Review

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Selenium compounds for cancer prevention and therapy – human clinical trial considerations

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Abstract: Selenium (Se) is an essential trace mineral crucial for human health. Nearly a dozen human clinical trials with seleno-methionine (SeMet) and selenized-yeast (contains mostly SeMet) for the prevention of non-cutaneous solid organ cancers in North America and European countries conclusively refuted their utility. We have articulated two lessons from these trials: (1) the anti-oxidant hypothesis was tested in inappropriate Se-adequate populations, and (2) the selection of these Se forms was not supported by cell culture and animal efficacy data. Nevertheless, preclinical studies of proximal methylselenol precursors ("methyl Se") have shown many desirable attributes, involving crucial molecules and pathways in cancer epithelial cells, vascular endothelial, immune and inflammatory cells in the tumor microenvironment, for potential use as chemopreventive and therapy agents. Methylseleninic acid and Se-methylselenocysteine are prototypical methyl-Se, yet not equal in their targets. Selenate, selenite and selenious acid had been recently studied in human clinical trials, providing novel safety data, but, missing critical genotoxicity assessments. Given the popularity of Se-enriched foods in China and a continued presence of nutritional Se deficiency in many localities, we discuss recommendations for clinical studies of Se forms for cancer therapy or chemoprevention in China and other countries with similar Se nutrition predicament.

Keywords: selenium; methylselenol; methylseleninic acid; human clinical trials

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Forms of selenium in food chain

The trace element selenium (Se) was named by Swedish chemist and physician Jons Jacob Berzelius more than 200 years ago, after the Greek moon goddess "Selene". Before the 1970s, Se was best known for semi-conductors in the electronic appliances until silicon took over. For its industrial applications, see a recent review of the Se global demand and supply [1].

Selenium initially attracted the medical profession's attention because of its toxicity to industrial workers. Selenium was also recognized as an important veterinary toxin, such that grazing herbivore animals who had consumed high-Se plants in spring time displayed "alkaline disease" in several northern US high plains states. Klaus Schwarz and co-workers at the US National Institutes of Health first reported Se as an essential trace element nutrient for mammalian life in 1957 as the essence of "factor 3", distinct from the nutritional function of vitamin E [2]. Since the 1970s, Se has been shown to be co-translationally incorporated into many Se-dependent proteins and enzymes (collectively known as selenoproteins SEPs), such as Se-dependent glutathione peroxidases (SeGPX), thioredoxin reductases (TrxRs), thyroxine deiodinases (TDIs) in the form of selenocysteine (SeCys or SeC). Selenoproteins now number 25 to date [3]. During the 1980s, SeCys, as the 21st amino acid, was shown to be encoded by the codon UGA (otherwise the protein translation stop codon) by co-translational incorporation specified through the SeCys insertion (SECIS) element, a unique mechanism that had been conserved from bacteria to mammals, including humans (Figure 1) [3].

Animals and humans absorb Se from their ingested gastrointestinal contents made of plants, animals and animal products or their mixtures. The Se level in the soil in which plants are grown largely determines the Se content in the food chain. Approximately two-thirds of Chinese land areas are low for soil Se, with some areas extremely deficient, leading to serious endemic health problems, such as Keshan Disease (KD, commonly known in China as "Ke-shan-bing") and Kashin-Beck Disease (KBD, commonly known in China as "Da-gu-jie-bing") [4–9]. First described in Keshan

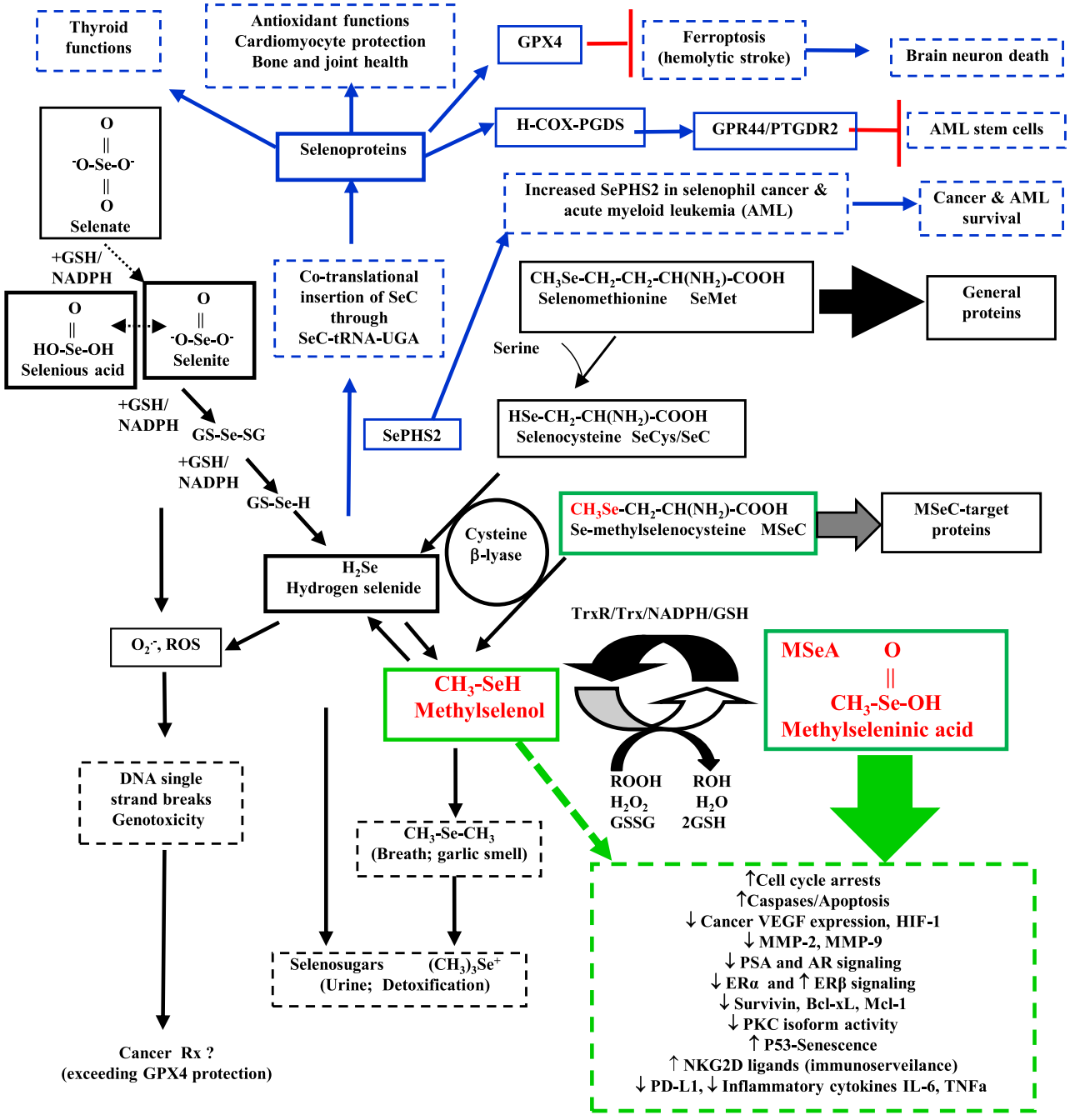

Figure 1: Structures and pathways for inorganic selenate/selenite and for methylseleninic acid (MSeA), Se-methylselenocysteine (MSeC) and other selenoanimo acids in nutritional functions and cancer chemoprevention and therapy contexts. Inorganic selenate (Se^{+6}) and selenite (Se^{+4}) are reduced by glutathione/NADPH to hydrogen selenide which is the substrate for selenophosphate synthetase 2 (SePHS2) to charge SeCys-tRNA for co-translational incorporation into selenoproteins (SEPs). Selenite (selenide) in excess of SEP synthetic capacity causes DNA damage, likely through reactive oxygen species (ROS). For selenoamino acids, tissue cysteine β-lyases release hydrogen selenide and methylselenol from SeC and MSeC, respectively. SeMet leads to massive tissue accumulation of Se due to its non-specific incorporation into general proteins in place of Met. Methylselenol pool may be selectively enriched by precursor compounds or functional foods such as Se-garlic, bypassing the hydrogen selenide pool. Depending on the entry point to a MSeA:MSeH redox cycle, the cellular effect and molecular targets of these methyl Se will be the integrative balance of MSeA, MSeH and their redox intermediary metabolites. Reported cellular effects and molecular targets of MSeA:MSeH are summarized. Due to the amino acid analog nature of MSeC, its *in vivo* "targets" mostly differ from those of MSeA. Modified and updated from Lu et al., *Nutri & Cancer* 2016 [43].

County in the far northeastern Hei-long-jiang Province, KD is a severe cardiomyopathy with high mortality [4]. KBD, which is another Se-deficiency driven disease of bone and joints, had been prevalent in the regions in the northwestern Shaanxi Province along with iodine deficiency [8, 9]. Nutritional intervention through fortification of common table salt with inorganic sodium selenite, if implemented sufficiently early before irreparable tissue damages had set in, had been very effective in preventing KD and KBD [4, 9]. A vast country of geological contrasts and diversities, some mountainous regions in Hubei and Sichuan Provinces in China contain high soil and environmental Se, especially in many coal mines [5]. Severe Se toxicity in people (e.g., hair loss, finger and toenail deformities), and livestock (e.g., hair brittleness and loss, hoof deformities) occur in these areas, especially in drought years when rice crops were replaced by draught-hardy crops that did not need as much water to cultivate.

In the US, environmental Se contamination from water irrigation evaporation and industrial waste runoff have caused irreparable damages and death to many fish and aquatic species and birds. In 2008 there was an outbreak of selenosis (Se toxicity) in several US states [10]. The source was identified as a liquid dietary supplement that contained 200 times the labeled Se in the form of sodium selenite. One person was hospitalized among 201 cases identified in 10 US States. The median estimated dose of Se consumed was ~42 mg/day (for reference, the nutritional Se requirement per US National Research Council (NRC) daily allowance is ~55 μg Se for adults [11]). Reported symptoms included diarrhea (78 %), fatigue (75 %), hair loss (72 %), joint pain (70 %), nail discoloration or brittleness (61 %), and nausea (58 %). Accidental poisoning of farm animals and race horses from mis-formulated mineral mix products had been documented in the US and other countries.

Dietary sources of Se through naturally-grown food ingredients likely provide the bulk of Se in the forms of SEPs, which are digested to yield seleno amino acids, such as SeMet and SeCys, for intestinal absorption. It is also possible some inorganic Se salts used in fertilizers or functional food fortifications could enter the food chain, if the Se was not incorporated into SEPs or bound to Se-binding proteins (SBPs). Some plants, their seeds or fruits accumulate Se. Brazil nut is known to contain high levels of Se, mostly as SeMet [12].

As dietary supplements, Se-enriched yeast (cultured with sodium selenite) and SeMet are marketed in US and European countries. Se-yeast contains mostly SeMet. Since Se is in the same family as sulfur (S) in the Periodic Table, S-compounds-rich allium species, such as garlic [13] and onion, and cruciferous vegetables, such as broccoli and Brussels sprouts were used to generate Se-enriched plants for research purposes by selenite fertilization in the US. Se-enriched eggs, tea, barley malts, bean sprouts, as well as high-Se crops from selenosis areas have been marketed in China as functional foods for Se health promotion.

Scope of review

Nutritional Se deficiency was not a prevalent issue in US [14, 15]. Therefore, the major focus of Se and cancer research in many US institutions in the past half century has been on the cancer chemopreventive efficacy of various inorganic, organic and synthetic Se forms in animal models and of SeMet and Se-Yeast in human cancer chemoprevention clinical trials. We will summarize and update the outcomes of these human clinical trials in the US and European countries and critique the reasons for their negative efficacy.

Nevertheless, many groups, including ours, have studied the possible cancer chemoprevention and therapeutic applications, especially of Se forms distinct from SeMet, such as methylseleninic acid (MSeA) and Se-methylselenocysteine (MSeC) which are considered proximal methylselenol precursors (Figure 1). We have and will refer them collectively as “methyl-Se”. We will examine and contrast pertinent studies with inorganic “genotoxic” Se pool (e.g., selenite) in the context of cancer chemoprevention and therapy. We shall discuss their potential mechanisms of actions. Given the popularity of Se-enriched foods and dietary supplement products in China and a continued presence of nutritional Se deficiency in many Chinese localities [7–9], we will discuss future research directions and recommendations for clinical translation studies in China and other countries with similar Se nutrition predicament.

Because we elected to focus on endogenous mammalian Se metabolism (methylselenol as a key anchor metabolite pool) and methyl-Se which are proximal methylselenol-precursors, we will not cover Se-substituted derivatives of S-containing drugs, Se-hybrid entities or aromatic synthetic Se compounds. Readers interested in these topics are referred to published expert reviews [16, 17].

Major clinical trials that failed to show cancer preventive activities of SeMet or Se-yeast

Table 1 summarizes the primary efficacy outcomes from major clinical trials in US & Canada, and European countries. Earlier intervention studies showed that Se supplementation

Table 1: Cancer intervention outcomes of clinical trials with SeMet or Se-yeast in North America and Europe.

