Review

Expert Opinion

informa healthcare

Methylselenocysteine – a promising antiangiogenic agent for overcoming drug delivery barriers in solid malignancies for therapeutic synergy with anticancer drugs

Arup Bhattacharya
Roswell Park Cancer Institute, Department of Cancer Prevention and Control, Buffalo, NY, USA

Introduction: Despite progress, chemotherapeutic response in solid malignancies has remained limited. Although initial results of the use of antiangiogenic agents in combination chemotherapy indicated an enhanced therapeutic response, recent data indicate that the surviving cancer is not only able to surmount therapy, but also 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 antiangiogenic agents used at present in the clinic, the putative benefits of the use of organoselenium 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 downregulates 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.

Keywords: antiangiogenic therapy, interstitial fluid pressure, intratumoral drug delivery, methylselenocysteine, selenomethionine, tumor vascular normalization

Expert Opin. Drug Deliv. (2011) *8*(6):749-763

1. Introduction

Cancer remains one of the leading causes of death in the US [1] and in many parts of the world. Although surgery remains the mainstay for removal of the bulk of the disease, systemic therapies including chemotherapy remain the treatment of choice in an adjuvant or neoadjuvant setting. Despite much progress in the development of new 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 the arrival of > 20 new anticancer agents over the past decade, the only exceptions being testicular cancer, gestational choriocarcinomas, Hodgkin's disease and high-grade lymphomas that, owing 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 new. Factors at coarser physiological levels can impede drug delivery and distribution and thereby critically influence therapy outcome [3,4].

10.1517/17425247.2011.571672 © 2011 Informa UK, Ltd. ISSN 1742-5247

Article highlights.

- Selenium downregulates key upstream angiogenic master regulators such as HIF-1α, COX2 and iNOS2.
- The organoselenium compound methylselenocysteine is an amino acid that is well tolerated in mammals. It is converted to the active methylselenol through one-stage β-lyase conversion and remains more bioavailable for anticancer effects compared with the other forms.
- Antiangiogenic oragno-selenium compounds have a low cost and toxicity advantage over other clinically used antiangiogenic agents. As a monotherapy, in the preclinical model it shows similar or better efficacy as other effective single antiangiogenic agents in the clinic.
- Tumor vascular maturation by Se enables a significantly enhanced tumor-specific drug delivery at the same systemic drug dose delivered in combination chemotherapy with Se compared with the drug alone. This results in an enhanced tumor response rates, including complete remission in the preclinical model.
- Use of Se as a therapeutic agent has more promise in the clinic for highly angiogenic tumors such as the clear cell renal cell carcinoma and in uniformly vascularized morphologically non-heterogeneous tumors such as in small cell lung cancer. Further clinical trials are warranted for use of Se in combination chemotherapy in these cancer types.
- Protection of normal healthy tissues from the cytotoxic effects of chemotherapy by Se makes its use attractive in the clinic in a combinatorial setting.

This box summarizes key points contained in the article.

Selenium (Se) is a natural element present in the earth's crust often in association with sulfur-containing compounds. Humans get their dietary requirements mainly from food. Dietary Se is in the range 29 – 152 μg/day in Europe and North America. Selenium 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 > 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 were 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/day L-selenomethionine (SLM) used in the SELECT trial [7], this 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 decreased chemotherapy-induced toxicity coupled with 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 seen 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 were 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]. The development of new anticancer agents and therapeutic combinations has focused almost exclusively on molecular targets, with often little concern about the architectural and morphologic heterogeneity that remain 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 the presence of large hypoxic, avascular necrotic or well-differentiated regions, expression of cell surface receptors, and 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**). Although tumor cell differentiation does not contribute directly to tumor propagation and growth, 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 region that actually sustains and protects proliferating cancer cells from receiving the cytotoxic doses of the anticancer drug as they are 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 owing to a non-uniform distribution of tumor blood vessels where large areas of tumors do not contain blood vessels (arrows, **Figure 1B – D, F**). Thus, whereas 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]. By 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, respectively [10]. Further, clinical samples of poorly differentiated human patient tumors #17073 (arrow, **Figure 1E**) growing in SCID mice showed a heterogeneous architecture with the presence of many large

