A Fully Human Insulin-Like Growth Factor-I Receptor Antibody SCH 717454 (Robatumumab) Has Antitumor Activity as a Single Agent and in Combination with Cytotoxics in Pediatric Tumor Xenografts

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Abstract

The insulin-like growth factor-I receptor (IGF-IR) and its ligands (IGF-I and IGF-II) have been implicated in the growth, survival, and metastasis of a broad range of malignancies including pediatric tumors. Blocking the IGF-IR action is a potential cancer treatment. A fully human neutralizing monoclonal antibody, SCH 717454 (19D12, robatumumab), specific to IGF-IR, has shown potent antitumor effects in ovarian cancer in vitro and in vivo. In this study, SCH 717454 was evaluated in several pediatric solid tumors including neuroblastoma, osteosarcoma, and rhabdomyosarcoma. SCH 717454 is shown here to downregulate IGF-IR as well as inhibit IGF-IR and insulin receptor substrate-1 phosphorylation in pediatric tumor cells. Combination of SCH 717454 with cisplatin or cyclophosphamide enhanced both the degree and the duration of the in vivo antitumor activity compared with single-agent treatments. Furthermore, SCH 717454 treatment markedly reduced Ki-67 expression and blood vessel formation in tumor xenografts, showing that the in vivo activity is derived from its inhibition of tumor cell proliferation and angiogenesis activity.

Introduction

In the United States, 12,400 children ages <20 years were diagnosed with cancer and 2,500 died of cancer in 1998 (1). For children between ages 1 and 19 years, cancer ranked fourth as a cause of death. The probability of developing cancer before age 20 years is 1 in 300. Neuroblastoma, bone tumors including osteosarcoma, and soft-tissue tumors including rhabdomyosarcoma represent three important groups of pediatric solid tumors and collectively account for nearly one-fifth of all pediatric malignancies (1). The currently available treatments include surgical resection, chemotherapy, and radiation. Despite recent advances in these therapeutic modalities, tumors frequently recur, and children with tumor metastasis have poor prognosis (2, 3). Therefore, there is an unmet need for new and effective treatment modalities for pediatric solid tumors.

The insulin-like growth factor-I receptor (IGF-IR), a transmembrane tyrosine kinase that is selectively activated by its ligands IGF-I and IGF-II, has been strongly implicated in the growth, survival, and metastasis of a wide variety of human tumors, including tumors in children and adolescents (4-9). Many pediatric tumors including neuroblastoma, Wilms’ tumor, osteosarcoma, and rhabdomyosarcoma are embryonal tumors of the childhood and exhibit increased expression of IGF-II and IGF-IR establishing autocrine loops to perpetuate growth and survival (10-15). The role of IGF-I in the development of Ewing’s sarcoma has also been well documented in addition to the induction of IGF-IR expression by the Ewing’s sarcoma fusion protein (16-19). Increased expression of IGF-IR in rhabdomyosarcomas has also been linked to chimeric transcription factor PAX3-FKHR (20). Highly metastatic neuroblastoma and osteosarcoma cells possess higher expression of IGF-IR than tumor cells that are less prone to metastasize (21). Furthermore, IGF-IR activation has been shown to generate strong antiapoptotic signals in various cancer cells including sarcoma and neuroblastoma cells, conferring resistance to the cytotoxic effects of chemotherapies (6, 21, 22). Epidemiologic data have linked elevated plasma levels of IGF-I and IGF-II with increased risk for major cancers such as breast, colon, lung, and prostate cancers, although no such data are currently available for pediatric cancers (6, 23-25). Importantly, selective impairment of IGF-IR using...
different approaches, including antisense technologies, dominant-negative mutants of IGF-IR, tyrosine kinase inhibitors, and neutralizing anti–IGF-IR antibodies, inhibited tumor cell growth, induced tumor cell apoptosis, increased tumor sensitivity to cytotoxic drugs, and decreased metastasis of cancer cells of both adult and pediatric origins (16, 26–31). These observations provide a strong rationale for targeting IGF-IR against pediatric cancers.

