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Methylselenocysteine - a Promising Antiangiogenic Agent for Overcoming Drug Delivery Barriers in Solid Malignancies for Therapeutic Synergy with Anticancer Drugs

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Abstract

Introduction—Despite progress, chemotherapeutic response in solid malignancies has remained limited. While initial results of the use of antiangiogenic agents in combination chemotherapy indicated an enhanced therapeutic response, recent data indicates that the surviving cancer is not only able to surmount therapy, but is actually able to adapt a more aggressive metastatic phenotype. Thus, selecting an antiangiogenic agent that is less likely to lead to tumor resurgence is a key to future therapeutic success of antiangiogenic agents, in a combinatorial setting.

Areas covered—Against the broad spectrum of currently used antiangiogenic agents in the clinic, the putative benefits of the use of organo selenium (Se) compounds, such as methylselenocysteine (MSC), are discussed in this review.

Expert opinion—MSC, being part of the mammalian physiology, is a well tolerated, versatile and economical antiangiogenic agent. It down regulates multiple key upstream tumor survival markers, and enhances tumor drug delivery, at a given systemic dose of an anticancer agent, while protecting normal tissue from cytotoxic adverse effects. Further clinical trials, especially in poorly differentiated cancers, are warranted.

1 Introduction

Cancer remains one of the leading causes of death in the United States ¹ and in many parts of the globe. While surgery remains the mainstay for removal of bulk of the disease, systemic therapies including chemotherapy remains the treatment of choice in an adjuvant or neoadjuvant setting. Despite much progress in the development of novel anticancer drugs and a general improved response to chemotherapy, curative treatment still remains elusive for most solid malignancies ². The therapeutic cure rates in most solid tumors remain unchanged despite arrival of more than 20 new anticancer agents over the past decade. The only exceptions being the testicular cancer, gestational choriocarcinomas, Hodgkin disease and high grade lymphomas that due to an innate high sensitivity to proapoptotic stresses, respond to chemotherapy in a routinely curative way ². Most other solid malignancies do not respond significantly to the various anticancer therapeutic strategies, both traditional and novel. Factors at coarser physiological levels can impede drug delivery and distribution and thereby critically influence therapy outcome ^{3, 4}.

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Selenium (Se) is a natural element present in the earth's crust often in association with sulphur containing compounds. Humans get their dietary requirements mainly from the food. Dietary Se is in the range of 29–152 µg/day in Europe and North America. Se is an essential trace element that is necessary for functioning of the enzyme glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). As selenocysteine, it is a constituent of over 25 selenoproteins in the mammalian system. Maximum expressions of these selenoproteins require a daily dietary intake of 0.1–0.2 mg/kg in animals and ~ 55 µg for humans ⁵. Deficiency symptoms occur such as in Keshan disease when the consumption is < 20 µg/day. The anticarcinogenic properties of Se was noticed as a result of the inverse association between cancer mortality and crop Se contents ⁶ which was subsequently supported by findings of an inverse relationship between blood Se levels and prevalence of several types of cancer ⁵.

Unlike the chemoprevention dose of 200 µg/d L-selenomethionine (SLM) used in the SELECT trial ⁷, the current article focuses on the therapeutic use of Se that uses ~72 fold higher daily dose of SLM ⁸ or methylselenocysteine (MSC) in order to obtain the threshold plasma Se concentration of 15 µM, a dose at which a decreased chemotherapy induced toxicity coupled with the enhanced antitumor efficacy of chemotherapeutic drug/s administered in combination with SLM/MSC in a dose dependent manner was observed in the preclinical animal models ^{9–11}. The antiangiogenic effects of Se were evidenced at this therapeutic dose in the preclinical system where tumor vascular normalization and a decrease in tumor interstitial fluid pressure (IFP) leading to a higher intratumoral drug delivery was observed ^{3, 12, 13}.

1.1 Tumor heterogeneity hinders chemotherapeutic efficacy

For chemotherapy to be effective, the cytotoxic agent should be able to reach the proliferating cancer cells in optimal concentration and be effective against the individual cancer cells in the *in vivo* microenvironment milieu. For a drug to be therapeutically effective in human tumors, after extravasation from the blood vessels, it must travel a distance of 200 µm in order to reach all viable cells in the tumor ¹⁴. Development of novel anticancer agents and therapeutic combinations has almost exclusively focused on molecular targets with often little concern about the architectural and morphologic heterogeneity which remains the hallmarks of most (>70%) solid malignancies in the clinic ³. Most spontaneous tumors originate from a single cell and yet at the time of clinical diagnosis display startling heterogeneity in many morphological and physiological features including presence of large hypoxic, avascular necrotic or well-differentiated regions, expression of cell surface receptors, proliferative and angiogenic potential ^{3, 15}. This architectural and morphologic heterogeneity is a critical barrier to optimal drug delivery and distribution in the tumor (*arrows*, Figure 1). Though tumor cell differentiation do not directly contribute to tumor propagation and growth, yet the tightly adhering differentiated cells do not allow tumor vasculature to grow within their midst ⁴, and thereby cause these differentiated (particularly terminally differentiated) regions to be bereft of blood vessels (*arrows*, Figure 1) with a peripheral rim regions that actually sustains and protects proliferating cancer cells from receiving the cytotoxic doses of the anticancer drug as they are the farthest from any blood vessels that can deliver the effective chemotherapeutic agent. The optimal distance of 200 µm that the drug/s need to travel in a morphologically heterogeneous tumor is unlikely to be attainable and may in fact be insufficient in a differentiated tumor due to a non-uniform distribution of tumor blood vessels where large areas of tumors do not contain blood vessels (*arrows*, Figure 1, B, C, D & F). Thus, while in the uniformly poorly differentiated human cancer xenografts FaDu and HCT-8, the maximum tolerated dose (MTD) of CPT-11 alone resulted in a cure of 30% and 20% respectively; in combination with the antiangiogenic agent SLM/MSC the cures increased to 100% ¹⁰. In contrast, in the well differentiated