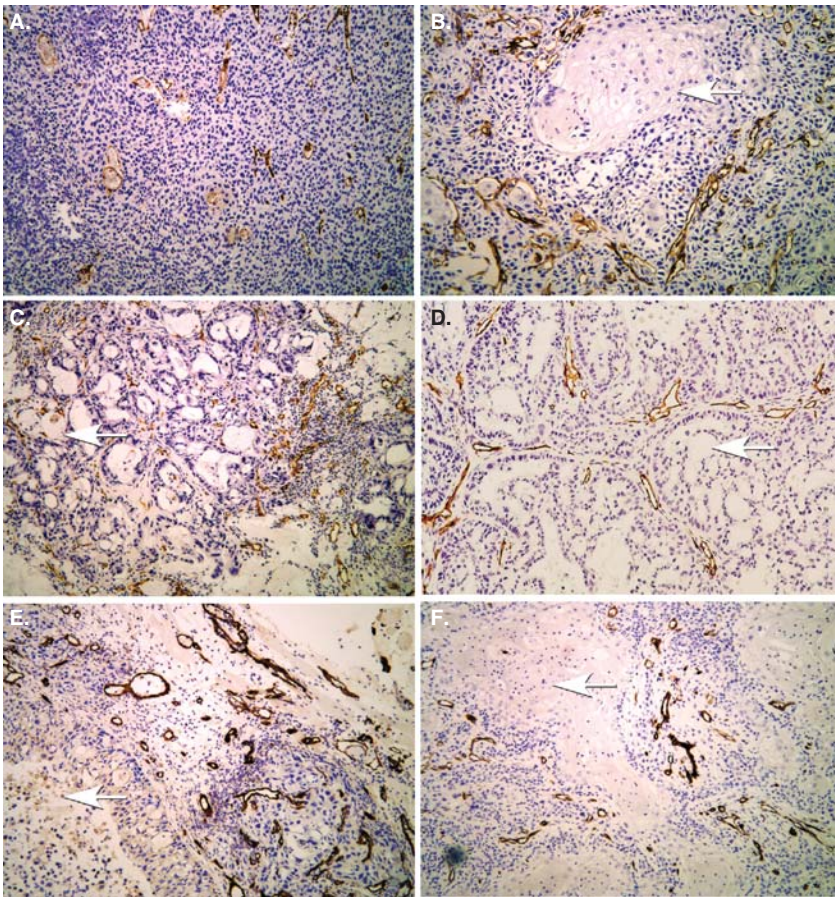

Figure 1. Microphotographs of CD31 immunostaining of tumor microvessels (brown) demonstrating tumor architectural and vascular heterogeneity that impedes chemotherapeutic efficacy by limiting intratumoral drug delivery and distribution (all magnifications × 100). Human HNSCC FaDu xenograft (**A**) is poorly differentiated and uniformly vascularized, whereas A253 (**B**) is well differentiated with differentiated islands (arrow) containing no vasculature. Similarly, human colorectal cancer xenograft HT-29 (**C**) and lung adenocarcinoma xenograft 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 cell. In human patients, this situation is often aggravated further with more heterogeneity, as can be seen in the patient samples of poorly differentiated HNSCC (**E**), where despite being poorly differentiated the widespread regions of necrosis (arrow) impart a heterogeneous vascular distribution that impedes chemotherapeutic efficacy. In the well-differentiated samples of patient HNSCC (**F**), the heterogeneity is increased further with the presence of many concentric keratin pearls of avascular differentiated regions.
HNSCC: Human head and neck squamous cell carcinoma.

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 alone [3]. A reduced interstitial space and extracellular matrix in such regions is likely to lower further the extravasation of the drugs from the blood vessels farther into the tumor by retarding convection and/or diffusion [16]. 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 owing to clonal selection of a resistant phenotype, as is seen in the clinic in many human cancers, such as small cell lung cancer, which often respond 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 is 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 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 impart an aberrant blood flow that, 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 become more compressed owing to increased IFP, which results in blood flow being diverted from the center of the tumor to the periphery. Drug delivery is retarded further 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 that often does not allow the drug to penetrate the required distance of 200 μm to reach most viable cells within human tumors [14]. 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 used to improve tumor drug delivery and efficacy, as summarized in **Table 1**. Of these, antiangiogenic agents and vascular disrupting agents (VDAs) affect 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 chemotherapy [21]. 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 are 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 now in clinical use, such as bevacizumab, a ligand-trapping monoclonal antibody against VEGF, and 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/fluorourail/leucovorin) alone to 20.3 months in the combination group in a Phase III study with previously untreated metastatic colorectal cancer [22]. 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 cancer [23]. 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 with 12.3 months in the combination group with bevacizumab [24]. Bevacizumab did not show any significant improvements in other Phase II and III clinical trials in previously untreated metastatic colorectal cancer [25] and metastatic breast cancer [26], 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 vatalanib [27]. A 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) alone [28]. Thus, most single target-based antiangiogenic agents seem to induce a transient functional normalization followed by chronic angiogenic inhibition that typically reduces uptake of the chemotherapeutic agent [29]. Improved survival is more likely to be obtained with broad spectrum multitargeted tyrosine kinase inhibitors such as sorafenib and sunitinib when used as monotherapy [30].

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 available antiangiogenic agents targeting VEGF is limited by 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 blockade [33]. Rebound hypervascularization promoting invasion and metastasis has also been reported in the mouse model after discontinuing the antiangiogenic drug [34]. 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 agents [35]. These evasive responses by tumors involve upregulation of alternative proangiogenic signals, such as platelet-derived growth factor (PDGF), placental growth factor (PIGF), angiopoietin-1, fibroblast growth factor family members and invasion of the tumor cells into local tissue to co-opt normal vasculature. Tumors at an advanced stage of progression in a patient with a history of multiple chemotherapeutic interventions are likely to foster further intrinsic resistance in the tumor microenvironment. Antiangiogenic agents cause tumor vascular maturation through increased pericyte coverage and result in 'normalization' of tumor vessels. The combined effect of a 'normalized' vessel and a consequently reduced tumor IFP is that the intratumoral drug delivery is enhanced significantly [12]. Use of antiangiogenic agents has also been reported to enhance therapeutic efficacy by reducing tumor stem-cell-like fraction [36]. A higher pericyte coverage that is responsible for vessel maturation can also lead to resistance

Table 1. 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, 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 lead to a reduced IFP	Similar effect is often achieved using tumor vascular maturation strategy [12]
Altering tumor ECM	Agents such as collagenase, relaxin, etc. that degrade 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/polymeric micellar carrier	Allows the drug to be 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 sites and used as in photodynamic therapies

ECM: Extracellular matrix; IFP: Interstitial fluid pressure; PDGF: Platelet-derived growth factor.

