A Feasibility Trial of Antiangiogenic (Metronomic) Chemotherapy in Pediatric Patients With Recurrent or Progressive Cancer

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Summary: Standard chemotherapeutic drugs, when modified by the frequency and dose of administration, can target angiogenesis. This approach is referred to as antiangiogenic chemotherapy, low-dose chemotherapy, or metronomic chemotherapy. This study evaluated the feasibility of 6 months of metronomic chemotherapy, its toxicity and tolerability, surrogate markers of activity, and preliminary evidence of activity in children with recurrent or progressive cancer. Twenty consecutive children were enrolled and received continuous oral thalidomide and celecoxib with alternating oral etoposide and cyclophosphamide every 21 days for a planned duration of 6 months using antiangiogenic doses of all four drugs. Surrogate markers including bFGF, VEGF, endostatin, and thrombospondin were also evaluated. Therapy was well tolerated in this heavily pretreated population. Toxicities (predominantly reversible bone marrow suppression) responded to dose modifications. Sixty percent of the patients received less than the prescribed 6 months of therapy due to toxicity (one case of deep vein thrombosis), personal choice (1 patient), or disease progression (10 patients). Forty percent of the patients completed the 6 months of therapy, resulting in prolonged or persistent disease-free status. One quarter of all patients continue to be progression free more than 123 weeks from starting therapy. Sixteen percent of patients showed a radiographic partial response. Only elevated thrombospondin-1 levels appeared to correlate with prolonged response. This oral antiangiogenic chemotherapy regimen was well tolerated in this heavily pretreated pediatric population, which showed prolonged or persistent disease-free status, supporting the continued study of antiangiogenic/metronomic chemotherapy in human clinical trials.

Key Words: angiogenesis, antiangiogenesis, clinical trial, metronomic, pediatric, therapy

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Angiogenesis, the process by which tumors induce new blood vessels for continued growth and spread, has attracted increasing attention since Folkman's original hypothesis.[1] His model of cancer-mediated angiogenesis contained several novel predictions. First, growing tumors require an expanding blood supply to obtain nutrients and oxygen as well as to remove waste products, a process mediated by inducers of angiogenesis. Second, tumor cells are genetically unstable and mutate rapidly to overcome therapy. In contrast, endothelial cells are under normal cellular control and thus lack the ability to become drug-resistant. Third, prolonged therapy would be required to maintain suppression of neovascularization. Since Folkman's initial observation, many angiogenesis pathways have been identified and have provided novel targets for clinical intervention. Newly developed drugs have focused on targeting angiogenesis by altering cytokines (eg, VEGF), altering the molecular environment in which angiogenesis occurs (eg, matrix-metalloproteinases [MMPs]), or attacking the cells needed to populate vascular growth (ie, endothelial cells).[2] Both synthetic drugs and naturally occurring proteins are undergoing clinical testing as single agents or in combination with standard treatment (chemotherapy or radiation therapy). The first major clinical trial of an angiogenesis inhibitor

(bevacizumab [Avastin]) showing significant activity has been reported.[3] Waiting for additional novel antiangiogenic drugs to become available and tested in appropriate combinations will take many more years, even though therapies are required now.

Most chemotherapeutic drugs used to target proliferating tumor cells have well-defined mechanisms of action and toxicities, raising the question of whether these same drugs could also target proliferating cells important in angiogenesis, including endothelium, pericytes, and circulating endothelial precursors.[4] We determined that whereas standard chemotherapy, given over 3 to 5 days, can kill dividing endothelial cells, it has little sustained antiangiogenic activity during the 3- to 4-week rest periods needed for organ recovery (usually bone marrow and gastrointestinal). During this recovery phase, the few endothelial cells that had been killed are more than made up for by aggressive endothelial proliferation. Thus, although traditional chemotherapy has some antiangiogenic effects, it is ineffective at the intermittent schedules used.[5] By contrast, tumors that have become resistant to high intravenous doses of chemotherapy, such as cyclophosphamide, when treated with low doses (making chronic therapy tolerable) and with frequent scheduling (to better target endothelial proliferation), showed dramatic responses.[4] The antiangiogenic activity was shown in that only after waves of endothelial cell apoptosis occurred did tumor cell death ensue. It was also shown that unlike tumor cells, which easily become resistant to repeated doses of a drug, endothelial cells appeared to be a stable target, as predicted by the original hypothesis. The use of existing chemotherapeutic agents administered in ways that increase their antiangiogenic activity is referred to as antiangiogenic chemotherapy,[4] low-dose chemotherapy,[6] or metronomic chemotherapy.[7]

