Pharmacodynamic and Antineoplastic Activity of BI 836845, a Fully Human IGF Ligand-Neutralizing Antibody, and Mechanistic Rationale for Combination with Rapamycin

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Abstract

Insulin-like growth factor (IGF) signaling is thought to play a role in the development and progression of multiple cancer types. To date, therapeutic strategies aimed at disrupting IGF signaling have largely focused on antibodies that target the IGF-I receptor (IGF-IR). Here, we describe the pharmacologic profile of BI 836845, a fully human monoclonal antibody that utilizes an alternative approach to IGF signaling inhibition by selectively neutralizing the bioactivity of IGF ligands. Biochemical analyses of BI 836845 demonstrated high affinity to human IGF-I and IGF-II, resulting in effective inhibition of IGF-induced activation of both IGF-IR and IR-A in vitro. Cross-reactivity to rodent IGFs has enabled rigorous assessment of the pharmacologic activity of BI 836845 in preclinical models. Pharmacodynamic studies in rats showed potent reduction of serum IGF bioactivity in the absence of metabolic adverse effects, leading to growth inhibition as evidenced by reduced body weight gain and tail length. Moreover, BI 836845 reduced the proliferation of human cell lines derived from different cancer types and enhanced the antitumor efficacy of rapamycin by blocking a rapamycin-induced increase in upstream signaling in vitro as well as in human tumor xenograft models in nude mice. Our data suggest that BI 836845 represents a potentially more effective and tolerable approach to the inhibition of IGF signaling compared with agents that target the IGF-I receptor directly, with potential for rational combinations with other targeted agents in clinical studies.

Introduction

Insulin-like growth factors I and II (IGF-I and IGF-II) are structurally related polypeptides that promote cell growth and survival (1). In normal physiology, IGFs have key roles in the control of cellular proliferation and survival and of organism growth (2). Their concentrations in blood are physiologically regulated and reflect hepatic production, but IGFs are also expressed locally in many tissues in a paracrine or autocrine manner (3). Tissue IGF bioactivity varies not only with circulating ligand concentration and with local ligand production, but also with the concentrations of the various serum IGF-binding proteins (4, 5).

IGF-I signals by binding to the IGF-I receptor (IGF-IR) or to hybrids of IGF-IR and insulin receptors (INSR; refs. 1, 6). IGF-II initiates signaling via these same receptor species, but can also activate the A isoform of the INSR (IR-A; refs. 7, 8). This is of particular interest from an oncologic perspective as IR-A is preferentially expressed in fetal and cancer cells (9, 10). In contrast with members of the EGF family, which are frequently amplified and/or mutationally activated in neoplasia, resulting in ligand-independent receptor activation, IGF-IR/INSR signaling always requires active ligands.

More than a decade of preclinical research has provided a rationale for targeting IGF signaling in the treatment of cancer (1, 11). Oncogenic transformation of mouse embryonic fibroblasts, for instance, has been described to be dependent on the expression of IGF-IR (12). Expression of IGF-II by transformed cells has been observed (13) and this can lead to activation of IGF-IR in an autocrine manner. In many preclinical cancer models, tumor growth can be stimulated by IGF-I or IGF-II, and tumors grow substantially slower in mice carrying a mutation that reduces IGF ligand levels (4). Moreover, a separate line of indirect evidence for the relevance of IGF signaling for neoplasia comes from the observation that there is considerable interindividual variation in circulating IGF-I levels, and that risk and prognosis of certain cancers are related to circulating IGF-I concentration (2, 14, 15). Finally, IGF signaling may also play a role as a resistance mechanism, which limits the effectiveness of cytotoxic or targeted anticancer agents, including rapalogs (16–21).
This preclinical evidence has motivated the development of several IGF-I receptor-targeted monoclonal antibodies (mAb; refs. 22–24). However, efficacy of anti-IGF-IR antibodies has been disappointing in clinical trials to date (25–30). One possible explanation for these results is that in clinical use, drugs studied to date do not adequately inhibit IGF signaling, especially in the context of the need to block autocrine loops in IGF-dependent cancers (31–33), particularly those driven by IGF-II, which can also signal via IR-A (34). For the alternative approach of targeting all receptor species in the insulin/IGF tyrosine kinase receptor family using small-molecule inhibitors, the dosing of these agents must be well balanced to avoid metabolic toxicity, and it remains to be demonstrated that clinical use at tolerable doses leads to adequate receptor blockade in neoplastic tissue.