to anti-VEGF therapy because it is not as dependent on VEGF-mediated survival signaling [35]. Pericytes, one of the key components of tumor vascular maturation, have been shown to allow some tumors to retain a core of pre-existing tumor vasculature live and functional despite the use of therapies that impair neoangiogenesis or promote vascular regression [37-39]. Therapeutically targeting pericytes is fraught with several problems. It can lead to an enhanced metastasis [40], take away the advantage of increased drug delivery within a tumor at a given systemic drug dose, and may lead to ascites or edema owing to increased vessel leakage [41]. Often the antiangiogenic therapy leads to acute reactive hypoxia at the peak of the response phase, which leads to upregulation of various tumor survival genes, including those of other proangiogenic signals. Tumor hypoxia, often accentuated by the use of antiangiogenic agents, leads to the recruitment of bone marrow-derived progenitor cells by the tumor for forming new vessels. This mechanism is generally through upregulation of hypoxia-inducible factor (HIF)-1α [42,43] signaling cascade, which is critical for tumor survival, proliferation and invasion, and can induce a vast array of > 100 genes that influence tumor survival, metabolism and neoangiogenesis [44]. In some solid malignancies, such as in clear cell renal cell carcinoma, where chemotherapeutic regimen has been primarily unsuccessful at retarding tumor growth, antiangiogenic agents sunitinib and bevacizumab are the first-line standards of care at present [45]. In others, such as in the colorectal cancers, antiangiogenic agent is now recommended only in metastatic disease and for use in the adjuvant setting [46].

2.2 Adverse factors affecting the use of antiangiogenic agents

The use of many 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]. Also, the use of antiangiogenic agents is contraindicated in patients with squamous cell lung cancer [52]. Other antiangiogenic agents such as thalidomide and lenalidomide are known to cause venous thromboembolism [53]. Antiangiogenic agents are known to have adverse renal effects such as proteinuria, acute kidney injury thrombotic microangiopathy, other glomerular lesions and interstitial nephritis [54]. 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

Selenium is an essential trace element required for the proper functioning of various selenoproteins, including GPx and

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 PIGF	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 (Gleevac)	Targets pericytes. Inhibits PDGF-R	Nausea, vomiting, diarrhea, edema, hemorrhage, fatigue, muscle cramps and rash
Interferon-α	Inhibits angiogenesis in part by downregulating bFGF expression	Flu-like symptoms, nausea, anorexia, diarrhea, fatigue, depression, dysrythmia, paresthesia, headache, alopecia and skin rash
LY317615	Inhibits protein kinase Cβ	Thrombocytopenia, fatigue, peripheral edema, nausea
2-Methoxyestradiol	Small molecule inhibitor that downregulates HIF-1α	
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, c-KIT, 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,↓ LVEF
KRN951	VEGFR, PDGFR, c-KIT	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
VDAs 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-1α, PHDs, VEGF, iNOS and Cox2	Garlic odor

TrxR, as it is an essential constituent of selenocysteine. Se plays a critical role as a cellular antioxidant, and detoxification with Se status has an inverse association with cancer risks [6,55,56]. Various natural and synthetic forms of Se, such as selenite, selenate, methylselininic acid (MSA), SLM, selenocysteine, MSC, selenobetaine, *p*-methoxybenzeneselenol, benzylselenocyanate, 1,4-phenylene-bis(methylene) selenocyante, 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 and growth, besides enabling the protection of healthy tissues from the cytotoxic effects of chemotherapeutic drugs. Selenium affects multiple targets in cancer and has been shown to affect cell cycle, induce apoptosis, and retard cell migration and invasion [57]. The active metabolites of some of the Se compounds are listed in **Table 3**. Whereas both inorganic selenite and selenide induce apoptosis through single-strand DNA breaks, the organoselenium 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 properties [62]. 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 is incorporated into cellular proteins, preventing release of its monomethylated Se moiety, is likely to reduce the anticancer potential of the Se compound. MSC has been reported to be a better anticancer agent compared with SLM, presumably because of its easy conversion to the active methylselenol. Stable monomethylated compounds such as MSC or selenobetaine act as precursors by releasing methylselenol through the action of lyases [63]. Selenium affects multiple anticancer pathways that hinder cancer initiation, growth and progression. Some of these are summarized as follows.

- Antiangiogenic and tumor vascular normalization effects leading to higher intratumoral drug delivery of anticancer drug(s) used in combination chemotherapy (**Table 4**) [3,12,13].
- Altered methionine metabolism leading to an increased sensitivity to caspase-mediated apoptosis.
- Retarding cell doubling time by increasing cell duration in G1, S and G2 phases of the cell cycle [61,64].
- Induction of genes involved in Phase II enzyme activity and downregulation of genes related to Phase I enzyme and cell proliferation [65].
- Inhibiting key targets such as cyclin A, cyclin D1, CDC25A, CDK4, PCNA, E2F gene expression and arresting cells in the G1 phase of the cell cycle [66].
- Inducing expression of P19, P21, P53, GST, SOD, NQO1, GADD153 and certain caspases [66].
- Possibly retarding metastasis because genes such as osteopontin are downregulated by Se [66].
- Modulating the immune system [67].

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 versus 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 is incorporated into selenoproteins [73]. Methylselenol and dimethylselenide are intermediate metabolites excreted through breath and urine [74]. The anticarcinogenic properties of organoselenium compounds have been attributed mainly to methylselenol [75-77]. Whereas inorganic selenite is toxic at doses beyond 5 p.p.m. [78,79], organoselenium compounds such as SLM are well tolerated in humans where a dose of 7200 μg 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 effects [8]. 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 treatment [8]. In the same studies all patients given 4800 μg twice daily had plasma Se levels > 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 men [80]. Dose-limiting toxicities for sodium selenite were fatigue 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 more cytotoxic properties when compared with selenate [81] or SLM/MSC. Among the organoselenium compounds, MSC is better than SLM in anticancer efficacy because it is converted to the active methylselenol through one-stage β-lyase conversion [82,83], whereas SLM is incorporated into proteins because it easily acylates Met-tRNA or is converted through the transsulfuration mechanism to selenocysteine, which is then degraded to hydrogen selenide by β-lyase [84]. Selenium compounds that directly enter the methylated pool are more likely to be effective than the Se compounds that are metabolized through the 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 setting [84].