human cancer xenografts A253 and HT-29 the combination could only increase the cures from 10% and 0% with CPT-11 alone to 60% and 20% with the combination therapy respectively¹⁰. Further, clinical samples of poorly differentiated human patient tumors #17073 (*arrow*, Fig 1E) growing in scid mice showed a heterogeneous architecture with the presence of many large necrotic avascular regions and did not respond to combination therapy at the usual MTD of CPT-11 but did respond with 75% cures to double the MTD of CPT-11 when used in combination with MSC compared with only 25% with the drug alone³. A reduced interstitial space and extracellular matrix in such regions is likely to further lower the extravasation of the drugs from the blood vessels farther into the tumor by retarding convection and/or diffusion¹⁶. Such avascular regions promote tumor hypoxia leading to clonal selection of a drug resistant phenotype in the surviving tumor cells. Cyclical repetition of the same initially effective chemotherapeutic drug in such cases is likely to meet with failure due to clonal selection of a resistant phenotype as is seen in the clinic in many human cancers such as small cell lung cancer that often responds to the initial chemotherapeutic regime while becoming resistant to it later on.

1.2 Tumor vasculature plays a critical role in chemotherapeutic efficacy

For a cytotoxic drug to be optimally effective; it must have access to each and every individual proliferating tumor cells. This is possible only in the presence of uniformly distributed tumor vasculature that are of 'normal' caliber across the tumor cross section allowing the effective anticancer drug to extravasate and traverse to different parts of the tumor by convection and/or diffusion. In reality, most solid malignancies in the clinic are fraught with heterogeneity in terms of tumor hypoxia, vasculature and interstitial fluid pressure (IFP)¹³. Tumor vasculature being without sufficient pericytes, perivascular smooth muscle is abnormal and leaky with an absent or discontinuous basement membrane. Further, the chaotic branching patterns with excessive loops imparts an aberrant blood flow which along with a limited or absent lymphatic system increases tumor IFP while retarding delivery of macromolecular drugs inside the tumor^{14, 17, 18}. Increased tumor IFP can result in lymphatic and vascular collapse and a reversal of arterial flow^{19, 20}. In the central part of the tumor, blood vessels get more compressed due to increased IFP which results in blood flow getting diverted from the center of the tumor to the periphery. Drug delivery is further retarded in tumors with a high packing density of constituent cells with reduced interstitial space and volume of extracellular matrix and a well organized, richly interconnected collagen network often that does not allow the drug to penetrate the required distance of 200 μm to reach most viable cells within human tumor¹⁴. Differentiated regions within tumors often show these characteristics with the presence of tightly packed cells that do not allow new vascular sprouts to penetrate these regions despite the presence of hypoxia and secretion of vascular endothelial growth factor (VEGF)⁴.

2. Strategies to enhance chemotherapeutic efficacy

Various strategies have been employed to improve tumor drug delivery and efficacy as summarized in Table 1. Of these, antiangiogenic agents and vascular disrupting agents (VDA) impact tumor vasculature directly. Unlike antiangiogenic agents, VDAs in general destroy blood vessels and often do not lead to a complete abrogation of tumor when used in combination chemotherapy²¹. Antiangiogenic agents often improve tumor blood flow and permeability while concurrently reducing tumor IFP. Other methods listed in Table 1 do not modulate tumor vasculature directly and is not within the purview of the subject matter of this paper.

2.1 Role of antiangiogenic agents in enhancing chemotherapeutic efficacy

Many antiangiogenic agents are currently in clinical use such as bevacizumab, a ligand-trapping monoclonal antibody against VEGF; the multi kinase inhibitors Sorafenib and Sunitinib. Clinical studies of antiangiogenic agent in combination chemotherapy have given only a modest improvement in terms of median survival. Bevacizumab had improved median survival from 15.6 months with the drug (Irinotecan/Fluorouracil/Leucovorin) alone to 20.3 months in the combination group in a phase III study with previously untreated metastatic colorectal cancer²². Similarly, it was found to improve the median survival from 10.8 months with the drug (Oxlaiplatin/Fluorouracil/Leucovorin) alone to 12.9 months in the combination group in a phase III trial with previously treated metastatic colorectal cancer²³. The results from a phase III clinical trial in recurrent or advanced non-small cell lung cancer showed a similar improvement in median survival from 10.3 months with paclitaxel/carboplatin compared to 12.3 months in the combination group with Bevacizumab²⁴. Bevacizumab did not show any significant improvements in other phase II and III clinical trials in previously untreated metastatic colorectal cancer²⁵ and metastatic breast cancer²⁶ respectively. Phase III clinical trials in previously treated colorectal cancer did not show any difference in overall survival between the drug (Oxlaiplatin/Fluorouracil/Leucovorin) alone and in combination with Vatalanib²⁷. Phase III clinical trial with Sorafenib in previously untreated non-small cell lung cancer did not show any survival advantage over the group treated with the drug (Carboplatin/Paclitaxel) alone²⁸. Thus, most single target based antiangiogenic agents seem to induce a transient functional normalization followed by chronic angiogenic inhibition that typically reduce uptake of the chemotherapeutic agent²⁹. Improved survival is more likely to be obtained with broad spectrum multi-targeted tyrosine kinase inhibitors such as Sorafenib and Sunitinib when used as monotherapy³⁰.