The activity and mechanism of antiangiogenic chemotherapy has been confirmed in animal models using different chemotherapy agents.[6,8] Recently, the mechanism by which metronomic chemotherapy mediates some of the antiangiogenic effect has been reported to be via increasing thrombospondin-1 levels in animals.[9] These preclinical metronomic experiments raise the question of whether a similar effect could be achieved clinically in patients.[10]

We report the results of an oral four-drug antiangiogenic chemotherapy regimen (thalidomide, celecoxib, etoposide, and cyclophosphamide) in a group of children with recurrent or refractory malignancies for which no curative therapy is available. The four agents were provided on an antiangiogenic schedule targeting non-overlapping aspects of neovascularization, taking into account different angiogenic pathways and possible non-endothelial-mediated resistance mechanisms.

METHODS

Eligibility

Between June 2001 and July 2002, eligible patients less than 22 years of age were enrolled after obtaining written informed consent, and patient assent when possible. The study was approved by the Dana-Farber Cancer Institute Institutional Review Board and was performed in accordance with the Declaration of Helsinki. Eligibility criteria included patients with recurrent or progressive poor prognosis tumors for which no curative therapy remained, Karnofsky performance status or Lansky Play status of 50 or greater, an estimated life expectancy of at least 2 months, no underlying organ toxicity of grade 3 or higher, and adequate organ function as defined by serum creatinine less than 1.5 mg/dL, total bilirubin 1.5 mg/dL or less, transaminases and alkaline phosphatase three times normal or less, hemoglobin at least 9.0 g/dL, platelets more than 75,000/mm^3, white blood cell count above 2,000/mm^3, and absolute neutrophil count above 1,000/mm^3. Patients agreed to practice appropriate contraception, and postpubertal females consented to a pregnancy test prior to enrollment. Patients could not be receiving any other concurrent radiation or chemotherapy, nor could they have received prior oral low-dose etoposide or cyclophosphamide. Use of prior standard intravenous etoposide and/or cyclophosphamide was permitted. Brain tumor patients receiving steroids and/or anticonvulsants were eligible for study.

Treatment

Patients received daily oral thalidomide (starting at 3 mg/kg for 1 week, then increasing the daily dose by 50 mg each week as tolerated to a maximum of 24 mg/kg or 1,000 mg, whichever was lower) and daily oral celecoxib (100 mg BID for patients $<$20 kg, 200 mg BID for patients 20–50 kg, and 400 mg BID for patients $>$50 kg), with alternating cycles of daily oral etoposide (50 mg/m^2/d) and daily oral cyclophosphamide (2.5 mg/kg/d to a maximum of 100 mg/d) every 21 days. The lower starting dose of thalidomide was related to a period of acclimatization to the sedative effects of the drug over the first few weeks of therapy and was not related to a dose-finding study. Therapy was planned for 6 months, although those wishing to continue therapy after completion of the 6 months were allowed to do so.

Surrogate Marker Analysis

VEGF, bFGF, endostatin, and thrombospondin-1 levels were evaluated from batched samples of serum, plasma, and urine (when available and with consent) using commercially available ELISA kits (VEGF and bFGF kits were obtained from R&D Systems, Minneapolis, MN; the endostatin and thrombospondin kits were obtained from Cytimmune Sciences Inc, College Park, MD) in accordance with the manufacturers' recommended methodology. MMPs were evaluated using reverse zymography as previously published.[11] Patients were requested to provide blood and/or urine before therapy and every ninth week, although samples were accepted whenever provided.

Statistical Analysis

The primary end point was the feasibility of this approach: it was scored positive if patients were alive, tolerating the therapy, and without progressive disease at 6 months. The proportion of patients off therapy within the first 3 months and between 3 and 6 months was also gathered to help identify populations that may be unsuitable for this type of therapy due to rapid disease progression. Results are reported as binomial point estimates with exact binomial confidence intervals.