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 is excreted in urine before being incorporated into protein. Activator of PP2A phosphatase
Methyl selininic 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

Further, selenobetaine must lose one methyl group first before dissociation of the Se-methylene carbon bond to form methylselenol [85], unlike MSC, which undergoes one-step conversion to methylselenol. Interestingly, in some of the preclinical animal studies, the use of the MTD of organoselenium compounds SLM and MSC did not show any difference in therapeutic efficacy [10]. Whereas MSC induces apoptosis in the G1 phase, selenite induces necrosis in the S/G2-M phase of the cell cycle [86]. MSA, another organoselenium 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 NADPH [87]. *In vitro*, MSA is more potent than MSC owing to its ability to deliver the monomethylated Se species directly to the cells, but *in vivo* both show similar efficacy [87], whereas MSA has a higher toxicity profile when compared with SLM/MSC [88].

3.2 Selenium as an antiangiogenic agent

Whereas 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 brain [89]. Though most selenocompounds (**Table 3**) have been reported to have antiangiogenic [90,91] and possibly vessel normalization effects in tumors, the organoselenium compounds MSA, SLM and MSC have been studied extensively in the preclinical system, where both the antiangiogenic and vascular normalization events have been confirmed and SLM has 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 cancer and lung cancer [3]. In addition to downregulating HIF-1α, Se has been shown to downregulate cyclooxygenase 2 (COX2) [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. Although as a single agent SLM and MSC had a modest ≥ 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 clinic [80] for its *in vivo* efficacy in retarding tumor growth, and has the clinical potential to improve 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 clear cell renal cell carcinoma.

3.3 Antiangiogenic selenium in combination chemotherapy

Selenium, when used in combination with chemotherapeutic agents in the preclinical models, gave complete remission in many tumor types [10,83]. This therapeutic response was found to be inversely associated with tumor morphologic heterogeneity, which translated to a corresponding heterogeneity in terms of tumor vascular distribution [3]. Well-differentiated tumor regions, despite being hypoxic and secreting VEGF, do not allow the formation of new tumor blood vessels [4]. 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 leads to a lower tumor microvessel density (MVD) and increased vascular maturation that often corresponds with an improved, reduced tumor IFP, resulting in increased drug delivery at a distance of ~ 90 μm from the blood vessel. Selenium-induced improvement in tumor drug delivery at the same administered systemic drug dose resulted in a fourfold increase in intratumoral drug concentration in the head and neck human tumor xenografts FaDu that were well vascularized with a uniform poorly differentiated histomorphology [12]. 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. Enhanced tumor drug delivery as a consequence of antiangiogenic effects of SLM/MSC [3,12].

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
	Drug alone	Combination with Se				
1. HNSCC:	± CPT-11 (100 mg/kg i.v. × 4 weekly)					
Uniformly poorly differentiated FaDu	30%	100%	40%	30%	NS	Significantly ↑
Well-differentiated A253	10%	60%	59%	31%	30%	Significantly ↑
2. Colorectal cancer:						
Uniformly poorly differentiated HCT-8	20%	100%	46%	48%	43%	Significantly ↑
Well-differentiated adenocarcninoma HT-29	0%	20%	52%	17%	NS	Significantly
3. Lung cancer:	± CPT (100 mg/kg i.v. × 4 weekly) or taxotere (60 mg/kg i.v. × 1)					
Poorly differentiated small cell lung cancer H69 (> 1000 – 1500 mm^3 at start of Rx)	0% with taxotere 20% with CPT-11	20% 60%	62%	50%	NS	Significantly ↑
Well-differentiated non- small cell lung cancer A549	0% with either	0%	NS	6%	NS	Significantly ↑
4. Human surgical samples of HNSCC growing in SCID mice:	± CPT (200 mg/kg i.v. × 4 weekly)					
Heterogeneous poorly differentiated #17073	25%	75%	NS	10%	ND	Significantly ↑
Well-differentiated #16653	0%	0%	NS	NS	ND	NS

HNSCC: Head and neck squamous cell carcinoma; IFP: Interstitial fluid pressure; MSC: Methylselenocysteine; MVD: Microvessel density; ND: Not done; NS: Not significant; SLM: L-selenomethionine.

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 seemed 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 enabling a better therapeutic response even in tumors that were otherwise not responsive to the combination at the usual MTD [10]. Thus, in the case of the surgical samples of head and neck squamous cell carcinoma (HNSCC) growing in SCID mice, this chemoprotective effect of Se allowed for dose escalation to double the MTD [10], and this escalated dose translated into higher (75% for Se + CPT-11 versus 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 enables administration of higher doses of the cytotoxic agent that otherwise would not be 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. Selenium-induced enhancement of chemotherapeutic efficacy was found to be independent of the host, tumor type and chemotherapeutic drug used. Further, reports suggest that the use of Se in combination chemotherapy is likely to prevent the development of a resistance tumor phenotype [96]. Benefits in an already concluded clinical trial with Se include disease stabilization, which has been reported for sodium selenate used as a monotherapy in asymptomatic chemotherapy-naive, castration-resistant prostate cancer [81]. In another Phase I clinical trial [8], SLM used in combination chemotherapy with a fixed dose of irinotecan gave two partial responses lasting 7 and 10 months in patients with colorectal cancers who had earlier progressed on 5-FU, leucovorin, oxaliplatin and bevacizumab but had not had any previous irinotecan treatment. Further, in the same study 12 patients had stable disease (SD), of which 6 were confirmed with subsequent CT scans and the SD in these 6 patients lasted between 7 and 12 months. Five of these six patients had metastatic colorectal cancer, whereas one patient had non-small cell lung cancer. Four patients with confirmed SD had previous irinotecan exposure. The use of Se in combination chemotherapy for 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 results described in the preclinical model, where a complete remission was seen even when the tumor was relatively larger (> 1000 – 1500 mm^3; Table 4) at the start of combination therapy with Se [3].

