Dual Targeting of the Type 1 Insulin-like Growth Factor Receptor and Its Ligands as an Effective Antiangiogenic Strategy

Hemant K. Bid1, Cheryl A. London2, Jin Gao3, Haihong Zhong4, Robert E. Hollingsworth4, Soledad Fernandez3, Xiaokui Mo3, and Peter J. Houghton1

Abstract

**Background:** In pediatric tumor xenograft models, tumor-derived insulin growth factor (IGF-2) results in intrinsic resistance to IGF-IR–targeted antibodies, maintaining continued tumor angiogenesis. We evaluated the antiangiogenic activity of a ligand-binding antibody (MEDI-573) alone or in combination with IGF-I receptor binding antibodies (MAB391, CP01-B02).

**Methods:** IGF-stimulated signaling was monitored by increased Akt phosphorylation in sarcoma and human umbilical cord vascular endothelial cells (HUVEC). Angiogenesis was determined in vitro using capillary tube formation in HUVECs and in vivo using a VEGF-stimulated Matrigel assay. Tumor growth delay was examined in 4 sarcoma xenograft models.

**Results:** The IGF ligand-binding antibody MEDI-573 suppressed Akt phosphorylation induced by exogenous IGF-I and IGF-2 in sarcoma cells. Receptor-binding antibodies suppressed IGF-I stimulation of Akt phosphorylation, but IGF-2 circumvented this effect and maintained HUVEC tube formation. MEDI-573 inhibited HUVEC proliferation and tube formation in vitro, but did not inhibit angiogenesis in vivo, probably because MEDI-573 binds murine IGF-1 with low affinity. However, in vitro antiangiogenic activity of MEDI-573 was also circumvented by human recombinant IGF-1. The combination of receptor- and ligand-binding antibodies completely suppressed VEGF-stimulated proliferation of HUVECs in the presence of IGF-I and IGF-2, prevented ligand-induced phosphorylation of IGF-IR/IR receptors, and suppressed VEGF/IGF-2–driven angiogenesis in vivo. The combination of CP1-B02 plus MEDI-573 was significantly superior to therapy with either antibody alone against IGF-I and IGF-2 secreting pediatric sarcoma xenograft models.

**Conclusions:** These results suggest that combination of antibodies targeting IGF receptor and ligands may be an effective therapeutic strategy to block angiogenesis for IGF-driven tumors. Clin Cancer Res; 19(11); 2984–94. ©2013 AACR.

Introduction

Many human cancers, including childhood sarcomas, exhibit autocrine or paracrine growth through secretion of insulin-like growth factors 1 and 2 (IGF-I, IGF-2) and signaling through the Type 1 receptor (IGF-IR) (1, 2). IGF-IR is a receptor tyrosine kinase that is widely expressed in multiple human cancers and is activated by its related ligands, IGF-I and IGF-2. This interaction activates intrinsic tyrosine kinase activity, resulting in receptor autophosphorylation and stimulation of signaling cascades that include the IRS-1/PI-3K/PI3K/mTOR, and Grb2/Sos/Ras/MAPK pathways (3). Dysregulation of IGFs has been associated with the pathogenesis of various pediatric cancers, such as osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma, thereby providing a potential therapeutic target for the treatment of these tumors (4). In preclinical models of childhood cancers, the prototypical anti-IGF-IR antibody, α-IR3, mediated downregulation of IGF-insulin receptor (IR), significantly retarded growth of several cell lines in vitro (5, 6), and inhibited the growth of rhabdomyosarcoma xenografts (7).

IGF-IR and its ligands play roles not only in tumor cell proliferation and survival, but also in tumor angiogenesis (8). Two studies have suggested that IGF-IR antibodies exert a strong effect on tumor angiogenesis (6, 9). Our data showed antiangiogenic activity of IGF-IR–binding antibody (SCH717454) both in vitro and in vivo but IGF-2...
Antibodies that bind the type I insulin-like growth factor receptor (IGF-IR), and prevent ligand association, have been tested in numerous clinical trials. Despite compelling data to indicate the importance of IGF-IR-mediated signaling, particularly in sarcoma cells, these antibodies have rarely induced objective tumor regressions either in sarcoma patients or in preclinical xenograft models of sarcoma. Our data suggest that IGF-IR–targeted antibodies are potent antiangiogenic agents. However, tumor-derived IGF-2 circumvents the IGF-IR antibodies, signaling through the insulin receptor. In contrast, antibodies that bind IGF ligands inhibit IGF-2–stimulated proliferation of human vascular endothelial cells, but the effect is reversed by exogenous IGF-1. Consequently, the antiangiogenic activity of a combination of IGF-IR–targeted and IGF-ligand–binding antibodies was examined in vitro and in vivo. Results indicate that the combination of antibodies effectively suppresses VEGF-stimulated angiogenesis both in vitro and in a mouse model, and significantly inhibits growth and angiogenesis of IGF-driven pediatric sarcoma xenograft models.