RESULTS

Patients

Twenty patients were enrolled with a median age at initiation of treatment of 8.5 years (range 0.8–19 years). Diagnoses included osteogenic sarcoma (n = 4), desmoplastic small round cell tumor (n = 1), rhabdomyosarcoma (n = 2), Ewing sarcoma (n = 1), ependymoma (n = 5), high-grade glioma (n = 3), diffuse pontine glioma (n = 1), refractory low-grade glioma (n = 1), glial sarcoma (n = 1), and medulloblastoma (n = 1). All patients had evidence of progressive or recurrent disease after prior optimal therapy. One patient had re-resection of recurrent disease before initiation of therapy and therefore could not be assessed for response. Patient characteristics, prior therapies, and treatment course with the four-drug antiangiogenic chemotherapy are detailed in Table 1. Patients with diverse tumor histologies were specifically included as they reflect the population in need of novel therapeutic approaches. Furthermore, because tumor-mediated cytokine production (eg, VEGF or bFGF), endothelial activation (eg, COX-2 signaling), and endothelial proliferation are common features of neovascularization, we hypothesized that a multipronged attack would likely be more effective than single-agent activity and would work in a broad variety of tumor types, where all of these mechanisms have been demonstrated.

Feasibility

Eight patients received less than 3 months of therapy due to rapid disease progression (n = 7) or toxicity (n = 1, thrombosis). Four of these eight patients discontinued treatment after less than 4 weeks of treatment. Four patients remained on therapy for 3 to 6 months before disease progression (n = 3) or withdrawal of consent (n = 1). Eight patients remained on therapy for the entire 6 months. Three patients demonstrated a radiographic partial response (1 glioma, 2 ependymomas), all of whom remained on therapy for the entire 6 months (Fig. 1). Of the eight patients who completed 6 months of therapy, seven chose to continue therapy beyond that point, supporting the notion that the therapy was well tolerated.

Given the heterogeneous patient population, an analysis of the time-to-progression (TTP) for patients in this trial was compared with their previous TTP. Ten of 20 patients (50%; 90% CI 30–70%) maintained longer TTP on this treatment than they had achieved on their most recent prior treatment regimen. Because most progression-free intervals decrease with subsequent recurrences, the ability to prolong this period in half of

TABLE 1. Patient Characteristics and Duration of Therapy on the Four-Drug Antiangiogenic Chemotherapy Protocol

Pt. No.	Diagnosis	Age At First Dx (yrs)	# of Surgeries	# XRT	# of Previous Chemos	# of Previous Therapies With VP16/Cyclo	Duration of Remission Prior to 4-Drug Therapy (wks)	6 Months on Study?	Time on 4-Drug Therapy (wks)	Progression-Free Survival (wks)
1	Glioblastoma	7	1	1	1	0	14	No	4	4
2	Ependymoma	2	1	0*	2	1	11	No	3	3
3	Optic glioma	0.5	2	0*	2	0	25	Yes	112	>167
4	Ependymoma	0.8	1	1	2	0	48	Yes	58	58
5	Osteosarcoma/retinoblastoma	1	2	1	3	1	372	No	5	5
6	Primitive neuroectomdermal tumor, then osteosarcoma	2	3	1	2	1	6	No	15	15
7	Osteosarcoma	14	3	0	1	1	61	Yes	28	28
8	Diffuse pontine glioma	14	0	1	1	0	3	No	23	23
9	Ependymoma	0.5	2	0*	0†	0	5	Yes	78	>152
10	Desmoplastic small round cell tumor	18	1	0	1	1	6	No	13	13
11	Rhabdomyosarcoma	7	1	2	2	2	30	No	12	12
12	Neurofibromatosis type 1; glioblastoma multiforme	5	2	1	1	0	31	No	3	3
13	Ewing sarcoma	8	1	2	2	2	248	No	15	15 (withdrew consent)
14	Osteosarcoma	6	1	1	2	0	29	No	2	2 (dose-limiting toxicity)
15	Spindle cell	2	2	1	2	1	36	Yes	83	83
16	Ependymoma	5	3	1	0†	0	9	Yes	96	>123
17	Rhabdomyosarcoma	14	1	3	6	2	18	No	9	9
18	Ependymoma	5	3‡	1	0	0	41	Yes	87	>124
19	High-grade glioma	16	1	1	1	0	46	No	9	9
20	Medulloblastoma	3	1	1	1	1	160	Yes	85	>126

*Patient did not receive radiation therapy to the brain due to age <3 years.
†Chemotherapy alone is ineffective treatment for ependymoma.
‡Gross total resection prior to starting therapy.