3.4 Protection from cytotoxicity in normal healthy tissues by selenium

Selenium use in combination chemotherapy has been found to have the extra 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 mechanism [102]. A significant reduction in hair loss, flatulence, abdominal pain, weakness, malaise and loss of appetite was noted when Se was given with chemotherapy in ovarian cancer [97,98]. Selenium was also found to be protective against cisplatin-induced hearing loss [100]. This effect is more likely to be when a higher dose of Se is given more frequently because the increase in plasma antioxidant concentration is otherwise not maintained during the chemotherapy phase [100]. Thus, when given as Seleno-Kappacarrageenan at a dose of 4000 μg/day starting 4 days before and continuing up to 4 days after chemotherapy with cisplatin, Se was found to protect against drug-induced nephrotoxicity and bone marrow suppression [99].

Selenium has been shown to downregulate HIF-1α [103], a key upstream regulator that influences > 100 genes involved in tumor survival, metabolism and neoangiogenesis [44]. This effect is due to Se-induced downregulation of reactive oxygen species (ROS) leading to stabilization of prolylhydroxylase (PHD) 2 and 3 and the consequent degradation of HIF-1α [103]. HIF-1α-independent proangiogenic factors have also been shown to be dependent on ROS-mediated activation and, because Se has been shown to downregulate ROS generation in cancer cells, it is likely that Se will also be able to inhibit a wide range of alternative angiogenic pathways. In addition to downregulating HIF-1α, Se has been shown to downregulate COX2 [92,93] and iNOS2 [93-95] – two additional and critical cellular regulators of tumor angiogenesis. Se has been shown to sensitize hypoxic cancer cells to cancer chemotherapy [103]. 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 trial [7] involving Se where the enrolment of participants irrespective of their baseline Se status was a major flaw in the study design [104], 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 do 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, have greater promise for use in the clinic for treating solid

malignancies as a cost-effective antiangiogenic agent in combination chemotherapy that the tumor is unlikely to be able to surmount easily owing to the targeting of different upstream angiogenic regulators such as HIF-1α, COX2 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 a high dose of SLM in the clinic [8]. Of the various organoselenium compounds, MSC is likely to be the most efficacious because it is stable at room temperature and is converted to the active intermediate methylselenol through one-step β-lyase conversion and is not as easily incorporated into normal cellular proteins.

5. Expert opinion

Antiangiogenic agents that target a single angiogenic molecule are likely to have limited clinical success owing to the ability of the tumors to surmount transiently such a 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 antiangiogenic agents used in the clinic at present (**Table 2**). By 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. Among the various solid malignancies, MSC is likely to be most effective in the treatment of uniformly poorly differentiated tumors that are uniformly well vascularized, 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 affect various tumor cell survival pathways, and this may enhance its clinical efficacy. This efficacy is likely to be more promising in chemo-naive cases where, unlike in the Phase I clinical trials, the tumors have not yet acquired resistance through clonal selection resulting from an aggressive and previous use of various chemotherapeutic regimens. Use of MSC is likely to be economically much more viable with an attractive cost–benefit compared with most other 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's morphological architecture and heterogeneity, failing which, such new agent(s) will at best be only partially successful. Also, the use of agents that can abrogate hypoxic, differentiated or necrotic avascular tumor regions, before the use of an antiangiogenic agent in combination chemotherapy, is likely to meet with better success in the clinic.

Acknowledgements

The author acknowledges the work of his mentor Dr Youcef M Rustum and his group [3,4,8,10,12,16,21,103], which has provided the main impetus for the insights presented in this article.

Declaration of interest

The author confirms no conflict of interest and has received no payments in preparation of this manuscript. This paper is supported by Grant 1R21 CA 133682-01A2, National Cancer Institute, Bethesda, MD, USA.

Bibliography

Papers of special note have been highlighted as of interest (•) or of considerable interest (••) to readers.

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2009. CA Cancer J Clin 2009;8(4):225-49
2. Savage P, Stebbing J, Bower M, et al. Why does cytotoxic chemotherapy cure only some cancers? Nat Clin Pract Oncol 2009;6(1):43-52
3. Rustum YM, Toth K, Seshadri M, et al. Architectural heterogeneity in tumors caused by differentiation alters intratumoral drug distribution and affects therapeutic synergy of antiangiogenic organoselenium compound. J Oncol 2010;2010:396286

•• **Report on the antiangiogenic and vessel maturation effects of Se on various solid tumor xenografts from different disease sites and the influence of tumor morphologic architecture on therapy response.**

4. Bhattacharya A, Toth K, Mazurchuk R, et al. Lack of microvessels in well-differentiated regions of human head and neck squamous cell carcinoma A253 associated with functional magnetic resonance imaging detectable hypoxia, limited drug delivery, and resistance to irinotecan therapy. Clin Cancer Res 2004;10(23):8005-17

•• **A study on how the tumor differentiation renders a heterogeneous tumor architecture that fosters tumor resistance to therapy.**

