Chelators at the Cancer Coalface: Desferrioxamine to Triapine and Beyond

Yu Yu, Jacky Wong, David B. Lovejoy, Danuta S. Kalinowski, and Des R. Richardson

Abstract The importance of iron and copper in cancer biology has been well established. Iron plays a fundamental role in cellular proliferation and copper has been shown to be a significant cofactor for angiogenesis. Early observations with the chelator used for the treatment of iron overload, desferrioxamine, showed that it had promise as an anticancer agent. These results sparked great interest in the possibility of developing more effective iron chelators for cancer therapy. The recent entry into clinical trials of the iron-binding drug, Triapine, provides evidence of the potential of this antitumor strategy. Likewise, chelators originally designed to treat disorders of copper overload, such as penicillamine, trientine, and tetrathiomolybdate, have also emerged as potential anticancer drugs, as they are able to target the key angiogenic cofactor, copper. In this review, we will discuss the development of these and other chelators that show potential as anticancer agents.

Iron (Fe) and copper (Cu) are fundamental for life (1, 2). The presence of Fe in ribonucleotide reductase is vital for proliferation owing to its role in catalyzing the rate-limiting step of DNA synthesis (i.e., the conversion of ribonucleotides into deoxyribonucleotides; review ref. 1). Ultimately, the importance of Fe is highlighted by the fact that Fe deprivation leads to G_1-S arrest and apoptosis (1). Neoplastic cells, in particular, have a high Fe requirement due to their rapid proliferation (1). To attain more Fe, neoplastic cells express higher levels of the transferrin receptor 1 (TfR1) and take up Fe at a greater rate than their normal counterparts (1). This is reflected by the ability of tumors to be radiolocalized using ^{67}Ga, which binds to the Fe transport protein, transferrin, for delivery via TfR1 (3, 4). Similarly, phosphorothiolated antisense TfR1 oligonucleotides targeted to *TfR1* mRNA showed selective anticancer activity (5), confirming the importance of Fe in cancer cell growth. These observations show how Fe chelation maybe a suitable therapeutic strategy for cancer treatment.

In addition to Fe, Cu is also a promising therapeutic target due to its key role in angiogenesis (6). Chelation of Cu suppresses several angiogenic mediators, including vascular endothelial growth factor-1, fibroblast growth factor-1, interleukin (IL)-1, IL-6, IL-8, and nuclear factor-κB (7). Because angiogenesis is critical for tumor metastasis (6), the ability of Cu chelators to inhibit angiogenesis represents a novel therapeutic strategy for cancer chemotherapy. This is confirmed by the recent entry into clinical trials of the Cu chelator tetrathiomolybdate (6).

Iron and Copper Metabolism

Iron transport into cells occurs by the binding of diferric transferrin to the TfR1 followed by receptor-mediated endocytosis (review ref. 8; Fig. 1). The subsequent release of Fe from transferrin occurs by a decrease of endosomal pH to 5.5 (8). The divalent metal transporter 1, an endosomal membrane protein, is then responsible for transporting free Fe out of the vesicle (Fig. 1; ref. 9). Once transported into the cytoplasm, Fe enters the putative labile Fe pool where it may be distributed to heme-containing proteins; non-heme-containing proteins, such as ribonucleotide reductase; or stored in the Fe storage protein, ferritin (Fig. 1; ref. 8). Intracellular Fe can be released via the ferroportin 1 transporter (10). Iron efflux is a particularly important function of enterocytes, hepatocytes, and macrophages, which are involved in Fe absorption, metabolism, and recycling, respectively (8). This process is linked with the ferroxidase activity of the Cu-containing proteins ceruloplasmin and hephaestin (11). Thus, Fe and Cu metabolism are associated.

The mechanism of Cu transport into human cells involves the high-affinity Cu transporter-1 (Fig. 1). The ATPase enzymes ATP7A and ATP7B, which are mutated in Menke's and Wilson's disease, respectively, are involved in Cu efflux and metabolism (Fig. 1; ref. 2). Extracellular Cu exists as Cu(II) and is reduced to Cu(I) before being transported into cells by the high-affinity Cu transporter-1 (2). A membrane-bound reductase is responsible for Cu(II) reduction (2). Upon entering cells, Cu binds to

Authors' Affiliation: Iron Metabolism and Chelation Program, Department of Pathology, University of Sydney, Sydney, New South Wales, Australia
Received 8/7/06; revised 9/15/06; accepted 9/21/06.
Grant support: National Health and Medical Research Council, Australian Research Council (D.R. Richardson), Cure Cancer Australia Foundation Grant, Cancer Research Fund University of Sydney, and Australian Rotary Health Research Fund, (D.R. Richardson and D.B. Lovejoy).
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Requests for reprints: Des R. Richardson, Iron Metabolism and Chelation Program, Department of Pathology, University of Sydney, Sydney, NSW 2006, Australia. Phone: 61-2-9036-6548; Fax: 61-2-9036-6549; E-mail: d.richardson@med.usyd.edu.au.