List of agents targeting tumor vasculature that are in clinical trial are listed in Table 2 highlighting the mode of action along with toxicity considerations to normal tissues/organs. Use of currently available antiangiogenic agents targeting VEGF is limited by a low cost-benefit and significant adverse effects^{31,32}. Blocking VEGF alone (e.g. with bevacizumab) has met with limited success and in fact a more malignant progression with increased local invasion and distant metastasis has been reported in the clinic on withdrawal of VEGF blockade³³. Rebound hypervascularization promoting invasion and metastasis has also been reported in the mouse model after discontinuing the antiangiogenic drug³⁴. Thus, with anti-VEGF therapy, some patients show limited survival advantage, other cases show no such benefits or an inevitable disease progression indicating an intrinsic resistance and or an adaptive response of tumors against such agents³⁵. These evasive responses by tumor involve up-regulation of alternative proangiogenic signals such as platelet derived growth factor or PDGF, placental growth factor or PIGF, angiopoietin-1, fibroblast growth factor family members and invasion of the tumor cells into local tissue to co-opt normal vasculature. Further, tumors at an advanced stage of progression in a patient with history of multiple chemotherapeutic interventions are likely to foster further intrinsic resistance in the tumor microenvironment. Antiangiogenic agents causes tumor vascular maturation through increased pericyte coverage and results in 'normalization' of tumor vessels. The combined effect of a 'normalised' vessel and a consequently reduced tumor IFP is that the intratumoral drug delivery gets significantly enhanced¹². Further, use of antiangiogenic agents has been reported to enhance therapeutic efficacy by reducing tumor stem-cell like fraction³⁶. A higher pericyte coverage that is responsible for vessel maturation can also lead to resistance to anti-VEGF therapy since it is not as dependent on VEGF-mediated survival signaling³⁵. Pericytes, one of the key components of tumor vascular maturation, has been shown to allow some tumors to retain a core of pre-existing tumor vasculature alive and functional despite the use of therapies that impair neoangiogenesis or promote vascular regression³⁷⁻³⁹. Therapeutically targeting pericytes itself is fraught with several problems. It can lead to an

enhanced metastasis⁴⁰; take away the advantage of increased drug delivery within a tumor at a given systemic drug dose; and, may lead to ascites or edema due to an increased vessel leakage⁴¹. Often the antiangiogenic therapy leads to an acute reactive hypoxia at the peak of the response phase that leads to upregulation of various tumor survival genes including those of other proangiogenic signals. Tumor hypoxia, often accentuated by use of antiangiogenic agents, lead to recruitment of bone marrow derived progenitor cells by the tumor for forming new vessels. This mechanism is generally through up-regulation of Hypoxia inducible factor (HIF)-1 α ^{42, 43} signaling cascade which is critical for tumor survival, proliferation and invasion, and, can induce a vast array of more than 100 genes that influences tumor survival, metabolism and neoangiogenesis⁴⁴. In some solid malignancies such as in clear cell renal cell carcinoma where chemotherapeutic regimen has been primarily unsuccessful in retarding tumor growth, antiangiogenic agents Sunitinib and Bevacizumab are currently the first-line standards of care⁴⁵. In others, such as in the colorectal cancers, antiangiogenic agent is currently recommended only in metastatic disease and for use in adjuvant setting⁴⁶.

2.2 Adverse factors affecting the use of antiangiogenic agents

Use of many currently clinically active antiangiogenic agents can lead to proteinuria, hypertension, hemorrhage, arterial and venous thromboembolism, bowel perforation, renal thrombotic microangiopathy, myocardial infarction, fatigue, mucositis, diarrhea, erythema, alopecia, hair depigmentation and wound healing complications^{24, 47–51}. Additionally use of antiangiogenic agents is contraindicated in patients with squamous cell lung cancer⁵². Other antiangiogenic agents such as thalidomide and lenalidomide are known to cause venous thromboembolism⁵³. Antiangiogenic agents are known to have adverse renal effects such as proteinuria, acute kidney injury thrombotic microangiopathy, other glomerular lesions and interstitial nephritis⁵⁴. Thus, most clinically used antiangiogenic agents are not well tolerated with various adverse effects on normal tissues and organs (Table 2).