5. Jackson MI, Combs GF Jr. Selenium and anticarcinogenesis: underlying mechanisms. Curr Opin Clin Nutr Metab Care 2008;11(6):718-26
6. Shamberger RJ, Frost DV. Possible protective effect of selenium against human cancer. Can Med Assoc J 1969;100(14):682
7. Lippman SM, Klein EA, Goodman PJ, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2009;301(1):39-51
8. Fakih MG, Pendyala L, Brady W, et al. A Phase I and pharmacokinetic study of selenomethionine in combination with a fixed dose of irinotecan in solid tumors. Cancer Chemother Pharmacol 2008;62(3):499-508
9. Azrak RG, Cao S, Pendyala L, et al. Efficacy of increasing the therapeutic index of irinotecan, plasma and tissue selenium concentrations is methylselenocysteine dose dependent. Biochem Pharmacol 2007;73(9):1280-7
10. Cao S, Durrani FA, Rustum YM. Selective modulation of the therapeutic efficacy of anticancer drugs by selenium containing compounds against human tumor xenografts. Clin Cancer Res 2004;10(7):2561-9

• **Report of MSC and SLM in the preclincal model using human tumor xenografts.**

11. Fakih M, Cao S, Durrani FA, Rustum YM. Selenium protects against toxicity induced by anticancer drugs and augments antitumor activity: a highly selective, new, and novel approach for the treatment of solid tumors. Clin Colorectal Cancer 2005;5(2):132-5
12. Bhattacharya A, Seshadri M, Oven SD, et al. Tumor vascular maturation and improved drug delivery induced by methylselenocysteine leads to therapeutic synergy with anticancer drugs. Clin Cancer Res 2008;14(12):3926-32

• **For details on tumor vascular maturation effect of MSC in a poorly differentiated human head and neck squamous cell carcinoma growing in nude mice.**

13. Bhattacharya A, Toth K, Sen A, et al. Inhibition of colon cancer growth by methylselenocysteine-induced angiogenic chemomodulation is influenced by histologic characteristics of the tumor. Clin Colorectal Cancer 2009;8(3):155-62
14. Tredan O, Galmarini CM, Patel K, et al. Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst 2007;99(19):1441-54
15. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 2010;1805(1):105-17
16. Rustum Y, Pendyala L, Creaven P, et al. A phase I dose escalation study of selenomethionine (SLM) in combination with fixed dose Irinotecan in patients with solid tumors. ASCO annual meeting proceedings. J Clin Oncol 2007;25(18S):2574
17. Jain RK. Delivery of molecular and cellular medicine to solid tumors. Adv Drug Deliv Rev 2001;46(1-3):149-68
18. Munn LL. Aberrant vascular architecture in tumors and its importance in drug-based therapies. Drug Discov Today 2003;8(9):396-403
19. Mollica F, Jain RK, Netti PA. A model for temporal heterogeneities of tumor blood flow. Microvasc Res 2003;65(1):56-60
20. Peterson HI. Modification of tumour blood flow–a review. Int J Radiat Biol 1991;60(1-2):201-10
21. Bhattacharya A, Toth K, Durrani FA, et al. Hypoxia specific drug tirapazamine does not abrogate hypoxic cells in combination therapy with irinotecan and methylselenocysteine in well-differentiated human head and neck squamous cell carcinoma A253 xenografts. Neoplasia 2008;10(8):857-65
22. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350(23):2335-42
23. Giantonio BJ, Catalano PJ, Meropol NJ, et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 2007;25(12):1539-44
24. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med 2006;355(24):2542-50
25. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 2003;21(1):60-5
26. Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med 2007;357(26):2666-76
27. Kohne C, Bajetta E, Lin E, et al. Final results of CONFIRM 2: a multinational, randomized, double-blind, phase III study in 2nd line patients (pts) with

metastatic colorectal cancer (mCRC) receiving FOLFOX4 and PTK787/ZK 222584 (PTK/ZK) or placebo. J Clin Oncol 2007;25(18S): ASCO Ann Meet Proc. Part I; 4033