Monoclonal antibodies and tyrosine kinase inhibitors are being vigorously pursued as potential anticancer therapies with antibodies promising to provide greater specificity and safety. To minimize the potential for immunologic liabilities of non-human antibodies, several fully human monoclonal antibodies against IGF-IR several fully human monoclonal antibodies against IGF-IR have recently been generated (32–35). These antibodies exhibit potent antitumor effects in breast, colon, prostate, ovarian, and pancreatic cancers in vitro and in vivo and offer great potential for success as safe and effective human anticancer therapeutics. We and others have previously described SCH 717454 (19D12, robatumumab), a fully human neutralizing anti–IGF-IR antibody that potently inhibits ligand binding and signaling (34, 36, 37). SCH 717454 inhibits the growth of various mouse xenograft tumor models. In the current study, we evaluated the in vivo effects of SCH 717454 in mouse xenograft models of neuroblastoma, osteosarcoma, and rhabdomyosarcoma. Our results show that SCH 717454 alone is efficacious in inhibiting tumor growth in these models and that its combination with cytotoxic agents (cyclophosphamide and cisplatin) results in a greater antitumor effect than that observed for either agent used alone.

Materials and Methods

Reagents

SCH 717454 is a fully human neutralizing IgG1 antibody raised against human IGF-IR using Medarex’s HuMab mouse technology (34). SCH 717454 was prepared, stored, and diluted in sodium acetate buffer [20 mmol/L sodium acetate, 150 mmol/L NaCl (pH 5.0–5.5)]. Control human IgG1 was purchased from The Binding Site. Cyclophosphamide and cisplatin were obtained from Sigma. Vehicle for these cytotoxic agents (cyclophosphamide and cisplatin) results in a greater antitumor effect than that observed for either agent used alone.

Cell Lines

Neuroblastoma cell lines (SK-N-AS and SK-N-FI), rhabdomyosarcoma cell line SJCRH30 and RD), and the osteosarcoma cell line SJSA-1 were obtained from the American Type Culture Collection. These cell lines were maintained in the RPMI 1640 supplemented with 10% fetal bovine serum (Invitrogen/Life Technologies). Animals used for tumor xenografts in the study were 6- to 15-week-old female nude mice (nu/nu) weighing 18 to 24 g (Charles River Laboratories). Mice were housed at Schering-Plough Research Institute according to the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care. Conventional animal chow and water were provided ad libitum. Animals were handled according to the protocols approved by the Schering-Plough Institutional Animal Care and Use Committee. For all studies, mice were allowed to acclimate for at least 3 days after receipt, and test animals were randomized into groups before the start of the treatments.

Measurements of IGF-I and IGF-II

Tumor cells were seeded on in RPMI 1640 containing 10% fetal bovine serum. After cells were attached, medium was changed to serum-free medium. After 24 h, cell medium was collected, debris was spun down, and supernatants were lyophilized. Cells on the plates were trypsinized and counted. Water was added to each lyophilized supernatant sample (1 mL/2 × 107 cells). IGF-I and IGF-II were measured using ELISA kits from Diagnostic System Laboratories.

Western Blot Analysis of IGF-IR and Insulin Receptor Substrate-1

Cells were treated as described and then lysed in lysis buffer [50 mmol/L HEPES (pH 7.4), 100 mmol/L NaCl, 10% glycerol, 1% Triton X-100, 1.5 mmol/L MgCl2, protease inhibitors, and 2 mmol/L sodium vanadate]. The blot was probed with anti–IGF-IR antibody (Santa Cruz Biotechnology), anti-phosphorylated IGF-IR (Cell Signaling Technology), anti-phosphorylated insulin receptor substrate-1 (IRS-1) antibody (Cell Signaling Technology), and anti-total IRS-1 antibody (Santa Cruz Biotechnology) and visualized by enhanced chemiluminescence.

In vivo Tumor Growth Assessments

For tumor implantation, specific cell lines were grown in vitro, washed once with PBS, and resuspended in 50% Matrigel (BD Biosciences) to a final concentration of 4 × 106 to 5 × 106 cells/mL. Nude mice were injected with 0.1 mL of this suspension s.c. in the flank region. Tumor volumes were measured by caliper twice a week. When tumor sizes reached 75 to 150 mm3, the animals were randomized to appropriate treatment groups, and i.p. treatments with either control human IgG1, SCH 717454, chemotherapeutic agents, or combinations were initiated. All in vivo efficacy studies were done with 10 mice per treatment group unless specifically indicated. At various intervals of treatment (usually twice per week), tumor volumes were measured, and antitumor activity was expressed as the percent inhibition of tumor size. Body weight was recorded at the time of tumor measurement. Animals were euthanized according to the Institutional Animal Care and Use Committee guidelines. Statistical analysis by Student’s t test was used to compare treatments.

