Clinical Development of VEGF Signaling Pathway Inhibitors in Childhood Solid Tumors

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Identify the mechanism, specificity, relative potency, dosing schedule, important pharmacokinetic characteristics, and agent-specific side effects of the VEGF signaling pathway inhibitors currently in pediatric development.
2. Describe the different concerns between children and adults regarding the common class side effects of the VEGF pathway inhibitors.

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ABSTRACT

Angiogenesis is a target shared by both adult epithelial cancers and the mesenchymal or embryonal tumors of childhood. Development of antiangiogenic agents for the pediatric population has been complicated by largely theoretical concern for toxicities specific to the growing child and prioritization among the many antiangiogenic agents being developed for adults. This review summarizes the mechanism of action and preclinical data relevant to childhood cancers and early-phase clinical trials in childhood solid tumors. Single-agent adverse event profiles in adults and children are reviewed with emphasis on cardiovascular, bone health, and endocrine side effects. In addition, pharmacological factors that may be relevant for prioritizing clinical trials of these agents in children are reviewed. Considerations for further clinical evaluation should in-

clude preclinical data, relative potency, efficacy in adults, and the current U.S. Food and Drug Administration approval status. Toxicity profiles of vascular endothelial growth factor (VEGF) signaling pathway inhibitors may be age dependent and ultimately, their utility in the treatment of childhood cancer will require combination with standard cytotoxic drugs or other molecularly targeted agents. In combination studies, toxicity profiles, potential drug interactions, and late effects must be considered. Studies to assess the long-term impact of VEGF signaling pathway inhibitors on cardiovascular, endocrine, and bone health in children with cancer are imperative if these agents are to be administered to growing children and adolescents with newly diagnosed cancers. *The Oncologist* 2011;16:1614–1625

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INTRODUCTION

Antiangiogenesis as a strategy for cancer therapy was proposed in 1971 by Judah Folkman [1]. Forty years later, numerous antiangiogenic drugs have been approved for colon, renal, and other adult malignancies. Dependence on new blood vessel formation is not restricted to epithelial cancers. Angiogenesis is a target shared by both adult cancers and the mesenchymal or embryonal tumors in children and adolescents. Pediatric development of antiangiogenic agents has been complicated by theoretical concerns for unique toxicities during growth and development as well as issues in prioritization among the many agents being developed in adults. This review summarizes the clinical development of vascular endothelial growth factor (VEGF) signaling pathway inhibitors in childhood solid tumors by: (a) reviewing mechanism of action or toxicity and preclinical data relevant to childhood solid tumors, (b) summarizing the pediatric clinical trial experience to date, and (c) comparing the adverse event profile in adults and children focusing on target or off-target effects and differences in the toxicity profiles. The review concludes with a discussion of challenges and considerations for future trials of antiangiogenic agents in children and adolescents.

VEGF

In the late 1960s, evidence emerged that tumor neoangiogenesis was mediated by diffusible molecules isolated from tumors including neuroblastomas, hepatoblastomas, and Wilms' tumors [1, 2]. This tumor-derived factor was subsequently purified, sequenced [3], and identified as VEGF. The biology of VEGF receptor (VEGFR) signaling has been reviewed previously [4, 5]. Briefly, the endothelial cell mitogenic and survival functions of VEGF are mediated primarily by the tyrosine kinase receptor VEGFR-2 (Flk-1/KDR). VEGF-VEGFR-2 binding, autophosphorylation, and downstream Src family kinase activation mediate disruption of the endothelial barrier, resulting in increased vascular permeability [6]. Signaling through VEGFR-1 (Flt-1) is more complex, with potential roles in modulation of response, chemotaxis, and recruitment of bone marrow-derived cells to the tumor vasculature. In addition, VEGFR-1 has been associated with premetastatic niches, the distant sites made "fertile" for development of metastases [4, 7, 8]. VEGF expression is induced by hypoxia via the transcriptional activator hypoxia-inducible factor (HIF)-1 α , which, in turn, is negatively regulated by the von Hippel-Lindau (VHL) tumor suppressor gene [9, 10].

VEGF IN PEDIATRIC SOLID TUMORS

VEGF and angiogenesis have pivotal roles in tumor growth and metastasis in children. Prior to primary tumor resection, children with cancer have increased circulating VEGF levels [11–13]. Primary tumor tissue from neuroblastoma [14–17] and Wilms' tumor [18, 19] expresses VEGF, with higher expression correlating with invasion, metastasis, and the risk for recurrence. VEGF overexpression can be demonstrated in primary tumors from patients with Ewing's sarcoma, although the prognostic implication is disputed [20, 21]. In experimental

Figure 1. Targeting VEGF signaling.

Abbreviations: TK, tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

models of Ewing's sarcoma, the isoform VEGF₁₆₅ is a critical driver of vasculogenesis [22, 23] and the EWS-ETS fusion protein may indirectly upregulate VEGF expression [20]. Similarly, in osteosarcoma, VEGF₁₆₅ appears to be necessary for the development of pulmonary metastasis, and primary tumors expressing multiple ligands and receptors in the VEGF signaling pathway are associated with adverse clinical outcomes [24–26]. In rhabdomyosarcoma, laboratory evidence suggests the presence of an autocrine loop, wherein VEGF is secreted by tumor and the PAX3-FKHR fusion protein induces expression of VEGFR-1 [27, 28].