doi:10.1158/1078-0432.CCR-06-1954

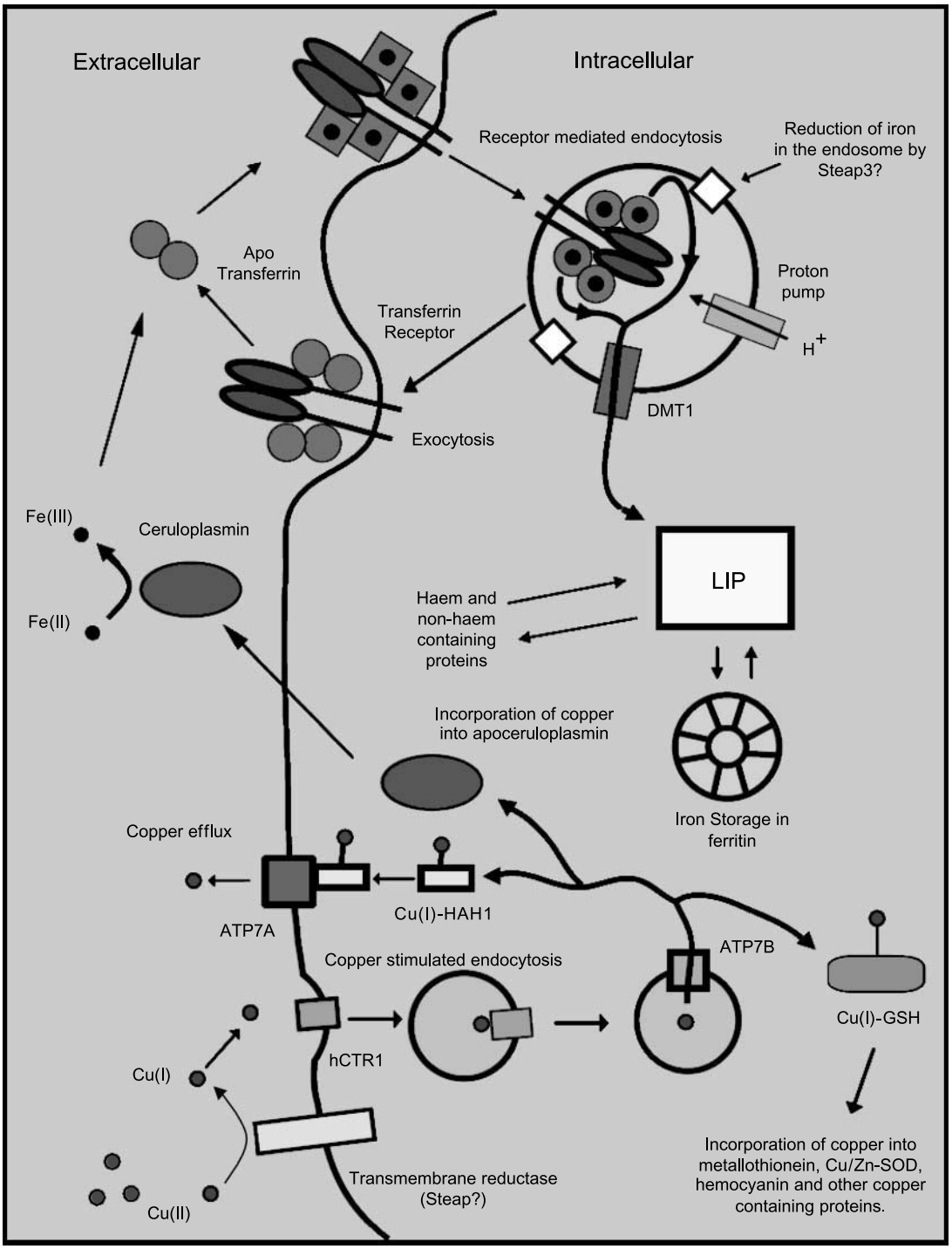

Fig. 1. A schematic diagram illustrating the mechanisms involved in Fe and Cu uptake. Copper(II) is reduced by a transmembrane reductase and then transported into cells by the high-affinity Cu transporter-1 (hCTR1). Intracellular Cu is bound to reduced glutathione (GSH) and HAH1 (also known as ATX1 antioxidant protein 1 homologue ATOX1), which act as Cu chaperones. Copper is transported out of cells by the Menke's P-type ATPase, ATP7A. Iron released from cells (e.g., enterocytes, hepatocytes, or macrophages) is oxidized by hephaestin and ceruloplasmin to Fe(III), which then binds to apo-transferrin. Diferric transferrin is endocytosed upon binding with TfR1. Iron released from transferrin is mediated by a decrease in endosomal pH and is transported out into the cytoplasm by the divalent metal transporter-1 (DMT1), where it then enters the putative labile iron pool (LIP) and is subsequently incorporated into ferritin or Fe-containing proteins.

reduced glutathione and HAH1 (also known as ATX1 antioxidant protein 1 homologue ATOX1) that act as chaperones to distribute Cu to proteins including metallothionein, ceruloplasmin, and hemocyanin (2).

The essential roles of Fe and Cu in key metabolic processes suggest that these nutrients are targets for anticancer therapy. However, the potential of these strategies has only recently been systematically explored.

Iron and Cancer Therapy: Desferrioxamine as an Anticancer Agent

The Fe chelator desferrioxamine (Fig. 2) is used clinically for the treatment of Fe-overload disorders (12, 13). Desferrioxamine was the first commercially available Fe chelator to be assessed in cancer therapy, where, for example, it was shown to reduce bone marrow infiltration of tumor cells in seven of nine neuroblastoma patients (Table 1; ref. 12).

Although desferrioxamine has some antiproliferative activity, it suffers serious limitations (13). It is highly hydrophilic, orally inactive, has poor membrane permeability, and is expensive to produce (13). It also possesses a short plasma half-life due to rapid metabolism (13). These limitations indicate why desferrioxamine failed to show anticancer activity in some studies (14, 15). Indeed, the failure of this drug in several clinical trials relates to the fact that it was never designed for cancer treatment, its purpose being as a therapeutic for Fe overload. As a result of the limited success of desferrioxamine, more effective Fe-binding ligands have been designed with a particular emphasis on chelator lipophilicity and membrane permeability. These are discussed below.

New-Generation Lipophilic Fe Chelators

Triapine. Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone; 3-AP; Fig. 2) is a tridentate chelator that ligates Fe via a sulfur and two nitrogen donor atoms (16). Triapine has been suggested to be one of the most potent inhibitors of ribonucleotide reductase yet identified (17). Human ribonucleotide reductase is a tetramer of two nonidentical homodimers, R1 and either R2 or p53R2 (18). R2 is involved in DNA synthesis for housekeeping, whereas *p53R2* is a p53-targeted gene that is transactivated by DNA damage (19). Similar to R2, p53R2 possesses an Fe-binding site important for enzymatic function (18). Triapine can equally inhibit both R2 and p53R2, whereas the clinically used ribonucleotide reductase inhibitor, hydroxyurea, was relatively ineffective at inhibiting ribonucleotide reductase activity of the p53R2 subunit (18).

