BMS-754807, a small molecule inhibitor of insulin-like growth factor-1R/IR

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Abstract

BMS-754807 is a potent and reversible inhibitor of the insulin-like growth factor 1 receptor/insulin receptor family kinases (Ki, < 2 nmol/L). It is currently in phase I development for the treatment of a variety of human cancers. BMS-754807 effectively inhibits the growth of a broad range of human tumor types *in vitro*, including mesenchymal (Ewing's, rhabdomyosarcoma, neuroblastoma, and liposarcoma), epithelial (breast, lung, pancreatic, colon, gastric), and hematopoietic (multiple myeloma and leukemia) tumor cell lines (IC_{50}, 5–365 nmol/L); the compound caused apoptosis in a human rhabdomyosarcoma cell line, Rh41, as shown by an accumulation of the sub-G_1 fraction, as well as by an increase in poly ADP ribose polymerase and Caspase 3 cleavage. BMS-754807 is active *in vivo* in multiple (epithelial, mesenchymal, and hematopoietic) xenograft tumor models with tumor growth inhibition ranging from 53% to 115% and at a minimum effective dose of as low as 6.25 mg/kg dosed orally daily. Combination studies with BMS-754807 have been done on multiple human tumor cell types and showed *in vitro* synergies (combination index, < 1.0) when combined with cytotoxic, hormonal, and targeted agents. The combination of cetuximab and BMS-754807 *in vivo*, at multiple dose levels, resulted in improved clinical outcome over single agent treatment. These data show that BMS-754807 is an efficacious, orally active growth factor 1 receptor/insulin receptor family–targeted kinase inhibitor that may act in combination with a wide array of established anticancer agents. [Mol Cancer Ther 2009;8(12):3341–9]

Introduction

Type I insulin-like growth factor-I receptor (IGF-1R) is a transmembrane tyrosine kinase growth factor receptor that plays an essential role in the establishment and maintenance of the transformed phenotype; activation results in mitogenesis and survival for cancer cells (1–4). Binding of its ligands, IGF-1 and IGF-II to IGF-1R, initiates a cascade of events that leads to activation of downstream signal transduction pathways, primarily the mitogenic signaling pathway mitogen-activated protein kinase (MAPK), and the antiapoptotic/survival pathway (phosphatidylinositol 3′-kinase/AKT; refs. 5, 6). IGF-1R is closely related to the insulin receptor (IR). In spite of their structural similarities, activation of IGF-1R leads to cell proliferation, motility, and metastasis, whereas the actions of insulin on IR are directed primarily at regulation of glucose homeostasis. However, it is clear that insulin increases the proliferation of neoplastic cell lines as well (7–9). Although the binding affinities vary, ligands directed to IR and IGF-1R cross-react (10, 11).

In recent years, IR has been shown to be significantly increased in primary malignant breast tumors when compared with normal breast tissue (12–14), and increased expression levels of total IR are correlated with poor survival (15). Although the classic insulin receptor IR-B mediates glucose uptake, it has been shown that stimulation of IR as well IR/IGF-1R hybrid receptors (IR-A/IGF-1R hybrid receptors) by insulin, IGF-1, and/or IGF-II may contribute to cancer growth, suggesting that both IR and IGF-1R may be targets for cancer therapy, and that it may be necessary to effectively block the oncogenic potential of these two pathways (9, 11). The challenge in targeting both IGF-1R and IR is that it has the potential to result in dysregulation of glucose homeostasis due to its effects on IR. Due to this potential complication, the development of IGF-1R inhibitors as anticancer therapeutics has focused mainly on the identification of antibodies specific for IGF-1R. According to first reports on clinical studies, administration of IGF-1R antibodies was well tolerated and responses were observed alone (16) and in combination with chemotherapy in solid tumors (17). Interestingly, hyperglycemia was among the observed toxicities of IGF-1R antibody treatment (18). Owing to the ever-increasing importance of IGF-1R and IR in cancer, we identified an oral and potent small

molecule inhibitor having both *in vitro* and *in vivo* activity in multiple human tumor types. BMS-754807, a pyrrolotriazine and reversible ATP-competitive antagonist of IGF-1R, inhibits the catalytic domain of the IGF-1R (19), and has been shown to block the activity of both IGF-1R and IR in *in vitro* kinase assays. Because the antibodies bind specifically to IGF-1R and not to IR, this could potentially be an escape mechanism for IGF-II and insulin signaling. The advantage of an inhibitor of both IGF-1R and IR signaling in cancer cells will have to be balanced with the potential for metabolic side effects. The aim of this article is to describe the *in vitro/in vivo* pharmacology of a dual IGF-1R/IR inhibitor that is currently in phase I clinical trials in a variety of human cancers.

Materials and Methods

Compound Preparation and Administration

BMS-754807 is a pyrollotriazine (pyrrolo[1.2-f][1,2,4]triazine; Fig. 1) that was synthesized by the Medicinal Chemistry group at Bristol-Myers Squibb Pharmaceutical Research Institute (19). Clinical vials of cetuximab (Erbitux, BMS-576365) were supplied by ImClone Systems. For oral administration to rodents, BMS-754807 was dissolved in a mixture of polyethylene glycol 400 (PEG400/water (4:1; vol/vol) facilitated by stirring and used through the duration of dosing. The volume of BMS-754807 administered was 0.01 mL/g of body weight. Cetuximab was dissolved in PBS and administered i.p. at a volume of 0.5 mL per mouse, so as to deliver 1 mg per mouse of drug.