VEGF SIGNALING PATHWAY INHIBITORS

Antiangiogenic agents can target ligands, receptors, or protein expression in the VEGF signaling pathway (Fig. 1). Each agent has unique biologic and pharmacologic properties that influence dosing, schedule, toxicity, efficacy, and the potential for combination with other drugs. Agents that target ligands include bevacizumab (Avastin®; Genentech Inc., South San Francisco, CA) and aflibercept (VEGF Trap®; Regeneron, Tarrytown, NY). Bevacizumab is a humanized monoclonal neutralizing antibody that binds with high affinity ($K_d = 1.8$ nM) all five human VEGF isoforms. Bevacizumab was the first U.S. Food and Drug Administration–approved VEGF-specific blocking agent. Aflibercept is a potent ($K_d = 1.5$ pM) composite decoy receptor, in which the extracellular domains of VEGFR-1 and VEGFR-2 are fused to an Fc segment of IgG₁. Aflibercept sequesters VEGF-A, VEGF-B, and placental growth factor.

Antibodies that result in VEGFR-2 blockade include the fully human monoclonal antibody ramucirumab (IMC-1121B; Imclone Systems, New York, NY) and its murine counterpart for preclinical studies, DC101 [29]. Ramucirumab binds VEGFR-2 with high affinity ($K_d = 50$ pM), blocks VEGF binding, and prevents activation of VEGF signaling pathways. Currently, ramucirumab is being evaluated in clinical trials in a variety of malignancies in adults [30].

Table 1. Comparison of VEGF TKIs evaluated in clinical trials of childhood cancer

	Cediranib	Pazopanib	Sorafenib	Sunitinib	Vandetanib
In vitro IC ₅₀ , nM					
RET	–	2800	47	41	100
MEK-1	10,000	–	>10,000	–	>10,000
Raf-1	–	–	6	>10,000	–
Protein binding, %	95	>99	99.5	95	99
Terminal half-life, hours	20	31	25–48	40–60	110
Adult steady-state C _{trough} , μM	0.04	50	13	0.08	2,100
Adult recommended dose	20–30 mg daily, continuous	800 mg daily, continuous	400 mg twice daily, continuous	50 mg daily × 4 wks every 6 wks	300 mg daily, continuous
Adult approved indication	None	Renal cell carcinoma	Renal cell and hepatocellular carcinoma	Renal cell carcinoma, GIST, pancreatic neuroendocrine tumors	Medullary thyroid cancer
Commercially available dosages, mg	None	200, 400	200	12.5, 25, 50	100, 300
Metabolism	UGT1A4	CYP3A4, CYP1A2, CYP2C8, UGT1A1, OATP1B1	CYP3A4, UGT1A9	CYP3A4	CYP2D6, CYP1C9, CYP2C19, CYP1A1/2, NAT2, DPD
References	[87, 101, 102]	[103–105]	[105–107]	[105, 107–111]	[86, 112, 113]
Abbreviations: C _{trough} , trough concentration; CYP, cytochrome P450; DPD, dihydropyrimidine dehydrogenase; GIST, gastrointestinal stromal tumor; IC ₅₀ , 50% inhibitory concentration; MEK, mitogen-activated protein kinase/extracellular signal-related kinase kinase; NAT2, N-acetyltransferase 2; RET, REarranged during Transfection; UGT, uridine 5'-diphospho-glucuronosyltransferase.					

Small molecule tyrosine kinase inhibitors (TKIs) of the VEGF signaling pathway, including cediranib, pazopanib, sorafenib, sunitinib, semaxanib, and vandetanib, are being evaluated in early-phase clinical trials in childhood cancer. Each inhibit VEGFR-1, VEGFR-2, and VEGFR-3 downstream signaling and can inhibit other kinases, including c-kit, b-Raf, PDGFR, FGFR, RET, Flt-3, ERK, and MEK, which may contribute to both antitumor effects and toxicity (Table 1, Fig. 2).

The small molecule PTC299 (PTC Therapeutics, South Plainfield, NJ) selectively inhibits post-transcriptional regulation of VEGF, resulting in inhibition of VEGF production by the tumor. PTC299 inhibits tumor cell division at the G₁/S phase of the cell cycle, providing an alternative mechanism of action. Early-phase clinical trials of PTC299 in adults with cancer and related disorders have been completed [31, 32]. A clinical trial in children with brain tumors is ongoing by the Pediatric Brain Tumor Consortium.

PRECLINICAL ACTIVITY OF VEGF SIGNAL PATHWAY INHIBITORS

The anti-VEGF antibody A.4.6.1, later humanized as bevacizumab [33], suppressed tumor angiogenesis and growth in murine xenograft models of rhabdomyosarcoma [34], neuroblastoma [35], and hepatoblastoma [36]. Aflibercept decreased tumor vasculature and resulted in regression of established tumors and lung micrometastases in orthotopic xenografts of a pediatric renal cancer that harbors the EWS–FLI translocation [37]. In neuroblastoma, aflibercept substantially decreased coopted vessels [38].

VEGF inhibition may augment antitumor effects when combined with cytotoxic chemotherapy [39–41]. Tumor vasculature is disorganized and hyperpermeable, resulting in increased interstitial fluid pressure and poor intratumoral perfusion with resultant hypoxia and acidosis, which can impair cytotoxic drug delivery and efficacy. VEGF blockade may transiently normalize tumor vasculature [42]. In neuroblastoma xenografts, VEGF neutralizing antibody induced a rapid decrease in tumor microvascular density, vascular permeability, and interstitial fluid pressure and transiently improved intratumoral perfusion assessed by intravital microscopy and contrast enhanced ultrasonography. These structural and functional changes induced by VEGF blockade correlated with enhanced tumor suppression and greater tumor penetration of topotecan [41].