Ex vivo Immunohistochemical Analysis of Tumor Ki-67

Xenograft tumor samples were collected after treatment with SCH 717454. Tumor samples were fixed in
10% buffered formalin, embedded in paraffin, and cut into 4 to 6 μm sections with Microtome onto slides. After deparaffinization and rehydration, antigens were retrieved with 1× Cytomation target retrieval solution from Dako in a decloaker chamber at 95°C for 20 min and then at room temperature for 20 min followed by sequential rinsing with distilled water and PBS at room temperature. Slides were incubated with hydrogen peroxide for 5 min to quench endogenous hydrogen peroxidase activity. After rinsing twice with TBS-0.1% Tween 20, slides were incubated with mouse anti-Ki-67 antibody (BD Pharmingen) and mouse control IgG (Vector Laboratories), each at a concentration of 5 μg/mL, for 15 min and then rinsed again with TBS-0.1% Tween 20. Slides were incubated sequentially with biotinylated goat anti-mouse IgG for 15 min, peroxidase-labeled streptavidin (Dako) for 15 min, and dianinobenzidine/hydrogen peroxide chromogen substrate (Vector Laboratories) for 5 min. All incubation steps were done at room temperature. Slides were counterstained with hematoxylin for 2 min and rinsed with distilled water. Slides were evaluated under an optical light microscope, and photographs were obtained showing brown staining of Ki-67 in nuclei of cells. Ki-67 signal was analyzed and quantified using ImagePro Plus 5.0 (Media Cybernetics).

**Analysis of Tumor Blood Vessel Formation**

To visualize blood vessel within the tumors, xenografted mice were treated with either control IgG1 or SCH 717454 when tumor size reached ~150 to 200 mm³. Xenograft mice were treated twice (on days 0 and 3). On day 5 after the initiation of SCH 717454 treatment (day 16 post-inoculation), mice were anesthetized, and FITC-labeled *Lycopersicon esculentum* (tomato lectin; FITC-lectin), an endothelial cell selective reagent (Vector Laboratories), was injected slowly via tail vein (0.1 mL of 1 mg/mL FITC-lectin solution). Cardiac perfusion was done with 10 mL of 4% paraformaldehyde followed by 10 mL PBS. Tumor was dissected and immersed sequentially in 12%, 15%, and 18% sucrose for 1 h each and then

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**Figure 1.** SCH 717454 downregulates IGF-IR and inhibits both basal and IGF-I–induced phosphorylation of IGF-IR and IRS-1 in pediatric tumor lines.

A, SK-N-FI cells either untreated or treated with control IgG1 (lane 2; 80 nmol/L) or various concentrations of SCH 717454 (0.2-80 nmol/L) for 0.5 or 4 h before stimulation with IGF-I (50 ng/mL) for 10 min. Cell lysates were subjected to Western analysis and probed with phosphorylated IGF-IR–specific antibody (top). The filter was then reprobed with an antibody that recognizes total IGF-IR. For the 4 h time point, the same blot was stripped and reprobed with anti-actin antibody as loading control. B, SK-N-FI cells were either untreated or treated with control IgG1 (lane 2; 80 nmol/L) or various concentrations of SCH 717454 (0.2-80 nmol/L) for 0.5 h before stimulation with IGF-I (50 ng/mL) for 10 min. Cell lysates were probed with anti-phosphorylated IRS-1 antibody (pY612). C, various pediatric tumor cell lines were either untreated (control; lane 1) or treated with SCH 717454 (20 nmol/L, 4 h; lane 2) or IGF-I (100 ng/mL, 20 min; lane 3). Cell lysates were then probed with anti-phosphorylated IRS-1 antibody (pY612). The blots were stripped and reprobed with anti-total IRS-1 antibody.
30% sucrose overnight at 4°C. Tumor samples were embedded in Tissue-Tek OCT from Sakura, and 20 μm tissue sections were prepared. Tissue sections were analyzed using a Leica confocal microscope. The fluorescent lectin signal intensity and total area (pixels) were analyzed and quantified using ImagePro Plus 5.0.