Trial	Se form & dose	Baseline Se µg Se/day	Post-Rx Sea ng/mL	Outcomeb ng/mL (max)	RR parameter	1° Ref Rx/placebo
Clark NPCT	Se-yeast, 200	113 (median)	190	PCa (2°)	0.37 (p=0.002)	[20]
Clark NPCT (follow-up)		1 tertile, <106		PCa (2°)	0.14	[22]
		2 tertile, 107–123		PCa (2°)	0.33	
		3 tertile, >123		PCa (2°)	1.14	
SWOG SELECT	SeMet, 200	136 (median)	251 (max283)	PCa (1°)	1.04 (p>0.15)	[24]
SELECT-BLDC	SeMet, 200			Bladder Ca (2°)	1.13 (p=0.52)	[33]
SELECT-CRC	SeMet, 200			Colorectal Ca (2°)	0.96 (p=0.94)	[34]
SWOG9917	SeMet, 200	137 (median)	261 (max305)	PCa (1°)	0.97 (p=0.73)	[26]
ECOG NBT[c]	Se-yeast, 200	126 (median)	Not available	PCa (1°)	0.94 (p=0.18)	[27]
	Se-yeast, 400		Not available	PCa (1°)	0.90 (p=0.17)	
ECOG5597	Se-yeast, 200	Not available		Lung Ca (1°)	1.25 (p=0.294)	[28]
Sel/Cel trial	Se-yeast, 200			Colo adenoma (1°)	1.03 (p=0.68)	[35]
				Recurrence (2°)	0.82 (p=0.01)	
Belgium-SELEBLAT	Se-yeast, 200			Bladder Ca (1°)	0.85 (p=0.44, ITT[d])	[29]
				Recurrence (1°)	0.96 (p=0.93, PPA[d])	
UK-SELENIB	Se-yeast, 200			Bladder Ca (1°)	0.92 (p=0.65)	[30]

[a]Highest median/mean reported for that study. [b]Outcome parameter: planned primary endpoint (1°) vs. added secondary endpoint (2°) of the respective trial. [c]The Negative Biopsy Trial (NBT)-US, New Zealand. [d]ITT, intention to treat analysis; PPA, per protocol analysis; PCa, prostate cancer.

as Se-yeast decreased the risk of cancers in China [18, 19], where the majority of agricultural land areas was deficient in soil Se. The Nutritional Prevention of Cancer Trial (NPCT, also known as the "Clark Trial" for its principal investigator the late Dr. Larry Clark) in the US reported supplementation with Se-yeast reduced risk of cancers of the prostate (Table 1), lung and colon and all cancer-mortality, all as secondary endpoints [20–23]. Sub-group analyses in NPCT [20, 21] indicated that risk of prostate cancer was reduced in a baseline Se-specific manner in subjects in the lower 2 tertiles of serum Se (<106 and 107–123 ng Se/mL) but there was no risk reduction for the highest tertile (>123 ng Se/mL) (Table 1). Such finding was in seeming agreement with a potential deficiency of anti-oxidants, thereby the rationale for testing Se and vitamin E each alone and in combination in the Selenium and vitamin E Cancer Prevention Trial [SELECT], which was by far the largest in terms of number of participants [24, 25]. The NPCT also motivated a number of other Phase III clinical trials in US, Canada and Costa Rica against prostate, lung cancer as primary endpoints [24–28] and other trials in European countries [29, 30]. Key limitations of the NPCT were (1) non-cutaneous solid cancers including prostate cancer (PCa) were secondary endpoints not planned in the original design, (2) the small number of cancer cases for each organ site.

However, all these subsequent trials with cancer incidence as primary endpoint showed no efficacy for SeMet or Se-yeast (Table 1). Follow-up analyses of SELECT have even shown a slight enhancement of PCa risk associated with α-tocopheryl acetate form of vitamin E [31] and in a subgroup of men on SeMet that had high baseline Se status [32]. The bladder [33] and colorectal cancer [34] responses to SeMet in SELECT as secondary efficacy outcomes were negative, as was for PCa. Clinical trials with Se-yeast for colorectal adenoma in US [35], bladder cancer recurrence prevention in Belgium [29] and UK [30] yielded negative efficacy outcomes, as well (Table 1).

In addition to the lack of cancer control efficacy, increase in Type 2 diabetes in participants taking SeMet or Se-yeast had now been reproducibly documented (Table 2). A negative risk for longevity was reported in a Denmark cohort of Se-yeast supplementation [36].

These negative outcomes with SeMet, Se-yeast and α-tocopherol acetate had seriously hampered the field of cancer chemoprevention research. To make matters worse, the Alpha-Tocopherol Beta-Carotene (ATBC) trial prior to these negative Se trials showed an enhancement of risk of lung and prostate cancer in male smokers given β-carotene supplementation [37]. Not surprisingly, the prominent nutritional epidemiologist John Potter authored a 2014 review article, declaring "the failure of cancer chemoprevention" [38]. Consequently, the US biomedical community has not been enthusiastic about new human clinical trials to test any new Se candidates.

Lessons from these failed trials

Based on the metabolic, biochemical and cellular functional differences between SeMet and other forms of Se [39, 40]

Table 2: Non-oncologic health outcomes of clinical trials with SeMet or Se-yeast in North America and Europe.

Trial	Se form & dose µg Se/day	Outcome# parameter	RR Rx/placebo	Ref
Clark NPCT	Se-yeast, 200	Type 2 diabetes (2°)	1.55 (p=0.03)	[20]
SELECT	SeMet, 200	Type 2 diabetes (2°)	1.07 (p=0.16)	[24]
Sel/Cel trial	Se-yeast, 200	Type 2 diabetes	1.25 (p=0.41)	[35]
		Type 2 diabetes (older pts)	2.21 (p=0.03)	
Denmark Se trial	Se-yeast, 100	All-cause mortality	0.75 (5 year); 1.15 (entire)	[36]
	Se-yeast, 200	All-cause mortality	0.64 (5 year); 0.99 (entire)	
	Se-yeast, 300	All-cause mortality	1.62 (5 year); 1.59 (entire)	

(Figure 1), we had argued that the failure of SeMet in SELECT or Se-yeast in other trials should not and could not be taken to indicate that all Se forms were ineffective for cancer chemoprevention [41]. We articulated two key factors for the failure of SELECT and other Se trials a decade ago [42, 43], worthy a timely reminiscence: 1) an inappropriate choice of Se-adequate human subjects for testing the Se anti-oxidant hypothesis which could and would have manifested in Se deficiency subjects, and 2) an incorrect choice of Se forms for cancer chemoprevention in Se-adequate populations. Other scientists have voiced their opinions, authors of the original paper reporting the outcome of SELECT [24] and their subsequent reviews, including baseline Se status of subjects, dosage and chemical forms of Se, and stage of cancer development targeted [44–46]. In the following sections we offer an updated and integrated discussion of these points.

Lesson 1: the antioxidant hypothesis was tested in wrong subjects/patient populations

The best studied biochemical activity of Se is its function as an integral part of GPXs, which are antioxidant enzyme SEPs neutralizing reactive hydrogen peroxide or phospholipid peroxides [3]. It has been estimated that achieving 1 µmol/L or 80 ng Se/mL plasma or serum is the upper limit for a maximal SeGPX response to supplemental Se in healthy adults [43, 47] (Figure 2), which is more than sufficiently provided by the National Research Council's recommended daily allowance of 55 µg Se for adults [11]. A study in Se-deficient subjects in China with SeMet supplementation provided a similar estimate of Se intake needed to support maximal plasma levels of SEP-P (SEPP1) [48]. The authors extrapolated 75 µg Se/day as the SeMet dose for US residents required to ensure full expression of SEPs, based on SEPP1 optimization with adjustments for body weight and individual variation [48].

Pertinent to participant nutritional status of the US trials, the third National Health and Nutrition Examination Survey (NHANES III) documented mean Se intake in the US of individuals of all ages in 1999–2000 of 103 µg [49], nearly twice the National Research Council (NRC) daily allowance of 55 µg Se for adults [11]. The mean serum Se concentration in US adults was 1.58 µmol/L and the median was 1.56 µmol/L in NHANES III [50, 51]. In the NPCT study the placebo group had a similar baseline plasma Se level of 113 ng Se/mL (1.4 µmol/L) (Table 1), and the plasma Se level in the supplemented group was increased to 190 or 2.4 µmol/L [20, 22]. Only 1.5 % of all subjects had Se levels lower than 80 ng/mL [22]. In spite of the increase of total Se level in the plasma, the plasma GPX3 activity of selected subjects before and after Se-yeast supplementation was not increased in NCPT [52].

In SELECT, the median baseline plasma Se level was 136 ng/mL (1.72 µmol/L), higher than that in the NPCT, and SeMet supplementation increased median levels to 223, 232, 228 and 251 ng/mL in four subsequent years (2.82–3.17 µmol/L) [24] (Table 1). In the SWOG9917 trial in men with high grade prostatic intraepithelial neoplasia (HG-PIN), the baseline median plasma/serum Se level was 135–138 ng/mL in the placebo and Se supplemented group, respectively [26] (Table 1). In the NBT study with Se-yeast in men with elevated PSA but negative prostate biopsies, the mean baseline Se values were 125, 127, and 127 ng/mL or 1.58, 1.61 and 1.61 µmol/L, respectively, for the placebo, 200-µg and 400-µg cohort [27]. In the lung cancer trial with Se-yeast, the baseline Se values were not presented, but only fewer than 1 % of subjects were rated below the normal range [28]. In all these Se trials, the baseline Se status was therefore more than nutritionally adequate. Thus, the hypothesis of cancer prevention by dietary Se can only be tested in populations with deficient Se intake, such as Chinese living in endemic low-Se locals, in spite of governmental Se supplementation drives (table salt) for the last 5 decades [7].

Lesson 2: the dose levels and selection of Se agents were not supported by cell culture and animal efficacy data

Se exposure levels in cell culture vs. human studies

In cell culture studies, SeMet exposure levels of 1–2 orders of magnitude higher than circulating total Se level were needed

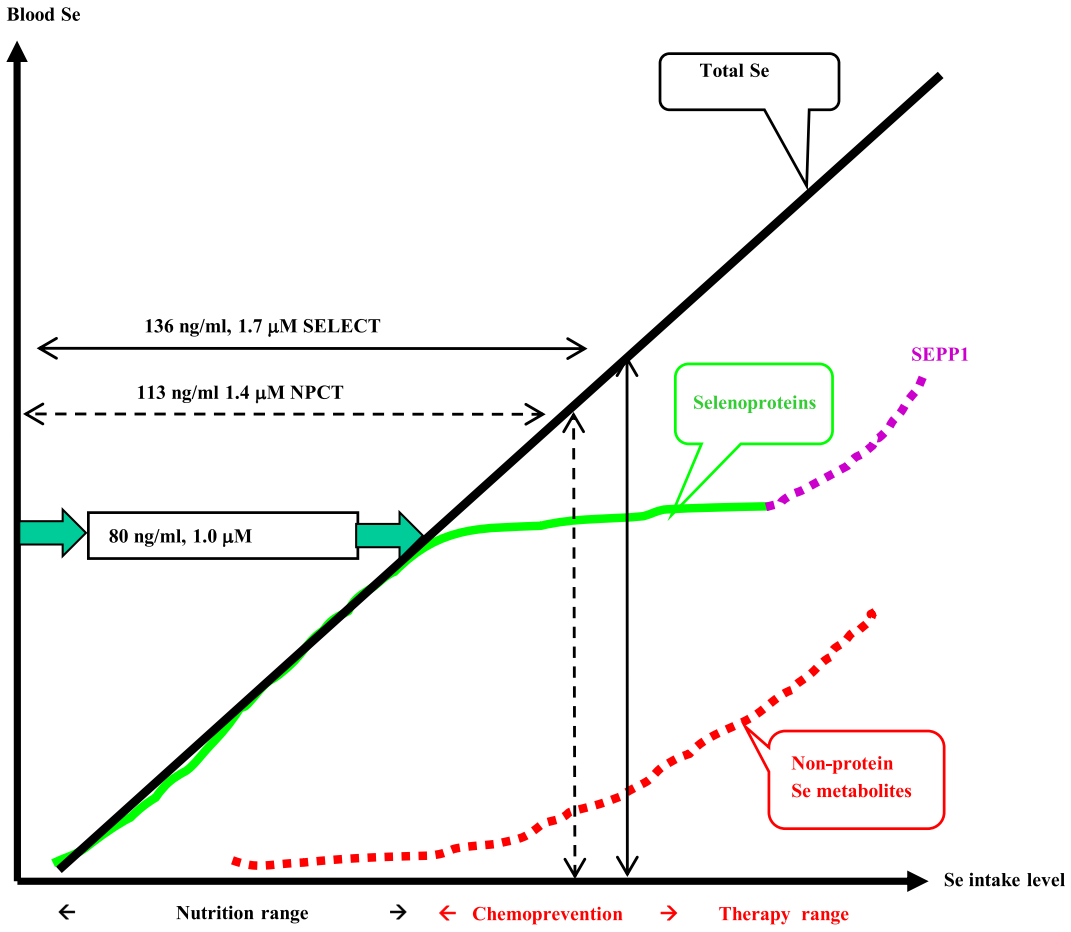

Figure 2: Schematic relationship of different pools of Se in blood circulation as a function of Se intake level in the nutritional range and in chemoprevention and therapy range. Mean or medium baseline plasma Se at enrollment for NPCT vs. SELECT was marked in reference to plasma Se threshold value to support full selenoprotein enzyme activities. Plasma selenoprotein-P (SEPP1) as Se storage protein had been shown to further elevate beyond the plateau level with pharmacological selenite exposure [158]. Modified from Lu et al., *Nutri & Cancer* 2016 [43].

to show a modest growth inhibitory effect in some cancer cell lines. For example, SeMet exposure inhibited the growth of A549 lung cancer cells with an IC_{50} of 65 µM and of HT29 colon cancer cells with an IC_{50} of 130 µM [53]. In PCa cells, 100–500 µM SeMet were needed to induce growth suppression and apoptosis [54]. Two additional studies with colon cancer cell lines reported cell cycle arrest with SeMet levels above 100 µM [55, 56].