A third therapeutic strategy is to target the IGF ligands rather than the receptors, aiming to abrogate signaling via IGF-IR and IR-A as well as their hybrid receptors, without impact on circulating insulin and glucose levels and their metabolic functions (30). Two agents, BI 836845 and MEDI-573 (35), with this mode of action have been developed. Here, we describe the pharmacologic activity of BI 836845, a fully human mAb that neutralizes the activities of both IGF-I and IGF-II. In contrast with MEDI-573, BI 836845 cross-reacts with mouse and rat IGF-I and IGF-II, allowing a comprehensive in vitro characterization of the pharmacodynamic properties of the antibody in preclinical rodent models.

Materials and Methods

Reagents and cell lines

BI 836845 was isolated by selection of specific Fab fragment clones from the human combinatorial antibody phage display library (HuCAL Gold; ref. 36) that bind human IGF-I with low nanomolar affinity, in three phage display library (HuCAL Gold; ref. 36) that bind human IGF-I with low nanomolar affinity, in three phasing cycles as previously described (37) and subsequently subjected to in vitro affinity maturation (38). IGF-IR antibodies used were dIR-3 (Calbiochem, No. GR11L) and one which was generated on the basis of sequence ID 2.13.2 of the patent WO 02/053596 A2 (termed IGF-IR mAb). Other reagents included rapamycin (LC laboratories) and purified IGF-I (CM001) and IGF-II (FM001; GroPep Bioreagents Pty Ltd.).

Recombinant mouse cell lines expressing human IGF-IR and IR-A, respectively, were kindly provided by R. Vigneri (University of Catania, Italy). GEO cell line (originally established by M. Brattain; Roswell Park Cancer Institute, Buffalo, NY and Baylar College of Medicine, Houston, TX) was obtained from S. Pepe (Università degli Studi di Napoli, Italy). RD-ES [American Type Culture Collection (ATCC) #HTB-166], SK-Es-1 (ATCC, #HTB-86), and all other cell lines used were purchased from ATCC, Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Japanese Collection of Research Bioresources Cell Bank (JCRB), or the Memorial Sloan-Kettering Cancer Center (New York, NY). Cell line authentication was conducted in-house or by the provider using short tandem repeat PCR. Upon delivery, cell lines were expanded and low-passage vials stored in liquid nitrogen. Experiments were carried out within 12 weeks after resusculation.

Biacore analysis

Binding affinity (Kd) was determined using a Biacore 3000 high-resolution surface plasmon resonance system in HEPES Buffer Saline containing EDTA and Surfactant P-20 (HBS-EP) buffer + 1 mg/mL CM-Dextran and 0.25 mg/mL bovine serum albumin (BSA) as running buffer at a flow rate of 20 μL/minute. The sensor chip was coated with approximately 1,000 RU of an unspecified reference antibody in flow cell 1 and approximately 1,000 RU of a rabbit-anti-human Fcγ–specific antibody in flow cell 2 using reagents from an amine coupling kit. The test antibody was captured by running a 1 μg/mL solution over the sensor surfaces for 3 minutes. IGF antigens were diluted to 500, 250, 125, 62.5, and 31.3 nmol/L and measured in random order using 5 minutes for association and dissociation. Data evaluation was performed using the BIAevaluation software, version 4.1. Biacore 3000 instrument; HBS-EP buffer, amine coupling kit, and BIAevaluation software were provided by Biacore/GE Healthcare.