Cellular Proliferation Assays

The majority of the cell lines were obtained from American Type Culture Collection with the exception of several sarcoma lines that were kindly provided by Dr. Lee Helman, Center for Cancer Research, National Cancer Institute, Bethesda, MD; cells were grown at their optimal density in RPMI+GlutaMax supplemented with 10% heat-inactivated fetal bovine serum (FBS), 10 mmol/L Hepes, penicillin, and streptomycin. Cell proliferation was evaluated by incorporation of ^{3}H-thymidine (GE Healthcare) into DNA after exposure of cells to BMS-754807 for 72 h. Results are expressed as an IC$_{50}$, which is the drug concentration required to inhibit cell proliferation by 50% compared with untreated control cells.

Western Blot Analysis

Cells or tumors were lysed in TTG buffer [20 mmol/L Tris-HCl (pH 7.6), 1% Triton X-100, 5% glycerol, 0.15 mol/L NaCl, 1 mmol/L EDTA, and Complete Tablets (Roche Diagnostics)] and Phosphatase Inhibitor Cocktail 2 (Sigma). Protein concentrations of total cell lysates or total tissue lysates were determined using a BCA assay kit (Pierce) with bovine serum albumin as the standard. Lysates were resolved by SDS-PAGE (Invitrogen) and transferred to nitrocellulose membranes (Bio-Rad Laboratories). After blocking in Odyssey Blocking Buffer with 0.1%Tween 20 (Li-Cor Biosciences), membranes were probed with the antibodies specific for the following: pIGF-1R (Tyr1135/1136)/IR (Tyr1150/1151; #3024), pAkt(Ser473), p-p44/42 MAPK (Thr202/Tyr204; #4051), total IGF-1R (Santa Cruz, sc-713), cleaved poly ADP ribose polymerase (#9542), and Caspase 3 [#9662; all antibodies were purchased from Cell Signaling Technology except actin (Chemicon International). Membranes were incubated with the appropriate IR-labeled secondary antibodies and protein was visualized using Li-Cor Biosciences Odyssey Infrared Imaging System (Li-Cor Biosciences). Inhibition was calculated by comparing the ratio of phosphorylation signals in untreated versus treated samples after normalization to actin, which is used as a loading control.

Cell Cycle

Rh41 cells (2×10^5) were seeded in six-well plates over night before treatment with reagents at 10× the cellular IC$_{50}$. Cells and supernatants were collected at 24, 48, and 72 h and fixed with 70% ethanol and stored overnight at −20°C. Cells were washed with cold PBS and then rehydrated in PBS containing propidium iodide (25 μg/mL; Sigma, P-4864). After 30 min of incubation at room temperature, samples were analyzed by FACscan (FACS Calibur).

Pharmacokinetic/Pharmacodynamic Study for IGF-1R Inhibition

Nude mice bearing IGF-1R-Sal–dependent salivary gland tumors were dosed with either 3.125 mpk (minimal efficacious dose in IGF-1R-Sal) or 12.5 mpk (minimal efficacious dose in GEO), and samples were evaluated at multiple time points up to 24 h (15, 30 min, 1, 2, 4, 6, 8, 16, and 24 h). Drug concentrations in serum and tumors were determined by liquid chromatography tandem mass spectrometry. Tumors from the same set of samples were pooled and analyzed by Western blot to determine the extent of inhibition of IGF-1R phosphorylation.

Antitumor Activity in Preclinical Models

All animal studies were done according to the USPHS Policy on Humane Care and Use of Laboratory Animals in an American Association for Laboratory Animal Care–approved site. All rodents were obtained from Harlan Sprague-Dawley Company. The required numbers of animals needed to detect a meaningful response were pooled at the start of the experiment and each was given a subcutaneous implant of a tumor fragment (∼20 mg) with a 13-gauge trocar. Tumors were allowed to grow to the predetermined size window (75–200 mg; tumors outside the range were excluded), and animals were evenly distributed to various treatment and control groups. There were typically eight mice per treatment and control groups, with the exception
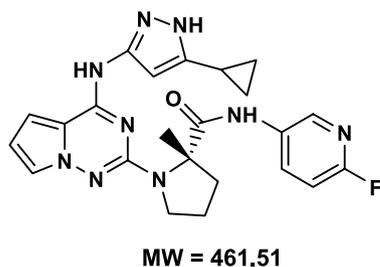

MW = 461.51

Figure 1. Structure of BMS-754807, a pyrrolotriazine (pyrrolo[1.2-f][1,2,4]triazine). *MW*, molecular weight.

of experiments conducted in the Sal-IGF (same as IGF-1R-Sal) tumor model, in which there were typically five mice per treatment and control group. Treatment of each animal was based on individual body weight. Treated animals were checked daily for treatment-related toxicity/mortality. Each group of animals was weighed before the initiation of treatment (Wt1) and then again following the last treatment dose (Wt2). The difference in body weight (Wt2 − Wt1) provides a measure of treatment-related toxicity.