3 Selenium as an anticancer agent

Se is an essential trace element required for the proper functioning of various selenoproteins including GPx and TrxR by being an essential constituent of selenocysteine. Se plays a critical role as a cellular antioxidant and detoxification with Se status having an inverse association with cancer risks^{6, 55, 56}. Various natural and synthetic forms of Se such as selenite, selenate, methylseleninic acid (MSA), SLM, selenocysteine, MSC, selenobetaine, p-methoxybenzeneselenol, benzylselenocyanate, 1,4-phenylene-bis (methylene) selenocyanate, diphenylselenide, methylphenylselenite and gamma-glutamyl MSC have been used to study the anticancer efficacy in terms of its' ability to prevent and retard cancer formation, growth besides enabling to protect healthy tissues from the cytotoxic effects of chemotherapeutic drugs. Se affects multiple targets in cancer and has been shown to affect cell cycle, induce apoptosis, retard cell migration and invasion⁵⁷. The active metabolite of some of the Se compounds is listed in Table 3. While both inorganic selenite and selenide induces apoptosis through single strand DNA breaks, the organo-selenium compounds induce apoptosis without DNA strand breaks^{58–61}. The active metabolite for organoselenium compounds is methylselenol. The most efficacious Se with anticancer properties are the mono- or dimethylated selenium compounds such as MSC and selenobetaines followed by selenite, selenocystine and dimethyl selenoxide in that order of decreasing cancer preventive properties⁶². A chemical form of Se that allows for rapid elimination as in the case of dimethyl selenoxide or where it is too far along the methylation pathway or gets incorporated into cellular proteins preventing release of its monomethylated Se moiety, is likely it reduces the anticancer potential of the Se compound. MSC has been reported to be a better anticancer agent compared to SLM presumably due to its easy conversion to the active methylselenol. Stable monomethylated compounds such as MSC or

selenobetaine acts as precursors by releasing methylselenol through action of lyases⁶³. Se impacts multiple anticancer pathways that hinder cancer initiation, growth and progression. Some of these are summarized below:

1. Antiangiogenic and tumor vascular normalization effects leading to higher intratumoral drug delivery of anti cancer drug/s used in combination chemotherapy (Table 4)^{3, 12, 13};
2. Altered methionine metabolism leading to an increased sensitivity to caspase mediated apoptosis;
3. Retarding cell doubling time by increasing cell duration in G1, S and G2 phases of the cell cycle^{61, 64};
4. Induction of genes involved in phase II enzyme activity and down-regulation of genes related to phase I enzyme and cell proliferation⁶⁵;
5. Inhibiting key targets such as cyclin A, cyclin D1, CDC25A, CDK4, PCNA, E2F gene expression and arresting cells in G1 phase of cell cycle⁶⁶;
6. Inducing expression of P19, P21, P53, GST, SOD, NQO1, GADD153 and certain caspases⁶⁶;
7. Possibly retarding metastasis since genes such as osteopontin is down-regulated by Se⁶⁶; and,
8. Modulating the immune system⁶⁷.

Selenomethionine has also been used in the preclinical animal model with methioninase protein or gene for cancer therapy with encouraging results^{68–70}.

3.1 Inorganic vs. organic selenium compounds – which form is better?

In humans, Se retention from SLM or MSC is much better than that from inorganic Se^{71, 72}. Selenide generated from the consumed Se compound is involved in cotranslational biosynthesis of selenocysteine which gets incorporated into selenoproteins⁷³. Methylselenol and dimethylselenide are intermediate metabolites excreted through breath and urine⁷⁴. The anticarcinogenic properties of organo-Se compounds have been attributed mainly to methylselenol^{75–77}. While inorganic selenite is toxic at dose beyond 5 ppm^{78, 79}, organo-selenium compounds such as SLM are well tolerated in humans where a dose of 7200 µgm twice daily for a total of 7 days followed by a daily single maintenance dose for a few weeks did not result in any adverse effects⁸. SLM was well tolerated with no skin or nail toxicities and the only toxicity attribute to SLM was the garlic-like odor in breath and urine that was seen more commonly during the induction SLM week and was found to disappear with prolonged treatment⁸. In the same studies all patients given 4800 µgm twice daily had a plasma Se levels exceeding 15 µM, the dose level required for decreased chemotherapy induced toxicity and an enhanced antitumor efficacy of chemotherapeutic drug/s administered in combination in the preclinical animal models^{9–11}. The plasma Se dose levels exceeded 30 µM by day 28 thereby confirming the feasibility of using SLM/MSC in the clinic. The MTD of inorganic Sodium selenate was found to be 60 mg given daily in men⁸⁰. Dose limiting toxicities for sodium selenite were fatigues and diarrhea while adverse events included nail disorders, muscle spasms, alopecia and nausea. Much of these were attributed to the accumulation of the inorganic metabolite selenite which has a more cytotoxic properties when compared to selenate⁸¹ or SLM/MSC. Amongst the organoselenium compounds, MSC is more efficacious than SLM in anticancer efficacy since it is converted to the active methylselenol through one-stage β -lyase conversion^{82, 83} while SLM gets incorporated into proteins since it easily acylates Met-tRNA or gets converted through trans-sulfuration mechanism to selenocysteine which then gets degraded to

hydrogen selenide by β -lyase⁸⁴. Se compounds which directly enters the methylated pool are more likely to be effective than the Se compounds that are metabolized through hydrogen selenide pool. Selenobetaine and MSC generate monomethylated Se, whereas sodium selenite and SLM are metabolized to hydrogen selenide and are thus less efficacious in a chemopreventive and possibly, therapeutic setting⁸⁴. Further, selenobetaine must lose one methyl group first before dissociating of the Se-methylene carbon bond to form methylselenol⁸⁵, unlike MSC which undergoes one-step conversion to methylselenol. Interestingly, in some of the preclinical animal studies, the use of MTD dose of organo selenium compounds SLM and MSC did not show any difference in therapeutic efficacy¹⁰. While MSC induces apoptosis in the G1 phase, selenite induces necrosis in the S/G2-M phase of the cell cycle⁸⁶. MSA, another organo-Se compound with anticancer efficacy, does not require β -lyase conversion to the monomethylated Se form as it is easily reduced intracellularly to the monomethylated form non-enzymatically in the presence of GSH and NADPH⁸⁷. *In vitro*, MSA is more potent than MSC due to its' ability to deliver the monomethylated Se species directly to the cells but *in vivo* both show similar efficacy⁸⁷ while MSA has a higher toxicity profile when compared to SLM/MS⁸⁸.