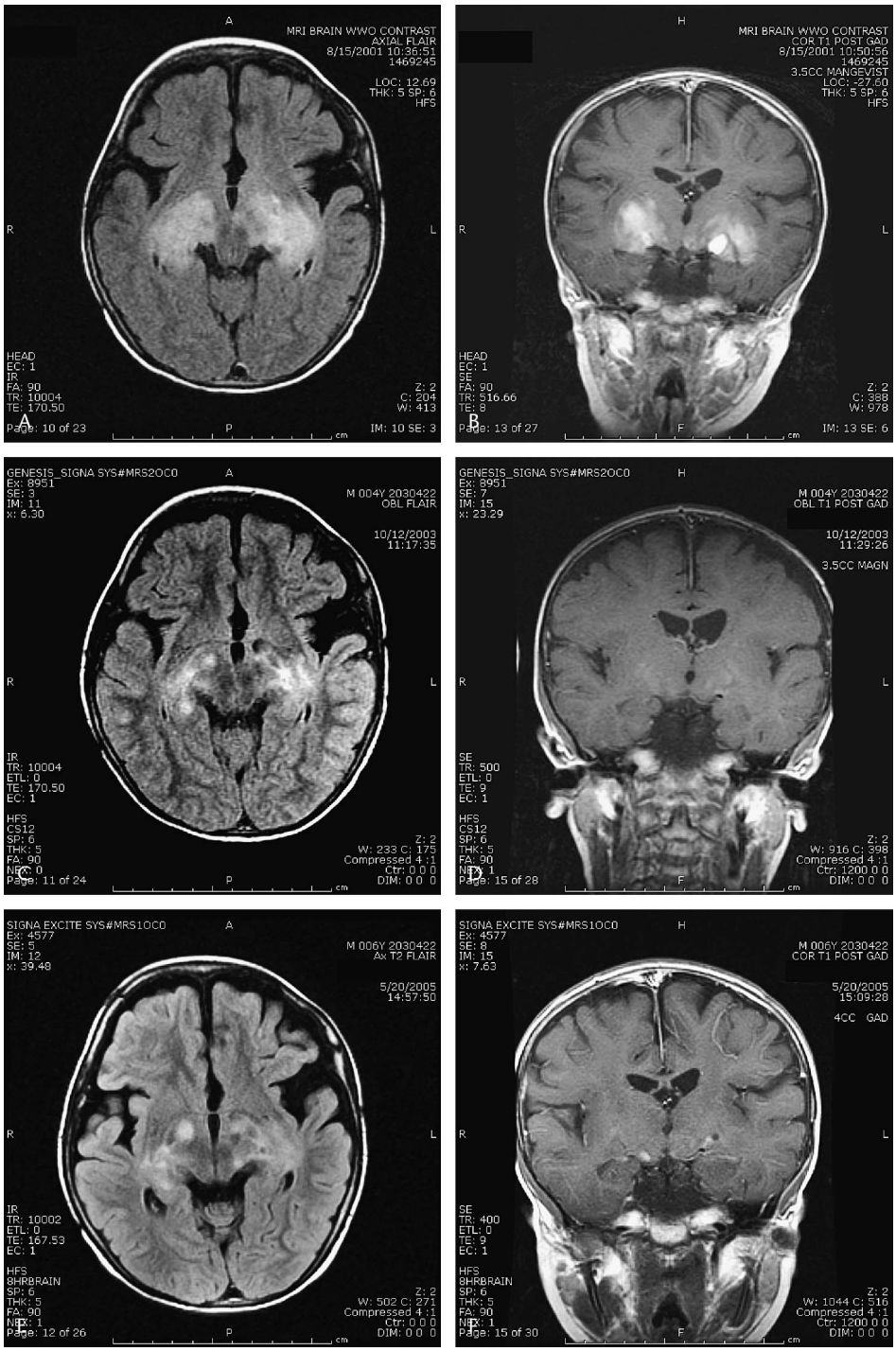

FIGURE 1. MRI axial flair (A, C, E) and coronal T1 with gadolinium (B, D, F) images of patient 3 before initiation of antiangiogenic therapy (A, B), showing a large bilateral supratentorial heterogeneous and contrast-enhancing glioma. C and D show the corresponding images after completion of 24 months of therapy. The response has persisted for 2 years off therapy (E, F).

the patients strongly supports the activity of this regimen, even in patients who did not demonstrate radiographic response.