Tumor response was determined by measurement of tumors with a caliper twice a week, until the tumors reached a predetermined "target" size of 0.5 or 1 g depending on the tumor type. Tumor weights (mg) were estimated from the formula:

$$Tumor\ weight = (length \times width^2) \div 2$$

Tumor response criteria are expressed in terms of tumor growth inhibition (%TGI).

TGI is calculated as follows:

$$\% \ TGI = \frac{\left(1 - \frac{T_t}{T_0} * \frac{C_0}{C_t}\right)}{\left(1 - \frac{C_0}{C_t}\right)}$$

where,

C_t is the median control tumor size at end of treatment
C_0 is the median control tumor size at treatment initiation
T_t is the median tumor size of treated group at end of treatment
T_0 is the median tumor size of treated group at treatment initiation

Significant antitumor activity is defined as the achievement of durable TGI of 50% or greater (i.e., TGI ≥ 50%) for a period equivalent to at least one tumor volume doubling time and drug treatment must be for a period equivalent to at least two tumor volume doubling time.

In combination therapy, tumor response was also expressed in terms of tumor growth delay (TGD value), defined as the difference in time (days) required for the treated tumors (T) to reach a predetermined target size of 500 mg compared with those of the control group (C).

Whenever possible, antitumor activity was determined at a range of dose levels up to the maximum tolerated dose (MTD), which is defined as the dose level immediately below which excessive toxicity (i.e., more than one death) occurred. When death occurred, the day of death was recorded. Treated mice dying before having their tumors reach target size were considered to have died from drug toxicity. Treatment groups with more than one death caused by drug toxicity were considered to have had excessively toxic treatments and their data were not included in the evaluation of a compound's antitumor efficacy.

Potential drug toxicity interaction affecting treatment tolerability is an important consideration in combination chemotherapy trials. Therefore, interpretation of combination therapeutic results must be based on comparison of antitumor activity of the best possible response for the single agents versus the combination at comparably tolerated doses. Therefore, therapeutic synergism was defined as a therapeutic effect achieved with a tolerated regimen of the combined agents that exceeded the optimal effect achieved at any tolerated dose of monotherapy. Statistical evaluations of data were done using Gehan's generalized Wilcoxon test (20). Statistical significance was declared at $P < 0.05$.

In vitro Drug Combination Study

A dilution of ratios drug combination method (21) was used in cellular proliferation assays to determine whether there was synergy, additivity, or antagonism when two compounds were added simultaneously to a variety of human tumor cells *in vitro*. Drug stock solutions for two compounds were combined in ratios of 10:1, 5:1, 3:1, 1:1, 1:3, and 1:5. These ratios, as well as the individual compound stock solutions, were diluted in a serial manner using 70% DMSO. These serial dilutions were then mixed with RPMI growth medium and added to cells to test the IC_{50} values of single agent as well as two compounds in the cellular proliferation assays. Combination indices, SEMs, and 95% confidence intervals were calculated for the different ratios and used to determine if the combination results represented synergistic, additive, or antagonistic effects. Combination index values below 1 would be considered to be synergistic when the 95% confidence interval (approximately the combination index value ± 2× SEM) did not exceed the value of 1.

Results

Biochemical Characterization and Selectivity of BMS-754807

Initial characterization of BMS-754807 included an *in vitro* assay to evaluate the inhibition of recombinant human IGF-1R in biochemical assays using synthetic peptide KKSRGDYMTMQIG as a phosphoacceptor substrate. Potency was characterized by inhibition of substrate phosphorylation and reported as an IC_{50} value, defined as the concentration of compound required to achieve 50% inhibition of enzyme activity. Given the high homology of the kinase domains (~85%), it is not surprising that BMS-754807 has equipotent activity against IGF-1R and IR (IC_{50} of 1.8 versus 1.7 nmol/L, respectively). Testing against a broad panel of 27 kinases (including receptor and nonreceptor tyrosine as well as serine/threonine kinases) identified additional off-target activities with the most potent activities against Met, RON, TrkA, TrkB, AurA, and AurB with IC_{50} values of 6, 44, 7, 4, 9, and 25 nmol/L, respectively (Supplementary Table S1). All other kinases were >100-fold less sensitive to inhibition by BMS-754807. Both cellular growth (thymidine incorporation) and pharmacodynamic (receptor autophosphorylation) cellular assays, however, showed a markedly higher degree of selectivity of IGF-1R over Met (≥29- and 9-fold, for growth and pharmacodynamic effects, respectively), and/or TrkA (≥25- and 64-fold, for growth and PD effects, respectively; Supplementary Table S2).

To further characterize BMS-754807 against a wider range of protein kinases as possible and beyond what was available in-house, BMS-754807 was submitted to Ambit Biosciences (22) for evaluation using a proprietary phage–based competition assay. BMS-754807 was tested against an additional 317 serine/threonine and tyrosine kinases and shown to have a predicted target activity of (K_d < 100 nmol/L) in 29 additional kinases, albeit at a reduced potency relative to the IGF family (Supplementary Fig. S1).