3.2 Selenium as an antiangiogenic agent

While Se deficiency can lead to an altered and increased permeability of the normal vessels of the heart and the eye, toxic levels of Se can decrease vascular permeability in the kidney, liver and eye while increasing vascular permeability in the brain⁸⁹. Though most selenocompounds (Table 3) have been reported to have antiangiogenic^{90,91} and possibly induce vessel normalization effects in tumors, the organoselenium compounds MSA, SLM and MSC have been extensively studied in the preclinical system where both the antiangiogenic and vascular normalization events have been confirmed and SLM have been used in clinical trials. In the preclinical system MSA, SLM and MSC have been found to lead to antiangiogenic effects in a variety of tumor xenografts including human head and neck squamous cell carcinoma, colorectal and lung cancer³. In addition to down regulating HIF-1 α , Se has been shown to down regulate cyclooxygenase (COX) – 2^{92,93} and inducible nitric oxide synthase 2 (iNOS2)^{93–95}. Thus, Se targets multiple major upstream angiogenic key players and different angiogenic pathways in the tumor that the tumor is likely to find difficulty in surmounting. Though as a single agent, SLM and MSC had a modest $\geq 30\%$ tumor growth inhibition, no complete remissions of tumors were observed *in vivo* in the preclinical studies. This tumor growth inhibition of Se as a monotherapy compares favorably with other effective single-agent antiangiogenic agents in the clinic⁸⁰ for its' *in vivo* efficacy in retarding tumor growth and has the clinical potential for improving overall median survival in patients. Thus, use of Se as a high dose therapeutic antiangiogenic agent used alone is promising in the highly angiogenic malignancies such as the clear cell renal cell carcinoma.

3.3 Antiangiogenic selenium in combination chemotherapy

Se, when used in combination with chemotherapeutic agents in the preclinical models, gave a complete remission in many tumor types^{10,83}. This therapeutic response was found to be inversely associated with tumor morphologic heterogeneity that translated to a corresponding heterogeneity in terms of tumor vascular distribution³. Well differentiated tumor regions despite being hypoxic and secreting VEGF do not allow formation of new tumor blood vessels⁴. As shown in Table 4, SLM or MSC in combination with cancer chemotherapy in solid malignancies from different disease sites growing subcutaneously (s.c) as xenografts in nude mice often lead to a lower tumor microvessel density (MVD) and an increased vascular maturation that often corresponded with an improved, reduced tumor IFP, resulting in an increased drug delivery at a distance of $\sim 90\ \mu\text{m}$ from the blood vessel. Se-induced improvement in tumor drug delivery at the same systemic drug dose

administered results in a 4-fold increase in intratumoral drug concentration in the head and neck human tumor xenografts FaDu that were well-vascularised with an uniform poorly differentiated histomorphology¹². This improved intratumoral drug delivery and distribution led to a higher response in terms of a complete cure in FaDu (from 30% with drug alone to 100% with the combination, Table 4) and in general in the poorly differentiated tumor xenografts with the tumor not recurring even after 3–6 months of remission. In general, where a significant reduction in MVD was seen, the tumor seems to respond better to combination chemotherapy (Table 4). Interestingly, SLM/MSc was found to be protective against the cytotoxic effects of anticancer drugs on normal tissues allowing for a scaling up of the MTD to double the usual MTD and enabled a better therapeutic response even in tumors that were otherwise not responsive to the combination at the usual MTD¹⁰. Thus, in the case of the surgical samples of HNSCC growing in scid mice, this chemoprotective effect of Se allowed for dose escalation to double the MTD¹⁰ and this escalated dose translated into a higher (75% for Se+CPT-11 vs. 25% with CPT-11 alone, Table 4) cure rates in the heterogeneous but poorly differentiated #17073 despite only a 10% improvement in tumor normalization and no significant effects on tumor MVD or IFP by Se. The ability to protect normal healthy tissues from the cytotoxic effects of a chemotherapeutic drug is an attractive feature offered by Se that allows for protection from toxicity and enable administration of higher doses of the cytotoxic agent otherwise not feasible. This chemoprotective ability of Se over healthy tissues while chemosensitizing the tumor is a feature not available with other antiangiogenic agents that are in fact poorly tolerated when compared with the organoselenium compounds in mammals including humans. Se-induced enhancement of chemotherapeutic efficacy was found to be independent of the host, tumor type and the chemotherapeutic drug used. Further, reports suggests that use of Se in combination chemotherapy is likely to prevent development of a resistance tumor phenotype⁹⁶. Benefits in already concluded clinical trial with Se includes disease stabilization that has been reported for sodium selenate used as a monotherapy in asymptomatic chemotherapy-naïve, castration-resistant prostate cancer⁸¹. In another phase I clinical trial⁸, SLM used in combination chemotherapy with fixed dose of irinotecan gave two partial responses lasting 7 and 10 months in patient with colorectal cancers who had earlier progressed on 5-FU, leucovorin, oxaliplatin, and bevacizumab but had no prior irinotecan treatment. Further, in the same study, twelve patients had stable disease (SD) of which six were confirmed with subsequent CT scans and the SD in these six patients lasted between 7 and 12 months. Five of these six patients had metastatic colorectal cancer while one patient had non-small cell lung cancer. 4 patients with confirmed SD had prior irinotecan exposure. The use of Se in combination chemotherapy for an enhanced therapeutic efficacy in various malignancies is promising especially in tumors with a more uniform morphology such as in small cell lung cancer where initial response to chemotherapy often is followed by drug resistance and tumor re-growth. Use of Se in combination chemotherapy is likely to mimic the result described in the preclinical model, where a complete remission was seen even when the tumor was relatively larger (>1000–1500 mm³, Table 4) at the start of combination therapy with Se³.