Results

**In vitro Characterization of Tumor Cell Lines**

Upon stimulation by its ligands (IGF-I and IGF-II), the activated (phosphorylated) IGF-IR phosphorylates the IRS-1 (the immediate downstream signaling molecule), which results in further downstream events leading to increased cell proliferation and cell survival. To study the IGF-IR signaling pathway in the neuroblastoma, osteosarcoma, and rhabdomyosarcoma, we looked at the status of total IGF-IR as well as IGF-IR phosphorylation in response to SCH 717454 in these tumor cell lines. We discovered that, shortly after treatment with SCH 717454 (0.5 h), the IGF-I–stimulated phosphorylation of IGF-IR was significantly inhibited in the neuroblastoma SK-N-FI cells, whereas the level of total IGF-IR protein remains unchanged (Fig. 1A, top). Extended treatment with SCH 717454 (4 h) resulted in both inhibition of IGF-IR phosphorylation and receptor downregulation (Fig. 1A, bottom). Similar responses were observed in the other pediatric tumor cell lines used in this study (data not shown). Next, we looked at signaling molecule downstream of the receptor by analyzing the phosphorylation status of IRS-1 in these tumor cells. The SK-N-FI neuroblastoma cells exhibited high basal expression of phosphorylated IRS-1, and this signal is further enhanced by IGF-I. Treatment with SCH 717454 results in a dose-dependent inhibition of the IGF-I–stimulated IRS-1 phosphorylation (Fig. 1B), confirming that the IGF-I signaling pathway is intact in this pediatric tumor cell line and that this pathway can be effectively inhibited by anti–IGF-IR antibody SCH 717454. Phosphorylated IRS-1 was also detected in other unstimulated pediatric tumor cell lines, and this basal phosphorylation was inhibited by SCH 717454 (Fig. 1C). These pediatric tumor cell lines produced IGF-II in large amounts, with SJCRH30 cells releasing IGF-I in substantial quantities (Table 1). The data shown here show that pediatric tumor cells used in the present study possess the functional IGF-IR and have the capacity to establish autocrine loops in promoting tumor growth in vivo.

**Inhibition of Neuroblastoma Growth In vivo**

After SK-N-FI tumor cells were inoculated s.c. to nude mice, treatment was initiated when the average tumor size reached 150 mm³ (~21 days post-inoculation). Mice in various groups were received either control human IgG1 (0.1 mg/mouse), SCH 717454 (0.04 or 0.1 mg/mouse), cisplatin (2 mg/kg i.p. twice per week), cyclophosphamide (100 mg/kg i.p. once per week), or combination of SCH 717454 with either cisplatin or cyclophosphamide on the same dosing schedules and frequency. At day 21 after treatment initiation, the SK-N-FI xenograft tumor was inhibited by 96% in the 0.04 mg SCH 717454 dose group and resulted in 11% tumor regression in the 0.1 mg dose group (Fig. 2A). In contrast, treatment with cisplatin or cyclophosphamide resulted in only 54% or 39% tumor growth inhibition, respectively, in this neuroblastoma xenograft model.

The effect of combined administration of SCH 717454 and cisplatin on SK-N-FI tumor xenografts was evaluated. The combined administration of 0.04 mg SCH 717454 and cisplatin was significantly more effective (34% tumor regression) than either agent alone (Fig. 2A). The combination of 0.1 mg SCH 717454 with cisplatin (38% tumor regression) was not better compared with 0.1 mg SCH 717454 used alone (11% tumor regression) based on statistical analysis. This is probably due to the fact that treatment with 0.1 mg SCH 717454 had already resulted in significant tumor regression by itself, that suggesting maximal in vivo response was reached at 0.1 mg SCH 717454 in this xenograft model. Similar results were observed when SCH 717454 was combined with cyclophosphamide (Fig. 2B).

**Table 1. Secretion of IGF-I and IGF-II by pediatric tumor lines**

<table>
<thead>
<tr>
<th>Cell</th>
<th>IGF-I</th>
<th>IGF-II</th>
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<tbody>
<tr>
<td>SK-N-AS</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>SK-N-FI</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>SJSA-1</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>SJCRH30</td>
<td>++++</td>
<td>++++</td>
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<td>RD</td>
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NOTE: Cells were cultured in serum-free medium for 24 h. Medium was collected, and each medium sample was assayed for IGF-I and IGF-II by ELISA. +, 0.1 to 1 ng/10⁶ cells; ++, 1 to 10 ng/10⁶ cells; ++++, 10 to 50 ng/10⁶ cells; ++++, >50 ng/10⁶ cells.