A recent study reported that the Triapine-Fe(II) complex was significantly more active at inhibiting ribonucleotide reductase than free Triapine (20). Triapine did not remove Fe from the active site of R2 or p53R2. Instead, this chelator formed a complex with Fe(III), which was reduced to Fe(II) that generated reactive oxygen species and quenched the ribonucleotide reductase tyrosyl radical (20). This inactivated the enzyme and prevented DNA repair and synthesis (20). These results were consistent with prior observations by our laboratory showing that the Triapine-Fe complex was redox active (21).

Building on the idea that a ribonucleotide reductase inhibitor may act as a radiosensitizer due to inhibition of DNA repair, Triapine has been examined in conjunction with radiotherapy (22). In mice bearing a U251 glioma or PSN1 pancreatic carcinoma, radiotherapy followed by Triapine was the most effective combination at inhibiting tumor growth (22).

Triapine is one of the most comprehensively assessed Fe chelators with antitumor activity. A number of phase I clinical trials have been reported and are summarized in Table 1. The most recent trial as a single agent showed that Triapine decreased serum tumor markers associated with stable disease in 4 of 21 patients (Table 1; ref. 17). At a dose of 120 mg/m^2/d every 2 weeks, Triapine was well tolerated, whereas a dose of 160 mg/m^2/d resulted in dose-limiting toxicities in three of six patients (17). The hematologic toxicities were anemia, thrombocytopenia, leucopenia, and met-hemoglobinemia (17). Glucose-6-phosphate dehydrogenase deficiency in patients has been reported to complicate Triapine treatment (23). Patients carrying glucose-6-phosphate dehydrogenase deficiency developed severe met-hemoglobinemia and hemolysis upon treatment (23). This observation may be linked to Triapine redox activity (23) and the inability of patients with glucose-6-phosphate dehydrogenase deficiency to effectively reduce methemoglobin to its ferrous state (23).

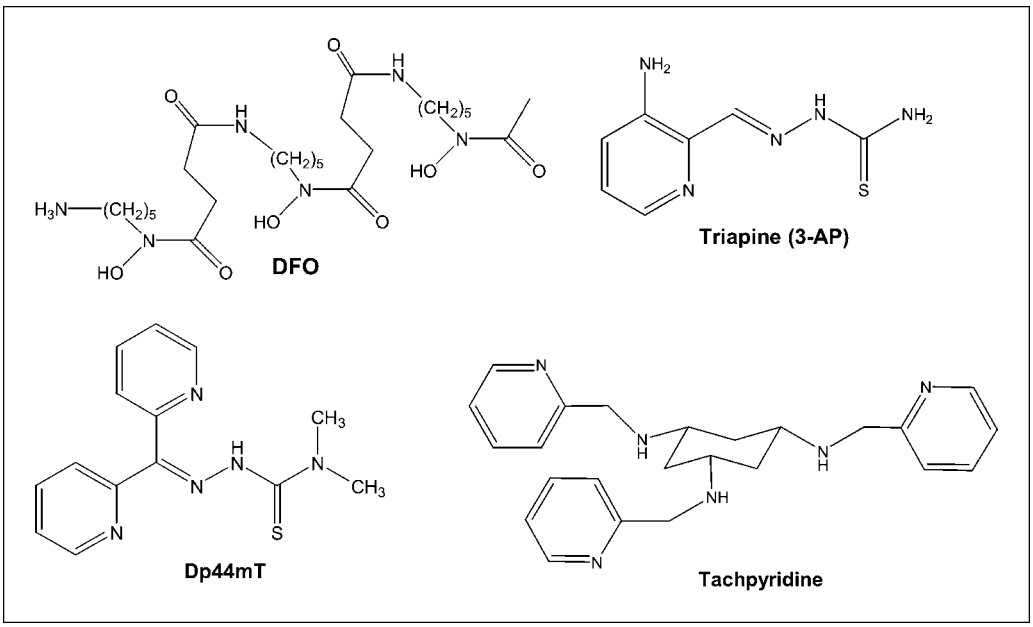

Fig. 2. Chemical structures of the Fe chelators desferrioxamine (DFO), 3-aminopyridine-2-carboxaldehyde (3-AP or Triapine), Dp44mT, (di-2-pyridylketone-4, 4-dimethyl-3-thiosemicarbazone) and *N,N′, N″*-tris(2-pyridylmethyl)-*cis, cis*-1,3,5-triaminocyclohexane (tachpyridine or tachpyr).

Table 1. Summary of clinical trials with the Fe chelators, desferrioxamine and Triapine, and Cu chelators, tetrathiomolybdate and D-penicillamine