For solid cancers other than those arising in the gastrointestinal tract, the only likely route of exposure to SeMet as dietary supplement is through absorption and delivery via the blood stream. It is very unlikely that such extreme high SeMet levels are achievable through oral SeMet supplements. Indeed, the highest mean Se level resulting from supplementation of SeMet or Se-yeast in the reported trials was 305 ng Se/mL (3.9 µM) (Table 1). In a Phase I dose escalation study by Roswell Park researchers, 4,800 and 7,200 µg SeMet/dose (twice per day for 7 days) resulted in serum Se levels of 15 and 20 µM on day 8, respectively [57]. The 7,200 µg SeMet dose-twice per day regimen represented a Se intake that was 29 fold higher than that used in the SELECT and other PCa trials (Table 1), but the resulting blood level was lower than that needed in cell culture experiments to reduce cancer cell growth.

Combs and coworkers [58] evaluated the relationship between serum levels of GPX3 and SEPP1 and plasma Se in a cohort of healthy, Se-replete men (n=106) and women (n=155) from North Dakota in a SeMet supplementation study. The baseline plasma Se was 142 ng/mL, with GPX3 and serum-derived SEPP1 constituting 20 and 34 %, respectively, of the total plasma Se. The non-specific Se components (calculated from difference between total Se minus Se in GPX3 and SEPP1 pools) accounted for virtually all of the inter-individual variation in total plasma Se. After a year-long supplementation with SeMet at doses of 50, 100 or 200 µg Se/day vs. placebo [59], GPX3 activity or SEPP1 concentrations

were not increased, but the Se contents of plasma, urine and buccal cells were increased dose-dependently and each of these plateaued after 9 months and were linearly related to the "effective" Se dose defined as μg/day per $kg^{0.75}$ (metabolic body weight). The authors concluded that the most responsive Se intake biomarkers in this Se-sufficient cohort were those related to body Se pools, i.e., plasma, buccal cell and urinary Se concentrations. As the plasma SEP pools were saturated even without supplementation, the percentage represented by the non-specific Se pool increased with increasing doses of supplementation as stylized in Figure 2.

Pennsylvania State University researchers Richie et al. directly compared SeMet and Se-yeast supplement in men and measured serum Se forms [60]. Less than 1 ng/mL SeMet was observed in the circulation of either SeMet- or Se-yeast-supplemented subjects. Non-specific incorporation of SeMet into general proteins is the most probable fate of the supplemented SeMet. Therefore, it is likely that SeMet *in vivo* will be in nM range and cannot elicit cellular responses observed in cell culture models by the triple digit μmol/L range of exposure.

Animal models showed no efficacy of SeMet

Two general types of animal models had been used for efficacy assessment: primary carcinogenesis induced by chemical-hormonal carcinogenesis in immune-competent rodents and "take" and growth of primary and metastatic PCa xenografts in immunocompromised mice or allografts in syngeneic mice. For the former category, a couple of studies conducted in the late 1990s did not find any efficacy of SeMet and α-tocopheryl acetate alone or in combination, or, of Se-yeast on a prostate carcinogenesis model in rats and these negative results were published a decade later after SELECT results had been published [61, 62]. Xenograft studies conducted before or since SELECT was initiated did not support any *in vivo* anti-cancer activity of SeMet [63, 64]. In a study with orthotopic PC3 xenograft tumors in the prostates of 6-week-old male nude mice fed a Se adequate diet (0.07 mg/kg), supplementation with different Se forms at two different concentrations (0.3 and 3.0 mg/L) in drinking water, SeMet, Se-yeast, or MSeC did not retard the growth of primary prostatic tumors and the development of retroperitoneal lymph node metastases, yet surprisingly sodium selenate did [63]. We evaluated the growth inhibitory effects of SeMet and selenite in comparison to MSeA and MSeC (see Figure 1 for structure and metabolism) on DU145 and PC-3 human PCa xenografts in athymic nude mice [64]. Each Se was given by a daily single oral dose regimen starting the day after subcutaneous inoculation of cancer cells. Serum, liver, and tumor Se content confirmed supplementation status. SeMet did not have any inhibitory effect in spite of an order of magnitude higher Se retention in liver and tumors of the SeMet-treated mice than in those from mice treated with selenite or MSeA and MSeC in either model. MSeA and MSeC each exerted a dose-dependent inhibition of DU145 xenograft growth. Selenite treatment increased DNA single-strand breaks in peripheral lymphocytes, whereas the other Se forms did not. In the PC-3 xenograft model, only MSeA was growth inhibitory at a dose of 3 mg/kg body weight. These data demonstrated superior *in vivo* growth inhibitory efficacy of MSeA over SeMet and selenite, against two human PCa xenograft models without the genotoxic effects of selenite.

Yan and DeMars compared the effects of dietary supplementation with SeMet vs. MSeA on metastasis of murine Lewis lung carcinoma in syngenic male C57BL/6 mice [65]. Mice were fed AIN93G control diet or that diet supplemented with MSeA or SeMet at 2.5 mg Se/kg for 4 weeks at which time they were given either an intramuscular or subcutaneous injection of the tumor cells. Dietary MSeA decreased lung metastasis yield when assessed at 2 weeks after intramuscular injection in one model or after surgical removal of the subcutaneous tumors in the other model. However, SeMet did not have any inhibitory effect in either metastasis models.

In summary, the data from cell culture studies and the animal models contemporaneous with SELECT decisions do not support cancer preventive activity of SeMet or Se-yeast, even with doses much higher than those tested in multiple clinical trials.

Methylselenol: MSeA redox pair and potential mechanisms of action

As discussed in preceding sections, Se deficiency is not a health concern in the US (see schematic in Figure 2). Thus, most animal models and cell culture studies since the mid-1980s by US cancer researchers had dealt with supra-nutritional or therapeutic levels of Se. The cell culture studies had used cancerous cell lines as the cell targets due to the lack of appropriate precancerous cell culture models. Most animal models had shown cancer chemopreventive and interception activity of Se intake that is 20–50 times greater than the rodent nutritional requirement [40]. Ip and Ganther as well as Combs [14, 40] articulated that cancer chemoprevention by Se in the nutritionally adequate subjects was independent of the antioxidant activity of plasma or tissue GPXs. This paradigm was based on the observation that the dietary level of Se (2 ppm [mg/kg] or greater as selenite or other Se forms) needed to achieve a significant anti-cancer activity in rodent animal models far exceeded

that was required (i.e., 0.1 ppm) to support maximal GPX3 expression in the blood or the target tissues from which the experimental cancers arose. This view had been extended to the other SEPs identified subsequently, including phospholipid glutathione peroxidase (Ph-GPX, also known as GPX4), SEPP1, SEP-W (aka Sel-W), and thyroixine de-iodinases (TDI) and thioredoxin reductases (TrxR) [39, 42, 43, 66, 67].

Novel functions of SEPs and carcinogenesis risk

Earlier studies with genetic manipulation approaches for individual SEPs yield mixed risk modification outcomes. Increased prostate and colon cancer tumorigenesis was observed when GPX was knocked out in the presence of adequate dietary Se, yet decreased lung cancer cell growth was observed when TrxR1 was knocked down [68]. Pertinent to the role of SEPs in mouse prostatic epithelium, Luchman et al. [69] created mice with a prostate-specific deletion of SeC tRNA gene (Trsp) that was required for the insertion of SeCys residues into SEPs during their synthesis. By 6 weeks of age, these Trsp-deficient mice exhibited widespread prostatic intraepithelial neoplasia (PIN) lesions in all prostatic lobes, which then progressed to high-grade dysplasia and microinvasive carcinoma by 24 weeks with increased lipid peroxidation markers in Trsp-deficient epithelial cells, in spite of adequate dietary Se. This novel model of prostate neoplasia is consistent with the idea that GPXs and possibly SEPs functioning collectively (net flux) as tumor suppressors in the murine prostate. Dietary Se deficiency thus conceivably increases prostate cancer risk, and correction of Se deficiency should lower cancer risk (anti-oxidant hypothesis).

Recent studies have produced greater molecular insights concerning key SEPs and key components of SEP synthesis pathways in promoting neuronal survival beyond a similar role in cancer cells. Two papers were published in *Cell* re-defining the function of the membrane bound GPX4 in protection against ferroptosis and its cerebral neuronal pathologic manifestations [70, 71]. As amino acid, SeCys resembles Cys, differing only by the substitution of Se for S. The authors demonstrated that selenolate-based catalysis in GPX4 was dispensable for normal embryogenesis, because the Cys-containing GPX4cys/cys expressing embryos produced live birth. Yet the survival of brain interneurons exclusively depended on SeCys-containing SEP GPX4, preventing fatal epileptic seizures that killed the postnatal GPX4cys/cys mice [70]. Mechanistically, the authors showed that SeCys in GPX4 conferred resistance to irreversible overoxidation compared to GPX4cys/cys neuronal cells which are highly susceptible to phospholipid peroxide-induced ferroptosis. Under cell culture conditions, concomitant deletion of all SEPs in GPX4cys/cys cells (GPXcys/cys proteins are stable and partially active) did not adversely affect cell viability because of the protection from the partial GPX4cys/cys activity. The indispensable role of Se, through co-translational SeC insertion, was pin-pointed to the protection against ferroptosis in crucial neurons after birth *in vivo*. Ferroptosis, a non-apoptotic form of programmed cell death, is triggered by oxidative stress in hemorrhagic stroke, and in some cancers and pathological conditions.

The second paper reported that mouse brain neurons responded to ferroptotic stimuli by induction of GPX4 and select SEPs (which they named selenome) [71]. Pharmacological intervention by sodium selenite exposure of cultured neurons or by intracerebral injection, increased GPX4 and the selenome, via coordinated activation of the transcription factors TFAP2c and Sp1 to protect the neurons. Even a single dose of Se delivered into the brain increased GPX4 expression, protected neurons, and improved behavior in a hemorrhagic stroke model. Selenite supplementation effectively inhibited not only GPX4-dependent ferroptotic death but also neuronal cell death triggered by either excitotoxicity or endoplasmic reticulum stress, both through GPX4 independent signaling. The authors developed a “safer” delivery approach for Se to cross the blood brain barrier with the use of HIV-Tat transduction domain cross-linked to plasma SelP (SEPP1) domain that contains multiple SeCys. This peptidyl Se delivery approach was shown to be efficacious to induce the “selenome” and through the TFAP2C-Sp1 mechanism to inhibit ferroptosis and protected neurons with a much wider therapeutic window than selenite.

Two independent groups have shown an increased expression of selenophosphate synthase 2 (SEPHS2), but not SEPHS1, in a majority of solid cancer cell lines [72] and in acute myeloid leukemia (AML) [73]. Because hydrogen selenide is the substrate of SEPHS2 to generate SeCys on SeC-tRNA for co-translational incorporation into SEPs, especially GPX4, SEPHS2 overexpression promoted survival of malignant cells (solid cancer cells and AML). Carlisle et al. [72] showed that breast and other cancer cells were selenophilic, due to a secondary function of the overactive cystine/glutamate antiporter SLC7A11 that promoted Se uptake and SeCys biosynthesis, in turn promoting synthesis of SEPS such as GPX4 and protecting the malignant cells against ferroptosis. They showed that loss of SEPHS2 impaired growth of orthotopic mammary-tumor xenografts in mice. With respect to hematologic malignancies, deregulation of transcription is a hallmark of acute myeloid leukemia (AML). Eagle et al. [73] found a MYB-regulated AML-enriched enhancer regulating SEPHS2. This enhancer upregulated SEPHS2, promoted SEP production and antioxidant function

and AML survival. SEPHS2 knockout and feeding a Se deficient diet significantly delayed leukemogenesis *in vivo* with little effect on normal hematopoiesis. These independent studies therefore suggest a risk enhancement action of nutritional Se supplementation in the form of selenite in some solid cancers and in AML. These data are congruent with observations of nanomolar to sub-micromolar selenite exposure in cancer cell culture studies led to about 30 % increase in cell growth compared to basal growth.

Contrary to these findings, a recent example for nutritional Se prevention was reported by researchers at Pennsylvania State University (PSU) in murine AML models, focusing on leukemia-initiating stem cells (LICs), which are typically not targeted by most existing therapies [74]. The PSU work showed that AML LICs were sensitive to endogenous and exogenous cyclopentenone prostaglandin-J (CyPG), Δ^{12}-PGJ_2, and 15d-PGJ_2. These PG species were increased upon dietary Se nutritional supplementation (i.e., Se adequate vs. deficient group, but not supra-nutritional Se vs. Se adequate group) via the cyclooxygenase-hematopoietic PGD synthase pathway. CyPGs are endogenous ligands for peroxisome proliferator-activated receptor gamma and GPR44 (CRTH2; PTGDR2) receptor. Deletion of GPR44 in a mouse model of AML exacerbated the disease suggesting that GPR44 activation mediated Se-driven apoptosis of LICs. Transcriptomic analysis of $GPR44^{-/-}$ LICs indicated that GPR44 activation by CyPGs suppressed Ki ras-mediated MAPK and PI3K/AKT/mTOR signaling pathways, to enhance apoptosis. Therefore, GPR44 receptor played a crucial role in the Se nutritional prevention of AML stem cells via CyPGs. Together, these new studies from different groups highlight need for additional in-depth studies with respect to organ site specific cancer risk for Se supplementation, especially Se forms beyond selenite.