The small molecule VEGFR TKIs cediranib, sorafenib, and sunitinib were evaluated against a panel of pediatric solid tumor cell lines in the Pediatric Preclinical Testing Program (PPTP). Consistent with an antiangiogenic mechanism, the class lacked in vitro activity. The PPTP evaluates in vivo antitumor activity by enumerating objective responses in s.c. xenografts and categorizing tumor control volumes and event-free survival as low, intermediate, or high activity [43, 44]. Results for cediranib [45], sorafenib [46], and sunitinib [47] are summarized in Table 2. All three agents exhibited growth inhibitory activity, inducing significant prolongation of the time to event in pediatric solid tumor models. Complete responses were rare, with most of the response activity rated as intermediate.

Figure 2. Relative potency of TKIs based on achievable steady-state C_{trough} in adults. Relative potency is plotted as the in vitro IC_{50} for selected kinases divided by C_{trough} at steady state (steady-state C_{trough}) in adults receiving the recommended dose. References for IC_{50} and C_{trough} are presented in Table 1. Steady-state C_{trough} values are total parent drug concentrations with no correction for protein binding or active metabolites.

Abbreviations: C_{trough} , trough concentration; EGFR, epidermal growth factor receptor; FGFR-1, fibroblast growth factor receptor 1; FLT-3, FMS-like tyrosine kinase 3; IC_{50} , 50% inhibitory concentration; PDGFR- β , platelet-derived growth factor receptor β ; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

CLINICAL TRIALS AND CLINICAL EXPERIENCE

Numerous reviews chronicle the clinical development of VEGF signaling pathway inhibitors in adults with cancer [42, 48–51]. Table 3 outlines published clinical trial data in the pediatric population. Generally, for agents with sufficient data in children, the pharmacokinetics in the adult and pediatric populations are similar. Direct comparison of the recommended fixed dose in adults (mg) with allometric dosing in children (mg/m² or mg/kg) indicates that the recommended doses of most VEGF signaling pathway inhibitors are comparable. However, current fixed tablet and capsule dosage formulations of the TKIs have rendered body size–based dosing difficult, particularly in young children. Class toxicity has been similar, with an apparent lower incidence of hypertension in the pediatric population and fewer than anticipated reports of growth plate toxicity. The recommended dose in children may depend on specific disease populations and concomitant medications, such as corticosteroids.

Because most of the agents have only completed pediatric phase I evaluation, there is insufficient data on their antitumor activity. Nonetheless, there have been early signals of single-agent activity, including partial and minor responses and stable disease for >6 months in soft tissue sarcoma, Ewing's sarcoma, osteosar-

coma, Wilms' tumor, hepatoblastoma, ependymoma, and high- and low-grade glioma [52–57].

Experience with adults suggests that aside from renal cell carcinoma (RCC), which harbors *VHL* mutation and HIF-1 α dysregulation, a VEGF sequestering agent like bevacizumab is unlikely to have single-agent activity. However, neutralizing antibody does not affect the pharmacology of concurrently administered cytotoxic agents and may actually improve drug delivery to the tumor by vascular normalization. Based on this experience, there are numerous pilot pediatric trials under way combining bevacizumab with other agents and some novel randomized selection phase II designs to help elucidate signals of efficacy in a particular disease (Table 4).

Monotherapy with TKIs has shown broader clinical activity in adults, including those with RCC, hepatocellular carcinoma, gastrointestinal stromal tumors (GISTs), medullary thyroid carcinoma, high-grade glioma, and sarcoma. Some of this activity may be a result of additional pathway inhibition, notably c-KIT and PDGFR for GIST and RET for medullary thyroid carcinoma. Given similarities among agents, prioritization for phase II evaluation of the TKIs in pediatrics should consider issues of availability, toxicity, and relative potency for each known kinase target (e.g., the inhibitory concentration versus exposures anticipated to be readily achieved in patients). A comparison of relative potency for cediranib, sorafenib, sunitinib, pazopanib, and vandetanib based on in vitro kinase inhibition and the steady-state concentration reported in adults at the recommended dose is shown in Figure 2.

In general, TKIs have been more difficult to combine with cytotoxic agents because of drug–drug interactions and greater toxicity [58]. Given the remarkable minor and partial responses in pulmonary metastases of adolescent patients with Ewing's sarcoma, synovial sarcoma, and osteosarcoma seen during a single-agent phase I study with cediranib [55], the TKIs will be developed in pediatrics for the most part using standard disease-based, single-arm phase II studies. However, successful adult trials have used a time to progression endpoint, which is uncommon in the pediatric setting, where objective response has been the standard. In addition, trials in adults have studied large patient populations in order to determine statistically significant, but small, absolute differences in progression-free survival times, which tempers enthusiasm in the pediatric setting. As a result, it is reasonable to study more sensitive markers of disease response in pediatrics.