3.3 Protection from cytotoxicity in normal healthy tissues by selenium

Se use in combination chemotherapy has been found to have the additional advantage of protecting healthy tissues from specific drug induced toxicities^{97, 98} such as in cisplatin^{11, 99–101} presumably at least partly through a p53 dependent mechanism¹⁰². A significant reduction in hair loss, flatulence, abdominal pain, weakness, malaise, loss of appetite was noted when Se was given with chemotherapy in ovarian cancer^{97, 98}. Se was also found to be protective against cisplatin-induced hearing loss¹⁰⁰. This effect is more likely to be when higher dose of Se is given more frequently since the increase in plasma antioxidant concentration is otherwise not maintained during the chemotherapy phase¹⁰⁰.

Thus, when given as Seleno-Kappacarrageenan at a dose of 4000 µg per day starting four days before and continuing up to 4 days after chemotherapy with cisplatin, Se was found to protect from drug induced nephrotoxicity and bone marrow suppression⁹⁹.

Se has been shown to down regulate HIF-1α¹⁰³, a key upstream regulator that influences >100 genes involved in tumor survival, metabolism and neoangiogenesis⁴⁴. This effect is due to Se-induced down regulation of reactive oxygen species (ROS) leading to stabilization of prolylhydroxylase (PHD) 2 & 3 and the consequent degradation of HIF-1α¹⁰³. HIF-1α independent proangiogenic factors have also been shown to be dependent on ROS-mediated activation and since Se has been shown to down regulate ROS generation in cancer cells, it is likely that Se will be also able to inhibit a wide range of alternative angiogenic pathways. In addition to down regulating HIF-1α, Se has been shown to down regulate COX2^{92, 93} and iNOS⁹³⁻⁹⁵ - two additional and critical cellular regulators of tumor angiogenesis. Se has been shown to sensitize hypoxic cancer cells to cancer chemotherapy¹⁰³. This may have implications in retarding tumor resistance and metastasis. Thus, Se affects multiple anticancer targets with different mechanisms of action that the tumor is unlikely to circumvent completely. In view of the negative results from the recently concluded SELECT trial⁷ involving Se where the enrolment of participants irrespective of their baseline Se status was a major flaw in the study design¹⁰⁴, it should be pertinent to point out that the dose of Se used in the SELECT trial is not a chemotherapeutically relevant dose for use in combination therapy and thus the results from that trial does not alter the prospective use of Se as an antiangiogenic agent as a monotherapy or in combination therapy at the higher doses described here.

4 Conclusions

The organoselenium forms of Se, specifically MSC, has a greater promise for use in the clinic for treating solid malignancies as a promising cost-effective antiangiogenic agent in combination chemotherapy that the tumor is unlikely to be able to surmount easily due to targeting of different upstream angiogenic regulators such as HIF-1α, COX-2 and iNOS2. Besides, Se targets various independent anticancer pathways further enhancing its anticancer properties. Further, being part of the mammalian system, it is very well tolerated as was evidenced by the use of high dose of SLM in the clinic⁸. Of the various organoselenium compounds, MSC is likely to be the most efficacious since it is stable at room temperature and gets converted to the active intermediate methylselenol through one-step β-lyase conversion and does not get as easily incorporated into normal cellular proteins.

5. Expert opinion

Antiangiogenic agents that target a single angiogenic molecule are likely to have a limited clinical success due to the ability of the tumors to transiently surmount such blockade. Further, their use is limited by the risk of adverse effects on normal tissues and organs as has been the experience with many of the currently used antiangiogenic agents in the clinic (Table 2). In contrast, MSC as an antiangiogenic agent targets multiple upstream angiogenic molecules and anticancer pathways that a tumor is unlikely to surmount easily. MSC being tolerated at the higher therapeutic doses, at least in the preclinical model, is likely to be more successful in treating solid malignancies either as a monotherapy as in clear cell renal cell carcinoma or in a combination therapeutic setting. It may not lead to an immediate rebound hypervascularization in the tumor after therapy withdrawal as seen with monoclonal antibodies against VEGF. Further, by sensitizing hypoxic tumor cells to anticancer drugs, it may retard tumor resistance to therapy. Amongst the various solid malignancies, MSC is likely to be most effective in the treatment of uniformly poorly differentiated tumors that are uniformly well vascularised as for example is the case of small cell lung cancers that are