Se metabolism and detoxification pathways

As outlined in Figure 1, hydrogen selenide (H_2Se) is a key intermediate metabolite, produced from inorganic selenate (Se^{+6}) and selenite (Se^{+4}) through the NADPH-glutathione and thioredoxin redox systems, and released from selenocysteine (SeCys) via cysteine β-lyase action [75, 76]. At a nutritional intake level, the H_2Se generated through these redox systems or enzymatic reactions is used for synthesizing SEPs through selenophosphate synthetase 2 (SEPHS2) to catalyze the synthesis of selenophosphate which in turn selenizes the SeCys-tRNA-Ser into SeCys for co-translational incorporation to elongate the growing SEP polypeptides. Above and beyond SEP synthesis capacity, excess H_2Se is converted to methylselenol (CH_3SeH), dimethylselenide ($(CH_3)_2Se$), and trimethylselenonium ion ($(CH_3)_3Se^+$) via sequential methylation, or to selenosugar(s) via conjugation with a sugar catalyzed by Se-carbon bond-forming enzymes [77–79]. Methylselenol can be also formed by Se-methylselenocysteine (MSeC) through the action of cysteine β-lyase or related lyases (1), which can be either demethylated to hydrogen selenide, or further methylated to $(CH_3)_2Se$ or $(CH_3)_3Se^+$), depending on the Se nutritional status [75, 80]. Dimethylselenide is excreted via the breath (garlic smell), while $(CH_3)_3Se^+$) and selenosugars are excreted in urine. Selenosugars are the key urinary Se metabolite within the non-toxic dose range, whereas $(CH_3)_3Se^+$ ion is the major form of Se metabolite at toxic dose levels, and both of them can be used as biomarkers of Se exposure status [81]. Both $CH_3)_3Se^+$ and selenosugar(s) pathways are considered to be an important process for Se detoxification, since these metabolites are not cytotoxic [82].

SeMet is predominantly incorporated into general proteins in place of Met (non-specific substitution) or metabolized to SeCys through a trans-selenation pathway similar to the transulfuration pathway in Met-Cys cycle. The efficiency of the latter pathway will be dependent on the metabolic capacity of the cell types and organs.

Methylselenol hypothesis

Many decades ago, Ip and Ganther originated the active anticancer metabolite hypothesis based on data from mammary chemical carcinogenesis models [40, 75] which speculated a mono-methylated Se species, presumably methylselenol as the active Se metabolite. They proposed that the chemopreventive efficacy of a given Se compound depends on the rate of its metabolic conversion to this active Se pool. Their supporting data were based on comparing the cancer chemopreventive efficacy of forms of Se that fed into different Se metabolite pools, with methyl-Se precursors of methylselenol displaying greater preventive efficacy than those for hydrogen selenide or dimethylselenide in the rodent mammary carcinogenesis model [83, 84]. They further showed that the alkylselenol and allylselenol precursor compounds were more active against mammary carcinogenesis than methylselenol precursors on an equal molar basis of dietary Se intake [85, 86]. However, these structure-activity studies have not been extended beyond the mammary carcinogenesis model for assessing the general applicability of this hypothesis for other organ sites.

Cell culture studies by us and others have shown that Se compounds that are proximal precursors of methylselenol induce numerous cellular, biochemical and gene expression

responses that are distinct from those induced by the forms of Se that enter the hydrogen selenide pool [39, 40, 42, 43, 66, 67]. These major cellular and biochemical effects are schematically summarized in Figure 1 and detailed in earlier reviews [39, 42, 43, 66]. A more recent study included COVID-related cytokine storm molecules IL-6 and TNFα [87].

Sodium selenite (Se^{+4}) and selenide (Se^{+2}) are examples of genotoxic forms of Se that feed into the hydrogen selenide (H_2Se) pool (Figure 1). While selenate (Se^{+6}) was almost biologically inert (for example, mM exposure to affect cell viability, likely due to greater demand for NADPH and glutathione (GSH) to bioactivate), selenite rapidly (within minutes to a few hours of Se exposure) induced DNA single strand breaks (SSBs), S phase or G2/M cell cycle arrest, and subsequent cell death by apoptosis or necrosis, depending on p53 status [39, 88–90]. Sodium selenide and SeCys recapitulated the DNA SSB and apoptosis inducing effects of selenite in mouse mammary carcinoma cells [91]. An MnSOD mimetic compound, copper dipropylsalicylate, blocked DNA SSBs and apoptosis, indicating that ROS, but not selenite *per se* triggered these events [92]. More studies have provided further support for ROS (superoxide generation) as intermediates for activating p53 by serine phosphorylation in apoptosis induction by selenite in LNCaP PCa cells [93, 94]. We have further shown that selenite at a daily oral dose of 3 mg/kg body weight to xenograft tumor bearing nude mice increased DNA SSBs in peripheral lymphocytes, whereas the same dose of MSeA or MSeC did not have this effect [64]. Sufficient dosages of these "genotoxic" Se that overwhelm the survival protection afforded by GPX4 may underlie their application to cancer therapy (Figure 1). The *in vivo* genotoxicity of selenite and related inorganic Se forms needs to be monitored in human clinical trials for benefit-to-risk assessment.

Proximal methylselenol precursors

We have shown that putative methylselenol precursors such as methylselenocyanate (MSeCN) and MSeC induce apoptosis of mammary tumor epithelial cells and leukemia cells without the induction of DNA SSBs [91, 95, 96]. Furthermore, another prototypical methyl-Se MSeA induced cancer cell apoptosis was caspase-dependent, whereas caspase involvement in selenite-induced cell death appeared to vary with the p53 status. MSeA or methylselenol generated enzymatically led to G_1 arrest [88, 95–99] or G2 arrest [100] in many cancer cells. Inhibitory effects on cyclin dependent kinases [99, 101] and protein kinase C [102] have been attributed to the methyl-Se pool. With regards to genotoxicity, a daily oral dose of 3 mg/kg body weight of MSeA and MSeC significantly suppressed DU145 human PCa xenograft growth without increasing DNA SSBs in the peripheral lymphocytes of the host mice, whereas the same dosage of selenite caused increased DNA SSBs and was ineffective for suppressing xenograft growth [64].

In addition to these cellular effects, MSeA exerts a rapid inhibitory effect on the expression of key molecules involved in angiogenesis regulation, tumor cell invasion, immune and inflammation in the tumor microenvironment (TME) (Figure 1). For example, we have shown that sub-apoptotic concentrations of MSeA inhibited the expression and secretion of the angiogenic factor VEGF in several cancer cell lines [103] and inhibited the expression of matrix metalloproteinase (MMP)-2 in the vascular endothelial cells [103, 104]. These effects in combination with a potent inhibitory effect on cell cycle progression of vascular endothelial cells [97, 98] indicate a key inhibitor function of MSeA on angiogenic switch regulation in early neoplastic lesions and tumors [39]. Furthermore, we and others have shown that MSeA and methylselenol released by methioninase from SeMet inhibited expression of the androgen receptor and its signaling to regulate prostate specific antigen (PSA) expression [105–107] as well as PSA protein stability [105] in PCa cells. MSeA inhibited estrogen receptor (ER-α) signaling in breast and endometrial cancer cells [108–111] and was a novel suppressor of aromatase expression in human ovarian tumor cells [112]. MSeA also potentiated apoptosis signaling induced by chemotherapeutic drugs or biologics in various cancer cell types through inhibition of survival molecules such as survivin, Bcl-XL [113], and Mcl-1 [114, 115]. A common thread with rapid down regulation and/or degradation of key proteins reported so far is secretory proteins (VEGF, MMP2, PSA, IL6, TNFα, PD-L1) proteins with intramolecular di-sulfide bonds. A tantalizing hypothesis is increased methyl-Se uptake increased mixed peptide-S-Se-CH_3/R bond formation due to a stronger reducing power of R/CH3-SeH than peptidyl-SH to create mis-folded proteins (unfolded protein responses UPR) in the endoplasmic reticulum (ER) and ensuing ER stress responses.

Methylselenol: MSeA redox cycle through thioredoxin reductase

Gromer and Gross [116] examined Ganther's hypothesis [75] that methylselenol and MSeA might exert their effects by inhibition of the selenoenzyme thioredoxin reductase (TrxR) via irreversible formation of a diselenide bridge. However, they showed that MSeA did not act as an inhibitor of mammalian TrxR but instead was an excellent substrate

for reduction by this enzyme. Nascent methylselenol efficiently reduced both H_2O_2 and glutathione disulfide (GSSG). They also found that MSeA was a poor substrate for human glutathione reductase, which is not a SEP, and that the catalytic SeCys residue of mammalian TrxR was essential for MSeA reduction to methylselenol.

Gopalakrishna's group investigated PKC isoforms as potential targets of MSeH:MSeA redox pair and extending on the role of TrxR [117–119]. They first showed that MSeA, but not methylselenol, inactivated specific protein kinase C (PKC) isoforms, especially those involved in tumor progression [117]. They showed that MSeA inactivated pure PKC enzyme activity, which could be reversed by the TrxR system or thiol agents, but methylselenol did not. In DU145 and LNCaP human PCa cell lines under serum-starved conditions, MSeA (at 5 μmol/L) decreased PKC activity within 5–15 min. The extent of PKC inactivation in these cell lines was observed to be less than that of the pure PKC enzyme, possibly due to TrxR-mediated MSeA:MSeH redox cycling to remove MSeA from the site of action. The increase in PKC inactivation, in particular PKCε isoform, was associated with increased apoptosis and cell arrest. As an illustration of selectivity, a 10-times higher concentration of MSeA was required to inactivate protein kinase A. The PKC inactivation was further enhanced when MSeA was converted from methylselenol by PKC-bound phospholipid peroxides within close proximity to PKC thioclusters. Nanomolar Se concentrations were needed for oxidation of the catalytic unit of PKC by the MSeA:MSeH redox cycle, highlighting the specificity of MSeA in inactivating PKC.

The same group subsequently showed that MSeA at submicromolar concentrations prevented the transformation of prostate epithelial cells but micromolar levels were required to inhibit cell growth, invasion and induce apoptosis in PCa cells [118]. Over-expression of PKCε attenuated MSeA inhibition of epithelial cell transformation and PCa cell apoptosis. In addition, increased TrxR expression caused resistance to MSeA treatment and inhibition of TrxR increased the sensitivity of cancer cells to MSeA [118]. These studies suggest that both PKCε and TrxR can negate the anticancer efficacy of MSeA. Therefore, MSeH, MSeA, and their redox cycling intermediates, especially in the localized protein microenvironment of thioclusters, may provide specific targeting niches to negatively regulate enzymatic activities involved in cancer promotion or growth. In retrospect, many of the reported activities that we and others attributed to the "methylselenol pool" likely represent the summation of actions of the dynamic MSeA:MSeH redox pair (Figure 1).

P53-dependence senescence as a barrier to cancer progression

Recent findings that MSeA induced senescence of normal primary lung fibroblasts suggest a possible mechanism to strengthen a barrier against early cancer lesion progression [120]. In this study, normal primary fibroblasts and PC3 PCa and HCT116 colon cancer cells were treated with low micromolar concentrations of MSeA for 48 h, followed by a recovery of 1–7 days. Cellular senescence, as evidenced by senescence-associated β-galactosidase staining and lack of 5-bromo-2-deoxyuridine incorporation, was observed in normal fibroblastic cells, but not in the two cancer cells. In addition, the ataxia telangiectasia mutated (ATM) DNA damage response protein was rapidly activated by MSeA and its kinase activity was required for the induced senescence response. Subsequently depletion of p53 was demonstrated to attenuate senescence, disrupt the MSeA-induced cell cycle arrest, and increased genome instability [121]. Pretreatment with KU55933, an ATM kinase inhibitor, or NU7026, an inhibitor of DNA-dependent protein kinase, desensitized MSeA cytotoxicity in normal fibroblasts but not in fibroblasts with knocked down p53. These results suggest that ATM-p53 DNA damage response is critical for senescence induction by MSeA in normal cells.

Human prostate cancer is believed to arise from precancerous lesions such as high-grade prostatic intraepithelial neoplasia (HG-PIN), which frequently have lost phosphatase and tensin homolog (PTEN) tumor suppressor permitting phosphatidylinositol-3-OH kinase (PI3K)-protein kinase B (AKT) oncogenic signaling. In particular relevance to a PCa chemoprevention context, we have shown that MSeA treatment of Pten-KO mice super-activated p53 and senescence *in vivo* in the early prostate lesions [122]. We observed that short-term (4 weeks) oral MSeA treatment significantly increased expression of P53 and P21Cip1 proteins and senescence-associated-β-galactosidase staining, and reduced Ki67 cell proliferation index in Pten KO prostate epithelium. Long-term (25 weeks) MSeA administration significantly suppressed HG-PIN phenotype, tumor weight, and prevented emergence of invasive carcinoma in Pten KO mice. Mechanistically, the long-term MSeA treatment not only sustained P53-mediated senescence, but also markedly reduced AKT phosphorylation and androgen receptor (AR) abundance in the Pten KO prostate. Importantly, these cellular and molecular changes were not observed in the prostate of wild-type littermates which were similarly treated with MSeA. Because p53 signaling is likely to be intact in HG-PIN compared with advanced prostate cancer, the selective superactivation of p53-mediated senescence by MSeA suggested a new paradigm of cancer chemoprevention

by strengthening a cancer progression barrier through induction of irreversible senescence with additional suppression of AR and AKT oncogenic signaling.