Treatment	No. patients	Outcome	References
DFO	9 patients with neuroblastoma	7 patients showed decrease in bone marrow infiltration; 1 patient showed a 50% reduction in tumor mass	12
DFO	10 children with recurrent neuroblastoma	No partial or complete responses, although decreased serum ferritin were noted in 4 patients	14
DFO	14 patients with advanced hormone-refractory prostate cancer	13 patients had disease progression, although 9 had stable measurable or evaluable disease	62
DFO Cyclophosphamide Etoposide Carboplatin Thiotepa (D-CECaT)	23 patients with advanced neuroblastoma and 2 patients with PNET	In previously untreated patients, there were 15 complete responses and 2 partial responses. In patients who had a different drug regimen previously, there were 2 very good partial responses and 4 partial responses. Median survival for most patients was 22 mo	63
D-CECaT	57 patients with advanced neuroblastoma	Following four treatment courses, almost all patients underwent surgery. After surgery, there were 24 complete responses, 26 partial responses, 3 minor responses and 4 with disease progression	64
DFO IFNα (Roferon) Adriamycin Tamoxifen Ascorbic acid	7 patients with inoperable hepatocellular carcinoma	Compared with 5 untreated patients, the treated patients had a longer survival rate, increased tumor regression and less progressive disease	65
DFO Doxorubicin or CHOP regimen Iron sorbitol citrate	9 patients with refractory malignant disease	Partial responses were observed in 2 of 4 patients with refractory non–Hodgkin's lymphoma	66
Triapine	27 patients with advanced cancer	8 patients experienced stabilization of disease for 2-4 mo, the remaining patients experienced progression. No objective tumor responses were observed	67
Triapine	24 patients with refractory leukemia	No patient had an objective response. Over 70% patients had >50% reduction of WBC count	68
Triapine	32 patients with different tumor types	No partial or complete responses were observed; 5 patients showed a positive antitumor effect, in which 2 achieved disease stabilization; 4 of the 5 patients had metastatic disease	69
Triapine gemcitabine	26 patients with progressive metastatic or locally advanced cancer	3 patients had objective responses; 2 other patients achieved a partial response; another patient achieved tumor size reduction without meeting the criteria for a partial response	25
Triapine	21 patients with advanced or metastatic cancer	No partial or complete responses of tumor size reduction were observed; 2 patients remained progression-free for 6 and 10 mo, whereas 4 others achieved stable disease for 3-4 mo	17
Triapine Cytarabine (ara-C)	31 patients with refractory acute leukemia and high-risk MDS	4 patients achieved a complete response after the first cycle of therapy	24
Tetrathiomolybdate	18 patients with metastatic cancer (14 evaluable for response)	Progression or stable disease for <90 d in 8 of 14 patients. In the remaining 6 patients, there was stable disease (5/6) or progression at one site (stable elsewhere; 1/6)	61
Tetrathiomolybdate	15 patients with advanced kidney cancer (13 evaluable for response)	Disease progression before 12 wk in 5 patients, disease progression at 12 wk in 4 patients, disease progression at 28-45 wk in 4 patients	51
Penicillamine	40 patients with newly diagnosed glioblastoma multiforme	Median survival of 11.3 mo and progression-free survival of 7.1 mo (no significant difference to reference group), Pencillamine did not improve survival in these patients	54

Abbreviations: DFO, desferrioxamine; ara-C, 1-β-D-arabinofuranosylcytosine; CHOP, cyclophosphamide-Adriamycin-vincristine-prednisone; PNET, primitive neuroectodermal tumors; MDS, myelodysplastic syndrome; D-CECaT, deferoxamine, cyclophosphamide, etoposide, carboplatin, and thiotepa.

The effect of combining Triapine with cytarabine (24) and gemcitabine (25) have also been investigated (Table 1). These trials showed a number of positive results; in particular, the study with gemcitabine resulted in some objective responses. Triapine is currently in phase II clinical trials in combination with a range of chemotherapeutics. Considering these results, Triapine may become the first Fe chelator to be widely used as an anticancer drug.

Pyridoxal isonicotinoyl hydrazone analogues. Pyridoxal isonicotinoyl hydrazone is a tridentate ligand that was first reported as a biologically effective Fe chelator by Ponka et al. (26). Major advantages of this chelator relative to desferrioxamine include oral availability, high membrane permeability, and simple synthesis (16, 27). Studies of the structure-activity relationships of pyridoxal isonicotinoyl hydrazone analogues identified 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone (311) as one of the most active ligands (28). Subsequent studies with 311 found that it inhibits growth of many human cancer cells (29). The ability of 311 to mobilize Fe results in the depletion of Fe pools necessary for ribonucleotide reductase activity, leading to apoptosis (27, 29). Indeed, electron paramagnetic resonance studies showed that 311 decreased the ribonucleotide reductase tyrosyl radical that leads to inhibition of this enzyme (29).

Apart from the activity of chelators at inhibiting ribonucleotide reductase, several studies have shown that their effect on the expression of molecules involved in cell cycle control could be a factor in their antitumor activity. For instance, desferrioxamine and 311 decreased levels of the cell cycle regulators cyclins D1, D2, and D3 (30). These proteins bind with cyclin-dependent kinase 4 to form a catalytically active dimer that phosphorylates the retinoblastoma protein, which is necessary for cell cycle progression (30). Additionally, 311 reduced expression of cyclin-dependent kinase 2 and the cyclins A and B1 (30). Inhibition of expression of these molecules would be effective in inducing cell cycle arrest. This activity was not observed after incubation of cells with the Fe complexes of desferrioxamine or 311, or the ribonucleotide reductase inhibitor hydroxyurea (30). This suggested that it was the ability of chelators to bind Fe that leads to these results rather than some other effect of the compound or its ability to inhibit ribonucleotide reductase (30).

The significantly greater antitumor activity of 311 and the success of Triapine led to studies assessing the structure-activity relationships of several new series of aroylhydrazone/thiosemicarbazone hybrid ligands. These included the 2-hydroxy-1-napthylaldehyde isonicotinoyl hydrazone series (31), di-2-pyridylketone isonicotinoyl hydrazone series (32), and, more recently, the di-2-pyridylketonethiosemicarbazone series (33). The latter compounds showed the greatest anticancer activity and are described below.

Di-2-pyridylketone thiosemicarbazone series of chelators. Of the di-2-pyridylketone thiosemicarbazone chelators, the analogue di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT; Fig. 2) was the most active against neoplastic cell lines (34). In fact, over 28 cell types, the average IC_{50} was 0.03 ± 0.01 μmol/L, being significantly more effective than the established cytotoxic agent, doxorubicin ($IC_{50} = 0.62 \pm 0.35$ μmol/L; ref. 34). Moreover, Dp44mT showed selective activity against tumor cells compared with normal cells such as fibroblasts, where the IC_{50} was >25 μmol/L (33). Dp44mT has high Fe chelation efficacy, with its ability to inhibit Fe uptake from transferrin and increase Fe release from cells being greater than desferrioxamine and similar to 311 (33). The Dp44mT-Fe complex was also capable of inducing intracellular reactive oxygen species, which probably plays a role in its antitumor activity (33). Importantly, the antiproliferative activity of Dp44mT was not affected by p53 status (34). This serves as a therapeutic advantage, as >50% of tumors contain functionally defective p53, which inhibits apoptosis induction by chemotherapeutics (34). Studies examining the mechanism of action of Dp44mT showed that it caused apoptosis (33) and overcomes resistance to established cytotoxics (34).