Toxicity

Therapy was well tolerated in this cohort of heavily pretreated patients. Toxicity (grade I–IV based on the Common Toxicity Criteria version 2.0) was monitored throughout the trial. Prophylactic medication was given for constipation, and sedation lasting a few weeks after initiation of thalidomide was anticipated. Although transient grade I and II toxicities were observed, only one patient discontinued therapy due to a grade III toxicity (deep venous thrombosis, an uncommon but well-described toxicity of thalidomide). Grade III and grade IV toxicities associated with the treatment are listed in Table 2.

TABLE 2. Grade III/IV Toxicities Observed on the Four-Drug Antiangiogenic Chemotherapy Protocol

	Toxicity	Episodes Within First 6 Months of Therapy	# of Patients*	Total # of Occurrences Beyond 6 Months of Therapy	# of Patients†
Grade III	ALT (>5.0–20.0 times normal)	0	0	1	1
	Amenorrhea	1	1	0	0
	Absolute neutrophil count/neutrophils (≥500–1,000)	21	10	18	7
	Diarrhea	2	1	0	0
	Hemoglobin (6.5–8.0 g/dL)	4	3	3	2
	Infection w/neutropenia	1	1	0	1
	Leukocytes (1,000–2,000/mm^3)	36	17	27	7
	Level of consciousness	1	1	0	0
	Lymphopenia (<500/mm^3)	1	1	0	0
	Cranial neuropathy	1	1	0	0
	Platelets (10,000–50,000/mm^3)	0	0	1	1
	Seizure	0	0	1	1
	Syncope	1	1	0	0
	Deep venous thrombosis	1	1	0	0
Grade IV	Absolute neutrophil count/neutrophils (<500/mm^3)	0	0	3	2
	Hypokalemia (<2.5 mmol/L)	0	0	1	1
	Leukocytes (<1,000/mm^3)	0	0	4	3

*20 patients completing 321 weeks of therapy.
†8 patients completing an additional 419 weeks of therapy.

To assess for longer-term toxicities of this approach, we evaluated aspects of growth and development. As noted in Table 2, amenorrhea was observed in one of three female patients menstruating at the time of study entry and was felt likely related to therapy. Because this trial included children undergoing periods of expected growth and development, we were able to assess growth in the context of stable or responding disease and identified no growth retardation, even in those who received prolonged therapy. For example, patient 3, who received over 2 continuous years of treatment and remains in a prolonged partial response, began therapy at a height of 94.1 cm (50th percentile) at 2.75 years of age and reached a height of 106 cm (50th percentile) at 4.75 years of age, supporting the lack of interference with normal growth. Similar maintenance of growth was found in the other patients in whom this could be evaluated.

Serum, plasma, and urine levels of VEGF, bFGF, endostatin, and thrombospondin-1 were evaluated in patients who consented to the biologic analysis. Although VEGF, bFGF, and endostatin levels did not demonstrate a response pattern when comparing baseline to on-therapy values, baseline thrombospondin-1 levels appeared to correlate with prolonged response. Of the seven patients for whom baseline values were obtained, three of three patients with baseline elevated thrombospondin-1 levels in serum above 75 μg/mL had prolonged disease-free periods of greater than 1 year when compared with the four patients with baseline levels below this threshold, all of whom demonstrated early progression (Fig. 2). However, once therapy was initiated, thrombospondin-1 levels did not demonstrate further increases, regardless of radiographic response, stable disease, or rapid tumor progression. Other than the baseline elevation of thrombospondin-1 in three of three patients with a prolonged response, none of the other markers demonstrated a response pattern that correlated with patient outcome. IL-8 and MMP levels were not available from a sufficient number of patients to draw conclusions.