Immune surveillance and immune oncology enhancement

Pertinent to the MSeA:MSeH hypothesis, MSeA, MSeC and dimethylselenide (DMSe) were observed to stimulate the cell surface expression of ligands for the lymphocyte receptor NKG2D in Jurkat T cells, specifically inducing the expression of MICA/B major histocompatibility complex class I related chain genes, which are up-regulated in stressed cells for immune system recognition [123]. MSeA and DMDSe induced a maximal MICA/B response at 5 μM, whereas selenite, selenate, SeMet, selenocysteine, and hydrogen selenide had no effect on the cell surface expression of MICA/B at the protein and mRNA level. These data suggest that methyl Se could improve NKG2D-based cancer immune surveillance and prevention. Programmed death-ligand 1 (PD-L1)-mediated resistance poses a great challenge for cancer immune-oncology treatment. Hu's lab explored [124] the effects of MSeA on the PD-L1-mediated resistance using both *in vitro* and *in vivo* models. Results showed that MSeA substantially attenuated cisplatin-induced PD-L1 expression via inhibiting AKT phosphorylation, thereby potentiated cisplatin cytotoxicity in prostate and lung cancer cell models. In lung cancer xenograft model, MSeA significantly suppressed cisplatin-induced PD-L1 expression, consequently enhanced T-cell immunity, ultimately improved the therapeutic efficacy of cisplatin. Moreover, IFN-γ-induced tumor PD-L1 expression was remarkably reduced by MSeA, with correlated reductions in Janus kinase (JAK) 2 and signal transducer and activator of transcription (STAT)-3 phosphorylation in prostate and lung cancer cell models. These effects were not observed with selenite exposure.

Taken together, cell culture studies suggest that methyl-Se affects multiple cellular processes related not only to pre/cancerous epithelial cells but also to their microenvironments including endothelial cells and immune cells conducive for chemopreventive action and even immuno-oncology therapy.

Efficacy studies of methyl Se to inhibit prostate and other preclinical cancer models

Whereas both MSeC and MSeA have been shown to inhibit chemically-induced mammary carcinogenesis in the 1990s [84, 125], their anti-cancer efficacy in prostate or other non-mammary organs has only recently been tested by us and others.

Xenograft models

We have shown that orally-administered MSeA and MSeC dose-dependently (1 and 3 mg Se/kg) inhibited the growth of DU145 human PCa xenografts in athymic nude mice, whereas selenite and SeMet did not [64]. MSeA was more active than MSeC against PC-3 xenograft growth. Measurement of tissue Se content showed that SeMet treatment led to 9.1-fold more liver Se retention and approximately 3.6 times higher tumor Se than mice treated with an equal dose of methyl-Se, despite no potency of SeMet to inhibit DU145 or PC-3 xenograft growth. The observed massive tissue Se accumulation supports non-specific incorporation of SeMet into proteins. The lack of anti-cancer efficacy of SeMet in the presence of elevated circulating and tissue Se accumulation agreed well with earlier work with SeMet in conventional rodent models [84, 126]. Oral bolus administration of MSeA activity against breast cancer xenograft growth has also been reported [127].

Allograft models

Lindshield and coworkers [128] evaluated the effects of dietary MSeC (1 mg/kg diet, 1 ppm), lycopene (250 mg/kg diet), and γ-tocopherol (200 mg/kg diet) alone and in combination on the growth of androgen-dependent Dunning R3327-H rat prostate adenocarcinomas in male, Copenhagen rats. AIN-93G diets containing these micronutrients were fed for 4–6 weeks prior to subcutaneous tumor implantation. After 18 weeks, MSeC consumption significantly ($p=0.003$) decreased final tumor area and tumor weight, but lycopene and γ-tocopherol did not alter these parameters. Tumor growth inhibition by MSeC was not associated with circulating male hormone level: none of these agents consumed alone or in combination altered serum testosterone or dihydrotestosterone concentrations.

Metastatic cancer models

Yan and DeMars [65] showed that AIN93G diet supplemented with MSeA at 2.5 mg Se/kg (2.5 ppm) significantly reduced pulmonary metastatic yield compared with controls, but SeMet did not. MSeA also decreased plasma concentrations of urokinase-type plasminogen activator and plasminogen activator inhibitor-1. Furthermore, MSeA reduced plasma

concentrations of VEGF, bFGF, and PDGF. SeMet did not affect any of these measurements. Thus, inhibition of tumor cell invasion and angiogenesis by MSeA might have contributed to inhibiting tumor metastasis.

Primary prostate carcinogenesis in transgenic/knockout mice models

We used the transgenic adenocarcinoma mouse prostate (TRAMP) model to test the efficacy of MSeA and MSeC against prostate carcinogenesis and to characterize potential mechanisms [41]. TRAMP mice (8 weeks old) were given a daily oral dose of MSeA or MSeC at 3 mg Se/kg body weight and were euthanized at either 18 or 26 weeks of age. By 18 weeks of age, the dorsolateral prostate weights for the MSeA- and MSeC-treated groups were lower than for the water control ($p<0.01$) and at 26 weeks genitourinary weight (reflecting tumor load) was much lower. The efficacy was accompanied by delayed lesion development, increased apoptosis, and decreased proliferation without changes of T-antigen expression in the dorsolateral prostate of Se-treated mice. In another experiment, MSeA given to TRAMP mice from 10 or 16 weeks of age increased their survival to 50 weeks of age and delayed the time of death due to neuroendocrine carcinomas and other lesions in the prostate and seminal vesicle hypertrophy. Wild-type mice receiving MSeA from 10 weeks did not exhibit decreased body weights or genitourinary weight compared with the control mice. Serum alanine aminotransferase (a marker of liver damage) was not increased. Therefore, these Se compounds selectively inhibited prostate carcinogenesis without general toxicity or observable effects on the normal prostate.

Christensen and coworkers studied the interaction of MSeC with isoflavones in TRAMP mice [129]. They did not observe efficacy of MSeC (at 3 ppm Se in diet) against TRAMP tumor readouts (urogenital tract weight, lesion severity). They pointed out several differences from the studies done by our group. These include route/dose of MSeC delivery (~15 μg Se per mouse throughout a day by diet vs. ~75 μg Se per mouse as daily bolus dose) and mouse genetic background (C57/B/FVB hybrid vs. C57B background). Additionally, the small number of mice per group and inability to separate NE-Ca lineage from epithelial lesions limited their statistical power [129].

Similarly, we did not detect inhibitory efficacy of MSeA or MSeC by providing them in the diet (3 ppm Se) to rats treated with methylnitrosourea (MNU) and testosterone promotion [130]. Male rats were treated with MNU, and 1 week later, slow-release testosterone implants, when they were randomized to groups fed AIN-93M diet supplemented with 3 ppm Se as MSeA or MSeC or control diet. Mean survival, tumor incidence in all accessory sex glands combined (dorsolateral and anterior prostate plus seminal vesicle) and the incidence of tumors confined to dorsolateral and/or anterior prostate were not statistically significantly different among the groups. The contrast with the inhibitory effects of MSeA and MSeC in mouse models might be due to differences in carcinogenic mechanisms, Se dosage, delivery mode, and pharmacokinetics or fundamental rat-mouse differences in Se metabolism.

Molecular targets of methyl Se in cancer models

Signaling pathway-based surveys

We examined the impact of acute high dose Se treatments (i.e., daily single oral gavage of 2 mg Se per kg body weight for 3 days) of female Sprague-Dawley rats bearing MNU-induced mammary carcinomas to increase the probability of detecting *in vivo* apoptosis and the associated gene/protein changes in the cancer cells [131]. Whereas control carcinomas doubled in volume in 3 days, MSeC and selenite treatments caused regression of approximately half of the carcinomas, accompanied by a 3- to 4-fold increase of morphologically detectable apoptosis and approximately 40 % inhibition of 5-bromo-2′-deoxyuridine incorporation index of the cancer epithelial cells. The mRNA levels of growth arrest-DNA damage inducible 34 (*gadd34*), *gadd45*, and *gadd153* genes were not higher in the Se-treated carcinomas than in the gavage or diet restriction control groups, contrary to our expectation [96]. The Gadd34 and Gadd153 proteins in the Se-treated carcinomas were localized in the nonepithelial stromal cells and not induced in the cancer cells. On the other hand, both Se forms decreased the expression of cyclin D1 and increased levels of P27Kip1 and c-Jun NH2-terminal kinase activation in a majority of the mammary carcinomas. The lack of induction of *gadd* genes *in vivo* by MSeA was confirmed in a human PCa xenograft model in athymic nude mice [131]. Collectively, these experiments showed the induction of cancer epithelial cell apoptosis and inhibition of cell proliferation by Se *in vivo* through the potential involvement of cyclin D1, P27Kip1, and c-Jun NH2-terminal kinase pathways, but cast doubt on the *gadd* genes as mediators of Se action *in vivo*.

Proteomic profiling revealing differential target profiles among MSeA and MSeC

We applied iTRAQ-proteomic approaches to profile protein changes of the TRAMP dorsolateral prostate and to characterize their modulation by MSeA and MSeC to identify potential molecular targets of Se [132]. Out of 75 proteins differing between TRAMP and wild-type mice. MSeA mainly affected those related to prostate functional differentiation, androgen receptor signaling, protein (mis)folding, and endoplasmic reticulum-stress responses (e.g., GRP78), whereas MSeC affected proteins involved in phase II detoxification (e.g., GSTM1) or cytoprotection, and in stromal cells. Although MSeA and MSeC were about equally efficacious against tumor development in the TRAMP model, their distinct affected protein profiles suggest biological differences in their molecular targets.

We also analyzed the proteome signatures of prostate from mice treated with MSeA, MSeC, SeMet and selenite [133]. Nude mice bearing subcutaneous PC-3 xenografts were treated daily with each Se form (3 mg Se/kg) orally for 45 days. Among 72 proteins significantly modulated by one or more Se forms, MSeA and MSeC each induced separate sets of tumor suppressor proteins and suppressed different onco-proteins. A similar spectrum of proteins was induced by selenite and MSeC which were related to energy metabolism (e.g., fatty-acid synthase), whereas those induced by SeMet included vimentin and heat-shock protein-70. While the proteome changes induced by MSeA were associated with PCa risk reduction, MSeC induced both desirable risk-reducing signatures (e.g., GSTM1 and GPX-3) and risk-promoting patterns in common with selenite and SeMet (e.g., inducing fatty acid synthase, a known metabolic oncoprotein for PCa).

While MSeH has been assumed an *in vivo* active anticancer metabolite pool [39, 40, 42] [43], the proteomic data revealed surprisingly little "protein targets" overlap between MSeA and MSeC (Figure 1). Therefore, MSeA and MSeC are not interchangeable and MSeC might be double edged through onco-proteins such as fatty acid synthase. These data, when considered in the framework of dynamic MSeA: MSeH redox cycle, would be reasonable depending on the entry point of the methyl-Se to fuel the redox cycle. Whether such findings are relevant for the mammary gland and other organ sites should be examined to assess their generalizability. IND-enabling toxicology and toxico-omic investigations of the tissues/organs exposed to these two Se forms in non-rodent mammalian species may help to inform the potential adverse impacts of each methyl Se form for human translational consideration.

Combination therapy applications in animal models and human trials

Animal models

Roswell Park researcher Rustum and colleagues carried out extensive studies on this topic. They initially [134] used athymic nude mice bearing human non-small cell carcinoma HNSCC (FaDu and A253) and colon carcinoma (HCT-8 and HT-29) xenografts to evaluate the potential role of Se compounds as selective modulators of the toxicity and anti-tumor activity of selected cancer drugs: fluorouracil, oxaliplatin, cisplatin, taxol, doxorubicin and in particular irinotecan, a topoisomerase I poison. They showed that a sub-lethal dose of Se either as MSeC or SeMet was highly protective against toxicity induced by these chemotherapeutic drugs. Furthermore, MSeC significantly increased the cure rate (defined as no detectable tumor at the transplant site for up to 3 months after treatment was terminated) of xenografts bearing human tumors that were either sensitive (HCT-8 and FaDu) or resistant (HT-29 and A253) to irinotecan. 100 % cure was achieved in nude mice bearing HCT-8 (20 % with irinotecan alone) and FaDu xenografts (30 % with irinotecan alone) treated with the maximum tolerated dose (MTD) of irinotecan (100 mg/kg/week for 4 weeks) when combined with MSeC. Administration of higher doses of irinotecan (200 and 300 mg/kg/week for 4 weeks) achieved high cure rate for HT-29 and A253 xenografts and was made possible due to selective protection of normal tissues by pretreating with MSeC. The observed *in vivo* protective action against drug toxicity was highly dependent on the schedule of Se, which required a minimum of 3 days ahead of the first drug treatment.

They next [135] studied the effect of MSeC on the pharmacokinetic and pharmacogenetic profiles of genes relevant to irinotecan metabolic pathway to identify possible mechanisms associated with the observed combinational synergy. Nude mice bearing tumors (FaDu and A253) were treated with MSeC, irinotecan, and their combination. Samples were collected and analyzed for plasma and intra-tumor concentration of irinotecan and its active form 7-ethyl-10-hydroxyl-camptothecin (SN-38). After MSeC treatment, the intra-tumor concentration of SN-38 increased to a significantly higher level in A253 than in FaDu tumors and was associated with increased expression of carboxyesterase CES1 (involved in the de-esterification of irinotecan) in both tumor models. Combined MSeC/irinotecan treatment, compared with irinotecan alone, decreased ABCC1 and DRG1 (multi-drug resistant associated proteins, drug efflux pumps) in FaDu tumors and increased CYP3A5 and TNFSF6 (involved in increased drug

metabolism and inducing apoptosis respectively) in A253 tumors. MSeC/irinotecan did not affect other investigated variables such as transporters, degradation enzymes, DNA repair, and cell survival/death genes when compared to irinotecan alone.