In vivo studies in mice showed that Dp44mT, administered at 0.4 mg/kg twice daily, reduced M109 lung carcinoma growth to 47% of the control after 5 days of treatment (33). A subsequent investigation in nude mice with human melanoma xenografts showed that Dp44mT (0.4 mg/kg/d) over 7 weeks decreased tumor growth to 8% of the control. At this dose, Dp44mT was well tolerated with no hematologic or biochemical abnormalities (34). However, fibrotic myocardial lesions were identified at the higher dose of 0.75 mg/kg and to a lesser extent at 0.4 mg/kg. This effect may be due to the marked redox activity of the Dp44mT-Fe complex (33, 35) and suggests that further optimization of the dose and mode of administration is necessary (34).

Dp44mT and other Fe chelators, including 311 and desferrioxamine, markedly up-regulate the expression of the metastasis suppressor gene *N-myc downstream regulated gene-1* (*Ndrg1*) in tumor cells *in vitro* (36) and *in vivo* (34). Increased *Ndrg1* expression was correlated to chelator antiproliferative activity and was reversed by Fe repletion (36). The fact that Fe deprivation leads to up-regulation of this metastasis suppressor suggests another link between Fe metabolism and proliferation and points to a novel mode of anticancer activity.

Tachypyridine. *N,N′,N″*-tris(2-pyridylmethyl)-*cis,cis*-1,3,5-triaminocyclohexane (tachpyridine or tachpyr; Fig. 2) is a hexadentate chelator (37). Tachpyridine is cytotoxic to bladder cancer cells with an IC_{50} of 4.6 μmol/L compared with 70 μmol/L for desferrioxamine (37). Although tachpyridine binds Ca(II), Mg(II), Mn(II), Cu(II), and Zn(II), toxicity studies with tachypyridine complexes suggest that Fe depletion mediates its cytotoxic effects (37).

Similar to Triapine and Dp44mT, tachpyridine induces apoptotic death independent of functional p53 (38, 39). Furthermore, tachpyridine-induced death was prevented in cells microinjected with Bcl-X_L and a dominant-negative caspase-9 expression vector, suggesting activation of the mitochondrial apoptotic pathway (39). In addition, tachpyridine-Fe complexes produce $^{\bullet}OH$ or hypervalent Fe through the Haber-Weiss reaction, which contributes to its antitumor activity (40).

Interestingly, tachpyridine arrests cells at G_2, which is a radiosensitive phase of the cell cycle (41). In fact, radiation increased the sensitivity of tumor cells to the action of tachpyridine (41). This result was unexpected because most Fe chelators arrest cells at the G_1-S interface due to inhibition of ribonucleotide reductase (16). Tachpyridine binds Fe, but it can also bind Cu(II) and Zn(II), which may underlie its

ability to arrest cells in G_2 (37). Currently, tachpyridine is in preclinical development with the National Cancer Institute (41), and evaluation of tachpyridine derivatives, such as trenpyr, are under way (42).

Copper and Cancer Therapy

The dependence of tumor growth on angiogenesis was first hypothesized by Folkman (43). This theory suggested that angiogenesis inhibitors might be useful cancer chemotherapeutics. In fact, angiogenesis was found to be important for metastasis (44, 45). It has long been known that Cu plays an essential role in angiogenesis (6). Pioneering studies showed that Cu became concentrated in the rabbit cornea during neovascularization (46). More recently, Cu was found to be important in the expression or function of many angiogenic factors, including fibronectin; collagenase; gangliosides; prostaglandin E_1; angiogenin; S100A13; fibroblast growth factor-1; secreted protein, acidic, cysteine-rich; synaptotagamin; vascular endothelial growth factor-1; IL-1, IL-6, and IL-8; and nuclear factor-κB (review ref. 6).

A recent study has linked Cu with the activation of hypoxia inducible factor-1α (47). This potentially holds great significance, as hypoxia inducible factor-1α regulates the expression of the proangiogenic molecules nitric oxide synthase and vascular endothelial growth factor-1 (48, 49). Another study suggested that ceruloplasmin, the proangiogenic serum Cu transport protein, is also partly regulated by hypoxia inducible factor-1α–dependent promoter activation (47).

Collectively, the requirement of Cu for angiogenesis suggests a role for Cu-chelating drugs in targeting tumor angiogenesis and metastasis, particularly in hypervascularized tumors (7, 50, 51).

Penicillamine and trientine. The copper chelators penicillamine and trientine (Fig. 3) are used in the treatment of the copper-loading disease, Wilson's disease (52). The success of these chelators in treating copper toxicity has led to their examination as angiogenesis inhibitors against cancer.

A landmark study compared the invasiveness of the VX2 rabbit brain carcinoma in normocupremic animals relative to rabbits copper-depleted by diet and penicillamine (53). Normocupremic rabbits developed large vascularized VX2 carcinomas, whereas small and relatively avascular tumors were found in copper-depleted rabbits (53). However, in a phase II clinical trial, penicillamine, in combination with a low Cu diet, resulted in no improvement in survival of patients suffering glioblastoma multiforme (54). Hence, further, studies with penicillamine are necessary to judge whether it has any place in chemotherapy.

Studies with the Cu chelator, trientine, also showed suppressed tumor development and angiogenesis *in vivo* (55). In comparison with penicillamine, trientine was more effective at inhibiting growth of a murine hepatocellular carcinoma xenograft model and resulted in marked suppression of neovascularization (55). More recently, trientine, in combination with methotrexate, exerted a tumoricidal effect in a human colorectal carcinoma xenograft in mice and led to "tumor dormancy" (56). However, these promising results remain to be confirmed in clinical trials.

Tetrathiomolybdate. The Cu chelator tetrathiomolybdate is another example of a ligand originally developed for Wilson's disease that inhibits angiogenesis and reduces tumor growth (7, 57). This promising antiangiogenesis agent induced Cu deficiency and suppressed tumor growth in the SUM 149 murine breast cancer xenograft model to 31% of untreated controls (7). Reduction in vascular density and tumor metastases had also been reported in tetrathiomolybdate-treated mice bearing SUM149 breast cancer xenografts (7).