BMS-754807 Has Potency in Multiple Human Cell Lines *In vitro*

In cellular proliferation assays, BMS-754807 was found to effectively inhibit the growth of a broad range of human tumor cell lines of different histologic origins including mesenchymal (Ewing's, rhabdomyosarcoma, neuroblastoma, and liposarcoma), epithelial (breast, lung, pancreatic, colon, and gastric), and hematopoietic (multiple myeloma and leukemia; Fig. 2). The IC_{50} values ranged from 5 to 365 nmol/L for the most sensitive cell lines; there were also potencies above 365 nmol/L in a variety of cell types (Supplementary Table S3).

BMS-754807 Inhibits IGF-1–Induced Receptor Activation

BMS-754807 was assessed for its ability to inhibit IGF-1R phosphorylation in an IGF-1R–driven cell line, IGF-1R-Sal (Fig. 3A). IGF-1R-Sal is a cell line derived from a xenograft tumor expressing a chimeric receptor that was constructed by an in-frame fusion of the cytoplasmic sequence of the human IGF-1Rβ subunit to the extracellular and transmembrane sequences of the human T-cell antigen, CD8α (23). In addition, BMS-754807 was evaluated in human rhabdomyosarcoma tumor cells Rh41 (Fig. 3B) and human colon carcinoma Geo (Fig. 3C), which require ligand occupancy for full activation of IGF-1R. The cellular IC_{50} values for inhibition of proliferation by BMS-754807 in IGF-1R-Sal and Rh41 were in the low nmol/L range (7 and 5 nmol/L, respectively), compared with the weak activity observed in Geo cells (365 nmol/L) tested in media containing 10% FBS (which includes a variety of growth factors; Supplementary Table S4). Inhibition of phosphorylation activity was measured by exposing cells to BMS-754807 for 1 h followed by probing Western blots with antibodies specific for pIGF-1R, pAkt, and pMAPK. Despite the differences in cellular potencies among the three cell lines, the IC_{50} values for inhibition of the pIGF-1R by BMS-754807 and downstream components (e.g., pAkt) were very similar (Supplementary Table S4). In contrast, there was greater inhibition against pMAPK in IGF-1R-Sal cells compared with Rh41 and Geo, indicating that additional compensatory pathways such as epidermal growth factor receptor (EGFR) might be important in driving signals in both Rh41 and Geo cell types.
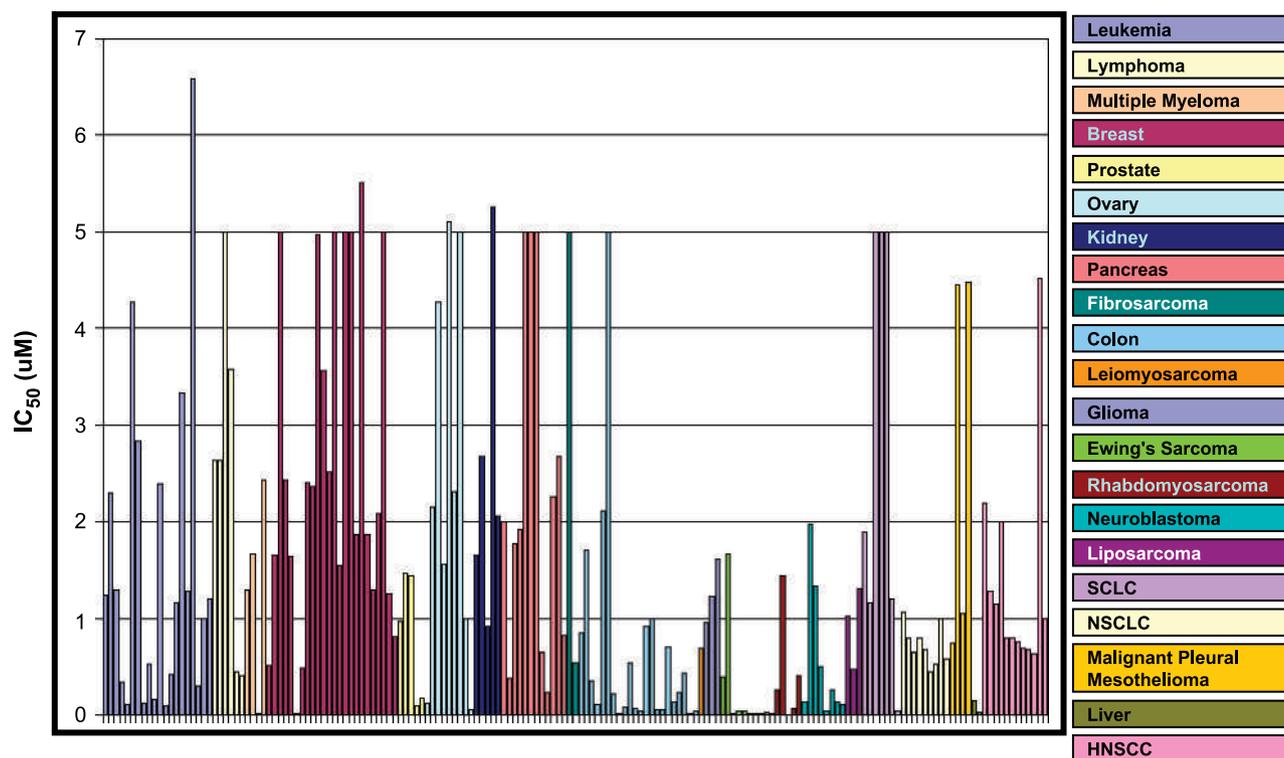

Figure 2. Broad range of antiproliferative activity in multiple human tumor cell lines: cell proliferation was evaluated in cells by [^{3}H]-thymidine incorporation after exposure to BMS-754807 for 72 h in media supplemented with 10% FBS. Results are expressed as an IC_{50}, which is the drug concentration required to inhibit cell proliferation by 50%.