Cell-based IGF-IR and IR-A phosphorylation assays

Mouse embryonic fibroblast cell lines derived from IGF-IR–deficient mice and engineered to overexpress human IGF-IR or human IR-A were used to measure IGF bioactivity, defined here as the ability of a sample (serum, plasma, cell culture media) to stimulate receptor phosphorylation as determined by ELISA. The cell lines were maintained in Dulbecco’s modified Eagle medium supplemented with 10% heat-inactivated FBS, 1 mmol/L sodium pyruvate, 0.075% sodium bicarbonate, nonessential amino acids (Gibco), and 0.3 μg/mL puromycin at 37°C and 5% CO2. The ELISA was performed following standard procedures. Briefly, 10,000 cells/well were seeded on 96-well plates followed by a 24-hour incubation. Cells were starved in medium with 0.5% FBS overnight before a dilution range of BI 836845 or IGF-IR antibodies was added to the cells, followed by either IGF-I (20 ng/mL), IGF-II (100 ng/mL), or a human serum pool (20%). Cells were incubated for 30 minutes at 37°C and 5% CO2 and subsequently fixed in 4% formaldehyde/PBS (20 minutes, room temperature), quenched (1.2 wt% hydrogen peroxide in wash buffer, 30 minutes, room temperature), and blocked (5% BSA in wash buffer, 60 minutes, room temperature with agitation). Subsequently, cells were incubated with primary antibody [phospho-IGF-IRβ (tyr1135/1136)/insulin receptor-β (tyr1150/1151) antibody; Cell Signaling Technology #3024, 1:1,000 in blocking buffer, incubation overnight at 4°C with agitation] and secondary antibody (anti-rabbit immunoglobulin G goat immunoglobulins conjugated with horseradish peroxidase; Dako #PO448, 1:500 in blocking buffer for 60 minutes at room temperature with agitation) followed by substrate solution (tetramethylbenzidine;
Bender MedSystems #BMS406.100; for 5 to 20 minutes with agitation). Finally, the reaction was stopped by adding 1 mol/L phosphoric acid, and the absorbance was read using a photometer (OD 450 nm, OD 650 nm as reference).

**Rat studies**

Six to 8-week-old male rats (Crl:WI[Han]) were treated intravenously once weekly for 13 weeks with 20, 60, or 200 mg/kg of BI 836845 and compared with vehicle-treated animals. The treatment phase was followed by a 12-week recovery phase. Plasma samples were taken at time points before, during, and after treatment. Rat body weight was measured twice weekly throughout the study and tail length at the end of the treatment period.

**Xenograft studies**

Tumors (<100 mm³) were established from cultured GEO or RD-ES cells (5 × 10⁶ in Matrigel) by subcutaneous injection into the right flank of female B6C3F1 Foxn1nu mice. Animals were randomized (n = 7/group) and treated with vehicle (0.5% Natrosol) or rapamycin (5 mg/kg) intraperitoneally (i.p.) once a day for 5 consecutive days per week, twice weekly with BI 836845 (100 mg/kg), or a combination of both agents. Tumor volumes were determined three times a week using a digital caliper. Body weights of the mice were measured daily as an indicator of tolerability of the compounds. Four hours after the last treatment (day 20), serum was sampled and RD-ES tumors were explanted, lysed in Bio-Plex Cell Lysis Kit (Bio-Rad #171-304012) containing 2 mmol/L Lysis Buffer, and treated with vehicle (0.5% Natrosol) or neutralizing recombinant IGF-I and IGF-II. As shown in Fig. 1A and B, BI 836845 inhibited both IGF-I (20 ng/mL) and IGF-II (100 ng/mL)-induced IGF-IR phosphorylation with an IC₅₀ of 90 ng/mL (0.6 nmol/L) and 1,120 ng/mL (7.5 nmol/L), respectively. In the same assay, two IGF-IR mAbs were less effective with respect to IGF-I–induced activation and displayed only weak effects on IGF-II–induced activation with more than 30% of IGF-II–induced IGF-IR phosphorylation remaining even at an antibody concentration of 100 nmol/L (Fig. 1B). Figure 1C demonstrates that BI 836845 also potently inhibited IGF-II (100 ng/mL)-induced IR-A activation with an IC₅₀ of 815 ng/mL (5.4 nmol/L), unlike IGF-IR–targeted antibodies that had no effect. When cell culture medium containing 20% human serum was used to stimulate receptor phosphorylation, this could be completely neutralized by BI 836845 with an IC₅₀ of 246 ng/mL (1.6 nmol/L). In contrast, an IGF-IR–targeted antibody showed only partial inhibition of IGF bioactivity (Fig. 1D).