The activity of tetrathiomolybdate has been attributed to its ability to form a high-affinity tripartite complex with copper and albumin, to chelate copper from the bloodstream, and to suppress the nuclear factor-κB signaling cascade (58). This reduces expression of angiogenic mediators, including vascular endothelial growth factor-1, fibroblast growth factor-1, IL-1a, IL-6, and IL-8 (58).

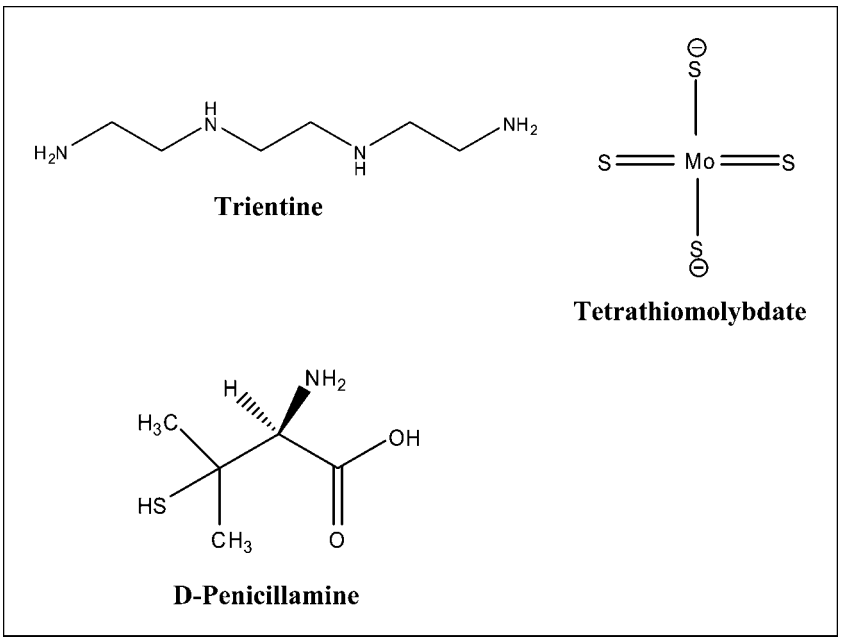

Fig. 3. Chemical structures of the copper-binding chelators, trientine (tetraethylene tetramine), tetrathiomolybdate, and D-penicillamine.

Further studies with tetrathiomolybdate assessed its antiangiogenic activity in combination with other treatments. One study showed that tetrathiomolybdate and doxorubicin was more effective than either single agent in the murine SUM149 xenograft model (59). Most recently, tetrathiomolybdate was combined with radiation treatment and assessed against tumor growth in a mouse model of head and neck squamous cell carcinoma (60). Mice receiving tetrathiomolybdate plus radiotherapy showed a significant decrease in tumor growth compared with mice receiving either tetrathiomolybdate or radiotherapy alone (60).

The promising activity of tetrathiomolybdate has led to its investigation in clinical trials (Table 1; refs. 51, 61). In the most recently published trial, tetrathiomolybdate suppressed the proangiogenic factors: basic fibroblast growth factor 1, IL-6, IL-8, and vascular endothelial growth factor-1 in patients with advanced metastatic kidney cancer (51). As a single agent, stable disease was observed in 31% of patients, but it was concluded that this response was not significant (51). These authors further concluded that tetrathiomolybdate may need to be combined with other antiangiogenic therapies to be effective (51). Indeed, clinical trials with tetrathiomolybdate in combination with other agents are under way.

Conclusions

Observations that rapid neoplastic cell proliferation requires Fe have led to the demonstration that some Fe chelators may be useful against cancer. Currently, the Fe chelator Triapine is being examined in clinical trials, with focus on its potential in combination therapy. The search for more effective chelators has led to the development of other potent ligands, including Dp44mT and tachpyridine. However, further *in vivo* and preclinical studies will be necessary to build on their promise.

The discovery of the role of Cu in angiogenesis led to the postulation that Cu chelators maybe useful angiogenesis inhibitors. The most promising of these is tetrathiomolybdate, which has produced significant *in vitro* and *in vivo* results. However, trials with tetrathiomolybdate as a single agent have not been highly successful. Further studies may identify an effective combination with other agents or may point to the need to develop more effective Cu chelators for antiangiogenesis therapy.

Irrespective of the fate of the chelators discussed in this review, it is apparent that "proof of principle" data exist to justify positioning these agents at the cancer treatment coalface.