In a subsequent paper [136], they further refined the parameters for MSeC to increase the therapeutic index of irinotecan against human tumor xenografts in the FaDu and A253 models. Starting 7 days prior to irinotecan treatment and continuing for 28 days with the drug, they titrated MSeC dose to a minimum effective dose of 0.01 mg/day (per mouse) and established the MTD as 0.2 mg Se/day. As MSeC dosage was increased to the MTD, the cure rate in the combination with irinotecan increased in lock step. MseC alone did not have any effect on the cure rate but reduced tumor growth up to 30 %. The plasma Se concentration peaked at 1 h after a single dose and 28 days after daily treatment of MSeC. FaDu tumors retained more Se A253 tumors. Liver attained the highest Se contest among normal murine tissues. Peak plasma and tissue Se concentrations increased with the dose and duration of MSeC treatment. The MSeC-dependent increase in Se level in normal murine tissues was associated with the protective effect against irinotecan toxicity observed in those tissues. Surprisingly, the tumoral total Se concentration did not correlate with the combination therapy cure rates.

The same group [137] demonstrated that one mechanism of selectivity was the differential impact of MSeC on the content of irinotecan and its active metabolite SN-38 between tumors of HNSCC and the normal tissue. The *in vivo* anti-tumor synergy between MSeC and irinotecan was influenced by treatment schedule. For the FaDu tumors, the concurrent combination (MSeC and irinotecan administered for 2 h) did not increase irinotecan response rate. However, the sequential combination of MSeC administered for 7 days before irinotecan resulted in a 65 % increase in irinotecan cure rate. Similar findings were made in the A253 xenograft. MSeC/irinotecan regimen enhanced tumor vessel maturation, increased intra-tumor concentration of SN-38 and apoptotic death of tumor cells. Yet, in the normal tissues, the SN-38 drug concentrations were not impacted by MSeC treatment, supporting tumor-selective enhancement of drug retention to improve cancer cell killing.

Our group established the enhancement of paclitaxel efficacy by MSeA in AR-negative PCa model [113]. In nude mice, the paclitaxel and MSeA combination inhibited growth of the DU145 subcutaneous xenograft with the equivalent efficacy of a four-time higher dose of paclitaxel alone. MSeA decreased the basal and paclitaxel-induced expression of Bcl-XL and survivin cell survival proteins *in vitro* and *in vivo*. Ectopic expression of Bcl-XL or survivin attenuated MSeA/paclitaxel-induced apoptosis. The sensitization effect of MSeA on paclitaxel has been confirmed independently in a triple-negative breast cancer xenograft model [127]. The synergism was attributable to a more pronounced induction of caspase-mediated apoptosis, arrest of cell cycle progression at the G_2/M checkpoint, and inhibition of cell proliferation. Treatment of SCID mice bearing MDA-MB-231 triple-negative breast cancer xenografts for 4 weeks with MSeA (4.5 mg/kg/day, orally) and paclitaxel (10 mg/kg/week, *i.p.*) inhibited tumor growth more profoundly than either agent alone. The combination of MSeC with estrogen receptor positive breast cancer chemotherapy drug tamoxifen also resulted in synergistic tumor growth inhibition in the MCF-7 breast xenograft tumors in ovariectomized female athymic nude mice [138]. In this model, sustained-release estradiol was implanted into the ovariectomized mice to stimulate MCF-7 tumors to grow. Tamoxifen pellets were implanted subcutaneously while MSeC was administered *i.p.* after the tumors reached 100 mm^3. For the tumors in mice given estradiol implant, tamoxifen and MSeC combination achieved the greatest tumor suppression, while each agent alone was modestly suppressive. Cell proliferation and angiogenesis were reduced as early as 7 days after MSeC treatment was initiated.

Zhan et al. [139]studied the efficacy of MSeA and androgen receptor degrader drug enzalutamide (formerly known as MDV3100) both *in vitro* and *in vivo* as well as the MSeC and MDV3100 combination *in vivo*. Using prostate cancer 22Rv1 cells in androgen-deprived conditions, combination of MSeA and MDV3100 suppressed dihydrotestosterone (DHT)-stimulated trans-activating activity of AR more profoundly than each individual agent. The synergistic suppression action applied to the mRNA levels of AR targets PSA and KLK2. These two compounds inhibited cell growth synergistically with a combination index of less than 1. In the 22Rv1 tumor xenograft model, MDV3100 was dosed at 10 mg/kg. MSeC and MSeA were each dosed at 3 mg Se/kg/day. The combination of MSeC and MDV3100 led to the smallest tumors, smaller than any of the single agent treatments. The tumor growth in the animals treated with MSeA and MDV3100 combination was not different from the group treated only with MDV3100. The authors attributed the lack of MSeA enhancement of MDV3100 efficacy *in vivo* to possible MSeA and MDV3100 conjugation when prepared in the same dosing solution.

Cisplatin in medical oncology use is largely limited by its severe side effects including gastrointestinal toxicity and nephrotoxicity. For testing the utility of sodium selenosulfate to attenuate cisplatin side effect, Li and co-workers treated mice by *i.p.* with 9 µmol sodium selenosulfate/kg for 11 days [140]. On days 5 and 7, they gave the mice an injection of cisplatin of 8 mg/kg 1 h after sodium selenosulfate treatment. Sodium selenosulfate decreased the incidence of diarrhea as a

measure of gastrointestinal toxicity from 88 to 6 %. Such a prominent protective effect promoted them to evaluate the safety potential of long-term sodium selenosulfate application in comparison with sodium selenite. Mice were administered with each Se form for 55 days at the doses of 12.7 μmol/kg and 19 μmol/kg (1.0 and 1.5 mg Se/kg by *i.p.* injection). The low-dose selenite caused growth suppression and hepatotoxicity and the high-dose selenite caused a 40 % mortality rate. In contrast, no toxicity signs were observed in the two sodium selenosulfate groups. Their results suggest sodium selenosulfate at a safe dose can markedly prevent cisplatin-induced gastrointestinal toxicity while improving its cancer "cure" rate.

Se-yeast combination with platinum drug therapy trial

Chinese researchers recently reported a beneficial effect of Se-yeast in the prevention of adverse reactions related to platinum-based combination therapy in patients with malignant tumors [141]. A total of 86 patients with malignant tumors under care in Eastern China Anhui Province (No. 2 Provincial People's Hospital) were randomized to receive either platinum-containing combined regimen with Se-yeast at a dose of 200 μg Se daily or just the platinum-containing combined regimen as control group (43 patients in each group). The platinum-containing combined regimen exhibited similar total cancer treatment efficacy (25.58 %) with vs. without (23.26 %) Se-yeast ($p>0.05$). However, patients with Se-yeast treatment had better appetites and more stable body weights than those without ($p<0.05$). The platinum-containing combined regimen significantly improved the Karnofsky Performance Status (KPS) scores of the two groups over baseline, and Se-yeast potentiated this improvement ($p<0.05$). Se-yeast treatment reduced the incidence of adverse reactions in patients after chemotherapy by 23.26 % ($p<0.05$), and patients also experienced milder adverse reactions after Se-yeast ($p=0.015$). Chemotherapy with Se-yeast treatment provided better pain mitigation for patients vs. without Se-yeast ($p=0.041$). They suggest that a 200 μg dose of Se-yeast might be a viable alternative for the management of cancer patients undergoing chemotherapy to reduce adverse events when the patients' Se nutritional supply may not be adequate.

Potential mechanisms of anti-cancer efficacy enhancement

Using androgen-independent and p53 non-functional prostate cancer cell culture models, we investigated the Se specificity and signaling pathways underlying the enhancement action on apoptosis-induced by different classes of chemotherapeutic drugs [142]. DU145 and PC3 human AR-negative PCa cells were exposed to minimal apoptotic concentrations of Se and/or the topoisomerase I inhibitor SN38 (irinotecan active metabolite), the topoisomerase II inhibitor etoposide, or the microtubule inhibitor paclitaxel. The results showed that sub-lethal MSeA increased the apoptosis potency of SN38, etoposide, or paclitaxel more than additive manner than the expected sum of the apoptosis induced by MSeA and each drug alone. The combination treatment did not further enhance JNK1/2 phosphorylation that was induced by each drug in DU145 cells. The JNK inhibitor SP600125 substantially decreased the activation of caspases and apoptosis induced by MSeA combination with SN38 or etoposide and completely blocked these events induced by MSeA/paclitaxel. A caspase-8 inhibitor completely abolished apoptosis and caspase-9 and caspase-3 cleavage, whereas a caspase-9 inhibitor significantly decreased caspase-3 cleavage and apoptosis but had no effect on caspase-8 cleavage. None of these caspase inhibitors abolished JNK1/2 phosphorylation. In contrast to MSeA, selenite did not show any enhancing effect on the apoptosis induced by these drugs. The results that support the enhancing effect was primarily through interactions between MSeA and JNK-dependent targets to amplify the caspase-8-initiated activation cascades in a p53-defective background.

In a follow up study, our group established the enhancement of paclitaxel efficacy by MSeA *in vivo*, and investigated Bcl-XL and survivin as molecular targets of MSeA to augment apoptosis in PCa [113]. MSeA decreased the basal and paclitaxel-induced expression of Bcl-XL and survivin *in vitro* and *in vivo*. Ectopic expression of Bcl-XL or survivin attenuated MSeA/paclitaxel-induced apoptosis. Along the line of suppression of survival molecules, Hu lab had shown that MSeA enhanced ABT-737 apoptosis in several cancer cell lines: breast, prostate and colon cancer cell lines [115]. MSeA suppressed Mcl-1 (another Bcl family prosurvival protein) expression both at the basal level and after ABT-737 induction. MSeA re-activated Bad, a pro-apoptotic protein which was suppressed by ABT-737. The synergistic effect was dependent on Bax expression in the model system, suggesting a central role of mitochondria apoptosis through Bcl family protein interactions.

Hu's lab recently compared gefitinib-resistant HCC827GR cells with parental cells for changes in amino acid metabolism [143]. The results showed that HCC827GR cells expressed higher level of ASCT2 (responsible for uptake of glutamine), SLC7A11 (solute transporter for exchange of intracellular glutamate for extracellular cysteine, also used

for transporting selenide), and ASNS (an enzyme that catalyzes the conversion of aspartate and glutamine to asparagine and glutamate) than parent cells. They demonstrated that MSeA led to a concentration-dependent inhibition of ASNS activity, accompanied by reduction in asparagine (ASN) availability, which in turn resulted in the suppression of MET kinase signaling in HCC827GR cells, supporting inhibiting ASNS-ASN-MET-TOPK signaling axis in MSeA-mediated efficacy enhancement of gefitinib.

Roswell Park researchers examined synergistic activity in the clonal TRAMP cell line C2G by MSeC and docetaxel [144]. Cells were treated with combinations of MSeC and/or docetaxel concurrently or sequentially with 24 h MSeC pretreatment. It was observed that the concurrent administration of MSeC and docetaxel in this cell line did not enhance docetaxel efficacy. On the other hand, the 24 h pretreatment of MSeC enhanced docetaxel growth inhibition synergistically. Under this treatment regimen, caspase-3 activity was significantly increased as early as 30 min. The caspase inhibitor z-VAD-fmk significantly attenuated the increased apoptosis induced by the combination treatment, indicating that the synergistic apoptosis is caspase-dependent. Survivin was decreased significantly by the combination treatment as compared to each drug individually.

In several breast cancer cell lines, MSeA with tamoxifen synergistically increased the caspase-mediated apoptosis as observed by the increased cleavage of caspases −7, −8 and −9 and PARP [145]. Cytochrome c and Bim were also increased by MSeA treatment. The general caspase inhibitor completely blocked MSeA induced apoptosis on its own and with tamoxifen. Specific caspase −8 and −9 inhibition suggested that the cleavage of caspase 9 was needed for the cleavage of caspase-8 by MSeA.

In cell culture models, the MSeA-specific enhancement action on drug-induced apoptosis was also found with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Yamaguchi et al. [146] demonstrated that the concomitant treatment with TRAIL and MSeA produced synergistic effects on the induction of apoptosis in androgen-dependent LNCaP and androgen-independent DU145 prostate cancer cells. MSeA rapidly down-regulated the expression of the cellular FLICE inhibitory protein, a negative regulator of death receptor signaling. In addition, they demonstrated that the synergistic effects of MSeA and TRAIL resulted from the activation of the mitochondrial pathway-mediated amplification loop. MSeA also effectively blocked TRAIL-mediated BAD phosphorylation at Ser^{112} and Ser^{136} in DU145 cells and induced the mitochondrial permeability transition and the release of cytochrome c and Smac/DIABLO proteins from the mitochondria into the cytosol. These results suggest that MSeA may help to enhance efficacy of and overcome resistance to drug-induced or TRAIL-mediated apoptosis in prostate cancer cells.

Whereas p53 was not required for the enhancement effect of MSeA on apoptosis induced by drugs or TRAIL as discussed above [142, 146], our group has shown a critical role of p53 and Bax/mitochondria pathway of caspases to mediate selenite's ability to enhance apoptosis induced by TRAIL in the LNCaP cells [94]. Selenite induced a rapid generation of superoxide and p53 Ser-15 phosphorylation, an indicator of DNA damage. It also increased Bax abundance and translocation into the mitochondria. Selenite and TRAIL combined treatment led to synergistic increases of Bax abundance and translocation into mitochondria, loss of mitochondrial membrane potential, cytochrome c release and the cleavage activation of caspases-9 and -3. Inactivating p53 with a dominant negative mutant abolished apoptosis without affecting superoxide generation, whereas a superoxide dismutase mimetic agent blocked p53 activation, Bax translocation to mitochondria, cytochrome c release and apoptosis induced by selenite/TRAIL. In support of Bax as a crucial target for crosstalk between selenite and TRAIL pathways, introduction of Bax into p53-mutant DU145 cells enabled selenite to sensitize these cells for TRAIL-induced apoptosis. The results indicate that selenite induces a rapid superoxide burst and p53 activation, leading to Bax up-regulation and translocation into mitochondria, which restores the crosstalk with stalled TRAIL signaling for a synergistic caspase-9/3 cascade-mediated apoptosis execution.