BIOMARKERS

To date, a consistent predictive biomarker of clinical response to VEGF signaling inhibitors has not been identified. In adult patients with metastatic GISTs treated with sunitinib, a rise in mature circulating endothelial cells was associated with clinical benefit [59]. Results from a pediatric trial of bevacizumab suggested that similar changes in circulating endothelial cells correlated with prolonged stable disease [52]. The assay, however, requires fresh blood, is complicated to export to large multicenter trials, and can be difficult to interpret. Plasma VEGF and soluble (s)VEGFR-2 are consistently modulated by treatment with the TKIs. In adults with early-stage non-

Table 2. Summary of *in vivo* Pediatric Preclinical Testing Program evaluations of vascular endothelial growth factor tyrosine kinase inhibitors

Overall EFS activity	Cediranib [45]			Sorafenib [46]			Sunitinib [47]		
	78%			75%			54%		
	CR	T/C activity	Response activity	CR	T/C activity	Response activity	CR	T/C activity	Response activity
Brain tumors	0/7	1/7	3/7	0/9	4/9	4/9	0/8	1/8	3/8
Ewing's sarcoma	0/3	3/3	3/3	0/5	1/5	2/5	0/5	4/5	4/5
Neuroblastoma	0/6	3/6	5/6	0/5	3/5	4/5	0/6	0/6	2/6
Osteosarcoma	1/5	3/5	4/5	0/5	4/5	4/5	0/4	1/4	3/4
Rhabdoid tumor	1/3	1/3	3/3	0/3	0/3	2/3	1/3	1/3	2/3
Rhabdomyosarcoma	0/5	2/5	5/5	0/6	0/6	0/6	0/6	5/6	5/6
Wilms' tumor	0/3	1/3	2/3	0/3	0/3	0/3	0/3	0/3	2/3

Cediranib, sorafenib, and sunitinib were tested against xenograft tumors from a broad range of pediatric tumors [45–47]. Three to nine cell lines were used for each tumor type. There were three CRs seen. T/C activity is derived from the ratio of mean tumor volume of treated tumors divided by mean tumor volume of control tumors at day 21. For T/C activity, agents producing a T/C $\leq 15\%$ are considered highly active, those with a T/C $\leq 45\%$ but $>15\%$ are considered to have intermediate activity. For response activity, agents inducing objective responses are considered highly active against the tested line, whereas agents inducing stable disease or delaying progression are considered to have intermediate activity. For T/C activity and response activity, agents are considered active if they have either intermediate or high activity. Abbreviations: CR, complete response; EFS, event-free survival; T/C, treated/control.

small cell lung cancer receiving preoperative pazopanib, post-treatment changes in plasma sVEGFR-2 were correlated with tumor shrinkage [60]. Current pediatric trials include a panel of cytokines and angiogenic factors but have yet to show a correlation with response [61]. Dynamic contrast imaging with measurements of changes in blood flow and permeability are currently being explored in pediatric trials [57].

MECHANISMS OF TOXICITY AND TOXICITY PROFILES IN ADULTS AND CHILDREN

When antitumor activity is anticipated to be modest, understanding the mechanism and impact of toxicity is critically important. The side effects of inhibition of the VEGF signaling pathway can be related to target inhibition or off-target effects of the particular agent. Mechanisms of toxicity have been evaluated in preclinical models. A class toxicity profile has emerged in adults with cancer and other diseases. In children and young adults, the toxicity spectrum is altered, in part, as a result of the role of angiogenesis in growth and development and differences in cardiovascular risk factors in children compared with adults.

Cardiovascular Toxicity

The acute and subacute cardiovascular toxicities of VEGF signaling pathway inhibitors include hypertension, left ventricular dysfunction, heart failure, conduction abnormalities (QTc prolongation), and thrombosis or acute coronary syndromes [62–66]. Hypertension and thrombosis are mechanism dependent and directly linked to inhibition of the VEGF signaling pathway. Left ventricular dysfunction and QTc prolongation may be associated with off-target or secondary effects. In

adults, the reported incidence of cardiovascular toxicity may differ among agents because of different potencies of target inhibition, off-target effects, differences in cardiovascular risk factors, and differences in protocol-specific cardiovascular monitoring and definitions of cardiovascular events [64]. In children, cardiovascular toxicities are influenced by prior therapy, including anthracyclines, the use of radiation for local control, and a lower incidence of pre-existing cardiovascular disease.

Hypertension

Hypertension is the most frequent cardiovascular effect of VEGF signaling pathway inhibitors for adults with cancer and the most frequent comorbid condition that impacts overall prognosis [67]. The mechanism of VEGF signaling pathway inhibitor–related hypertension is an area of ongoing research. VEGF functions to decrease vascular tone and reduce blood pressure by dilating arterioles and venules likely via downstream effects on endothelial nitric oxide synthase expression [68, 69]. Inhibition of VEGF results in constriction of the microcirculation, leading to microvascular rarefaction or extinction of the small vessels [70]. Hypertension may be a biomarker for VEGF signaling pathway inhibition, predict clinical benefit [71], and be related to VEGF polymorphisms [72].

Blood pressure norms in children are sex, age, and height specific. Pre-existing hypertension is rare in children enrolled in early-phase clinical trials. Many dose-finding studies have excluded children receiving antihypertensive medications at baseline. The incidence of drug-related hypertension reported from clinical trials of VEGF signaling pathway inhibitors in children has been low. Grading criteria for hypertension ac-