**Additional materials and methods**

Two-dimensional (2D) cell viability assays, Western blot, and quantitative real-time PCR (qRT-PCR) analyses (primers/probes listed in Supplementary Table S2), and determination of total rat serum IGF-I levels and ex vivo IGF-I bioactivity are described in the Supplementary Materials and Methods.

**Statistical analysis**

Statistical analysis was performed using the GraphPad Prism software (GraphPad Software Inc.). Control (pretreatment or vehicle-treated animals), and treatment groups were compared in one-way ANOVA followed by a Tukey post test if not indicated otherwise. P values less than 0.05 (*), 0.01 (**), and 0.001 (***)) were considered to be statistically significant.

**Results**

**Biochemical characterization of BI 836845**

BI 836845 is a fully human mAb selected from a naïve phage display library and affinity optimized for IGF-I binding. As shown in Supplementary Table S1, surface plasmon resonance analysis demonstrated that in addition to the high affinity for human IGF-I (0.07 nmol/L), BI 836845 also shows high affinity for human IGF-II (0.8 nmol/L). BI 836845 was also shown to strongly cross-react with mouse and rat IGF-I and IGF-II (Supplementary Table S1). No binding to human insulin was detected at up to 100-fold higher antibody concentrations (data not shown).

To measure IGF bioactivity (IGF-IR or IR-A kinase activating activity) in serum, plasma, or cell culture media, we developed a cell-based assay utilizing mouse fibroblasts engineered to express human IGF-IR or IR-A, in which receptor phosphorylation can be quantified by ELISA. This assay was initially used to determine the potency and effectiveness of BI 836845 in neutralizing recombinant IGF-I and IGF-II. As shown in Fig. 1A and B, BI 836845 inhibited both IGF-I (20 ng/mL) and IGF-II (100 ng/mL)-induced IGF-IR phosphorylation with an IC₅₀ of 90 nmol/L (0.6 nmol/L) and 1,120 ng/mL (7.5 nmol/L), respectively. In the same assay, two IGF-IR mAbs were less effective with respect to IGF-I–induced activation and displayed only weak effects on IGF-II–induced activation with more than 30% of IGF-II–induced IGF-IR phosphorylation remaining even at an antibody concentration of 100 nmol/L (Fig. 1B). Figure 1C demonstrates that BI 836845 also potently inhibited IGF-II (100 ng/mL)-induced IR-A activation with an IC₅₀ of 815 ng/mL (5.4 nmol/L), unlike IGF-IR–targeted antibodies that had no effect. When cell culture medium containing 20% human serum was used to stimulate receptor phosphorylation, this could be completely neutralized by BI 836845 with an IC₅₀ of 246 ng/mL (1.6 nmol/L). In contrast, an IGF-IR–targeted antibody showed only partial inhibition of IGF bioactivity (Fig. 1D).

**Pharmacodynamic effects of BI 836845 in vivo**

The cross-reactivity of BI 836845 to mouse and rat IGF-I and IGF-II has allowed a comprehensive preclinical characterization of the pharmacodynamic properties of the antibody in these species. Pharmacodynamic effects of BI 836845 were studied in 6- to 8-week-old rats treated once weekly for 13 weeks with 20, 60, or 200 mg/kg of the antibody (last treatment on day 85). Treatment with BI 836845 at all dose levels was well tolerated and resulted in a clear reduction in plasma IGF bioactivity as determined ex vivo by the bioassay described above (Fig. 2A). These effects of BI 836845 on plasma IGF bioactivity were seen despite a corresponding increase in total IGF-I plasma levels. Figure 2B shows that treatment with BI 836845 resulted in elevated total IGF-I from day 3 onward, with up to 25-fold increases seen for the groups treated with 60 and 200 mg/kg. Following cessation of treatment, a dose-dependent return of both plasma IGF bioactivity (Fig. 2A) and total IGF-I levels (Fig. 2B) toward control levels was seen by day 169. A dose-dependent effect of BI 836845...
treatment was observed on rat body weight gain (Fig. 2C). Compared with controls, the 60 and 200 mg/kg doses showed a similar reduction in body weight gain, which was less pronounced for the 20 mg/kg dose level (Fig. 2C). To confirm that the effect of BI 836845 on body weight was due to a reduction in body growth, tail length was monitored. Figure 2D shows that there was a dose-dependent reduction in tail length compared with vehicle controls on day 85 of the study. In addition to these findings, we noted that essentially all organ weights were reduced. Moreover, we observed a reduction in secondary spongiosa of bones, where IGF-I is known to play an important role (ref. 39; data not shown).