It is therefore possible that the p53 functional status of the cancer may influence the choice of Se forms to provide the most enhancement of efficacy to be balanced with an optimal reduction of side effects. Since the risk for selenite-induced DNA damage and genotoxicity in the treatment of a cancer patient is less of a concern than for primary prevention use, the combined use of selenite and methyl Se with chemotherapeutic drugs may target a broader spectrum of cancers.

Roswell Park researchers investigated the role of MSeC on increased drug delivery via tumor vascular maturation in mice with FaDu head and neck squamous cell carcinoma (HNSCC) xenografts after 2 weeks of oral MSeC treatment [147]. Changes in microvessel density (CD31), vascular maturation (CD31/alpha-smooth muscle actin), perfusion (Hoechst 33342/DiOC7), and permeability (dynamic contrast-enhanced magnetic resonance imaging) were determined at the end of the 14-day treatment period. Double immunostaining of tumor sections revealed approximately 40 % decrease in microvessel density following MSeC treatment along with a concomitant increase in the vascular maturation index (approximately 30 % > control) indicated by pericyte coverage of microvessels. Hoechst 33342/DiOC7 staining

showed improved vessel functionality, and dynamic contrast-enhanced magnetic resonance imaging using the intravascular contrast agent, albumin-GdDTPA, revealed a significant reduction in vascular permeability following MSeC treatment. They found a 4-fold increase in intratumoral doxorubicin levels with MSeC pretreatment compared with administration of doxorubicin alone. A similar conclusion was reached with a different drug irinotecan [137] in that its efficacy was influenced by treatment schedule with MSeC and associated with enhancement of tumor vessel maturation, intra-tumor concentration of active metabolite SN-38 and apoptotic death of tumor cells. Normal tissue drug concentrations were not impacted by MSeC, supporting a tumor selective action.

This group further examined the mechanism of enhanced irinotecan efficacy by MSeC in head and neck HNSCC cells with respect to the angiogenic master regulator hypoxia inducible factor 1 (HIF-1), which is an upstream-regulator of VEGF and carbonic anhydrase IX (CAIX) [148]. MSeA suppressed hypoxia-induced HIF-1. MSeA or shRNA knockdown of HIF-1α increased cell death under hypoxic but not normoxic conditions. In the animal model, the combination treatment of irinotecan and MSeC against the parental xenografts produced a similar therapeutic efficacy as the HIF-1α knockdown tumors treated with only irinotecan. HIF-1a is another probable target of MSeA to enhance irinotecan therapeutic effect.

Yet in another study from the Roswell Park investigators [149], the drug conjugating enzyme Ugt1a was found necessary for MSeC to protect against toxicity caused by irinotecan in rats. In the Ugt1a mutant rats, the irinotecan MTD was lower than wild-type rats. This was specific for irinotecan as no differences were observed for docetaxel and cisplatin which are not substrates for Ugt1a.

The majority of clear-cell renal cell carcinoma (ccRCC) tumors are characterized by the loss of Von Hippel-Lindau tumor suppressor gene function, a stable expression of HIF1α and 2α, an altered expression of tumor-specific oncogenic microRNAs (miRNAs), a clear cytoplasm with dense lipid content, and overexpression of thymidine phosphorylase. Rustum and co-workers (now at University of Iowa) [150, 151] further addressed HIF and miRNAs in down regulation of specific drug-resistant biomarkers in tumor cells by a defined dose and schedule of MSeC or SeMet for mechanism-based drug combination. The inhibition of HIFs by Se gavage was necessary for optimal therapeutic benefit. Durable responses were achieved only when MSeC was combined with sunitinib (a vascular endothelial growth factor receptor (VEGFR)-targeted biologic), topotecan (a topoisomerase 1 poison and HIF synthesis inhibitor), and S-1 (a 5-fluorouracil prodrug). The documented synergy was Se dose- and schedule-dependent and associated with enhanced prolyl hydroxylase-dependent HIF degradation, stabilization of tumor vasculature, downregulation of 28 oncogenic miRNAs, as well as the upregulation of 12 tumor suppressor miRNAs. They have launched Phase 1/2 clinical trials of SeMet in sequential combination with VEGFR PTKI drug axitinib in ccRCC patients refractory to standard therapies (NCT02535533) with acceptable safety and promising early efficacy signals with SeMet daily supplement dosages up to 4 mg.

In summary, methyl-Se compounds enhance therapeutic efficacy through several potential mechanisms of action, ranging from increased tumor vascular maturity and drug delivery and retention, reduction in the expression of pro-survival molecules, increased caspase-mediated apoptosis, to improved tolerance to chemotherapeutic drugs, which usually cause dose-limiting toxicity. These results indicate that there is a possible role for supplemental Se, especially methyl Se compounds in enhancing the therapeutic efficacy of the current approved drugs and other immuno-oncology biologicals. The scheduling optimization and mechanisms of action should be further investigated.

Human PK and PD studies of methyl Se compounds

The first human single-dose pharmacokinetic study of MSeC was conducted by Marshall and colleagues at Roswell Park [152]. They randomized healthy male volunteers in a double-blinded fashion to receive either a single oral dose of MSeC at one of 3 dosages or placebo in step cohorts. The results show that the most distinct concentration curve is for the 1,200 μg dose, with an apparent dose-response pattern for lower doses (400, 800 μg) over that of placebo. Across the three MSeC dose cohorts, t_{max} were similar, ranging between 3 and 5 h. The mean Se C_{max} increased from 10 ng/mL for placebo to 22.8, 30.75, and 63.2 ng/mL (~0.8 μmol/L) in the low, medium, and high dose subjects, respectively.

Marshall and colleagues then performed multiple-dose PK, PD studies with MSeC vs. SeMet [153]. In this work, 29 Se-replete patients were enrolled in a randomized, double-blind trial for 84 days of repeated dosing with either Se form. No toxicity was observed. SeMet supplementation (400 and 800 μg) increased blood Se concentration in dose-dependent and time-dependent manners and more than did the same dosages of MSeC, the latter did not display dose- or time-dependency. The data would be consistent with MSeC not being incorporated as an amino acid into general proteins as would SeMet in lieu of Met. Neither Se form impacted the

two major plasma SEPs: SEPP1 and GPX3, consistent with the nutritional adequacy threshold being met prior to either form of Se supplementation began.

Human clinical studies with inorganic Se (selenate, selenite, selenious acid, etc)

Given the shifting focus away from Se-yeast and SeMet for cancer prevention use, researchers have started to explore the human safety and clinical impacts of inorganic and other Se forms. Table 3 summarizes these human clinical trials with inorganic Se forms and their major findings.

Selenate

Australian researchers reported the safety, tolerability and PK of "mega-dose" sodium selenate in men with castration-resistant prostate cancer (CRPC) more than a decade ago [154]. Initially, sodium selenate was given as a single dose to one patient each at 5, 10, 15 or 30 mg (compared to 200 µg SeMet used in SELECT and other trials). However, after observing very short serum half-life for the administered Se, the daily dosing frequency was increased to 3 times per day at the same dosages (i.e., total daily intake of 15, 30, 45 and 90 mg) for 12 weeks. Overall, 19 patients were enrolled with a mean age of 72 years. 12 patients completed the treatment for 12 weeks. Of the other 7 who did not, 4 withdrew from the study due to cancer progression, 1 with grade-III fatigue, another with

Table 3: Human clinical trials with inorganic Se compounds sodium selenate, sodium selenite and selenious acid.

Trial [ref]	Se form tested	Route/frequency	Daily dose µg Se total	Duration	Outcomes/findings
Australia CRPC phase I trial [154]	Sodium selenate	Oral, trice per day	15,000 30,000 45,000 90,000	12 weeks	RP2D ~60,000 µg Se/day ~60 mg Se/day Likely acute renal damage in 1 pt @this dose
Australia AD phase II trial [156]	Sodium selenate	Oral, trice per day	960 30,000 vs. placebo	24 weeks	Safe at this dose. Increased CSF Se correlated with slowing AD memory deterioration
Sweden SeCAR phase I trial [157]	Sodium selenite	i.v. infusion, 5 day/week	Dose escalation 3 × 3 scheme	4 weeks	MTD = 10.2 mg Se/per meter squared ~19.5 mg Se for men, ~17.4 mg Se for women
German AKTE cancer phase III trials [159]	Sodium selenite	i.v. infusion	500 vs. placebo	Multiple cycles	No interference for radiotherapy alleviated loss of taste and appetite and diarrhea induced radiation
US UCSF phase I/PK trial [160]	Sodium selenite	Oral, once daily	5,500 11,000 16,500 33,000 49,500	Multiple cycles	PK modeling after single dose Radiation therapy sensitization in CRPC
Korea BCRL phase II trial [161]	Sodium selenite	i.v. infusion	500 vs. placebo	2 weeks	Relief of lymphedema from breast cancer chemotherapy
UK osteoporosis phase II trial [162]	Sodium selenite	Oral, once daily	200 50 vs. placebo	6 months	No detectable change in urine bone osteoclastic resorptive marker NTx
New Zealand cancer PD trial [163]	Sodium selenite MSeC SeMet	Oral, once daily	400 400 400	4, 8 weeks vs. baseline	No change in plasma VEGF or endoplasmic reticulum proteins by any Se No change in PBMCs GSH by any Se form
US Rutgers U phase I combo PK trial [164]	Selenious acid = (protonated selenite)	i.v. infusion Once per cycle 2 days before chemo	3 × 3 dose escalation 50–5,000	~6 cycles	No DLT for Se in combo therapy regimen RP2D=5 mg or 5,000 µg Se

RP2D, recommended phase 2 dose; CSF, cerebrospinal fluid; AD, Alzheimer's disease; MTD, maximally tolerated dose; CRPC, Castration resistant prostate cancer; PBMCs, peripheral blood mononuclear cells; GSH, glutathione; DLT, dose limiting toxicity.

concomitant grade-III diarrhea and muscle cramps, and the third patient with an acute renal impairment. This patient accounted for the only serious adverse event that could have been due to sodium selenate (increased creatinine level from 90 to 260 mmol/L on 60 mg dose). The patient's medical records noted a prior history of underlying kidney disease, even though the immediate cause could not be determined. The 2 other patients who did not complete the 12 weeks supplement were in the 90 mg dose group, receiving 3 × 30 mg daily.

The researchers suggested a recommended phase II dose (RP2D) of 3 × 20 mg daily. The t_{max} was ~2.5 h and post-peak $t_{1/2}$ half-life ~2.9 h. They identified selenite as the major metabolite in the plasma and reached steady-state levels by 3 weeks. In the urine, selenate, SeMet and other methyl Se species were identified, but selenite was hardly detectable. As a surrogate marker of tumor progression, PSA in 1 patient had a 57 % reduction in PSA. Two patients' PSA values were stabilized for 28 and 41 weeks. For all other patients who completed the 12-week scheduled treatment, the mean doubling time of PSA increased from 2.2 to 4.0 months, indicating a slowing down of the cancer growth.

It is not surprising that human patients tolerated such "mega" high doses of selenate when considering that selenate was almost biologically inert in cell culture models and in animal models of neurocytotoxicity based on findings of senior author Niall Corcoran's group who had discovered a selenate-specific activator activity for protein phosphatase A2 [155].

Australian researchers reported Se and SEPs and inorganic Se (difference between total Se and SEP-Se) in serum and cerebrospinal fluid (CSF) from a 24-week randomized controlled trial of sodium selenate in Alzheimer's disease (AD) patients that aimed to assess tolerability, and efficacy of selenate in modulating Se concentration in the central nervous system (CNS) [156]. Forty AD cases were randomized to placebo, nutritional Se (0.32 mg sodium selenate, 3 times daily), or supra-nutritional Se (10 mg, 3 times daily) groups. They showed that serum Se increased in dose-dependent manner (nutritional + 45 %, p<0.01, Student's *t* test; supra-nutritional + 504 %, p<0.001) from baselines of 122–145 ng/mL. The supra-nutritional selenate supplementation was well tolerated and yielded a significant (p<0.001) but variable (95 % CI=13.4–24.8 ng/mL) increase in CSF Se, from baseline level of 1.3–1.6 ng Se/mL. Reclassifying subjects as either responsive or non-responsive based on elevation in CSF Se concentrations revealed that responsive group did not deteriorate in Mini-Mental Status Examination (MMSE) vs. non-responsive group (p=0.03). Pooled analysis of all samples revealed that CSF Se could predict change in MMSE performance (Spearman's rho=0.403; p=0.023).

Selenite

I.v. infusion and oral supplementation of selenite (sodium salt) had been studied in multiple countries. In a Phase I trial in Sweden (Selenite in the Treatment of Patients with Carcinoma SECAR) [157], 35 patients with different therapy resistant tumors received *i.v.* infusion of sodium selenite daily for 5 consecutive days per week either for 2 or 4 weeks in a classical 3 + 3 dose escalation design. The primary endpoint was safety, dose-limiting toxicity (DLT) and the MTD. The most common adverse events were fatigue, nausea, and cramps in fingers and legs. DLTs were acute, of short duration and reversible. Biomarkers for organ functions indicated no major systemic toxicity. The authors suggested MTD of 10.2 mg/m^2 (i.e., ~19.5 mg for an adult male; 17.4 mg for an adult female). The calculated median plasma half-life for selenite was 18 h. The plasma C_{max} Se from a single dose of selenite increased in a nonlinear pattern.