**Materials and Methods**

**Reagents**

Medium 200, fetal bovine serum (FBS), and Alamar Blue (AB) were purchased from Invitrogen. Low serum growth supplement (LSGS) was obtained from Cascade Biologies Inc. Endothelial Tube Formation Assay Kits were from Cell Biolabs, Inc. Growth factor–reduced Matrigel for in vitro experiments and precoated Matrigel inserts for invasion assays were purchased from BD Biosciences. MedImmune generously provided MEDI-573 and CP1-B02 antibodies, and MAB391 antibody was purchased from R&D Systems. MEDI-573 binds human IGF-2 with high affinity, whereas its affinity for human IGF-1 is lower, and affinity for murine IGF-1 is very low (18). CP1-B02 and MAB391 antibodies bind the IGF-I receptor, preventing ligand binding. Human recombinant IGF-1 and IGF-2 were purchased from PeproTech Inc.. BMS754807 was purchased through Selleckchem.com.

**Cell culture**

Human umbilical vein endothelial cells (HUVEC) were obtained from the American Type Culture Collection. All experiments were done using endothelial cells between passages 3 and 8. HUVECs were maintained in medium M200 (Invitrogen) with 15% FBS, endothelial cell growth supplements (LSGS Medium, Cascade Biologies), and 2 mmol/L glutamine at 37°C with 5% CO2. All cells were maintained as sub confluent cultures and split 1:3, 24 hours before use. Rhabdomyosarcoma cell lines were cultured in RPMI-1640 supplemented with 10% FBS.

**Western blotting**

Cell lysis, protein extraction, and immunoblotting were as described previously (6, 9). We used primary antibodies to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), AKT, phospho-AKT (Ser473), IGF-IR, and phospho-IGF-IR (Tyr1131), IR, and phosphor-IR (Tyr1146; Cell Signaling). Immunoreactive bands were visualized by using Super Signal Chemiluminiscence substrate and Biomax MR and XAR film (Eastman Kodak Co.). Immunoprecipitations were conducted by adding either 2 μg of IGF-IR antibody (Santa Cruz biotechnology Inc.). Fifteen microliters of total sample was resolved on a 4% to 12% SDS-polyacrylamide gel. Proteins were transferred to a PVDF membrane and immune-detection was conducted with specific primary antibodies.

**RT-PCR analysis for IGF-IR and INS receptor in HUVEC cells**

HUVEC cells were cultured in M200 media. When cells were 80% confluent, then were washed with PBS and total RNA was harvested with the Qiagen RNAeasy mini Kit (Qiagen). One micrograms of RNA was reverse
transcribed with random primers and Superscript II Rnase H reverse transcriptase. The cDNA was stored in aliquots at \(-20^\circ C\) until use. For semiquantitative PCR, analysis was carried out using Platinum PCR Supermix (Invitrogen) as instructed by the manufacturer. The following primers were used: IGFI-I R forward primer: 5’-GAAATTCCATGGTACCCGAA-3’, IGFI-I R reverse primer: 5’-CTCTTGTGCCGTGATG-3’, (389bp) and IR forward primer: 5’-CATGGATGAGCGGATGTCTG-3’, IR reverse primer: 5’-gTTTTACCTCCCGTCCTCA-3’ (364bp). The PCR cycling conditions for the IGFI-IR and INS cDNA included preincubation for 5 minutes at 95°C and followed by 32 cycles of 30 seconds at 95°C, 60 seconds at 58°C, 60 seconds at 72°C, and a final extension for 10 minutes at 72°C. PCR products were identified using ethidium bromide (EtBr).