Table 3. Summary of clinical trials in children with refractory cancer

Agent (References)	Dose/ schedule	Patient characteristics, median (range)	PK at recommended dose	Adverse events			
				Bone	Cardiovascular	Endocrine	Other
Aflibercept (VEGF Trap) [74]	2, 2.5, 3 mg/kg i.v. every 2 wks; recommended: 2.5 mg/kg i.v. every 2 wks	Refractory solid tumors, $n = 18$; age, 13 (1.9–21.6) yrs	2.5 mg/kg free; $ssC_{trough} = 0.02 \mu M$; $T_{1/2} = NR$	NR	NR	NR	Intratatumoral bleeding
Bevacizumab [52]	5, 10, 15 mg/kg i.v. every 2 wks; recommended: 10 mg/kg every 2 wks, 15 mg/kg every 3 wks	Refractory solid tumors, $n = 18$; age, 13 (1–21) yrs; cycles, 3 (1–16)	15 mg/kg; $C_{max} = 2 \mu M$; $T_{1/2} = 12$ hours	No epiphyseal expansion ($n = 3$)	Median blood pressure increase in patients ($n = 11$) was 6 mmHg (systolic), 9 mmHg (diastolic)	Postmenarchal females ($n = 3$) had increased FSH and LH during therapy	
Cediranib (AZD2171) [55]	8, 12, 17 mg/m ² PO daily $\times$ 28 days; recommended: 12 mg/m ² PO daily $\times$ 28 days	Refractory extracranial solid tumors, $n = 16$; age, 15 (8–18) yrs; cycles, 4 (1–15)	12 mg/m ² ; $C_{max} = 0.2 \mu M$; $T_{1/2} = 13$ hours	No increase in growth plate volume ($n = 6$)	Hypertension (grade 3, $n = 1$; grade ≤ 2 , $n = 2$); LVEF (grade 1, $n = 2$; grade 2, $n = 3$); QTc prolongation (grade 3, $n = 1$)	Hypothyroidism (grade ≤ 2 , $n = 4$)	
Pazopanib [57]	275, 350, 450 mg/m ² PO daily $\times$ 28 days; recommended: 450 mg/m ² PO daily $\times$ 28 days	Refractory solid tumors, $n = 25$; age, 13 (5–21) yrs; cycles, 3 (1–13+)	Pending	Monitored but not yet reported	Hypertension (grade 3, $n = 2$); no QTc prolongation; LVEF monitored but incidence not yet reported	Thyroid function monitored but incidence not yet reported	Elevated amylase or lipase (grade 3, $n = 2$); increase in liver function tests (grade 3, $n = 2$)
Semaxanib (SU5416) [53]	(a) 110, 145, 190, 250, 330 mg/m ² i.v. twice weekly; (b) 48, 65, 85 mg/m ² i.v. twice weekly (patients on EIACD); ceased clinical development	Refractory CNS tumors: (a) $n = 23$; age, 8.5 (2.2–21) yrs; (b) $n = 10$; age, 10.6 (1.5–16.4) yrs	110 mg/m ² ; $C_{max} = 10 \mu M$; $T_{1/2} < 1$ hour	NR	NR	NR	Hallucinations (grade 3, $n = 1$); increased liver function tests (grade 3, $n = 3$); headache (grade 3, $n = 1$); rash (grade 3 or 4, $n = 1$)
Sorafenib [54]	105, 130, 150, 200, 250 mg/m ² PO daily $\times$ 28 days; recommended: 200 mg/m ² PO daily $\times$ 28 days	Refractory solid tumors, $n = 34$; age, 16 (5–21) yrs; cycles, 2 (1–22)	200 mg/m ² ; $C_{max} = 3 \mu M$; $T_{1/2} > 24$ hours	NR	Hypertension (grade 3, $n = 1$)	NR	Hand-foot skin reactions; increase in liver function tests
Sunitinib [56, 114]	15, 20 mg/m ² PO daily $\times$ 4 wks every 6 wks; recommended: NR	Refractory solid tumors, $n = 12$; age, 15 (10–20) yrs	20 mg/m ² ; $ssC_{trough} = 0.1 \mu M$; $T_{1/2} = NR$	No epiphyseal expansion ($n = 1$)	Decrease in LVEF (grade 3, $n = 1$; grade 2, $n = 1$); QTc prolongation (grade ≤ 2 , $n = 2$; max QTc = 490 msec)	Hypothyroidism (grade 2, $n = 5$)	
	25, 37.5, or 50 mg PO, daily $\times$ 4 wks every 6 wks	Imatinib-resistant GIST, $n = 7$; age, 15 (10–17) yrs; cycles, 14.6 (12–15)	NR	NR	NR	Hypothyroidism (grade ≤ 2 , $n = 2$)	
Vandetanib (ZD6474) [79, 80]	50, 65, 85, 110, 145 mg/m ² PO daily $\times$ 28 days; recommended: 145 mg/m ² PO daily $\times$ 28 days	Diffuse pontine glioma (with radiation), $n = 35$; age, 6.4 (2.8–16.4) yrs; cycles, 7.5 (<1–24)	145 mg/m ² ; $C_{max} = 0.7 \mu M$; $ssC_{trough} = 1.9 \mu M$; $T_{1/2} = NR$	Premature closure of growth plate (1 of 30)	Hypertension (grade 4, $n = 2$); asymptomatic QTc prolongation ($n = 9$)	NR	Posterior reversible encephalopathy syndrome
	100, 150 mg/m ² PO daily $\times$ 28 days; recommended: NR	Hereditary medullary thyroid carcinoma, $n = 7$; age, 16 (9–18) yrs; cycles, 14 (10–26)	NR	Linear growth continued; growth plate volume % change, 18% (–44% to 50%)	Hypertension (grade 2, $n = 1$)	Hypothyroidism ($n = 6$ athyreotic patients on levothyroxine)	