Of the eight patients who remained on therapy for at least 6 months, three had previously received intravenous

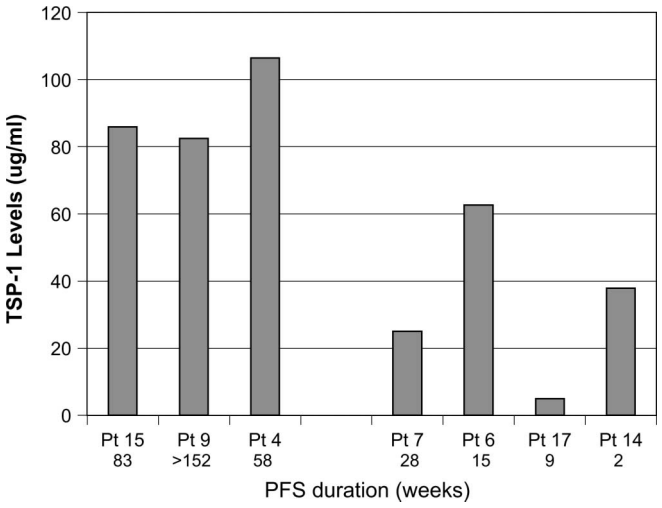

FIGURE 2. Pretherapy serum thrombospondin-1 levels and progression-free survival (PFS) duration >1 year.

etoposide or cyclophosphamide at standard dosing with evidence of progressive disease, suggesting that their tumors were resistant to these drugs by the time they initiated this treatment. This supports the novel mechanism of action of the low-dose oral administration of these agents to a target other than the tumor cell (see below).

DISCUSSION

Most cancer patients die of their disease. New therapeutic modalities are needed to alter the outcome for these patients. Ideally, novel strategies will use agents that can be easily administered, are well tolerated, are capable of inhibiting further tumor progression, have the potential to induce tumor shrinkage, and are commercially available. This latter point is important, for although many new and exciting antiangiogenic and other biologic agents will become available over the next decade, their use in active combinations will take many more years of clinical testing. For these reasons, we selected four orally administered and commercially available drugs that target different aspects of angiogenesis to formally test this approach in patients with recurrent or progressive cancer for whom no other available effective therapy remained.

Evidence for the antiangiogenic activity of this therapy is derived from a number of factors. Each drug in the combination has a proven effect on downregulation of angiogenesis in preclinical models. In addition, each has human data supporting a novel mechanism of action. For example, significant responses have been observed with low-dose daily oral etoposide[12–14] or oral cyclophosphamide[15,16] in humans whose tumors had already progressed on "chemotherapeutic" doses of these same agents.[17] These results are similar to experiments in animals whose tumors were made highly resistant to chemotherapy and for whom stabilization of tumor growth or shrinkage was proven to be the result of inhibition of tumor-mediated angiogenesis.[4,6] Finally, there is no indication that the effects observed are the result of another mechanism. "Maintenance" chemotherapy in patients with leukemia (one of the most angiogenic cancers[18]) may act via a similar mechanism.[19]

Numerous case reports or limited series of patients treated with any of the four drugs alone have been reported. In some of these cases, radiographic responses were observed. The combination of thalidomide (downregulation of VEGF and bFGF), celecoxib (interference with activated endothelial cell COX-2), and etoposide alternating with cyclophosphamide (inhibits endothelial cell proliferation needed for new vessel formation) therefore makes it difficult to ascribe the response or stable disease observed in a given patient to any one particular drug. This was not felt to limit the utility of this approach for two reasons. First, the majority of patients treated with the individual agents above tend to obtain only transient benefit, such that for most of these drugs, their use is considered temporary and palliative. For example, administration of oral etoposide in patients with recurrent brain tumor resulted in a stable disease or radiographic response rate of 42% but a median time to progression of only 8.8 weeks.[12] Second, given the broad antiangiogenic pathways targeted by this approach, synergistic activity might well occur in the context of the combination, without a significant increase in toxicity or difficulty tolerating the four drugs, as demonstrated in this trial.

We considered several factors in developing this combination of agents. Table 3 identifies seven characteristics that were emphasized in the treatment design. Oral administration, although not required for activity, was sought, given the need for prolonged and frequent dosing. Chronic low dosing (resulting in less toxicity) of drugs that could maintain activity against proliferating vascular cells was imperative. We specifically selected agents that could be combined without overlapping toxicities and with different mechanisms of antiangiogenic action so that simultaneous attack on different components of neovascularization could be achieved and tolerated. An important premise of the initial proposal regarding angiogenesis targeting was the stability of the tumor-induced endothelium such that resistance would not develop.[1] The published literature largely supports this concept, although

TABLE 3. Factors Considered in the Development of the Four-Drug Antiangiogenic Chemotherapy Protocol