Taking advantage of the blood samples from the Sweden SECAR trial [157] and the German High-dose Sodium Selenium Supplementation in Patients With Left Ventricular Assist Device (SOS-LVAD) study of cardiac patients with end-stage heart failure undergoing surgery for implementation of a ventricular assist device (ClinicalTrials.gov, Identifier: NCT02530788), Brodin et al. [158] investigated SEPP1 as a potential biomarker of Se supplementation status beyond its recognized role in the nutritional range such that SEPP1 becomes saturated with increasing Se intake, reaching maximal concentrations of 5–7 microg SEPP1/mL at intakes of ca. 100–150 µg Se/day. Blood samples from these two i.v. infusion of selenite trials dealt with dosages >1 mg/day. Total Se was quantified by spectroscopy, and SEPP1 by a validated ELISA. The high dosage selenite infusions increased SEPP1 in parallel to elevated Se concentrations within a couple of days to final values partly exceeding 10 µg SEPP1/mL, independent of age and sex. The authors concluded that the saturation of SEPP1 concentrations observed in prior studies with moderate Se dosages (<400 µg/day) might reflect an intermediate plateau of expression, rather than an absolute upper limit. Circulating SEPP1would seem to be a suitable biomarker for therapeutic applications of selenite exceeding the recommended upper intake levels.

German researchers have conducted clinical trials with Se *i.v.* infusion (500 µg Se) as sodium selenite [aka selenase, biosyn Arzneimittel GmbH, Fellbach, Germany] in a number of cancer trials [159]. The German Working Group Trace Elements and Electrolytes in Oncology (AKTE) aimed to stratify the patients with a potential need for supplemental Se and how best to monitor Se supplementation with respect to health effects and risks. Two randomized phase III clinical

studies were conducted to test a potential radioprotective effect of supplemental Se during radiation therapy in patients with uterine cancer (n=81) and head and neck tumor patients (n=39). Their results showed that a relative Se deficit in whole blood or serum was detected in the majority of tumor patients (carcinomas of the uterus, head and neck, lung, rectal or prostate cancer). In prostate cancer, tissue Se concentrations were relatively elevated in the carcinoma center as compared to the surrounding compartment or as compared to tumor samples from patients with benign prostatic hyperplasia. Adjuvant Se supplementation successfully corrected Se-deficiency in the patients analyzed and decreased radiotherapy-induced diarrhea in a randomized study of radiotherapy patients with carcinomas of the uterus. Survival data imply that Se supplementation did not interfere with radiation efficacy. They noted positive effects of supplemental Se in the prevention of ageusia (loss of taste) and dysphagia (loss of appetite) due to radiotherapy in a second randomized trial in patients with head and neck cancer. No adverse effects of supplemental Se (i.e., 500 μg) were observed in these studies.

University of California-San Francisco researchers completed PK modeling of oral selenite dosing, prompted by a finding of potential radiosensitizer role in a phase I study (NCT02184533) in 15 subjects with metastatic cancer receiving daily oral sodium selenite with palliative radiation therapy [160]. Disease stabilization was observed, as evidenced by tumor regression, marked reduction in pain symptoms, and decreased PSA levels in patients with castrate-resistant prostate cancer. They characterized population PK based on the Se plasma concentrations obtained from five dosing cohorts (5.5, 11, 16.5, 33, and 49.5 mg). The model described externally administered selenite (inorganic) with a baseline component for endogenous Se levels. A one-compartment model characterized selenite PK metrics: absorption rate constant (0.64 h^{-1}), apparent clearance (1.58 L/h), apparent volume of distribution (42.3 L), and baseline Se amount (5,270 μg). An inverse relationship was found between logarithmic function of selenite dose level and Se bioavailability. Whether a cancer-selective DNA single strand breaking activity of selenite was responsible for the sensitization of palliative radiation therapy action is an interesting possibility for further mechanistic research.

A Korean group assessed how sodium selenite *i.v.* injection would affect breast cancer-related lymphedema (BCRL) symptoms and parameters associated with antioxidant effects [161]. They performed a randomized, double-blind, controlled trial on 26 participants with clinical stage II–III BCRL. The control group (n=12) and Se group (n=14) underwent five sessions of 0.9 % saline and 500 μg sodium selenite (Selenase®) *i.v.* injections, respectively, within 2 weeks. Clinical diagnosis on lymphedema by physicians, bioimpedance data, blood levels of oxidative markers, including GSH, glutathione disulfide (GSSG), malondialdehyde (MDA), glutathione peroxidase activity (GPx), and serum oxygen radical absorbance capacity (ORAC) levels, were investigated at baseline, after completing 2 week injections, and at follow-up. As standard of care, patients were educated on self-administered manual lymphatic drainage to manage their lymphedema. The results showed that sodium selenite increased whole blood Se over baseline vs. no change in saline control group. Compared to the baseline, at 2 weeks, 75.0 % of participants showed improvement, while there was no change in the saline group. At follow-up, 83.3 % and 10.0 % of the Se and saline patients, respectively, showed down-staging from III to II (p=0.002). Extracellular water (ECW) ratios were significantly reduced at 2 weeks and follow-up, only in the Se group. Blood GSH, GSSG, GSH/GSSG ratio, MDA, and ORAC levels did not change by Se. They concluded that the selenite effect on lymphedema might be associated with non-antioxidant properties.

UK researchers [162] conducted EudraCT 2016-002964-15/ClinicalTrials.gov, NCT02832648 trial to test the hypothesis that selenite supplementation could reduce bone osteoclast resorptive actions of reactive oxygen species and improve physical function in post-menopausal women with a 6-month randomized, double-blind, placebo-controlled design. They recruited postmenopausal women older than 55 years with osteopenia or osteoporosis. Participants were randomly assigned 1:1:1 to receive selenite 200, 50 μg, or placebo orally once per day. The primary endpoint was urine N-terminal cross-linking telopeptide of type I collagen (NTx, expressed as a ratio to creatinine) at 26 weeks. Analysis included all randomly assigned participants who completed follow-up. Groups were compared with analysis of covariance with Hochberg testing. Secondary endpoints were other biochemical markers of bone turnover, bone mineral density, short physical performance battery, and grip strength. Mechanistic endpoints were glutathione peroxidase, highly sensitive C-reactive protein, and interleukin-6. 120 participants were recruited between Jan 23, 2017, and April 11, 2018, and randomly assigned to one of the 3 arms (n=40 per group). 115 (96 %) completed follow-up and were included in the primary analysis (200 μg Se [n=39], 50 μg Se [n=39], placebo [n=37]). Median follow-up was 25.0 weeks. In the 200 μg group, mean serum Se increased from 78.8 to 105.7 ng/mL. Urine NTx to creatinine ratio (nmol bone collagen equivalent:mmol creatinine) did not differ significantly between treatment groups at 26 weeks: 40.5 for placebo, 43.4 for 50 μg, and 42.2 for 200 μg. None of the secondary or mechanistic endpoint measurements differed between treatment groups at 26 weeks. Seven (6 %) of 120 participants were withdrawn from treatment at

week 13 due to abnormal thyroid-stimulating hormone concentrations (one in the 200 μg group, three in the 50 μg group, and three in the placebo group) and abnormal blood glucose (one in the 50 μg group). There were three serious adverse events: a non-ST elevation myocardial infarction at week 18 (in the 50 μg group), a diagnosis of bowel cancer after routine population screening at week 2 (in the placebo group), and a pulmonary embolus due to metastatic bowel cancer at week 4 (in the 200 μg group). All severe adverse events were judged as unrelated to trial medication. The UK researchers concluded that oral selenite supplementation at 50–200 μg/day doses did not affect musculoskeletal health in postmenopausal women in UK.

Selenite vs. MSeC, SeMet

A New Zealand phase I randomized double-blinded trial examined the PD profile of sodium selenite, MSeC and SeMet in two cohorts of 12 patients each, one cohort with chronic lymphocytic leukemia (CLL) and the other with solid malignancies [163]. All 24 patients were randomized to receive 400 μg of Se as one of the 3 forms, taken orally daily for 8 weeks. PD parameters were assessed before, during and 4 weeks after Se compound exposure in plasma and peripheral blood mononuclear cells (PBMCs). Their results showed no significant changes of the plasma concentrations of vascular endothelial growth factor-α (VEGF-α), expression of proteins associated with endoplasmic reticulum stress (the unfolded protein response) or in intracellular total glutathione in PBMCs, in either disease cohort or when grouped by Se form. Extrapolating from pre-clinical data, the dose examined was speculated too low to achieve the Se plasma concentration (≥5 μmol/L) expected to elicit significant PD effects.

Selenious acid (aka selenous acid = protonated selenite)

In a cancer combination chemotherapy context, Rutgers University scientists in the USA conducted a phase I trial for a combination of selenious acid with carboplatin/paclitaxel modality to determine the MTD, safety, and effects of Se on carboplatin PK in the treatment of chemo-naive women with gynecologic cancers [164]. Eligible patients received *i.v.* selenious acid on day 1 followed by *i.v.* carboplatin plus paclitaxel infusion on day 3. A standard 3 + 3 dose-escalating design was used for addition of selenious acid to the standard dose chemotherapy regimen. In 45 patients and 291 treatment cycles, selenious acid was infused to 9 cohorts of patients with Se ranging from 50 to 5,000 μg. The Se MTD was not reached within the context of 3-drug combination with reported grade-III/IV toxicities including neutropenia (66.7 %), febrile neutropenia (2.2 %), pain (20.0 %), infection (13.3 %), neurologic (11.1 %), and pulmonary adverse effects (11.1 %). None was considered DLT. The infused Se did not change carboplatin pharmacokinetics. Correlative studies showed post-treatment downregulation of RAD51AP1, a protein involved in DNA repair, in both cancer cell lines and patient tumors. They recommended a 5,000 μg dose of Se (5 mg) as selenious acid form as RP2D for future trials.

Conclusions and future directions

Summary and conclusions

The outcomes of the published randomized Phase III trials with SeMet or Se-yeast (200 or 400 μg Se per day) for prevention of prostate, lung, colorectal and bladder cancers in Northern America and European countries have had a negative impact on the field of Se cancer research. We elaborated two major reasons for the failure of these human studies to detect a preventive efficacy of SeMet or Se-yeast, namely, inappropriate Se-replete subject/patient populations selected for testing the anti-oxidant hypothesis and the forms of Se chosen for human clinical trials that were ineffective in cell culture and animal models.

Mechanistic studies have indicated that Se forms, dosages and dosing scheduling are critical factors for chemoprevention and therapy efficacy, depending on their entry into two distinct Se metabolite pools. It is likely that the MSeA:MSeH redox pair exert many desirable attributes of cancer chemoprevention and therapy, including targeting key signaling pathways, angiogenic switch regulators, immune surveillance and immuno-oncology therapy, and invasion and metastasis molecules, inflammation in the TME as well as sex hormone signaling in gender-specific cancers.

Accumulating data support MSeA and MSeC as more promising candidates than SeMet for future clinical investigations of cancer chemopreventive and therapy efficacy. Our proteomic analyses of prostate and their lesions from MSeA vs. MSeC-treated mice indicated these forms are non-exchangeable, not surprising given the amino acid analog nature of MSeC. Therefore, their safety and efficacy should be each rigorously studied and compared in animal models, eventually in humans.

A couple of published human PK studies [152, 153] with MSeC vs. SeMet had shown major differences for C_{max}, AUC between these amino acid forms, consistent with SeMet non-specific incorporation into general proteins in place of Met

and minimal, if not at all, of this activity for MSeC. The small number of human clinical trials with inorganic sodium selenate, sodium selenite and selenious acid (Table 3) have yielded important safety information of these non-amino acid Se forms, with selenate being the best tolerated among the 3 forms. However, none of the trials have assessed DNA damage or genotoxicity to PBMC or somatic cells in the human participants. So far, no human study has been reported for MSeA.

Recommendations

Given [a] the closer proximity of MSeA than MSeC to the MSeH:MSeA redox cycle, [b] the cheaper and simpler chemical preparation for MSeA than the production of MSeC at Good-Manufacturing-Practice scale, and [c] more favorable profile of PD molecular "targets" for MSeA than that for MSeC, we recommend more human cancer clinical studies on MSeA. We consider the classic 3 + 3 dose escalation study designs employed by Marshall et al. [152, 153] and in Phase I/II studies reported for selenate, selenite and other Se forms highly appropriate for DLT and MTD (as discussed in Human clinical studies with inorganic Se [selenate, selenite, selenious acid, etc], Table 3). We recommend that in future human clinical studies DNA damage and genotoxicity biomarkers be incorporated as important safety assessments for MSeA and other Se forms to provide critical risk-benefit tools for their precision use in cancer chemoprevention and therapy and to differentiate MSeA from selenite and related inorganic compounds.

In spite of impressive public health efforts in China to correct nutritional Se deficiency with selenite-enriched table salt and other supplement forms, Se nutritional adequacy for the billion-plus Chinese population can be a formidable and continuing undertaking for perpetuity. We therefore recommend that in future clinical studies in Chinese participants, the Se nutritional status be reliably monitored. By stratified recruitment based on the baseline Se status, in participants with deficient Se status, we suggest a combination approach between MSeA at variable dosages for anticancer efficacy and a steady maintenance dosage of selenite for nutritional support to maximize the entry into the MSeA:MSeH redox cycle.

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