28. Scagliotti G, Novello S, von Pawel J, et al. Phase III study of carboplatin and paclitaxel alone or with sorafenib in advanced non-small-cell lung cancer. J Clin Oncol 2010;28(11):1835-42
29. Ma J, Waxman DJ. Combination of antiangiogenesis with chemotherapy for more effective cancer treatment. Mol Cancer Ther 2008;7(12):3670-84
30. Jain RK, Duda DG, Clark JW, et al. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 2006;3(1):24-40
31. Verheul HM, Pinedo HM. Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition. Nat Rev Cancer 2007;7(6):475-85
32. Kamba T, McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. Br J Cancer 2007;96(12):1788-95
33. de Groot JF, Fuller G, Kumar AJ, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro Oncol 2010;12(3):233-42
34. Paez-Ribes M, Allen E, Hudock J, et al. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell 2009;15(3):220-31
35. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev 2008;8(8):592-603
36. Folkins C, Man S, Xu P, et al. Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. Cancer Res 2007;67(8):3560-4
37. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 2005;307(5706):58-62
38. Mancuso MR, Davis R, Norberg SM, et al. Rapid vascular regrowth in tumors after reversal of VEGF inhibition. J Clin Invest 2006;116(10):2610-21
39. Shaked Y, Ciarrocchi A, Franco M, et al. Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. Science 2006;313(5794):1785-7
40. Gerhardt H, Semb H. Pericytes: gatekeepers in tumour cell metastasis? J Mol Med 2008;86(2):135-44
41. Stoeltzing O, Ahmad SA, Liu W, et al. Angiopoietin-1 inhibits vascular permeability, angiogenesis, and growth of hepatic colon cancer tumors. Cancer Res 2003;63(12):3370-7
42. Ceradini DJ, Kulkarni AR, Callaghan MJ, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10(8):858-64
43. De Falco E, Porcelli D, Torella AR, et al. SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells. Blood 2004;104(12):3472-82
44. Manalo DJ, Rowan A, Lavoie T, et al. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 2005;105(2):659-69
45. Sun M, Lughezzani G, Perrotte P, et al. Treatment of metastatic renal cell carcinoma. Nat Rev Urol 2010;7(6):327-38
46. Hubbard J, Grothey A. Antiangiogenesis agents in colorectal cancer. Curr Opin Oncol 2010;22(4):374-80
47. Zhu X, Wu S, Dahut WL, et al. Risks of proteinuria and hypertension with bevacizumab, an antibody against vascular endothelial growth factor: systematic review and meta-analysis. Am J Kidney Dis 2007;49(2):186-93
48. Frangie C, Lefaucheur C, Medioni J, et al. Renal thrombotic microangiopathy caused by anti-VEGF-antibody treatment for metastatic renal-cell carcinoma. Lancet Oncol 2007;8(2):177-8
49. Hurwitz H, Saini S. Bevacizumab in the treatment of metastatic colorectal cancer: safety profile and management of adverse events. Semin Oncol 2006;33(5 Suppl 10):S26-34
50. Johnson DH, Fehrenbacher L, Novotny WF, et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. J Clin Oncol 2004;22(11):2184-91
51. Widakowich C, de Castro G Jr, de Azambuja E, et al. Review: side effects of approved molecular targeted therapies in solid cancers. Oncologist 2007;12(12):1443-55
52. van Heeckeren WJ, Sanborn SL, Narayan A, et al. Complications from vascular disrupting agents and angiogenesis inhibitors: aberrant control of hemostasis and thrombosis. Curr Opin Hematol 2007;14(5):468-80
53. Zangari M, Fink LM, Elice F, et al. Thrombotic events in patients with cancer receiving antiangiogenesis agents. J Clin Oncol 2009;27(29):4865-73
54. Gurevich F, Perazella MA. Renal effects of anti-angiogenesis therapy: update for the internist. Am J Med 2009;122(4):322-8
55. Clark LC, Combs GF Jr, Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional prevention of cancer study group. JAMA 1996;276(24):1957-63
56. Shamberger RJ, Willis CE. Selenium distribution and human cancer mortality. Crit Rev Clin Lab Sci 1971;2(2):211-21
57. Zeng H, Combs GF Jr. Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. J Nutr Biochem 2008;19(1):1-7
58. Lu J, Jiang C, Kaeck M, et al. Dissociation of the genotoxic and growth inhibitory effects of selenium. Biochem Pharmacol 1995;50(2):213-19
59. Lu J, Kaeck M, Jiang C, et al. Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. Biochem Pharmacol 1994;47(9):1531-5
60. Wilson AC, Thompson HJ, Schedin PJ, et al. Effect of methylated forms of selenium on cell viability and the induction of DNA strand breakage. Biochem Pharmacol 1992;43(5):1137-41
61. Kaeck M, Lu J, Strange R, et al. Differential induction of growth arrest inducible genes by selenium compounds. Biochem Pharmacol 1997;53(7):921-6
62. Ip C, Hayes C, Budnick RM, et al. Chemical form of selenium, critical

metabolites, and cancer prevention. Cancer Res 1991;51(2):595-600

• **A report summarizing the differences in the different chemical forms of selenium.**

63. Ganther HE. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis 1999;20(9):1657-66

64. Sinha R, Said TK, Medina D. Organic and inorganic selenium compounds inhibit mouse mammary cell growth in vitro by different cellular pathways. Cancer Lett 1996;107(2):277-84

65. Xiao H, Parkin KL. Induction of phase II enzyme activity by various selenium compounds. Nutr Cancer 2006;55(2):210-23

66. El-Bayoumy K, Sinha R. Molecular chemoprevention by selenium: a genomic approach. Mutat Res 2005;591(1-2):224-36

67. Combs GF Jr, Gray WP. Chemopreventive agents: selenium. Pharmacol Ther 1998;79(3):179-92

68. Gupta A, Miki K, Xu M, et al. Combination efficacy of doxorubicin and adenoviral methioninase gene therapy with prodrug selenomethionine. Anticancer Res 2003;23(2B):1181-8

69. Miki K, Xu M, Gupta A, et al. Methioninase cancer gene therapy with selenomethionine as suicide prodrug substrate. Cancer Res 2001;61(18):6805-10

70. Yamamoto N, Gupta A, Xu M, et al. Methioninase gene therapy with selenomethionine induces apoptosis in bcl-2-overproducing lung cancer cells. Cancer Gene Ther 2003;10(6):445-50

71. Bogye G, Alfthan G, Machay T. Bioavailability of enteral yeast-selenium in preterm infants. Biol Trace Elem Res 1998;65(2):143-51

72. Thomson CD, Robinson MF, Butler JA, et al. Long-term supplementation with selenate and selenomethionine: selenium and glutathione peroxidase (EC 1.11.1.9) in blood components of New Zealand women. Br J Nutr 1993;69(2):577-88

73. Gladyshev VN, Hatfield DL. Selenocysteine-containing proteins in mammals. J Biomed Sci 1999;6(3):151-60

74. Kobayashi Y, Ogra Y, Ishiwata K, et al. Selenosugars are key and urinary metabolites for selenium excretion within the required to low-toxic range. Proc Natl Acad Sci USA 2002;99(25):15932-6

75. Wang Z, Jiang C, Lu J. Induction of caspase-mediated apoptosis and cell-cycle G1 arrest by selenium metabolite methylselenol. Mol Carcinog 2002;34(3):113-20

76. Ip C, Ganther H. Efficacy of trimethylselenonium versus selenite in cancer chemoprevention and its modulation by arsenite. Carcinogenesis 1988;9(8):1481-4

77. Ip C, Ganther H. Biological activities of trimethylselenonium as influenced by arsenite. J Inorg Biochem 1992;46(3):215-22