	Thalidomide	Celecoxib	Oral Etoposide	Oral Cyclophosphamide
Oral administration	Yes	Yes	Yes	Yes
Mechanism of action	Inhibits VEGF- and bFGF-induced neovascularization	Inhibits COX-2 on immature endothelial cells	Inhibits topoisomerase II in dividing endothelial cells	Alkylates DNA in dividing endothelial cells
Toxicity as a single agent in humans	Sedation, constipation, peripheral neuropathy	Stomach upset, renal damage	Bone marrow suppression, second tumors	Bone marrow suppression, second tumors
Tolerability as single agent	Good	Good	Good	Good
Possible drug resistance	None known	None known	Hepatic autometabolism (CYP 3A4)	Hepatic autometabolism (P450 2B1, 2C11)
Preclinical data demonstrating antiangiogenic activity	Limited (metabolized differently in mice than humans)	Good	Good	Good
Human clinical data demonstrating activity in cancer	Good	Good	Good	Good

resistance mechanisms outside the realm of the genetic instability of the endothelium are still possible. For example, many drugs induce their own metabolism (autometabolism) within the liver, so that the effective circulating dose decreases over time. Therefore, agents that are active at the initiation of therapy can lose this activity over time, and the tumor, or in this case the vasculature, is assumed to have become resistance. To account for this possible "extrinsic" resistance (not mediated directly by the endothelium), dosing schedules were designed to help reduce the development of autometabolism by cycling the two agents where this mechanism is known to exist (CYP3A4 pathway for etoposide[20] and P450 2B1 and 2C11 for cyclophosphamide[21,22]). Finally, agents for which animal and/or human data were available that suggested a possible antiangiogenic mechanism of action were selected. The literature has many examples of cancer patients who responded to individual agents within the combination.[15,23–29] Furthermore, two of the agents selected (celecoxib and cyclophosphamide) had published animal data supporting the antiangiogenic activity of the schedule provided.[30,31] Data from our laboratory in murine models using a large number of tumor types have confirmed the antiangiogenic activity of oral etoposide (manuscript in preparation) and thus its inclusion in the cocktail. Although the human data for thalidomide were promising, and animal studies had documented its antiangiogenic activity in the eye assay,[32] the altered metabolism of this drug in mice and rats prevented detailed preclinical drug administration in a method comparable to that proposed in the trial.[33] However, the breadth of human data using chronic oral administration of thalidomide, and its proven role in downregulation of bFGF- and VEGF-mediated angiogenesis, supported its inclusion.

Fifty percent of patients entered onto this clinical trial had a progression-free period that was longer than the one they had achieved in their prior treatment. Although this type of analysis is not a standard method for chemotherapeutic drugs, it is our experience that progression-free intervals typically get shorter with subsequent relapses. As such, we feel that this further supports both the novel mechanism of this treatment as well as its activity.

The "response" rate, defined as radiographic response or stable disease after 6 months, was achieved in 40% of the patients enrolled. Sixteen percent of evaluable patients demonstrated a greater than 50% reduction in the size of their lesions (see Fig. 1), although the significance of these results is limited by the small patient numbers. Stable disease is not typically included in the response rate of most chemotherapeutic agents due to their direct effect on tumor cell kill. By contrast, antiangiogenic agents target different aspects of neovascularization. Because recurrent tumors already have an established vascularity, antiangiogenic agents may only be able to prevent recruitment of new vessels (resulting in stable disease) rather than killing off the vascularity already present (which would result in shrinkage). The effect observed depends on the specific inhibitors used as well as the angiogenic pathways used by the tumor. However, for most cancer patients in the terminal phase of disease, for whom no other effective therapy is known, stabilization of tumor growth would be considered a benefit.

Antiangiogenic therapy might well be expected to interfere with a variety of normal cellular and organ processes that also depend on neovascularization.[34,35] As expected, amenorrhea was noted in a single patient where this was evaluable. By contrast, growth was not inhibited in any of the children in this study where it could be evaluated. The explanation likely relates to the complexity by which angiogenesis is regulated. Approximately 25 different natural inducers of neovascularization (eg, VEGF, bFGF) and another 15 inhibitors of this process (eg, endostatin, tumstatin, thrombospondin) have been identified.[36] Biologic systems that use this many molecules are typically those in which a great deal of control, under a wide array of circumstances, is needed. In this way, the menstrual cycle, growth, development, wound healing, and inflammation can each be initiated, propagated, and stopped independently. This may have a significant impact for therapy, as not only does this complexity provide the specificity by which tumor-induced angiogenesis can be targeted without affecting normal function, but also means that different tumors can upregulate different inducers and/or downregulate different inhibitors of neovascularization to achieve their goals. Therapeutic interventions with antiangiogenic agents will have to take this heterogeneous and complex system into account if effective targeting of this process is to occur. This may well account for the limited activity of single-agent antiangiogenesis trials, as well as the ability of one tumor to respond to a given therapy while another apparently identical tumor does not. As such, the use of combinations of drugs with antiangiogenic activity, as performed in this trial, may well improve our ability to target a greater number of the abnormal signals induced by the tumor.

