionine content, stability, and composition of the latter and the inherent variability of natural products between lots.

Clinical Studies Issues

In prospective epidemiological studies, inconsistent results were be obtained possibly because the inverse association between Se levels and cancer risk is nonlinear, with the chemopreventive effect of supplementation limited to deficient or marginal populations. The ongoing NCI-funded Phase III trial (Dr. L. Clark, University of Arizona) is assessing the effect of 200 μg and 400 μg Se/day (*ca.* 0.04 and 0.07 μmol/kg-bw/day) as Se-enriched brewer's yeast (*ca.* 50% of Se as *l*-selenomethionine) on mortality and multiple cancer endpoints in subjects at high risk for skin cancer. Subjects are stratified by entering plasma Se levels to determine efficacy at marginal *vs.* adequate status. The results of this study should provide valuable safety and efficacy information which could be used to plan future NCI, Chemoprevention Branch clinical trials with *l*-selenomethionine.

The NCI, Chemoprevention Branch is planning a short-term Phase I/IIa trial of *l*-selenomethionine in combination with *d*-α-tocopherol acetate in presurgical breast cancer patients. Epidemiological evidence of the interaction of low Se status with low vitamin E status in increasing cancer risk, as well as the increased efficacy of racemic selenomethionine plus vitamin E acetate against rat mammary gland carcinogenesis, suggest that these agents might have synergistic activity in the breast. Mechanistically, the

antioxidant activities of GSH-Px in the cytosol would be combined with those of vitamin E in membrane lipids. Thus, the planned Phase I/IIa trial is a four-arm study to determine if *l*-selenomethionine with or without *d*-α-tocopherol acetate administered between biopsy and surgery can modulate intermediate biomarkers (proliferative index, DNA ploidy, and nuclear pleomorphism index) in patients with breast DCIS.

In the NCI, Cancer Prevention Studies Branch-sponsored trial in Linxian, China, the combination of high Se brewer's yeast, vitamin E, and β-carotene significantly reduced total mortality, which was attributed to decreased total and stomach cancer rates. A 42% decrease in esophageal cancer rates was not statistically significant. A Phase II trial is being considered to evaluate the effect of the *l*-selenomethionine/*d*-α-tocopherol acetate combination on intermediate biomarkers in esophageal dysplasia.

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Table I. Clinical Trials of *l*-Selenomethionine Sponsored/Funded by NCI, DCPC

Study No. Title (PI) Period of performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase II (Dose-titration, efficacy, intermediate biomarkers)					
Planned Study *l*-Selenomethionine and *d*-α-To-copherol Acetate in Breast Neoplasia 11/96–	Breast	Presurgical patients with possible ductal carcinoma *in situ* (DCIS) 50/arm (2x2 factorial)	400 µg Se as *l*-seleno-methionine + 400 IU *d*-α-vitamin E acetate/day between diagnosis and surgery (1–4 wk)	Intermediate biomarkers: Computer-assisted morpho-metry (ploidy, nuclear pleomorphism), proliferation	Protocol being finalized
Planned Study *l*-Selenomethionine and *d*-α-To-copherol Acetate in Patients with Esophageal Dysplasia	Esophagus	Patients showing evi-dence of esophageal dysplasia 50/arm (2x2 factorial)	400 µg Se as *l*-seleno-methionine + 400 IU *d*-α- vitamin E acetate/day for 6 mo	Intermediate biomarkers: Computer-assisted morpho-metry (ploidy, nuclear pleomorphism), proliferation	
Phase III (Efficacy, intermediate biomarkers)					
ZO1-CN-00112-10-CPSB Study of Effect of Nutritional Intervention on Esophageal Cancer in Linxian, People's Republic of China (Dr. Philip Taylor, NCI, CPSB, Dr. William Blot, NCI, DCE, and Linxian Nutrition Intervention Study Group) 1985–91	Esophagus	Commune residents 40–69 years of age in high-risk area for eso-phageal cancer (Linxian, China) 29,584 residents	50 µg Se as Se-enriched yeast + 15 mg β-carotene + 30 mg vitamin E qd for 5¼ yr vs. 3 other mineral/vitamin combinations and placebo	Efficacy: Esophageal cancer incidence, mortality	Study completed. Mortality reduction (9%) significant; attributed to lower total and stomach cancer rates Published reports: [61,64]

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Table I. Clinical Trials of *l*-Selenomethionine Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) *(continued)*					
UO1-CA-59338-01 PSA: an Intermediate Marker for Prostate Cancer (Dr. Larry Clark, University of Arizona) 9/93–7/96	Prostate	1. Retrospective: Skin cancer patients with PSA >4 ng/ml 1,000 frozen serum samples from RO1-CA-49764 2. Prospective: Skin cancer patients (from RO1-CA-49764) with normal PSA plus men from PSA study in intermediate U.S. Se region n=1,900 (1,000 + 900)	200 or 400 µg Se as Se-enriched brewer's yeast for 12 mo	Intermediate biomarkers: PSA	Study in progress Abstract: [55]

Table I. Clinical Trials of *l*-Selenomethionine Sponsored/Funded by NCI, DCPC *(continued)*

Study No. Title (PI) Period of performance IND No.	Cancer Target	Study Population No. of Subjects	Dose(s) Treatment Duration	Endpoint(s)	Remarks
Phase III (Efficacy, intermediate biomarkers) (continued)					
RO1-CA-49764-01 to -06 Nutritional Prevention of Cancer Project Original title: The Prevention of Non-Melanoma Skin Cancer with Nutritional Supplement of Selenium (Dr Larry Clark, University of Arizona) 8/88–11/98	Colon, lung, prostate, skin	Patients with single SCC (or SCCIS) or ≥2 BCC within previous 12 mo n=1,313 at 200 μg Se qd n=925 at 400 μg Se qd	Nutritional dose: 200 μg Se qd as Se-enriched brewer's yeast Supranutritional dose: 400 μg Se qd as Se-enriched brewer's yeast for at least 2 yr 200 or 400 μg Se as *l*-selenomethionine for those intolerant to brewer's yeast for at least 2 yr	Efficacy: Total cancer incidence; total cancer mortality; colon polyp incidence; melanoma and non-melanoma skin cancer incidence; lung, colon, and prostate cancer incidence; interaction with retinol, vitamin E, and β-carotene (retrospective for prostate cancer subjects) Long-term safety	1983–1988: Prevention of nonmelanoma skin cancer with nutritional dose, (200 μg qd Se); interaction with retinol, vitamin E, and β-carotene; 1990: Expanded to include prevention of colon cancer; long-term safety of supranutritional dose; 1992: Expanded to include *l*-selenomethionine for those intolerant to brewer's yeast; 1994: Expanded to include skin, lung, colon, prostate cancer endpoints; PSA levels. Interim results: No toxicity reported in subjects treated with 200 μg or 400 μg Se qd by analysis of hair and nail changes, and liver and kidney function tests after 8,250 person-years Abstract: [55]

l-SELENOMETHIONINE DEVELOPMENT STATUS

Task Name	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
PRECLINICAL EFFICACY														
PRECLINICAL TOXICOLOGY														
CLINICAL, PHASE III														

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