**Endothelial cell tube formation assay**

For the Endothelial Tube Formation Assay (CBA200, Cell Biolabs Inc.), ECM gel was thawed at 4°C and mixed to homogeneity using cooled pipette tips. Cell culture plates (96-well) were bottom-coated with a thin layer of ECM gel (50 μL/well), which was left to polymerize at 37°C for 60 minutes. HUVECs (2–3 × 10^4) were added to each well on the solidified ECM gel. Culture medium was added to each well in the presence or absence of ligand binding (MEDI-573) or receptor binding (MAB391 or CP01-B02). The plates were incubated at 37°C for 12 to 18 hours and the endothelial tubes were observed using a fluorescent microscope after staining with Calcein AM. Three microscope fields were selected at random and photographed. Tube-forming ability was quantified by counting the total number of cell clusters and branches under a 4 × objective and 4 different fields per well. The results are expressed as mean fold change of branching compared with the control groups. Each experiment was conducted at least 3 times.

**Cell viability/proliferation assay**

HUVECs were seeded on 6-well plates at a density of approximately 1 × 10^5 cells/well in M200 medium. Cells were treated with 10, 20, and 30 μg/mL of MEDI-573 1 day after seeding. After 4 days, Calcein AB (Invitrogen) was added directly into culture media at a final concentration of 0.1%. The plates were incubated at 37°C. Optical density was measured spectrophotometrically at 540 and 630 nm after adding Calcein AB for 3 to 4 hours. As a negative control, Calcein AB was added to medium without cells.

**Vascularization of Matrigel plugs in vivo**

To further characterize antiangiogenic properties of MEDI-573 in vivo, we conducted murine Matrigel plug experiments. An isotype-matched control MoAb/PBS was used as a negative control, and VEGF (100 ng/mL) as a positive control. Alternatively, plugs containing VEGF (100 ng/mL) and IGF-2 (50 ng/mL) were implanted and mice received MEDI-573 alone, CP1-B02 alone, or the 2 antibodies in combination. Matrigel was injected subcutaneously into CB17SCID/-/- female mice, forming semi-solid plugs. Mice received treatment of MEDI-573 (30 mg/kg; ref. 18), the dose for CP1-B02 (20 mg/kg) was based on previous studies with IGF-IR-binding antibodies (4, 13). Antibody concentrations were those causing maximal inhibition of angiogenesis or the combination intraperitoneally immediately after the Matrigel injection and on day 3. On day 7, plugs were excised under anesthesia, fixed in PBS-buffered 10% formalin containing 0.25% glutaraldehyde, and were processed for hematoxylin-eosin (H&E) and Masson’s Trichrome staining. Vascular identity of the infiltrating cells was established with CD34 immunostaining. Eight hot-spots were identified for each Matrigel or tumor section. The regions containing the most intense areas of neovascularization (“hotspots”) were chosen for analysis. Eight hot spots were identified for each Matrigel or tumor section. The ImagePro Plus analysis system was used to quantify the percentage of area occupied by the vessel-like structures in each field. The mean ± SE from each group were compared. The negative control was obtained by tissue staining with secondary antibody only.

**Evaluation of antibodies against pediatric tumor xenografts**

All experiments were conducted under Institutional Animal Care and Use Committee–approved protocol AR09-00036. Four tumor models were selected. Two Ewing’s sarcomas (EW5, EW8) that secrete IGF-1, and 2 rhabdomyosarcomas (Rh18, Rh30) that secrete IGF-2 (6) Tumor lines and methods have been reported previously (19). Treatment was started when tumors exceeded 100 mm^3. Mice received MEDI-573 (30 mg/kg), or CP01-B02 (20 mg/kg) or the combination twice weekly for a planned 6-weeks treatment and observation. Tumors were harvested when they reached 4-times the initial volume at start of therapy. Tumors were fixed and stained for CD34-positive cells to assess microvesSEL formation and for Ki67 staining for proliferation.

**Statistics**

Two-sample t tests were used for the analysis of the experiments with continuous outcomes using GraphPad Prism. Data were reported as mean ± SE. Type I error rate was set to 0.05. For in vivo testing against pediatric sarcoma xenograft models, criteria for defining an event (4 times the tumor volume at the start of treatment) was similar to that used by the PPTP (19). Log-rank tests were used to compare the time-to-event curves between groups and Holm’s method was used to adjust multiplicity within each xenograft model. SAS 9.3 was used for this analysis (SAS, Inc.).