Figure 3. Inhibition of IGF-1–induced receptor activation. **A,** Western blot analysis of inhibition of IGF-1R, Akt, and MAPK phosphorylation in (**A**) IGF-1R-Sal, (**B**) Rh41, and (**C**) Geo cells exposed to BMS-754807. Cells were exposed to no drug (DMSO), or to increasing concentrations of drug (0.1–1,000 nmol/L) as described. Cells were lysed in TTG lysis buffer [1% Triton X-100, 5% glycerol, 0.15 mol/L NaCl, 20 mmol/L Tris-HCl (pH 7.6), Complete tablet (Roche), and Phosphatase Inhibitor Cocktail 2 (Sigma)], and whole cell lysates (15 or 30 μg of protein) were analyzed for pIGF-1R (Tyr1135/1136)/IR (Tyr1150/1151), pAkt (Ser 473), and p-p44/42 MAPK (Thr202/Tyr204) using Western Blot analysis (all antibodies were from Cell Signaling Technology and actin antibody was from Chemicon International). Antibody detection was done using Li-Cor Odyssey Infrared Imaging System. Protein visualization was performed using the Odyssey Infrared Imaging System as described above.
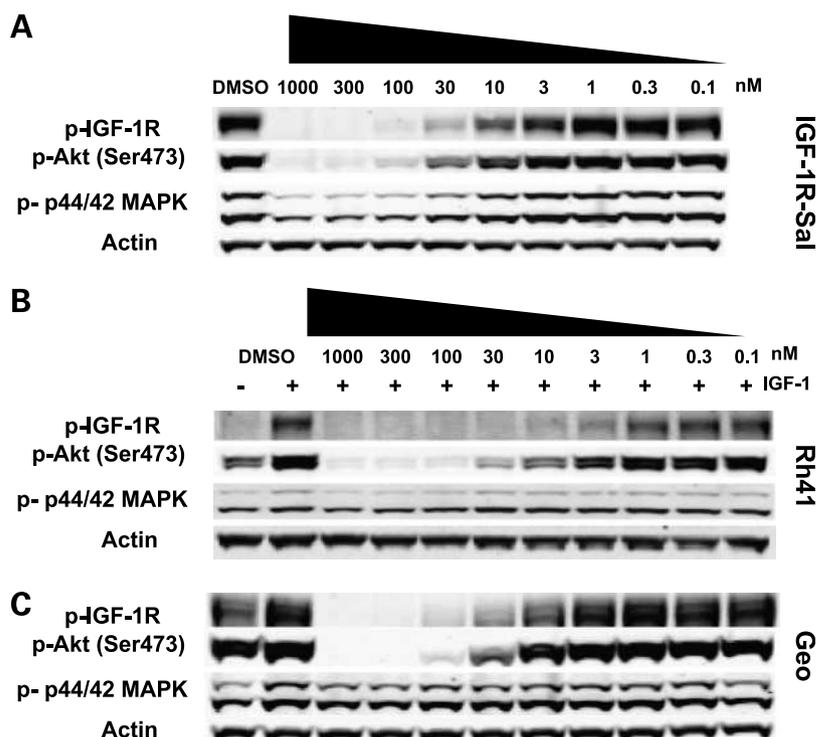
BMS-754807 Causes Cell Cycle Changes and Induces Apoptosis in Human Cell Lines

Treatment of cells with targeted agents, cytotoxics, and/or DNA-damaging agents differentially arrests the cell cycle at G_0-G_1, S, or G_2-M phases, which may be followed by induction of apoptotic cell death. Studies were designed to examine the apoptotic and cell cycle effects of BMS-754807 compared with a commercially available monoclonal antibody (mAb) against IGF-1R (mAb391, R&D Systems) that had similar potency in cellular proliferation assays in Rh41 cells (IC_{50} values for mAb391, 0.15 nmol/L; 5 nmol/L for BMS-754807). An Aurora kinase inhibitor, VX-680, which has an IC_{50} value of 93 nmol/L in a cellular proliferation assay in Rh41, was included as an internal control because BMS-754807 was shown to have activity against Aurora A and B kinases (with IC_{50} values of 9 and 25 nmol/L, respectively, in *in vitro* kinase assays; Supplementary Table S1) and Aurora B is known to arrest cells in G_2-M phases resulting in greater than 4N DNA (24). VX-680 has also been shown to inhibit other kinases (25) and these activities may be contributing to the inhibition of IGF-1R, as was observed in the current studies. For Western blot analysis (Fig. 4A), Rh41 cells were exposed to BMS-754807, mAb391, or VX-680 at 10 times the cellular IC_{50} for 30 minutes up to 48 hours. Samples were harvested for cell cycle distribution and collected at 24 hours (Fig. 4B), 48 hours (Fig. 4C), and 72 hours. Due to the high percentage of dead cells, the 72-hour time point was not included in the cell cycle analysis. Exposure of Rh41 cells to BMS-754807 resulted in immediate inhibition of pIGF-1R activity; treatment with mAb391 resulted in a diminution of