References

1. Le NT, Richardson DR. The role of iron in cell cycle progression and the proliferation of neoplastic cells. Biochim Biophys Acta 2002;1603:31–46.
2. Harris ED. Cellular copper transport and metabolism. Annu Rev Nutr 2000;20:291–310.
3. Chitambar CR, Massey EJ, Seligman PA. Regulation of transferrin receptor expression on human leukemic cells during proliferation and induction of differentiation. Effects of gallium and dimethylsulfoxide. J Clin Invest 1983;72:1314–25.
4. Chitambar CR. Gallium nitrate for the treatment of non-Hodgkin's lymphoma. Expert Opin Investig Drugs 2004;13:531–41.
5. Yang DC, Jiang XP, Elliott RL, Head JF. Inhibition of growth of human breast carcinoma cells by an antisense oligonucleotide targeted to the transferrin receptor gene. Anticancer Res 2001;21:1777–87.
6. Harris ED. A requirement for copper in angiogenesis. Nutr Rev 2004;62:60–4.
7. Pan Q, Kleer CG, van Golen KL, et al. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res 2002; 62:4854–9.
8. Richardson DR, Ponka P. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. Biochim Biophys Acta 1997; 1331:1–40.
9. Andrews NC. The iron transporter DMT1. Int J Biochem Cell Biol 1999;31:991–4.
10. Donovan A, Brownlie A, Zhou Y, et al. Positional cloning of zebrafish ferroportin 1 identifies a conserved vertebrate iron exporter. Nature 2000;403:776–81.
11. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. Annu Rev Nutr 2002;22439–58.
12. Donfrancesco A, Deb G, Dominici C, et al. Effects of a single course of deferoxamine in neuroblastoma patients. Cancer Res 1990;50:4929–30.
13. Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia. Blood 1997;89: 739–61.
14. Blatt J. Deferoxamine in children with recurrent neuroblastoma. Anticancer Res 1994;14:2109–12.
15. Selig RA, White L, Gramacho C, Sterling-Levis K, Fraser IW, Naidoo D. Failure of iron chelators to reduce tumor growth in human neuroblastoma xenografts. Cancer Res 1998;58:473–8.
16. Kalinowski D, Richardson DR. Evolution of iron chelators for the treatment of iron overload disease and cancer. Pharmacol Rev 2005;57:1–37.
17. Wadler S, Makower D, Clairmont C, Lambert P, Fehn K, Sznol M. Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-hour intravenous continuous infusion. J Clin Oncol 2004;22:1553–63.
18. Shao J, Zhou B, Zhu L, et al. *In vitro* characterization of enzymatic properties and inhibition of the p53R2 subunit of human ribonucleotide reductase. Cancer Res 2004;64:1–6.
19. Tanaka H, Arakawa H, Yamaguchi T, et al. A ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 2000; 404:42–9.
20. Shao J, Zhou B, Di Bilio AJ, et al. A ferrous-Triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. Mol Cancer Ther 2006;5:586–92.
21. Chaston TB, Lovejoy DB, Watts RN, Richardson DR. Examination of the anti-proliferative activity of iron chelators: multiple cellular targets and the different mechanism of action of triapine compared to desferrioxamine and the potent PIH analogue 311. Clin Cancer Res 2003;9:402–14.
22. Barker CA, Burgan WE, Carter DJ, et al. *In vitro* and *in vivo* radiosensitization induced by the ribonucleotide reductase inhibitor Triapine (3-aminopyridine-2-carboxaldehyde-thiosemicarbazone). Clin Cancer Res 2006;12:2912–8.
23. Foltz LM, Dalal BI, Wadsworth LD, et al. Recognition and management of methemoglobinemia and hemolysis in a G6PD-deficient patient on experimental anticancer drug Triapine. Am J Hematol 2006;81: 210–1.
24. Yee KW, Cortes J, Ferrajoli A, et al. Triapine and cytarabine is an active combination in patients with acute leukemia or myelodysplastic syndrome. Leuk Res 2006;30:813–22.
25. Yen Y, Margolin K, Doroshow J, et al. A phase I trial of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone in combination with gemcitabine for patients with advanced cancer. Cancer Chemother Pharmacol 2004;54:331–42.
26. Ponka P, Borova J, Neuwirt J, Fuchs O. Mobilization of iron from reticulocytes. Identification of pyridoxal isonicotinoyl hydrazone as a new iron chelating agent. FEBS Lett 1979;97:317–21.
27. Richardson DR, Milnes K. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents II:The mechanism of action of ligands derived from salicylaldehyde benzoyl hydrazone and 2-hydroxy-1-naphthylaldehyde benzoyl hydrazone. Blood 1997;89:3025–38.
28. Richardson DR, Tran EH, Ponka P. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents. Blood 1995; 86:4295–306.
29. Green DA, Antholine WE, Wong SJ, Richardson DR, Chitambar CR. Inhibition of malignant cell growth by 311, a novel iron chelator of the pyridoxal isonicotinoyl hydrazone class: effect on the R2 subunit of ribonucleotide reductase. Clin Cancer Res 2001;7:3574–9.
30. Gao J, Richardson DR. The potential of iron chelators of the pyridoxal isonicotinoyl hydrazone class as effective antiproliferative agents, IV: the mechanisms involved in inhibiting cell-cycle progression. Blood 2001;98:842–50.
31. Lovejoy DB, Richardson DR. Novel "hybrid" iron chelators derived from aroylhydrazones and thiosemicarbazones demonstrate selective antiproliferative activity against tumor cells. Blood 2002;100:666–76.
32. Becker EM, Lovejoy DB, Greer JM, Watts R, Richardson DR. Identification of the di-pyridyl ketone isonicotinoyl hydrazone (PKIH) analogues as potent iron chelators and anti-tumour agents. Br J Pharmacol 2003;138:819–30.
33. Yuan J, Lovejoy DB, Richardson DR. Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: *in vitro* and *in vivo* assessment. Blood 2004;104:1450–8.
34. Whitnall M, Howard J, Ponka P, Richardson DR. A novel class of iron chelators with a wide spectrum of potent anti-tumor activity that overcome resistance to chemotherapeutics. Proc Natl Acad Sci U S A 2006;103:14901–6.
35. Richardson DR, Sharpe PC, Lovejoy DB, et al. Dipyridyl thiosemicarbazone chelators with potent and selective anti-tumor activity form iron complexes with redox activity. J Med Chem 2006;49: 6510–21.
36. Le NT, Richardson DR. Iron chelators with high antiproliferative activity up-regulate the expression of a

growth inhibitory and metastasis suppressor gene: a link between iron metabolism and proliferation. Blood 2004;104:2967–75.

37. Torti SV, Torti FM, Whitman SP, Brechbiel MW, Park G, Planalp RP. Tumor cell cytotoxicity of a novel metal chelator. Blood 1998;92:1384–9.

38. Abeysinghe RD, Greene BT, Haynes R, et al. p53-independent apoptosis mediated by tachpyridine, an anti-cancer iron chelator. Carcinogenesis 2001;22:1607–14.

39. Greene BT, Thorburn J, Willingham MC, et al. Activation of caspase pathways during iron chelator-mediated apoptosis. J Biol Chem 2002;277:25568–75.

40. Samuni AM, Krishna MC, DeGraff W, et al. Mechanisms underlying the cytotoxic effects of Tachpyr—a novel metal chelator. Biochim Biophys Acta 2002;1571:211–8.

41. Turner J, Koumenis C, Kute TE, et al. Tachpyridine, a metal chelator, induces G_2 cell-cycle arrest, activates checkpoint kinases, and sensitizes cells to ionizing radiation. Blood 2005;106:3191–9.