Abbreviations: C_{max} , maximum concentration; CNS, central nervous system; EIACD, enzyme inducing anti-convulsant drug; FSH, follicle-stimulating hormone; GIST, gastrointestinal stromal tumor; LH, luteinizing hormone; LVEF, left ventricular ejection fraction; NR, not reported; PK, pharmacokinetics; PO, orally; ssC_{trough} , steady-state trough concentration; $T_{1/2}$, terminal half-life.

cording to the Common Terminology Criteria for Adverse Events (versions 3 and 4) and recommendations by the Angiogenesis Task Force of the National Cancer Institute Investigational Drug Steering Committee [73] are not easily applicable to children. Some trials in children have adopted an algorithm for blood pressure monitoring and management that permits management of mild to moderate hypertension with single-agent antihypertensive medication without dose reduction or interruption of the VEGF signaling pathway inhibitor [54, 55, 57, 74]. In children, correlation between blood pressure elevation and tumor response is limited to date because of the small sample sizes of the clinical trials.

Hemorrhage and Thrombosis

Hemorrhagic and thrombotic complications of VEGF signaling pathway inhibitors may be related to disruption of the delicate hemostatic balance and altered platelet–endothelial cell interactions. In animal models, the absence of VEGF increases endothelial cell apoptosis and compromises tight junctions, leading to exposure of the prothrombotic basement membrane of the vessel [75]. In adults treated with bevacizumab, the rates of thromboembolism were 11.9% (all grades) and 6.3% (grade 3 or 4) [73]. Hemorrhage is the most common fatal adverse event in adults receiving bevacizumab combined with chemo-

Table 4. Clinical trials of BV in children with cancer

Study	Design	Agents	Disease	Status (ClinicalTrials.gov identifier)
CCHMC Cincinnati, OH	Pilot	Vincristine, irinotecan, and temozolomide + BV	Recurrent solid tumors	Open to accrual (NCT00786669)
CCHMC, Cincinnati, OH	Pilot	Temozolomide + radiation + BV	Newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma	Open to accrual (NCT00890786)
SJCRH, Memphis, TN	Phase I	Everolimus + BV	Recurrent solid tumors	Open to accrual (NCT00756340)
All Children's Hospital, St. Petersburg FL	Phase I	Irinotecan + temozolomide + BV	Recurrent or refractory brain tumors	Open to accrual (NCT00876993)
Children's Hospital of Los Angeles, Los Angeles, CA	Phase I	Irinotecan + temozolomide + BV	Recurrent solid tumors	Open to accrual (NCT00993044)
SJCRH, Memphis, TN	Phase I	Sorafenib + cyclophosphamide + BV	Recurrent solid tumors	Open to accrual (NCT00665990)
NANT N2007-02	Phase I	Cyclophosphamide + zoledronic acid + BV	Recurrent high-risk neuroblastoma	Open to accrual (NCT00885326)
MSKCC, New York, NY	Phase I	¹³¹ I-3F8 + BV	Recurrent neuroblastoma	Open to accrual (NCT00450827)
PBTC-022	Single-arm phase II	Irinotecan + BV	Brain tumors	Completed (NCT00381797)
MSKCC, New York, NY	Single-arm phase II	Irinotecan and temozolomide + BV	Recurrent neuroblastoma	Open to accrual (NCT01114555)
CERN08	Single-arm phase II	Lapatinib + BV	Recurrent ependymoma	Open to accrual (NCT00883688)
ASSG01 Texas Children's Hospital, Houston, TX	Single-arm phase II	Valproate + BV + radiation	Newly diagnosed high-grade glioma	Open to accrual (NCT00879437)
MSKCC, New York, NY	Single-arm phase II/III	Irinotecan, temozolomide, high dose alkylator, + BV	Newly diagnosed desmoplastic small round blue cell tumor	Open to accrual (NCT01189643)
OS2008 SJCRH, Memphis, TN	Single-arm phase II/III	Standard therapy + BV	Newly diagnosed localized or metastatic osteosarcoma	Open to accrual (NCT00667342)
COG AEWS0521	Randomized selection phase II	Vincristine, topotecan, and cyclophosphamide with or without BV	Recurrent Ewing's sarcoma	Closed (NCT00516295)
COG ACNS0821	Randomized selection phase II	Irinotecan + temozolomide with or without BV	Recurrent PNET/medulloblastoma	Open to accrual (NCT01217437)
COG ACNS0822	Randomized selection phase II	Temozolomide versus BV versus vorinostat with local radiation	Newly diagnosed high-grade glioma	Open to accrual (NCT01236560)
COG ARST0921	Randomized selection phase II	Vinorelbine + cyclophosphamide + BV versus temsirolimus	Recurrent rhabdomyosarcoma	Open to accrual (NCT01222715)
ITCC 006/BO20924	Randomized phase II	Standard therapy with or without BV	Newly diagnosed metastatic rhabdomyosarcoma or soft tissue sarcoma	Open to accrual (NCT00643565)
Lawlor-1/DFCI and other sites	Biologically stratified	BV plus radiation with or without erlotinib or temozolomide based on EGFR/MGMT status	Newly diagnosed diffuse intrinsic pontine glioma	Approved, not yet activated (NCT01182350)

Trial status from ClinicalTrials.gov, February 28, 2011.