**Antineoplastic activity of BI 836845 in cancer models**

BI 836845 was assayed for in vitro growth inhibitory activity in cancer-derived cell lines grown in 10% FBS. The proliferation of several cell lines derived from different cancer types, including non–small cell lung cancer (NSCLC), small cell lung cancer (SCLC), Ewing sarcoma, and multiple myeloma, was found to be potently inhibited by BI 836845 (low nmol/L EC_{50} values; Table 1). Approximately one third of the responsive cell lines showed elevated levels of IGF-I and/or IGF-II mRNA expression compared with median expression of 625 cell lines (examples are shown in Fig. 3A), indicating that BI 836845 is capable of attenuating in vitro proliferation of cell lines showing autocrine ligand production. The colorectal carcinoma cell line GEO showed a particularly high level of IGF-II expression (50-fold over median; Fig. 3B), which was associated with constitutive IGF pathway activation in tumors in vivo (Fig. 3C). In addition, we assessed mRNA expression of INSR and its variants by qRT-PCR and identified expression of both INSR splice variants in GEO cells (Supplementary Fig. S1A). In contrast, the Ewing sarcoma cell line RD-ES exclusively
expressed the IR-A variant (Supplementary Fig. S1C), in line with previously published findings (40). In both cell lines, no insulin mRNA expression was detectable (Supplementary Fig. S1A and S1C), and INSR mRNA levels were significantly lower than those of IGF-IR (Supplementary Fig. S1B and S1D). This difference was also reflected at the protein level in RD-ES cells in vitro and in vivo (Supplementary Fig. S1E). The GEO cell line was established as a subcutaneous xenograft model in immunodeficient (nude) mice to determine whether BI 836845 could inhibit the in vivo growth of a tumor with high IGF-II expression. Figure 3D shows that treatment with 100 mg/kg of BI 836845 twice weekly resulted in 54% tumor growth inhibition (TGI) after 27 days of treatment.

BI 836845 enhances the antineoplastic efficacy of rapamycin

Previous studies have shown improved efficacy when combining IGF-IR mAbs with rapamycin (41, 42). We were therefore interested in assessing whether the combination of an IGF ligand-neutralizing antibody with rapamycin would be an effective antineoplastic strategy. We therefore tested the combined effect of BI 836845 and rapamycin on the viability and signaling of two BI 836845-sensitive Ewing sarcoma-derived cell lines, SK-ES-1 and RD-ES. Cell viability studies in vitro demonstrated that the combination of BI 836845 and rapamycin was more potent and effective than either single agent at inhibiting proliferation of both cell lines (Fig. 4A and C; P < 0.001 at concentrations ≥12.33 and ≥0.1 nmol/L,
respectively). To investigate the molecular mechanism behind the improved efficacy seen when both agents were combined, we assessed AKT phosphorylation levels (serine 473; pAKT) as a biomarker reflecting the consequences of rapamycin-induced relaxation of an inhibitory feedback loop (43). Treatment of SK-ES-1 (Fig. 4B) and RD-ES cells (Fig. 4D) with 100 nmol/L rapamycin resulted in increased pAKT compared with untreated cells. When BI 836845 was combined with rapamycin, phosphorylation of AKT was inhibited, indicating that the feedback increase in pAKT in the presence of rapamycin is dependent on IGF ligand-driven signaling (Fig. 4B and D). The inhibitory effects on cell proliferation of the combination versus each single agent were tested in 60 cancer cell lines (Supplementary Fig. S2A). In 13 out of 17 cell lines derived from different cancer types, the effects on proliferation were consistent with the effects on AKT phosphorylation (Supplementary Fig. S2B).