known to be sensitive to chemotherapeutic drug but nonetheless acquire resistance over time. Further, as a result of protecting the healthy tissues and organs from the cytotoxic effects of chemotherapy, a higher systemic dose of the drug is likely to be well tolerated when administered in combination with MSC. Besides being antiangiogenic, MSC is known to impact various tumor cell survival pathways and this may enhance its' clinical efficacy. This efficacy is likely to be more promising in chemo-naïve cases where unlike in the phase I clinical trials, the tumors have not yet acquired resistance through clonal selection resulting from an aggressive and prior use of various chemotherapeutic regimen. Use of MSC is likely to be more economically much more viable with an attractive cost-benefit compared to most other currently available antiangiogenic agents in the clinic. The use of MSC is not fraught with any known contraindications. For increased efficacy of MSC or any such other potent antiangiogenic agent, consideration needs to be given to the tumor morphologic architecture and heterogeneity, failing which, such new agent/s will at best be only partially successful. Additionally, the use of agents that can abrogate hypoxic, differentiated or necrotic avascular tumor regions, prior to the use of an antiangiogenic agent in combination chemotherapy, is likely to meet with better success in the clinic.

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Figure 1.

Microphotographs of CD31 immunostaining of tumor microvessels (*brown*) demonstrating tumor architectural and vascular heterogeneity that impedes chemotherapeutic efficacy by limiting intratumoral drug delivery and distribution (All magnifications, X100). Human head and neck squamous cell carcinoma (HNSCC) FaDu xenograft (A) is poorly differentiated and uniformly vascularised while A253 (B) is well-differentiated with differentiated islands (*arrow*) containing no vasculature. Similarly human colorectal cancer xenograft HT-29 (C) and lung adenocarcinoma xenografts A549 (D) are differentiated and the differentiated regions with glandular structures do not contain tumor vasculature (*arrows*) making it difficult for an effective chemotherapeutic drug to access each and every single proliferating tumor cells. In human patients, this situation is often further aggravated with more heterogeneity as can be seen in the patient samples of poorly differentiated HNSCC (E) where despite being poorly differentiated the wide spread regions of necrosis (*arrow*) imparts a heterogeneous vascular distribution which impedes chemotherapeutic efficacy. In the well-differentiated samples of patient HNSCC (F), the heterogeneity is further increased with the presence of many concentric keratin pearls of avascular differentiated regions.

Table I

Improving tumor drug delivery.

Strategy	Mode of Action	Remarks
Tumor vascular maturation	Antiangiogenic agents used to 'normalize' tumor blood vessels	Instead of obliterating tumor vasculature, the goal is to slow down angiogenesis allowing time for maturation through recruitment of proper pericyte coverage. It results in a lower tumor IFP ^{3,12}
Overcoming drug sequestration	Varying pH that allows for example to decrease uptake of basic drugs into acidic endosomes	Though helpful, yet by itself may not overcome the architectural morphologic intratumoral barrier ¹⁴
Lowering tumor IFP	Targeting important players such as VEGF, PDGF etc that affect tumor vasculature/stroma and leads to a reduced IFP	Similar effect is often achieved using strategy ¹²
Altering tumor ECM	Agents such as collagenase, relaxin etc which degrades ECM	Can possibly increase metastatic spread
Macromolecular drug carrier	Agents such as dextran to facilitate uptake of the drug by the tumor	Generally localizes close to the tumor blood vessels and ineffective against cancer cells farther away ¹⁴
Using liposomal/polymetric micellar carrier	Allows the drug to get concentrated inside the tumor in the initial 1–3 days. Later on the accumulated drug diffuses into the tumor	Various factors including affinity for anatomical location can hinder its antitumor efficacy
Nanotechnology-based systems	Can be programmed to facilitate better drug delivery by responding to physical or biological stimuli	The development of an optimal agent is still awaited
Modifying active drug or using a prodrug	Once the tumor retains sufficient therapeutic threshold, drug will be activated to its active form	Attractive strategy applicable to some cancer disease site and used as in photodynamic therapies