**Results**

**Effect of antibodies on ligand-induced IGF-IR signaling**

To determine whether MEDI-573 and MAB391 equally inhibited phosphorylation of Akt induced by IGF-I and IGF-2, 3 sarcoma cells lines (Rh30, EW8, Rh18) were
serum-starved overnight, incubated with or without antibody [MEDI-573 (30 μg/mL) and MAB391 (15 μg/mL)] for 5–60 minutes, then stimulated with IGF-I or IGF-2 for 5 minutes. As shown in Fig. 1A, IGF-I and IGF-2 induced phosphorylation of Akt (S473) in control (no antibody) cells, but MEDI-573 rapidly suppressed the stimulation in all tumor cell lines, with almost complete abrogation of phospho-Akt within 15 minutes. In contrast, MAB391 did not suppress IGF-2–induced phosphorylation of Akt in any of the sarcoma cell lines, but did suppress IGF-I–induced Akt phosphorylation in the same lines (Fig. 1B). This result is consistent with IGF-2 activating Akt via the IR (8). Thus, MAB391 had a similar activity to another IGF-IR–binding antibody, SCH717454, in that it failed to prevent IGF-2 binding and activating the IR (8).

**MEDI-573 potently inhibits angiogenesis by blocking HUVEC tube formation and proliferation in vitro**

We next examined whether MEDI-573 inhibited VEGF165–induced tube formation of HUVECs, focusing on an antibody concentration range of 10 to 30 μg/mL. HUVECs were stimulated with VEGF165 (10 ng/mL) on Matrigel to form tubes in the absence or presence of MEDI-573 (Fig. 2A). Reduction in tube formation by MEDI-573 was concentration-dependent; at 30 μg/mL the antibody significantly inhibited tube formation. For the proliferation assay, HUVEC cells were stimulated with VEGF165 in the absence or presence of MEDI-573 and cell number determined by Calcein AB staining after 4 days. As shown in Fig. 2B, MEDI-573 inhibited proliferation in a concentration-dependent manner with 70% or more inhibition at 30 μg/mL. An isotype matched (IgG2a) control antibody had no effect on either tube formation or cell proliferation (data not shown).

**MAB391 potently inhibits angiogenesis in vitro**

Previous studies (6, 9, 8) have shown that IGF-IR–targeted antibodies exert antiangiogenic effects, and that IGF-IR signaling is required for HUVEC proliferation when stimulated by VEGF (8). Results using MAB391 are consistent with these data, and show that MAB391 inhibits VEGF-stimulated tube formation of HUVECs in vitro at 15 μg/mL (Fig. 2C).

Figure 1. A, MEDI-573 inhibits both IGF-1 and IGF-2 stimulation of Akt phosphorylation. Sarcoma cells were grown for 24 hours under serum-free conditions, then incubated for 5 to 60 minutes with MEDI-573 (30 μg/mL), or 5 minutes without antibody, and stimulated for 5 minutes with IGF-I (10 ng/mL) or IGF-2 (50 ng/mL). B, MAB391 inhibits only IGF-I not IGF-2 stimulation of Akt phosphorylation. Sarcoma cells were grown for 24 hours under serum-free conditions, then incubated for 5 to 60 minutes with MAB391 (15 μg/mL), or 5 minutes without antibody, and stimulated for 5 minutes with IGF-I (10 ng/mL) or IGF-2 (50 ng/mL). Cells were harvested 5 minutes after IGF-stimulation at the times shown and phosphorylation of Akt(Ser473) and total Akt determined by immunoblotting.
Antiangiogenic activity of MEDI-573 in vivo

To directly test the antiangiogenic activity of MEDI-573 in vivo, mice were implanted subcutaneously with Matrigel plugs infused with PBS or VEGF165. Mice were treated with MEDI-573 (30 mg/kg) administered by intraperitoneal injection immediately after implantation of the plug and again after 3 days. Plugs were excised at day 7 and angiogenesis quantified as described in the Materials and Methods. VEGF165 increased the number of vessels detected in Matrigel plugs by more than 10-fold over that in PBS-infused (control) plugs. There was no observed reduction in vessel formation in the treated group as compared with controls (Fig. 2D). Thus, MEDI-573 did not have detectable antiangiogenic effects in the mouse. Similar to results with another IGF-IR–binding antibody (8) inclusion of IGF-2 into the Matrigel plug completely circumvented the antiangiogenic activity of CP01-B02 treatment, Supplementary Fig. S1.