This four-drug combination was very well tolerated in this heavily pretreated population of patients. Determination of the tolerability of therapy was influenced by a number of factors. Our ability to provide oral therapy at home and without the need for hospitalization or central venous access was important for many families, especially given the palliative nature of the population. The treatment produced only a single grade 3 toxicity necessitating stoppage of treatment. In total, the 20 patients received a total of 740 weeks of treatment (321 weeks within the first 6 months and an additional 419 weeks of therapy in the 7 patients who chose to continue treatment), and yet the patients experienced limited grade 3 and 4 toxicities that were easily managed as outpatients with dose reductions. No patient required transfusion or growth factor support. For many, hair regrew and they regained weight that had been lost secondary to previous chemotherapy or radiation treatments. Finally, although most patients are forced to discontinue standard-dose chemotherapy because of cumulative toxicity, even if they are responding, seven of the eight patients chose to continue their treatments after the protocol therapy was completed.

The use of lower-than-normal intravenous doses of chemotherapy, oral administration, or more frequent schedules does not necessarily make a drug antiangiogenic. We have reserved this term (as well as low-dose and metronomic chemotherapy) to refer to neovascularization as the primary target of treatment, rather than the tumor cell. Although endothelial cell proliferation is the primary target of this therapy, any ability of the drugs to also directly attack tumor cell proliferation would constitute an added, albeit nonessential,

function of this therapy. Thus, if we use these approaches earlier in the disease process, before the tumors have seen and become resistant to these drugs, rather than in end-stage patients, we may see added benefit.

It is imperative that surrogate markers of antiangiogenic activity be identified. A recent report on the mechanism by which antiangiogenic chemotherapy (including low-dose cyclophosphamide) mediated its effect in animals has been identified as the upregulation of thrombospondin-1,[9] and it may thus act as a surrogate marker of activity. Although the precise targets that upregulate thrombospondin-1 remain to be identified, MMP-9 is one pathway known to affect the production of thrombospondin-1.[37,38] We evaluated thrombospondin-1 (as well as VEGF, bFGF, and endostatin) in the seven patients where baseline levels and those obtained after treatment were available. In the three patients where testing was performed and where response or prolonged disease stabilization of at least 1 year's duration occurred, all patients had baseline thrombospondin levels above 75 μg/mL, whereas those with early progressive disease had levels below this range (see Fig. 2). These preliminary results may indicate that antiangiogenic chemotherapy works best in an environment of elevated thrombospondin rather than directly through upregulation of this molecule. Although the number of patients for whom samples were received preclude any firm conclusions, these results would be the first clinical support for the mechanism of action of antiangiogenic chemotherapy originally identified in preclinical models.[9] By contrast to the results for thrombospondin-1, the lack of correlation of response with VEGF, bFGF, or endostatin (there were insufficient samples for IL-8 or MMPs to draw any conclusions) is not surprising and may result from a number of factors. With over 25 inducers of angiogenesis and 15 known inhibitors, our current evaluation of 4 (2 stimulators and 2 inhibitors) falls short of describing the true net effect of all 40 molecules acting together. This likely is one reason that the literature contains contradictory results regarding tumor response to therapy.[39,40] Furthermore, the analysis of angiogenic factors in the blood and urine may not accurately reflect the local concentrations produced by the tumor cells into the adjacent space, where neovascularization occurs.

We provide the first human experience with multiagent antiangiogenic (metronomic) chemotherapy in a diverse population of patients, including those with tumors already resistant to conventional chemotherapy and radiation. The encouraging results of this trial demonstrate the tolerability of oral, daily dosing of combination metronomic chemotherapy using FDA-approved drugs in end-stage cancer patients.

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