78. Jacobs M, Forst C. Toxicological effects of sodium selenite in Swiss mice. J Toxicol Environ Health 1981;8(4):587-98

79. Jacobs M, Forst C. Toxicological effects of sodium selenite in Sprague-Dawley rats. J Toxicol Environ Health 1981;8(4):575-85

80. Corcoran NM, Hovens CM, Michael M, et al. Open-label, phase I dose-escalation study of sodium selenate, a novel activator of PP2A, in patients with castration-resistant prostate cancer. Br J Cancer 2010;103(4):462-8

81. Lu J, Berndt C, Holmgren A. Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase. Biochim Biophys Acta 2009;1790(11):1513-19

82. Foster SJ, Kraus RJ, Ganther HE. The metabolism of selenomethionine, Se-methylselenocysteine, their selenonium derivatives, and trimethylselenonium in the rat. Arch Biochem Biophys 1986;251(1):77-86

83. Li GX, Lee HJ, Wang Z, et al. Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. Carcinogenesis 2008;29(5):1005-12

84. Abdulah R, Miyazaki K, Nakazawa M, et al. Chemical forms of selenium for cancer prevention. J Trace Elem Med Biol 2005;19(2-3):141-50

85. Foster SJ, Kraus RJ, Ganther HE. Formation of dimethyl selenide and trimethylselenonium from selenobetaine in the rat. Arch Biochem Biophys 1986;247(1):12-19

86. Ip C. Lessons from basic research in selenium and cancer prevention. J Nutr 1998;128(11):1845-54

87. Ip C, Thompson HJ, Zhu Z, et al. In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Res 2000;60(11):2882-6

• **Study reporting the efficacy of MSA *in vitro* and *in vivo*.**

88. Spallholz JE, Hoffman DJ. Selenium toxicity: cause and effects in aquatic birds. Aquat Toxicol 2002;57(1-2):27-37

89. Demirel-Yilmaz E, Dincer D, Yilmaz G, et al. The effect of selenium and vitamin E on microvascular permeability of rat organs. Biol Trace Elem Res 1998;64(1-3):161-8

90. Jiang C, Jiang W, Ip C, et al. Selenium-induced inhibition of angiogenesis in mammary cancer at chemopreventive levels of intake. Mol Carcinog 1999;26(4):213-25

91. Jiang C, Ganther H, Lu J. Monomethyl selenium-specific inhibition of MMP-2 and VEGF expression: implications for angiogenic switch regulation. Mol Carcinog 2000;29(4):236-50

92. Hwang JT, Kim YM, Surh YJ, et al. Selenium regulates cyclooxygenase-2 and extracellular signal-regulated kinase signaling pathways by activating AMP-activated protein kinase in colon cancer cells. Cancer Res 2006;66(20):10057-63

93. Yin MB, Li ZR, Toth K, et al. Potentiation of irinotecan sensitivity by Se-methylselenocysteine in an in vivo tumor model is associated with downregulation of cyclooxygenase-2, inducible nitric oxide synthase, and hypoxia-inducible factor 1alpha expression, resulting in reduced angiogenesis. Oncogene 2006;25(17):2509-19

94. Ambs S, Merriam WG, Ogunfusika MO, et al. p53 and vascular endothelial growth factor regulate tumor growth of NOS2-expressing human carcinoma cells. Nat Med 1998;4(12):1371-6

95. Gallo O, Masini E, Morbidelli L, et al. Role of nitric oxide in angiogenesis and

tumor progression in head and neck cancer. J Natl Cancer Inst 1998;90(8):587-96

96. Caffrey PB, Frenkel GD. Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo. Cancer Chemother Pharmacol 2000;46(1):74-8

97. Sieja K. Protective role of selenium against the toxicity of multi-drug chemotherapy in patients with ovarian cancer. Pharmazie 2000;55(12):958-9

98. Sieja K, Talerczyk M. Selenium as an element in the treatment of ovarian cancer in women receiving chemotherapy. Gynecol Oncol 2004;93(2):320-7

99. Hu YJ, Chen Y, Zhang YQ, et al. The protective role of selenium on the toxicity of cisplatin-contained chemotherapy regimen in cancer patients. Biol Trace Elem Res 1997;56(3):331-41

100. Weijl NI, Elsendoorn TJ, Lentjes EG, et al. Supplementation with antioxidant micronutrients and chemotherapy-induced toxicity in cancer patients treated with cisplatin-based chemotherapy: a randomised, double-blind, placebo-controlled study. Eur J Cancer 2004;40(11):1713-23

101. Ohkawa K, Tsukada Y, Dohzono H, et al. The effects of co-administration of selenium and cis-platin (CDDP) on CDDP-induced toxicity and antitumour activity. Br J Cancer 1988;58(1):38-41

102. Fischer JL, Mihelc EM, Pollok KE, et al. Chemotherapeutic selectivity conferred by selenium: a role for p53-dependent DNA repair. Mol Cancer Ther 2007;6(1):355-61

103. Chintala S, Toth K, Cao S, et al. Se-methylselenocysteine sensitizes hypoxic tumor cells to irinotecan by targeting hypoxia-inducible factor 1alpha. Cancer Chemother Pharmacol 2010;66(5):899-911

- **Report on the effects of Se on HIF-1alpha and PHDs.**

104. El-Bayoumy K. The negative results of the SELECT study do not necessarily discredit the selenium-cancer prevention hypothesis. Nutr Cancer 2009;61(3):285-6

Affiliation

Arup Bhattacharya PhD
Roswell Park Cancer Institute,
Department of Cancer Prevention and Control,
Elm and Carlton Streets,
Buffalo, NY 14263, USA
Tel: +1 716 845 4944; Fax: +1 716 845 1144;
E-mail: arup.bhattacharya@roswellpark.org