pIGF-1R activity starting at 30 minutes followed by internalization of IGF-1R at 1 hour, as was first described for the anti–IGF-1R mAb, CP-751,871 (Figitumumab) in 3T3/IGF-1R cells (26). BMS-754807 has more potent effects at inhibiting pAkt activity than mAb391 in this cell line; it also caused a marked increase in apoptotic activity compared with mAb391 as shown by an increased cleavage of poly ADP ribose polymerase and Caspase 3 expression. In addition, BMS-754807 induced greater apoptosis in Rh41 cells by 24 hours as indicated by an increased sub-G_1 peak (23.1%), compared with control (2.4%) or mAb391 (13.4%). VX-680 arrested Rh41 cells in G_2-M phase of the cell cycle as expected, with increased DNA content (>4N) indicating a block in cytokinesis, but little to no apoptosis at the 24- and 48-hour time points (2.8%; Fig. 4B; ref. 24). Therefore, the effects of BMS-754807 and VX-680 produce different phenotypes in Rh41 cells. However, in less IGF-1R–dependent cell lines, such as HCT116 and A549, we have observed Aurora B phenotypic cell cycle effects with BMS-754807 (data not shown). Additional studies are under way to help clarify the observed cell line–dependent differences in cell cycle phenotypes caused by BMS-754807 (25).

BMS-754807 Inhibits Tumor Growth in a Pharmacokinetic/Pharmacodynamic Model, IGF-1R-Sal

A mechanistic pharmacokinetic/pharmacodynamic study was established to examine the temporal relationship between BMS-754807 and the inhibition of IGF-1R phosphorylation in IGF-1R-Sal tumor–bearing nude mice. After oral administration of BMS-754807 (3.125 and 12.5mg/kg, both efficacious doses), the time course of BMS-754807
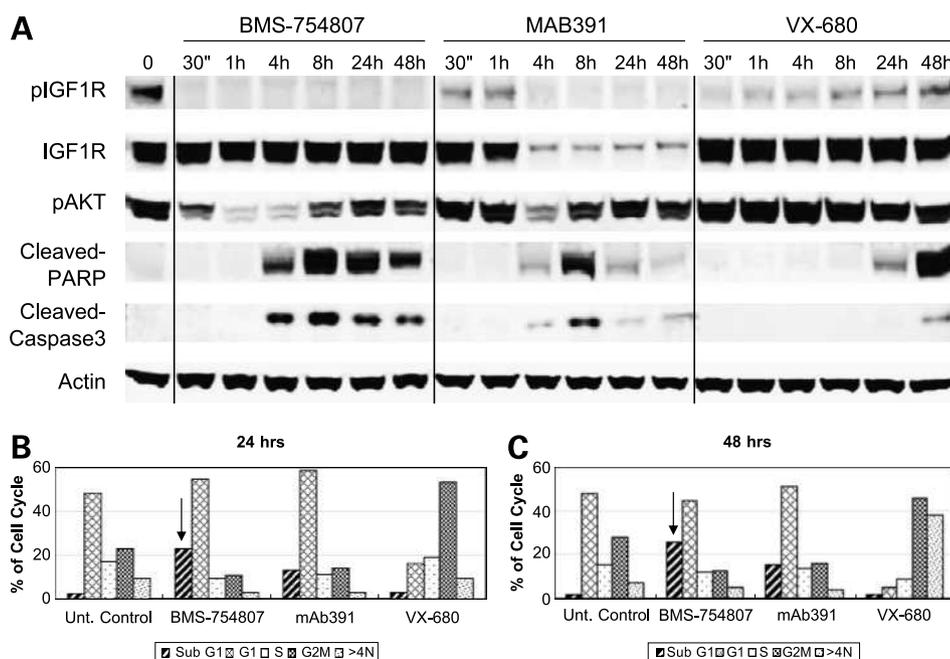
Figure 4. Cell cycle and induction of apoptosis in Rh41 cells exposed to BMS-754807, mAb391, and VX-680. **A,** Rh41 cells were grown in RPMI containing 10% FBS and exposed to compounds (BMS-754807, mAB391, VX-680) at 10× cellular IC_{50} for 30 min, 1, 4, 8, 24, or 48 h, at 37°C, 5% CO_2. Western blot analysis using whole cell lysates (25 μg) were analyzed as described. **B,** Rh41 cells were exposed to BMS-754807, mAb391, or VX-680 as described. Cells and supernatants were collected at 24 h (**B**) and 48 h (**C**), fixed with 70% ethanol and resuspended in PBS containing propidium iodide, and analyzed by FACscan. The experiment was repeated twice and a representative study is shown.

concentrations in serum and tumors was similar to the time course of pIGF-1R inhibition measured *ex vivo* (Fig. 5A–C). Inhibition of pIGF-1R was rapid beginning at 15 minutes, and correlated well with the drug concentrations in the tumor and serum (Fig. 5B and C shown at 12.5mg/kg) indicating target modulations.