IGF-I circumvents the antiangiogenic activity of MEDI-573 in vitro

To ascertain why MEDI-573 failed to inhibit angiogenesis in the Matrigel plugs, we further examined its activity in vitro. While MEDI-573 is a dual IGF-I/IGF-2-neutralizing antibody, it binds IGF-I with a lower affinity compared with its affinity for IGF2. Furthermore, the affinity of MEDI-573...
for murine IGF-I is far lower than that for human IGF-I (18). It was thus possible that exogenous murine IGF-I circumvented the antiangiogenic effect of MEDI-573 in the mouse model. To test this, we determined whether exogenous human IGF-I or IGF-2 could circumvent the effect of MEDI-573 in blocking VEGF-induced proliferation of HUVECs. Cells were grown under serum-depleted conditions with PBS (control), or VEGF without and with MEDI-573, or with VEGF, antibody and exogenous IGFs. As shown in Fig. 3A, VEGF-stimulated tube formation was completely inhibited by MEDI-573. Exogenous IGF-2 (50 ng/ml) did not significantly overcome the effect of MEDI-573, whereas IGF-1 (10 ng/ml) partially circumvented the blockade.

**IGF-2 circumvents the antiangiogenic activity of MAB391 in vitro**

Our previous results (8) showed that cells secreting IGF-2 could maintain angiogenesis in the presence of IGF-IR-targeted antibodies, whereas those secreting predominantly IGF-1 could not. We therefore tested whether exogenous IGF-2/2 could circumvent the MAB391-induced block of VEGF-induced tube formation of HUVECs. Cells were grown under serum-depleted conditions with PBS (control), or VEGF without and with MAB391, or with VEGF, antibody and exogenous IGFs. As shown in Fig. 3B, VEGF-stimulated tube formation was completely inhibited by MAB391. Exogenous IGF-2 did not significantly overcome the effect of MAB391, whereas IGF-2 circumvented the blockade.

**Effect of the combination of receptor-binding and ligand-binding antibodies on HUVEC tube formation**

We next examined the expression of IGF-IR and IR on HUVEC cells. As shown in Fig. 4A, both receptors were detected by RT-PCR and immunoblotting. Stimulation of HUVECs with VEGF induced a robust phosphorylation of IGF-IR at 24 hours that was blocked by the MAB391 receptor-binding antibody. Stimulation of cells by VEGF in combination with IGF-1 was also completely abrogated by MAB391, whereas VEGF combined with IGF-2 circumvented the block on signaling, consistent with IGF-2 signaling through the IR, and continued phosphorylation of Akt, Fig. 4B. In contrast, a small molecule inhibitor of IGF-IR and IN-R, BMS754807 (20) equally inhibited IGF-I and IGF-2–induced phosphorylation of Akt in HUVECs (Fig. 4C). A similar experiment was undertaken using MEDI-573 as the ligand-neutralizing antibody. Stimulation of cells by VEGF in combination with IGF-1 was also completely abrogated by MAB391, whereas MEDI-573 combined with IGF-2 circumvented the block on signaling with IGF-2 signaling through the IR, and continued phosphorylation of Akt, Fig. 4D. On the basis of these results, we speculated that the combination of IGF-IR–targeted antibody MAB391 with the MEDI-573 antibody that binds IGF-I/2 would suppress VEGF-stimulated tube formation of HUVECs in the presence of IGF ligands. As shown in Fig. 5A, VEGF stimulated...
tube formation was completely inhibited by the combination of MEDI-573 and MAB391 in presence of either exogenous IGF-I or IGF-2. We have shown previously that including IGF-2 with VEGF in Matrigel plugs circumvents the antiangiogenic effects of the IGF-IR–targeted antibody SCH717454 (8) and in vitro human IGF-I circumvents the antiangiogenic effects of MEDI-573, whereas murine IGF-I circumvents MEDI-573 in vivo. We next tested whether the combination of MEDI-573 with an IGF-IR–targeted antibody would effectively block VEGF or VEGF/IGF-2–driven angiogenesis in vivo. For these in vivo studies, that require large amounts of antibody, we used CP1-B02, an IGF-IR–binding antibody provided by Medimmune. Characterization of CP1-B02 showed that it was similar to MAB391 (or SCH717454) in that it blocked IGF-I stimulation of Akt phosphorylation, but not that induced by IGF-2, and potently blocked HUVEC tube formation and proliferation (Supplementary Fig. S2–S4). For in vivo evaluation, Matrigel plugs infused with PBS (control), VEGF (100 ng/mL), or VEGF + IGF-2 (50 ng/mL) were implanted subcutaneously. Mice received 2 administrations of MEDI-573, CP1-B02 or the combination of antibodies, and angiogenesis was
Effect of the combined of receptor-binding and ligand-binding antibodies on growth of IGF-secreting pediatric sarcoma xenografts