**Inhibition of Osteosarcoma Growth In vivo**

Subcutaneous inoculation of the SJSA-1 osteosarcoma line into nude mice resulted in a time-dependent growth of tumors. This tumor growth was effectively suppressed by both SCH 717454 and cyclophosphamide as single agents (Fig. 2C). At day 14 after treatment initiation, tumor growth was inhibited by 71%, 82%, and 88% at 0.02, 0.1, and 0.5 mg SCH 717454, respectively, and by 97% at 100 mg/kg cyclophosphamide. There was no discernible tumor regression by single-agent treatments. However, combinations of 100 mg/kg cyclophosphamide with SCH 717454 at doses of 0.1 and 0.5 mg regressed tumors...
by 41% and 53%, respectively ($P < 0.05$), on day 10 after the treatment initiation. To determine if combination treatments could affect the duration of the antitumor response, after the first week of treatment, SCH 717454 dosing (but not cyclophosphamide dosing) was continued until the mean tumor size reached 1,000 mm$^3$. In groups receiving SCH 717454 as a single agent, the tumors reached 1,000 mm$^3$ by day 21. In contrast, in cyclophosphamide and SCH 717454 combination groups where SCH 717454 treatment continued, tumor growth was substantially delayed with the mean tumor size reaching 1,000 mm$^3$ by day 38 (Fig. 2C). Quantitation by the log tumor cell kill method (value ≥ 2.0; Supplementary Table S1) showed the combination treatment elicited a further and substantial tumor response in this osteosarcoma model. No significant body weight loss was

Figure 2. SCH 717454 inhibits pediatric tumor growth in xenograft models as a single agent and in combination with chemotherapeutic agents. A and B, nude mice were inoculated s.c. with SK-N-Fi neuroblastoma cells ($5 \times 10^6$ per mouse). When the average tumor size reached 150 mm$^3$ (on day 21 post-inoculation), tumor-bearing mice were randomized to indicated treatment groups (10 mice per group) and treatments (i.p. route) with either 0.1 mg control IgG1 antibody, 0.04 mg or 0.1 mg SCH 717454 (twice weekly), 100 mg/kg cyclophosphamide (once weekly), 2 mg/kg cisplatin (twice weekly), or both SCH 717454 and cyclophosphamide were initiated. C, nude mice were inoculated s.c. with SJSA-1 osteosarcoma cells (7 $\times$ 10$^6$ per mouse). When tumor size reached 96 mm$^3$, mice randomized to indicated treatment groups (10 per group) were treated i.p. (twice weekly) with either control IgG1 antibody, SCH 717454, 100 mg/kg cyclophosphamide, or both SCH 717454 and cyclophosphamide. Note that cyclophosphamide dosing ceased after three doses, whereas SCH 717454 dosing continued until the tumor volume reached 1,000 mm$^3$. D, effects of SCH 717454 on RD rhabdomyosarcoma xenograft growth as a single agent and in combination with cyclophosphamide. Nude mice were inoculated s.c. with RD rhabdomyosarcoma cells (7 $\times$ 10$^6$ per mouse). Treatment (i.p. twice weekly for 0.5 mg control IgG1, 0.1 and 0.5 mg SCH 717454, and 100 mg/kg cyclophosphamide) was initiated when the average tumor size reached 125 mm$^3$. On day 18 after the treatment initiation, when control group tumor volume reached 660 mm$^3$, the study was terminated and tumor growth inhibition or regression in various groups was calculated. Bars, SE. *, $P < 0.05$, compared with IgG1 control; #, $P < 0.05$, compared with single treatments.
observed in both single-agent and combination groups during the course of study (Supplementary Fig. S2).