Table 2

Drugs targeting tumor vasculature 29,30, 51

Name	Target/Mode of action	Toxicity
Recombinant/Fusion protein:		
Avastin (Bevacuzumab)	VEGF-A	Hypertension and hemorrhage.
Angiostatin	Direct inhibitor of angiogenesis	Neutropenia, fatigue, dyspnea, transient ischemic attack, VTE, CVA and intracranial hemorrhage
Aflibercept	VEGF-Trap targets VEGF-A and PlGF	Hypertension, abdominal pain, anorexia, fatigue, headache, arthralgia, bowel perforation, diarrhea.
Endostatin	Direct inhibitor of angiogenesis	Abdominal pain, bowel obstruction, gastrointestinal hemorrhage and myocardial infarction
Atrasentan	Small molecule inhibitor of endothelin A receptor	Headache, peripheral edema and rhinitis
Imatinib (Gleevec)	Targets pericytes. Inhibits PDGFR- β	Nausea, vomiting, diarrhea, edema, hemorrhage, fatigue, muscle cramps and rash
Interferon-alpha	Inhibits angiogenesis in part by downregulating bFGF expression	Flu-like symptoms, nausea, anorexia, diarrhea, fatigue, depression, dysrhythmia, paresthesia, headache, alopecia and skin rash
LY317615	inhibits protein kinase C β	Thrombocytopenia, fatigue, peripheral edema, nausea
2-Methoxyestradiol	Small molecule inhibitor that downregulates HIF-1 α	
Neovastat (AE941)	Inhibits VEGF signaling and MMP activity. Induces endothelial cell apoptosis	Altered taste, nausea, vomiting, dyspepsia, constipation, diarrhea, anorexia
PI-88	Heparan sulfate mimetic that targets endothelial cells	
SU11248	Targets pericytes. Inhibits PDGFR- β	Fatigue, diarrhea, nausea, vomiting, heartburn, hypertension, low blood count
VEGF-Trap	Soluble VEGF receptor	Hypertension and proteinurea
Tyrosine kinase inhibitors:		
ZD6474	VEGF-R1, -2, -3 and EGFR	Nausea, diarrhea, hypertension, proteinuria, ataxia
Sorafenib	VEGFR, Raf, PDGFR, cKIT, FLT-3	Diarrhea, hypertension, hand-foot syndrome, facial erythema, alopecia, hypertension and hemorrhage
Sunitinib	VEGFR, FLT-3, CSF-1R, RET	Diarrhea, hypertension, hand-foot syndrome, hypertension, ATE, hemorrhage, periorbital edema and hair depigmentation.
Vatalanib	VEGFR, PDGFR	Nausea, diarrhea, hypertension, proteinuria, ataxia, ATE, VTE, hypertension
CHIR-258	VEGFR, PDGFR, FGFR	Hypertension, myelosuppression, fatigue, anorexia, elevated troponin I, $\downarrow$ LVEF
KRN951	VEGFR, PDGFR, cKIT	Hypertension, proteinuria, ataxia and intracranial hemorrhage
Pazopanib	VEGFR, PDGFR, c-Kit	Liver enzyme elevation, hypertension and diarrhea
Axitinib	VEGFR, PDGFR, c-Kit	Hypertension and stomatitis
Immunomodulatory derivative:		
Thalidomide	Inhibits NF- κ B, TNF- α , IL-6, and VEGF	Constipation, sedation, neuropathy and VTE
Lenalidomide	Unknown mechanism	Myelosuppression and VTE
Actimid	Unknown mechanism	VTE

<u>Name</u>	<u>Target/Mode of action</u>	<u>Toxicity</u>
VDA's such as:		
Combrestatin A4P	Tubulin	Cardiac ischemia, ataxia, tumor pain, thrombocytopenia, hypotension
TZT-1027	Tubulin	Neutropenia, neuropathy, nausea, vomiting, fatigue, tumor pain and phlebitis
ZD6126	Tubulin	Pulmonary embolism, myocardial ischemia and decreased LVEF
DMXAA	TNF- α	Confusion, tremor, headache, visual changes, myocardial ischemia
ADH-1	N-cadherin	Cardiac ischemia, flushing, fatigue, nausea, dysgeusia, hypertension and pulmonary hemorrhage
Selenomethionine	HIF-1 α , PHDs, VEGF, iNOS and Cox-2	Garlic odor

Table 3

Various Selenium compounds used in cancer therapy^{62, 84}

Se Compound	Active metabolites	Remarks
Sodium selenite	Hydrogen selenide	50% absorbed and retained. Genotoxic, induces single strand DNA breaks in vitro.
Sodium selenate	Hydrogen selenide	Almost completely absorbed but most gets excreted in urine before being incorporated into protein. Activator of PP2A phosphatase
Methyl seleninic acid	Methyl selenol	Have a toxic effect and low dose tolerance
Selenocysteine	Methyl selenol, Hydrogen selenide	
Methylselenocysteine	Methyl selenol	90% absorbed. Mode of action through retarding neoangiogenesis and inhibition of cell cycle regulatory proteins. Relatively less binding to plasma components and well tolerated. Single step conversion to methylselenol.
Selenomethionine	Methyl selenol, selenomethionine	Binds to plasma components. Well tolerated. Multiple step conversion to methylselenol.

Enhanced tumor drug delivery as a consequence of antiangiogenic effects of SLM/MS 3,12

Table 4

Human Tumor Xenografts growing subcutaneously in nude or scid mice	Response (cure) to chemotherapy		Reduction in MVD	Increase in Vascular maturation	Reduction in IFP	Improved Doxorubicin Delivery at ~90 μm from tumor vessel
	± CPT-11 (100 mg/kg i.v. × 4 weekly)					
	Drug alone	Combination with Se				
1. Head & Neck Squamous Cell Carcinoma (HNSCC):						
Uniformly Poorly differentiated	30%	100%	40%	30%	NS	Significantly ↑
FaDu, Well-differentiated A253.	10%	60%	59%	31%	30%	Significantly ↑
2. Colorectal Cancer:						
Uniformly poorly differentiated	20%	100%	46%	48%	43%	Significantly ↑
HCT-8; Well-differentiated adenocarcinoma HT-29.	0%	20%	52%	17%	NS	Significantly ↑
± CPT (100 mg/kg i.v. × 4 weekly) or Taxotere (60 mg/kg i.v. × 1)						
3. Lung Cancer:						
	Drug alone	Combination with Se				
Poorly differentiated small cell lung cancer H69 (>1000–1500 mm ³ at start of Rx); Well differentiated non small cell lung cancer A549.	0% with taxotere 20% with CPT-11 0% with either	20% 60% 0%	62% NS	50% 6%	NS NS	Significantly ↑ Significantly ↑
± CPT (200 mg/kg i.v. × 4 weekly)						
4. Human Surgical Samples of HNSCC growing in scid mice:						
	Drug alone	Combination with Se				
Heterogeneous poorly Differentiated #17073; Well-differentiated #16653.	25% 0%	75% 0%	NS NS	10% NS	ND ND	Significantly ↑ NS

NS – Not significant; ND – Not done