Effects of BMS-754807 in Oral Glucose Tolerance Test

The effects of BMS-754807 on glucose homeostasis was evaluated in rodents by conducting an oral glucose tolerance test. Fasted mice were treated with various doses of BMS-754807 for 30 minutes before glucose challenge. The results showed that serum glucose levels increased 1.1- to 1.6-fold at efficacious doses of 3.125 and 12.5 mg/kg, respectively, at time 0 and returned to baseline by 1 hour (Supplementary Fig. S2). Insulin levels remained elevated at both doses by 2 hours posttreatment (Supplementary Table S5).

### Antitumor Effects of BMS-754807 in Human Tumor Xenografts

BMS-754807 has shown *in vivo* antitumor activity as a single agent in a number of preclinical tumor models in mice (Table 1). *In vivo*, the IGF-1R-Sal xenograft was established as a 4-day *in vivo* model to rapidly and efficiently evaluate compound efficacy on inhibition of tumor growth (23). BMS-754807 was found to inhibit tumor growth in a selected group of epithelial (IGF-1R-Sal, GEO, and Colo205), hematopoietic (JJN3), and mesenchymal (RD1 and Rh41) xenograft tumor models with TGI ranging from 53% to 115%. In the highly sensitive Rh41 rhabdomyosarcoma, BMS-754807 was effective at a dose level of 3.125 mg/kg twice daily and as low as 6.25 mg/kg once daily. In separate experiments, exposure at 6.25 mg/kg in nude mice was estimated to be ~200 nmol/L/h with a C_{max} of ~71 nmol/L and a T_{max} of ~0.5 hour.

Effects of BMS-754807 in Combination with Multiple Agents in Human Tumor Cell Lines and Human Xenograft Models

Synergy has been shown by the combination of tyrosine kinase inhibitors in conjunction with targeted agents that act through different mechanisms and/or pathways to provide a cross-talk to improve outcome in patient diseases. Due to the wide expression of IGF-1R and the potential cross-talk between the IGF-1R pathway and other signaling pathways implicated in tumorigenesis (EGFR, HER-2/neu, and mammalian target of rapamycin), BMS-754807 has the potential to be combined with other therapies in a wide range of tumors (27–29). The reciprocity of inhibition of multiple signaling pathways is being tested in clinical studies by simultaneous inhibition of IGF-1R in combination with EGFR and HER2 antibodies and small molecule inhibitors (30–32). To explore the cross-talk with other signal transduction pathways, BMS-754807 was tested in combination with anticancer agents in multiple human tumor cell types *in vitro*. BMS-754807 showed synergy when combined with targeted agents such as cetuximab (colon cancer), trastuzumab (breast cancer), lapatinib (lung cancer), bicalutamide (prostate cancer), and dasatinib (sarcoma and colon cancer). Synergy was also shown with cytotoxic agents: paclitaxel (breast, lung), docetaxel (breast), and vincristine (colon, lung; Supplementary Table S6).

Therapeutic benefit has also been shown *in vivo* with BMS-754807 in combination with cetuximab (C225) compared with either single agent used at their MTD. Treatment of mice bearing GEO tumors (human colon cancer) with a combination of BMS-754807 and cetuximab produced therapeutically synergistic antitumor activities, achieving a TGD of 38 days (Table 2; Fig. 6). Both single agent BMS-754807 and cetuximab possess significant antitumor activity