The antitumor activity of CP01-B02, MEDI-573, or the combination was evaluated against 4 pediatric sarcoma models. Ewing sarcoma lines (EW-5, EW-8) secrete predominantly IGF-I, whereas rhabdomyosarcoma lines (Rh18, Rh30) secrete predominantly IGF-2 (6). MEDI-573 (30 mg/kg) or MEDI-573 with MAB391 and both IGF-1 and IGF-2. Tube formation was quantified as described in the Materials and Methods. Each result is the mean ± SE for 3 independent experiments. B, Matrigel plugs containing with PBS, VEGF (100 ng/mL) or VEGF (100 ng/mL) plus IGF-2 (50 ng/mL) were implanted subcutaneously in mice. Mice received no treatment, MEDI-573 (30 mg/kg), CP1-B02 (20 mg/kg), or both antibodies on day 0 (after Matrigel plug implantation) and day 3. The Matrigel plugs were excised on day 7, and processed as described in the legend to Fig. 3 (mean ± SE from 6 high-power fields), P < 0.05.

Discussion

Alterations in the IGF signaling axis have been linked to the pathogenesis of various cancers including sarcomas. Consequently, inhibiting IGF-mediated signaling has become an attractive therapeutic approach. Small molecule inhibitors, antibodies directed at the IGF-IR as well as IGF-1/2-neutralizing antibodies to block IGF-IR-mediated signaling have all entered clinical trials. While receptor-targeted antibodies have shown antitumor activity in mouse models of childhood sarcomas (6, 13, 14), in most cases they do not induce actual tumor regression. These preclinical results mirror emerging clinical response rates where IGF-IR–targeted antibodies may be overestimated. However, if tumor cells secreting IGF-2 are circumventing the antiangiogenic effects of receptor–targeted antibodies both in vitro and in vivo (8). In the mouse, circulating levels of IGF-2 are low or undetectable (21, 22), hence the antitumor and antiangiogenic activities of IGF-IR–targeted antibodies may be underestimated. However, if tumor cells secreting IGF-2 are circumventing the antiangiogenic effects of receptor–targeted antibodies by signaling through the IR (8), therapies that neutralize IGF-2 may be potentially important. IR-A (the high affinity IGF-2 receptor where exon 11 is spliced out) is expressed in most xenografts, and cell lines derived from childhood sarcomas, as well as human vascular endothelial cells (8). Supplementary Fig. S3). Hence, blocking potential IGF-2/IR-A signaling may be an important determinant of antiangiogenic or antitumor activity.
Figure 6. A, antitumor activity of the IGF-ligand–binding antibody (MEDI-573) or IGF-I receptor-binding antibody (CP01-B02) alone or in combination (MEDI + CP). Antibodies were administered twice weekly at 30 mg/kg (MEDI-573), or 20 mg/kg (CP01-B-02) for a planned treatment of 6 weeks. Treatment was initiated when tumors exceeded 100 mm³. B, tumors were harvested at “event” (4-fold tumor volume at treatment initiation), and stained for CD34 to determine tumor microvessels, and Ki67 to assess proliferation, P < 0.05.
Several approaches to inhibiting the IGF-I/IGF-IR axis have been proposed including ligand-binding antibodies and administration of IGF-binding proteins (IGFBP; ref. 18, 23). Here, we evaluated the antiangiogenic activity of the IGF-I/IGF-2 ligand neutralizing antibody, MEDI-573 (18). We also evaluated available receptor-binding antibodies for in vitro (MAB391) and in vitro (CP1-B02), in combination with MEDI-573 to block angiogenesis. Both MAB391 and CP1-B02 behaved similarly to the IGF-IR–targeting antibody SCH717454. Specifically, MAB391 or CP1-B02 potently inhibited IGF-I-stimulated phosphorylation of Akt, but not that induced by IGF-2. Both antibodies inhibited in vitro angiogenesis of HUVECs, and this was circumvented by IGF-2. MEDI-573 is a fully human antibody that neutralizes both IGF-1 and IGF-2 and inhibits IGF signaling through both the IGF-IR and IR-A pathways (18). In contrast to MAB391 and CP1-B02, MEDI-573 blocked both IGF-I– and IGF-2–induced phosphorylation of Akt in several sarcoma cell lines. MEDI-573 inhibited VEGF-stimulated HUVEC tube formation and proliferation in vitro, but surprisingly did not significantly suppress VEGF-stimulated angiogenesis in a mouse model. In part, failure to inhibit angiogenesis in the mouse model may be explained by the relatively low-binding affinity of the antibody for mouse IGF-1 (Kd ~2,000 pmol/L; ref. 18). To examine this further, we determined whether human recombinant IGF-I could circumvent the MEDI-573–mediated block on proliferation and differentiation of HUVECs in vitro. We found that exogenous human recombinant IGF-I could partially or completely restore normal proliferation and tube formation in the presence of MEDI-573. This may reflect the lower-binding affinity of this antibody for IGF1 compared with IGF-2 (Kd, 294 pmol/L versus 2 pmol/L) (18). As IGF-2 circumvented the antiangiogenic effect of MAB391 and IGF-1 circumvented the effect of MEDI-573, we considered combining the antibodies as a potential antiangiogenic strategy. Indeed, while each antibody alone blocked HUVEC proliferation and differentiation in the absence of exogenous IGFs, the combination of receptor-binding and ligand-neutralizing antibodies completely suppressed in vitro angiogenesis in the presence of exogenous IGFs, prevented ligand-induced phosphorylation of IGF-IR/IR and blocked activation of Akt. Similarly, the combination of CP1-B02 and MEDI-573 completely suppressed angiogenesis into Matrigel plugs containing both VEGF and IGF-2.