**Inhibition of Rhabdomyosarcoma Growth In vivo**

The efficacy of SCH 717454 as a single agent and in combination with cyclophosphamide was examined in s.c xenografts derived from SJCRH30 and RD rhabdomyosarcoma cell lines. In the RD rhabdomyosarcoma model (Fig. 2D), SCH 717454 alone inhibited tumor growth by 39% and 58% at 0.1 and 0.5 mg dose (twice per week), respectively, compared with control IgG1 group (0.5 mg twice per week). In this model, treatment with cyclophosphamide (100 mg/kg twice per week) alone inhibited tumor growth completely and caused a 15% tumor regression. This tumor regression was significantly enhanced to 65%, when 0.5 mg SCH 717454 was combined with 100 mg/kg cyclophosphamide. In the SJCRH30 model (Supplementary Fig. S3), SCH 717454 alone inhibited tumor growth by 37% and 53% at 0.1 and 1 mg dose (twice per week), respectively. Cyclophosphamide (100 mg/kg once per week) by itself was only inhibited by 37% of tumor growth compared with the 1mg control IgG1 group (twice per week). However, combination of 1 mg SCH 717454 with cyclophosphamide increased the tumor growth inhibition to 61%, better than either single agent used alone (P < 0.05).

**Inhibition of In vivo Tumor Cell Proliferation**

To further elucidate the mechanism of action of SCH 717454, we set to determine if reduction of tumor size is associated with inhibition of tumor cell proliferation. The nuclear proliferation marker Ki-67 was immunohistochemically evaluated ex vivo using tumor xenograft tissues collected after treatment. In SK-N-FI neuroblastoma xenograft tumor obtained after twice weekly treatment with 0.1 mg SCH 717454 for 2 weeks, the tumor Ki-67 staining was reduced by 38% and along with significant change in xenograft tumor morphology (shown in H&E staining; Fig. 3A). In the SJSA-1 osteosarcoma xenograft samples obtained 3 days after a single dose of SCH 717454, the staining of Ki-67 was reduced in a dose-dependent manner by 37% and 51%, respectively, after 0.1 and 0.5 mg SCH 717454 treatment (Fig. 3B). These data show that SCH 717454 can effectively block pediatric tumor cell proliferation in vivo. Moreover, Ki-67 and related proliferation biomarkers could be used as potential biomarkers in the clinical development of SCH 717454 and other anti–IGF-IR therapies (biologics and small molecules).

**Inhibition of In vivo Tumor Angiogenesis**

In addition to dysregulated growth signal, new blood vessel needs to be formed to feed the accelerated growth of tumor. To evaluate the effect of SCH 717454 on angiogenesis within the tumor tissue, blood vessel formation was studied in the SJSA-1 tumor xenograft mice (n = 5; Fig. 4A) using FITC-lectin, which specifically recognizes the vascular endothelial cells. Treatment (Fig. 4A, arrow) was initiated in the SJSA-1 tumor-bearing mice when the tumor size reached 150 to 200 mm³ on day 11 post-inoculation. Compared with control IgG1, 0.5 mg SCH 717454 treatment (two doses) reduced in the intensity of the fluorescent lectin staining by 74%, showing thinner blood vessels and reduced branches (Fig. 4B). This result suggests that SCH 717454 has antiangiogenesis effect and results in the reduction of blood vessel formation during tumor growth, thereby contributing to its antitumor effect in vivo. Taken together, SCH 717454 can both directly inhibit the IGF-1 signaling pathway and modulate the blood vessel formation via its antiangiogenesis effect.

**Discussion**

There is strong evidence supporting a critical role of IGF-IR in tumorigenesis, tumor survival, and metastasis in a wide variety of human cancers including pediatric tumors (3, 6). Our present study showed that SCH 717454, a fully human neutralizing monoclonal antibody specific for IGF-IR, inhibits tumor growth as a single agent. In addition, its combination with conventional chemotherapeutic agents (cyclophosphamide and cisplatin) results in a greater antitumor effect than that observed for either single agent alone in neuroblastoma, osteosarcoma, and rhabdomyosarcoma tumor xenograft models.
SCH 717454 inhibits rhabdomyosarcoma xenograft growth only partially while inhibiting the growth of neuroblastoma and osteosarcoma tumors almost completely. This finding is consistent with the reported existence in rhabdomyosarcoma cells of redundant growth factor autocrine loops (14) and suggests that the IGF/IGF-IR loop represents a dominant mechanism in the growth of neuroblastoma and osteosarcoma xenografts. The data presented here are also consistent with the results reported by the investigators of the Pediatric Preclinical Testing Program (36). In their study, SCH 717454 showed antitumor activity against a panel of pediatric tumor models with the most potent activities in Ewing’s sarcoma, osteosarcoma, and neuroblastoma models. Recently, several anti–IGF-IR monoclonal antibodies including SCH 717454 have shown single-agent activity in Ewing’s sarcoma in phase II clinical trials providing clinical proof of concept supporting importance of the IGF-IR pathway in Ewing’s sarcoma (37–40).