Abbreviations: BV, bevacizumab; CCHMC, Cincinnati Children's Hospital Medical Center; CERN, Collaborative Ependymoma Research Network; COG, Children's Oncology Group; DFCI, Dana-Farber Cancer Institute; EGFR, epidermal growth factor receptor; ITCC, Innovative Therapies for Children with Cancer; MGMT, O-6-methylguanine-DNA methyltransferase; MSKCC, Memorial Sloan-Kettering Cancer Center; NANT, New Approaches to Neuroblastoma Therapy; PBTC, Pediatric Brain Tumor Consortium; PNET, pediatric neuroendocrine tumor; SJCRH, St. Jude Children's Research Hospital.

therapy. In a meta-analysis of randomized clinical trials comparing chemotherapy with chemotherapy plus bevacizumab, the incidences of fatal hemorrhage were 0.5% (95% confidence interval [CI], 0.1%–1.7%) in controls and 1.3% (95% CI, 0.6%–2.9%) in patients treated with chemotherapy plus bevacizumab. The relative risk (RR) for fatal hemorrhage with chemotherapy plus bevacizumab was 2.77 (95% CI, 1.07–7.16) [76]. No hemorrhage or thrombosis occurred in children with solid tumors in monotherapy phase I studies of bevacizumab or cediranib [52, 55]. Dose-limiting intratumoral bleeding did occur in the pediatric phase I aflibercept trial [74]. Whether this is unrelated to aflibercept or reflects a 10-fold higher VEGF binding affinity than with bevacizumab and broader target inhibition remains unclear. Careful monitoring for the development of hemorrhage or thromboembolic events in future trials, particularly, combination trials with myelosuppressive agents, is warranted.

Left Ventricular Cardiac Dysfunction

VEGF knockout mice develop dilated cardiomyopathy, indicating that VEGF has an important function in cardiomyocyte integrity. Anthracycline-related cardiotoxicity results from direct toxicity of free radicals to myocytes. The ventricular dysfunction recognized during clinical trials of sunitinib, sorafenib, and other molecularly targeted anticancer agents appears to be a result of an indirect effect of VEGF-blocking agents. In adults with imatinib-resistant GISTs receiving sunitinib, a decrease in left ventricular ejection fraction (LVEF) to ≤50% was observed in 20% of patients, and 8% developed clinical congestive heart failure (CHF). Cardiac biopsies demonstrated cardiomyocyte hypertrophy and swollen mitochondria without evidence of edema, inflammation, or fibrosis [62]. Microvessel rarefaction and hypertension produce increased afterload that may contribute to ventricular dysfunction. PDGF improves heart failure in animal models [77], and

agents including sunitinib, sorafenib, cediranib, and pazopanib inhibit PDGFR, providing a possible link between off-target effects and cardiotoxicity. However, cardiotoxicity is not limited to TKIs. In women with breast cancer receiving bevacizumab, the RR for CHF was 4.74 (95% CI, 1.66–11.18), compared with women receiving placebo [78].

In children, the phase I trial of sunitinib was amended to restrict the cumulative prior anthracycline exposure, because of left ventricular dysfunction observed at doses below the adult maximum-tolerated dose [56]. Reversible, nondose-limiting decreased LVEF values also occurred in the cediranib and pazopanib studies in children. Ongoing TKI studies in children require periodic echocardiograms to assess LVEF.

Conduction Abnormalities

Prolongation of cardiac ventricular depolarization and repolarization (QT interval) is not unique to VEGF-related TKIs. Drugs that interfere with the transmembrane flow of ions in cardiac cells, increase depolarizing currents, or decrease repolarizing currents result in a prolonged action potential and QT interval on electrocardiogram. In adults, sunitinib and vandetanib have been associated with QTc prolongation. In children, all clinical trials of VEGF TKIs now include electrocardiogram screening. To date, a prolonged QTc interval has been observed in children receiving vandetanib [79, 80] and cediranib [55], but not pazopanib [57].

Epiphyseal Growth Plate and Bone Health in Children

During endochondral ossification, hyaline cartilage at the mineralizing front of the epiphyseal growth plate is replaced by bone. Chondrocytes proliferate, synthesize the avascular cartilagenous matrix, mature, and hypertrophy. VEGF and other factors secreted by hypertrophic chondrocytes facilitate capillary invasion and differentiation of VEGFR-expressing osteoblasts and osteoclasts. Chondrocytes undergo apoptosis, leaving a mineralized cartilagenous matrix scaffold on which osteoblasts deposit trabecular bone and osteoclasts remodel the mineralized matrix. Normally, the size of the epiphyseal growth plate remains relatively constant during linear growth. In early adulthood, chondrocytes in the epiphyseal growth plate degenerate, the growth plate ossifies, and linear growth ceases. However, throughout life, continued osteoblast and osteoclast activity for appositional bone growth occurs in response to changes in weight, muscle activity, stress, and injury [81]. The impact of VEGF signaling pathway inhibitors on healing of injured bone and remodeling of normal bone and on bone metastasis are areas of ongoing research [82, 83].

In animal models, VEGF inhibition results in thickening of the epiphyseal growth plate as a result of expansion of hypertrophic chondrocytes. This has been attributed to delayed vascular invasion, reduction in chondrocyte apoptosis, and impaired trabecular bone formation. VEGF signaling pathway inhibitors result in reversible inhibition of long bone growth in juvenile animals [84–87].