An RD-ES xenograft model was used to determine whether the additive effects of BI 836845 and rapamycin seen in vitro could be recapitulated in vivo. The combination of BI 836845 (twice weekly, 100 mg/kg i.p.) and rapamycin (5/2 weekly, 5 mg/kg i.p.) was more effective (TGI 93%) at inhibiting tumor growth than either single agent (rapamycin TGI 75%, BI 836845 TGI 53%) after 20 days of treatment (Fig. 5A). Single-agent treatments were significantly different from control treatment from day 6 onward (P < 0.05 for rapamycin and P < 0.01 for BI 836845 on day 6), whereas combination treatment differed significantly from controls from day 3 onward (P < 0.05 on day 3). Differences between single-agent treatments and combination treatment were statistically significant from day 8 onward (P < 0.05 on day 8). Further analyses of pAKT levels in the RD-ES tumors showed that rapamycin increased pAKT levels, and that this increase could be inhibited by BI 836845 (Fig. 5B). IGF bioactivity in the serum of RD-ES tumor-bearing mice was increased upon rapamycin treatment compared with control mice (Fig. 5C). BI 836845 treatment alone, and in combination with rapamycin, completely inhibited IGF bioactivity in plasma (Fig. 5C). To investigate the contribution of RD-ES tumors to the elevated presence of rapamycin and BI 836845...
levels of plasma IGF bioactivity, we treated non–tumor-bearing mice similarly and found that rapamycin induced a significant increase in plasma IGF bioactivity comparable with that seen in the RD-ES tumor-bearing mice (Fig. 5D).

Discussion

BI 836845, a fully human mAb, blocks the activation of IGF-IR and IR-A by binding to and neutralizing both IGF ligands. The preclinical data presented in this study contributed to the rationale for a clinical trial program, and phase I studies are now in progress.

The mechanism of action of BI 836845 is distinct from that of IGF-IR–targeted antibodies, which to date have proved disappointing in clinical studies (30). By binding to and neutralizing the IGF ligands, BI 836845 blocks activation not only of the IGF-IR, but unlike anti-IGF-IR antibodies also reduces IGF-II–stimulated activation of IR-A. This may be an important advantage, as the expression of this receptor variant is often increased in tumor cells (7, 8, 10, 44) and may play a role in autocrine IGF-II–mediated growth stimulation (34, 40). In addition, some IGF-IR antibodies have been shown to be weak inhibitors of IGF-II–driven IGF-IR signaling, which is thought to be due to differences in the binding sites of IGF-I and IGF-II on the IGF-I receptor (45).

The rodent models presented here are informative not only with regards to in vivo efficacy but also to tolerability of BI 836845, as this antibody blocks the bioactivity of both human and rodent IGF ligands. Long-term administration of BI 836845 to rats was well tolerated at doses sufficient to reduce somatic growth. Growth inhibition of rats was associated with a substantial decrease of serum IGF bioactivity, a relevant pharmacodynamic endpoint. This was achieved in the presence of...
of significantly elevated total IGF-I levels in the plasma following BI 836845 treatment. A plausible explanation for the increase in total serum IGF-I relates to a compensatory increase in growth hormone secretion, resulting in increased hepatic IGF-I expression (1). Although we did not observe a significant increase in growth hormone levels in BI 836845-treated rats, this does not exclude this mechanism, as the pulsatile nature of growth hormone secretion limits the utility of single growth hormone measurements (data not shown). A similar phenomenon has been observed with the anti-VEGF ligand-targeting approach of bevacizumab, where following treatment serum VEGF levels have been found to be increased, presumably as a result of an increase in VEGF synthesis and/or a decrease in VEGF clearance caused by complex formation between VEGF and the antibody (46).