Neither MEDI-573 nor CP01-B02 antibodies significantly inhibited growth of any of the 4 sarcoma xenograft models. In part, it was surprising that the IGF-IR–targeting antibody (CP-01B02) did not show activity against Ewing sarcoma models as these secrete predominantly IGF-1, although in vitro cell lines also secrete IGF-2. As expected, MEDI-573 did not inhibit growth, in part due to circulating mouse IGF-I for which it has low-binding affinity. However, combining the antibodies had significantly greater activity in each tumor model. In combination these antibodies significantly reduced CD34 staining and Ki67 staining, suggesting a marked antiangiogenic effect caused decreased tumor cell proliferation.

Our results clearly support the idea that dual targeting of IGF-IR and its ligands (IGF-I/IGF-2) may be an effective antiangiogenesis strategy. The xenograft studies verified the therapeutic efficacy of such combinations against IGF-I and IGF-2 secreting tumors, such as Ewing sarcoma and rhabdomyosarcoma, respectively. Together, these studies provide a sound scientific base for developing this novel strategy for treatment of childhood sarcomas.

Disclosure of Potential Conflicts of Interest
R.E. Hollingsworth is employed (other than primary affiliation; e.g., consulting) as a director, oncology research in MedImmune, LLC. No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: H.K. Bid, C.A. London, P.J. Houghton
Development of methodology: H.K. Bid, C.A. London, J. Gao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.) : H.K. Bid, C.A. London, H. Zhong, P.J. Houghton
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H.K. Bid, C.A. London, S. Fernandez, X. Mo
Writing, review, and/or revision of the manuscript: H.K. Bid, C.A. London, H. Zhong, S. Fernandez, X. Mo, P.J. Houghton
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Gao, R.E. Hollingsworth, P.J. Houghton
Study supervision: P.J. Houghton

Acknowledgments
MEDI-573 and CP1-B02 antibodies were generously provided by MedImmune.

Grant Support
This work was supported by USPHS grants CA77776, CA23099, and CA165995 from the National Cancer Institute, and the Pelotonia Foundation Fellowship (to H.K. Bid).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received June 20, 2012; revised March 18, 2013; accepted March 18, 2013; published OnlineFirst April 2, 2013.

References


Clinical Cancer Research

Dual Targeting of the Type 1 Insulin-like Growth Factor Receptor and Its Ligands as an Effective Antiangiogenic Strategy


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-2008

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/03/29/1078-0432.CCR-12-2008.DC1

Cited articles
This article cites 23 articles, 11 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/19/11/2984.full.html#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/19/11/2984.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.