42. Torti SV, Ma R, Venditto VJ, Torti FM, Planalp RP, Brechbiel MW. Preliminary evaluation of the cytotoxicity of a series of tris-2-aminoethylamine (Tren) based hexadentate heterocyclic donor agents. Bioorg Med Chem 2005;13:5961–7.

43. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 1971;285:1182–6.

44. Wiggins DL, Granai CO, Steinhoff MM, Calabresi P. Tumor angiogenesis as a prognostic factor in cervical carcinoma. Gynecol Oncol 1995;56:353–6.

45. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 1993;143:401–9.

46. Ziche M, Jones J, Gullino PM. Role of prostaglandin E1 and copper in angiogenesis. J Natl Cancer Inst 1982;69:475–82.

47. Martin F, Linden T, Katschinski DM, et al. Copper-dependent activation of hypoxia-inducible factor (HIF)-1: implications for ceruloplasmin regulation. Blood 2005;105:4613–9.

48. Palmer LA, Semenza GL, Stoler MH, Johns RA. Hypoxia induces type II NOS gene expression in pulmonary artery endothelial cells via HIF-1. Am J Physiol 1998;274:L212–9.

49. Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996;16:4604–13.

50. Moriguchi M, Nakajima T, Kimura H, et al. The copper chelator trientine has an antiangiogenic effect against hepatocellular carcinoma, possibly through inhibition of interleukin-8 production. Int J Cancer 2002;102:445–52.

51. Redman BG, Esper P, Pan Q, et al. Phase II trial of tetrathiomolybdate in patients with advanced kidney cancer. Clin Cancer Res 2003;9:1666–72.

52. Yarze JC, Martin P, Munoz SJ, Friedman LS. Wilson's disease: current status. Am J Med 1992;92:643–54.

53. Matsubara T, Saura R, Hirohata K, Ziff M. Inhibition of human endothelial cell proliferation *in vitro* and neovascularization *in vivo* by D-penicillamine. J Clin Invest 1989;83:158–67.

54. Brem S, Grossman SA, Carson KA, et al. Phase 2 trial of copper depletion and penicillamine as antiangiogenesis therapy of glioblastoma. Neuro-oncol 2005;7:246–53.

55. Yoshii J, Yoshiji H, Kuriyama S, et al. The copper-chelating agent, trientine, suppresses tumor development and angiogenesis in the murine hepatocellular carcinoma cells. Int J Cancer 2001;94:768–73.

56. Yoshiji H, Yoshii J, Kuriyama S, et al. Combination of copper-chelating agent, trientine, and methotrexate attenuates colorectal carcinoma development and angiogenesis in mice. Oncol Rep 2005;14:213–8.

57. Cox C, Merajver SD, Yoo S, et al. Inhibition of the growth of squamous cell carcinoma by tetrathiomolybdate-induced copper suppression in a murine model. Arch Otolaryngol Head Neck Surg 2003;129:781–5.

58. Pan Q, Bao LW, Merajver SD. Tetrathiomolybdate inhibits angiogenesis and metastasis through suppression of the NF-κB signaling cascade. Mol Cancer Res 2003;1:701–6.

59. Pan Q, Bao LW, Kleer CG, Brewer GJ, Merajver SD. Antiangiogenic tetrathiomolybdate enhances the efficacy of doxorubicin against breast carcinoma. Mol Cancer Ther 2003;2:617–22.

60. Khan MK, Mamou F, Schipper MJ, et al. Combination tetrathiomolybdate and radiation therapy in a mouse model of head and neck squamous cell carcinoma. Arch Otolaryngol Head Neck Surg 2006;132:333–8.

61. Brewer GJ, Dick RD, Grover DK, et al. Treatment of metastatic cancer with tetrathiomolybdate, an anticopper, antiangiogenic agent: phase I study. Clin Cancer Res 2000;6:1–10.

62. Dreicer R, Kemp JD, Stegink LD, et al. A phase II trial of deferoxamine in patients with hormone-refractory metastatic prostate cancer. Cancer Invest 1997;15:311–7.

63. Donfrancesco A, Deb G, Angioni A, et al. D-CECaT: a breakthrough for patients with neuroblastoma. Anticancer Drugs 1993;4:317–21.

64. Donfrancesco A, De Bernardi B, Carli M, et al. Deferoxamine followed by cyclophosphamide, etoposide, carboplatin, thiotepa, induction regimen in advanced neuroblastoma: preliminary results. Italian Neuroblastoma Cooperative Group. Eur J Cancer 1995;31A:612–5.

65. Kountouras J, Buora P, Karolides A, Zaharioudaki E, Tsapas G. Recombinant a2 interferon (a-IFN) with chemo-hormonal therapy in patients with hepatocellular carcinoma (HCC). Hepatogastroenterology 1995;42:31–6.

66. Voest E, Neijt JP, Keunen JE, et al. Phase I study using desferrioxamine and iron sorbitol citrate in an attempt to modulate the iron status of tumor cells to enhance doxorubicin activity. Cancer Chemother Pharmacol 1993;31:357–62.

67. Feun L, Modiano M, Lee K, et al. Phase I and pharmacokinetic study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) using a single intravenous dose schedule. Cancer Chemother Pharmacol 2002;50:223–9.

68. Giles FJ, Fracasso PM, Kantarjian HM, et al. Phase I and pharmacodynamic study of Triapine, a novel ribonucleotide reductase inhibitor, in patients with advanced leukemia. Leuk Res 2003;27:1077–83.

69. Murren J, Modiano M, Clairmont C, et al. Phase I and pharmacokinetic study of triapine, a potent ribonucleotide reductase inhibitor, administered daily for five days in patients with advanced solid tumors. Clin Cancer Res 2003;9:4092–100.

Clinical Cancer Research

Chelators at the Cancer Coalface: Desferrioxamine to Triapine and Beyond

Yu Yu, Jacky Wong, David B. Lovejoy, et al.

Clin Cancer Res 2006;12:6876-6883.