In human cancers, autocrine expression of IGF-I or IGF-II is not a rare event (2). It is unlikely that this represents a random deregulation of gene expression. Rather, a malignant clone that expresses both a growth factor and its receptor will likely have a proliferative advantage that...
will be selected for. Thus, autocrine expression of IGF may indicate a cancer that is responsive to and/or dependent on IGF-IR activation (1). However, this does not necessarily imply that such tumors will be responsive to any particular IGF-targeting strategy. Interruption of an autocrine loop in vivo requires a high drug concentration in the tumor, and it is conceivable that pharmacokinetic or tolerability issues may limit efficacy of IGF-targeting therapies even for the subset of cancers that depend on IGF stimulation. Therefore, it was of interest to observe that BI 836845 was capable of significantly inhibiting not only in vitro proliferation of IGF-secreting cell lines but also the in vivo growth of a colorectal cancer model that expresses high levels of IGF-II. Despite the important pharmacodynamic information that has been generated from the profiling of BI 836845 in rats and mice, the translational limitation of using rodent models for studying IGF physiology is that they express far less IGF-II than humans (47), and this needs to be taken into consideration when interpreting such studies in rodents.

Besides being potential drivers of tumor growth in certain subsets of cancer, IGFs may contribute to resistance mechanisms limiting the efficacy of other anticancer agents. Previous studies have shown a potential for IGF targeting in rational combinations, such as IGF-IR and IGF ligand antibodies (48), or combinations of IGF-IR mAbs with rapamycin to more efficiently block angiogenesis (41, 42). We studied the combination of BI 836845 with rapamycin in view of these prior preclinical and clinical data indicating that the efficacy of rapalogs may be limited because inhibition of mTOR complex (mTORC)-1 results in AKT activation due to IGF-IR–dependent interference with negative feedback loops constraining signaling downstream of the IGF-IR (21, 43, 49, 50). Our in vitro and in vivo findings provide evidence that the activity of rapamycin can be improved by coadministration of BI 836845 by abolishing AKT activation, suggesting a rational combination that deserves clinical evaluation. Apart from the known cell-autonomous actions (43, 49), we observed a novel homeostatic response to mTORC1 inhibition at the whole organism level, characterized by increases in serum IGF-IR activating activity, a phenomenon observed in nude mice in the presence or absence of xenograft tumors. This response was clearly attenuated by BI 836845, suggesting that rapamycin-induced AKT activation is dependent on IGF ligand activity (mechanistic model depicted in Supplementary Fig. S5). Interestingly, a similar effect was seen in a study using vascular smooth muscle cells (51). However, the precise definition of the mechanism by which rapamycin increases serum IGF bioactivity requires additional investigation and is an active area of research. Similarly, the evaluation of angiogenesis and proliferation markers, such as CD34 and Ki67, will be important because enhanced antiangiogenic effects may also play a role in explaining the improved efficacy of the combination (48).

Despite the recent clinical setbacks with agents targeting the IGF-IR (30), the data presented here support the differentiated therapeutic concept of IGF-I/II ligand neutralization, which warrants clinical investigation. Preclinical antiproliferative activity was observed with BI 836845 both in vitro and in vivo, and long-term exposure at levels sufficient to abolish IGF serum bioactivity and strongly attenuate somatic growth was not associated with significant toxicity in rats. The results presented also provide clues that may be relevant to the design of phase II clinical trials. Presuming that dosing of BI 836845 will not be limited by toxicity, a crucial issue will be defining a phase II dose. Our preclinical studies have identified measurable pharmacodynamic endpoints such as serum IGF bioactivity that will be useful for defining the pharmacokinetic/pharmacodynamic (PK/PD) relationship in humans. With regards to rational rather than arbitrary drug combinations, our findings indicate that coadministration of BI 836845 with rapalogs deserves consideration, and with respect to potential selection criteria for tumors that may be particularly responsive, autocrine expression of IGF-I or IGF-II may define a subpopulation of interest (52).

Disclosure of Potential Conflicts of Interest

Michael N. Pollak has commercial research grants from Pfizer and Novo Nordisk and is a consultant/advisory board member of Boehringer Ingelheim, Pfizer, Novo Nordisk, Sanofi Aventis, Bristol Myers Squibb, and Axelar. No potential conflicts of interest were disclosed by the other authors.

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Development of methodology: P.J. Adam

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References

34. Nagy ZA, Hubner B, Lohning C, Rauchenberger R, Tho-


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