Downregulation of the proliferation marker Ki-67 and histopathologic evidence for reduced numbers of cancer cells in SCH 717454–treated mice (Fig. 3) confirm inhibition by SCH 717454 of pediatric cancer cell proliferation in vivo. These observations are consistent with the demonstrated ability of SCH 717454 to downregulate IGF-IR and to inhibit both basal and IGF-I–induced IGF phosphorylation of IRS-1 in vitro (Fig. 1). Studies using fully human monoclonal antibodies including SCH 717454 have clearly shown that these antibodies inhibit the proliferation of adult tumor cells by inhibiting IGF-I binding (thereby preventing IGF-IR activation) and by inducing IGF-IR downregulation (32, 34, 36, 41). Notably, IGF-IR downregulation in the tumor and peripheral blood cells in vivo by CP-751,871 is evident on its administration and correlates with the antibody plasma level (33). These findings are consistent with our observations that a near-maximal downregulation of Ki-67 in neuroblastoma xenografts occurs within 1 day of SCH 717454 administration and persists for at least 3 days and suggest that the prolonged antiproliferative activity of SCH 717454 against neuroblastoma and osteosarcoma may be due, at least in part, to the sustained receptor downregulation. SCH 717454 was also shown here to suppress the formation of new blood vessels in the SJSA-1 tumor xenograft. These findings are consistent with reports showing that IGF-IR activation regulates vascular endothelial growth factor production by tumor cells (42, 43) and that inhibition of IGF-IR action by antisense, antibody, or tyrosine kinase inhibitor reduces both neovascularization and vascular endothelial growth factor expression in neuroblastoma and Ewing’s sarcoma tumors (28, 44). Importantly, vascular endothelial growth factor levels are elevated in sera from children with neuroblastoma, osteosarcoma, Ewing’s sarcoma, and rhabdomyosarcoma (45). Thus, inhibition of angiogenesis appears to be a contributing factor to the antitumor activity of SCH 717454.

IGFs are known to protect a variety of cancer cells from the cytotoxic effects of radiation and chemotherapies (22, 46, 47). Recent evidence suggests that IGF-IR activation can confer resistance to anti-HER2 antibody trastuzumab in breast cancer and to anti–epidermal growth factor receptor antibody in human glioblastoma cells (48, 49). Consistent with these findings are reports showing that sensitivity of various cancer cells (including some pediatric tumors) to chemotherapy and radiation can be enhanced by various approaches to block IGF-IR action (7, 47). Inhibition of IGF-IR signaling by kinase inhibitors increases the susceptibility of
rhabdomyosarcoma, osteosarcoma, and Ewing’s sarcoma to several different chemotherapeutic agents, including cisplatin, doxorubicin, and vincristine (29, 32), and expression of a dominant-negative mutant of IGF-IR induces apoptosis of Ewing’s sarcoma cells and enhances their sensitivity to the cytotoxicity of doxorubicin (49). Efficacy of other anti-IGF-IR antibodies in combination with various anticancer agents in preclinical mouse xenograft models was reported recently (33, 41, 50). We showed here that combination of SCH 717454 with either cyclophosphamide or cisplatin in the neuroblastoma, osteosarcoma, and rhabdomyosarcoma models results in a greater antitumor effect than that observed for either single agent used alone. We found that administration of SCH 717454 does not cause body weight loss or elicit overt toxicity during the entire course of treatment. More importantly, combination of SCH 717454 with various cytotoxics does not result in further body weight loss. This finding suggests that this anti-IGF-IR antibody can be used in the clinic with a full dose of standard chemotherapy regimen. Our studies provided mechanistic insight to use an anti-IGF-IR antibody in settings where tumor proliferation is dependent on the activated IGF-IR pathway. We conclude that anti-IGF-IR monoclonal antibody SCH 717454 is a promising therapeutic candidate for treating pediatric solid tumors as a single agent and in combination with chemotherapeutic agents.

**Disclosure of Potential Conflicts of Interest**


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**References**


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