In children with cancer enrolled in dose-finding studies of VEGF signaling pathway inhibitors, monitoring growth plates

has included assessment of left wrist radiographs for bone age, serial radiographs of the lower extremities, or volumetric measurements of the right distal femoral growth plate using magnetic resonance imaging. Epiphyseal growth plate thickening has not been detected in children enrolled in early-phase clinical trials of bevacizumab [52], bevacizumab in combination with ¹³¹I-3F8 antibody [88], sunitinib [56], or cediranib [55]. However, the duration of exposure to these antiangiogenic agents was limited. To date, there have been rare reports of skeletal toxicity. In the pediatric phase I/II trial of vandetanib in children (*n* = 10) with medullary thyroid cancer, the median percent change in growth plate volume during therapy was –18% (range, –44% to 50%), with all patients having linear growth during therapy [80]. One child with diffuse intrinsic pontine glioma treated with vandetanib had minimal closure of the growth plate [79]. An infant treated with bevacizumab for a proliferative vascular disorder had reversible radiographic changes of the metaphyses [89]. Because the duration of exposure to these agents in pediatric oncology studies has been limited, current TKI trials for other benign diseases involving children, such as neurofibromatosis type 1, may provide an opportunity for longitudinal data on effects on the growth plate and linear growth.

Endocrine Toxicity

In endocrine glands, vascularization and fenestrated endothelium facilitate transport of secretory peptides and hormones among interstitial fluid, parenchymal cells, and plasma. Hormones including thyroid-stimulating hormone (TSH), adrenocorticotrophic hormone, gonadotropins, and sex steroids are inducers of VEGF [4]. In animal models, paracrine and autocrine feedback of VEGF has been described in the ovary, testis, and pancreas [90, 91].

In clinical trials, endocrine side effects of VEGF signaling pathway inhibitors are consonant with the specialized function of the endothelium in endocrine glands. In females, physiological VEGF-mediated angiogenesis occurs in the endometrium during the menstrual cycle, corpus luteal development, and early pregnancy. The impact of VEGF signal inhibitors on long-term female reproductive function has not been systematically studied, but VEGF suppression would be predicted to cause reversible menstrual abnormalities resulting from intraovarian effects on periovulatory events [92]. Elevation in amylase and lipase during sunitinib therapy indicate toxicity to the exocrine pancreas. Effects on the endocrine pancreas have also been reported. In adults with RCC, sunitinib lowered blood sugars and improved glycemic control in 19 diabetic patients, with two patients able to discontinue antidiabetic medication during sunitinib administration [93].

Hypothyroidism has occurred in adults and children receiving bevacizumab or VEGF TKIs. Hypothyroidism was reported in 36%–46% of adults receiving sunitinib. The duration of sunitinib administration increased the risk for developing hypothyroidism, with a mean time to onset 12–50 weeks after therapy initiation [94]. A number of mechanisms have been proposed [95], including reduction in capillary blood flow, capillary regression and constriction within the thyroid [96],

immune and nonimmune thyroiditis, inhibition of iodine uptake [97, 98], and inhibition of thyroid peroxidase activity [99], but no correlation has been made with potency of RET inhibition. In athyreotic patients with thyroid carcinoma, sorafenib enhanced thyroxine and 3,5,3 tri-iodothyronine metabolism, possibly through increased type 3 deiodination, requiring increased levothyroxine supplementation [100]. Similarly, athyreotic children with medullary thyroid carcinoma required increased levothyroxine supplementation during vandetanib therapy [80]. Some children with cancer receiving cediranib or pazopanib have required levothyroxine supplementation because of drug-related elevations in TSH [55, 57].

CONCLUDING REMARKS

The goal of drug development for childhood cancers is to improve the overall survival rate and decrease the long-term side effects of treatment. Table 3 summarizes the published data from the single-agent, dose-finding clinical trials of specific antiangiogenic agents evaluated in children with refractory solid tumors. These preliminary studies have determined recommended phase II doses that are similar to those used in adults and have generally reassured the pediatric oncology community that the toxicity profile of this class of agent should not impede their further development in children. Prioritizing individual antiangiogenic compounds for further development should take into consideration drug availability, appropriate formulations, relative potency, antitumor activity in adults, and agent-specific side effects. Nonetheless, as with all novel agents, the single greatest challenge to deployment of antiangiogenic agents into the armamentarium of drugs for pediatric cancer is designing phase II trials that demonstrate sufficient antitumor activity to justify their placement in the upfront or early salvage therapy of childhood malignancy.

The ability to predict the relative activity of the antiangiogenic agents poised to move forward in the clinic based on their performance during preclinical testing is, as yet, an untested hypothesis. Screening multiple agents given the small

relapsed populations available for pediatric trials is likely unfeasible. As yet, biomarkers predictive of response to VEGF inhibitors have not been forthcoming. Finally, adult trials, with a few notable exceptions, including those in high-grade glioma and sarcoma, have shown benefits in terms of longer times to progression, not tumor response. Careful choice of tumor histologies for study, clinically relevant endpoints, and prospective definition of adequate signal to move an antiangiogenic agent to the first-line setting are critical. Ultimately, targeting the VEGF signaling pathway in children with cancer requires combining these agents with standard cytotoxic agents or other molecularly targeted agents. Initial combination studies for feasibility may be nonrandomized. However, to determine the impact of VEGF signaling inhibitors on outcome and toxicity, randomized comparison trials are necessary.

Ultimately, upfront childhood cancer therapy frequently includes surgery, radiation, and, in some cases, stem cell transplant therapy, which carries additional acute risks as well as delayed morbidity associated with the cardiovascular system, endocrine system, and growth. In the next generation of single-agent and combination studies, toxicity profiles, potential drug interactions, and both acute and late effects must be monitored closely. Further understanding of the mechanism and reversibility of these side effects is essential to define the role of antiangiogenic agents in the treatment of childhood cancer.

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