Figure 5. Effects of BMS-754807 *in vivo*. Pharmacodynamic and pharmacokinetic study in IGF-1R-Sal–tumored mice. Mice were dosed with either 3.125 or 12.5 mpk, and serum and tumor samples were evaluated at multiple time points up to 24 h. **A,** IGF-1R activity is inhibited in tumors of mice treated with 12.5 mpk of BMS-754807 as early as 30 min after dosing; pIGF-1R activity was inhibited for up to 8 to 16 h, and full activity was not restored even by 24 h. **C,** a good correlation was observed between serum drug levels and tumor drug concentration (4-fold higher than serum concentration), and (**B**) pIGF-1R inhibition was shown (i.e., as the plasma or tumor concentration is increased, pIGF-1R activity is decreased).
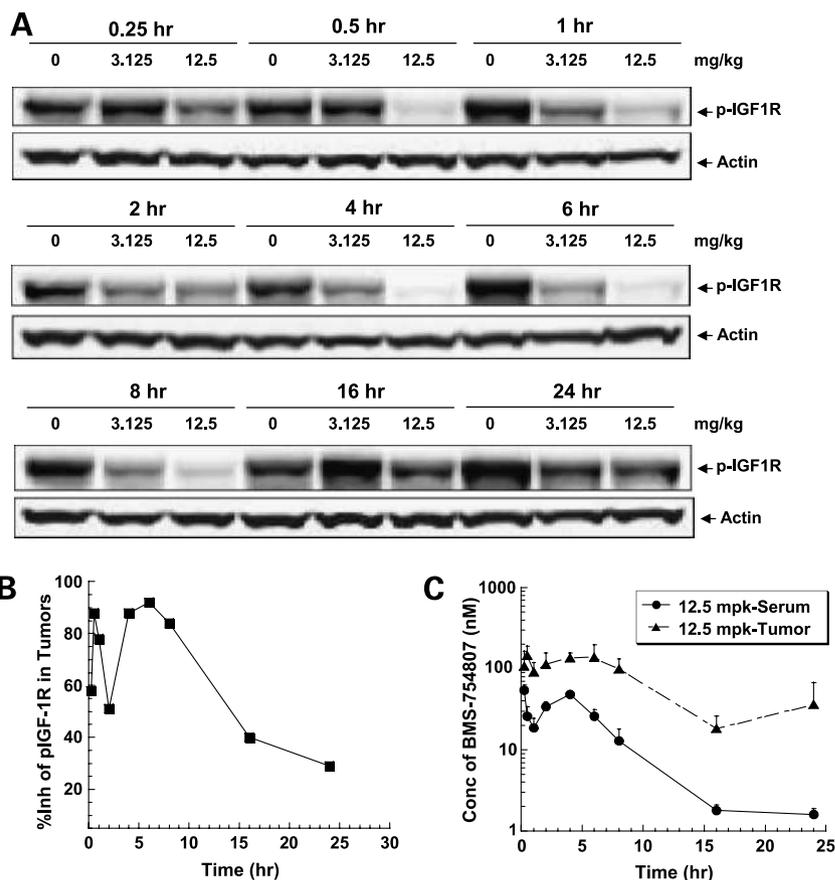
in the GEO tumor model. At its MTD (25 mg/kg, orally, twice daily for 17 days), BMS-754807 produced 61% TGI and TGD of 7 days. Cetuximab at its optimal dose (0.25 mg/mouse, i.p., every 3 days for 6 doses) in this study produced a TGI of 85% and growth delay of 14 days. Importantly, combination of BMS-754807 with cetuximab at a variety of dose levels produced antitumor activities (TGD, 23–38 days) that were generally greater than either agent alone (TGD, 15–21 days) or single-agent cetuximab (TGD, 14 days; Fig. 6), reaching statistical significance when cetuximab (0.25 mg/mouse) was combined with BMS-754807 at its MTD (25 mg/kg), as well as at a dose level 2-fold below

Table 1. Summary of tumor responses of selected tumor xenografts grown in mice

Models	Dose (mg/kg)	Regimen	Efficacy* (% TGI)
Sal-IGF	3.1–12.5	twice daily for 4 d	80–110
Sal-IGF	6.25–150	once daily for 4 d	67–115
GEO	25	twice daily dosing for 14 d	56–75
GEO	25–50	once daily for 14 d	41–60
Colo205	50	once daily for 23 d	53
JJN3	25	twice daily dosing for 14 d	53
Rh41	3.1–25	twice daily dosing for 14 d	65–115
Rh41	12–50	once daily for 21 d	45–97
RD1	50	once daily for 14 d	58

NOTE: Mice were dosed with BMS-754807 in PEG400:H2O (4:1) on multiple dosing schedules either once daily or twice daily. Activity was observed in epithelial, hematopoietic, and mesenchymal xenograft tumor models with TGI ranging from 53% to 115% and a minimum effective dose as low as 3.125 mg/kg, twice daily or 6.25 mg/kg, four times daily.
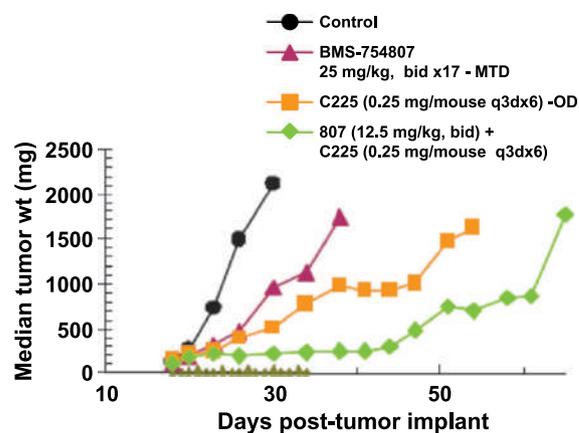*Efficacy (percent tumor growth inhibition).

Figure 6. Combination of BMS-754807 with cetuximab *in vivo*. Antitumor effect of BMS-754807 in combination with cetuximab on the growth of the GEO human colon carcinoma xenograft model in nude mice. Each symbol represents the median tumor weight of a group of eight mice. Triangles adjacent to the *X*-axis indicate the schedule of drug administration.

Table 2. Tabulated results for the data as shown in Fig. 6

Treatment	Dose (mg/kg or mg/mouse)	%TGI	Efficacy growth delay (d)	P*	Tolerability	
					AWC (g)	Mortality (%)
BMS-754807	25	61	7	0.3683	−1.5	0
	12.5	38	4	0.1177	−0.8	0
	6.25	24	3	0.0631	−0.4	0
Cetuximab	0.25	85	14	1	1.1	0
	0.125	74	12	0.8344	2.2	0
'807+cetuximab	25+0.25	88	24	0.0357	1.5	0
	12.5+0.25	88	38	0.0166	1.2	0
	6.25+0.25	86	23	0.0719	1.4	0
	25+0.125	89	21	0.2053	0.6	0
	12.5+0.125	86	30	0.2246	0.4	0
	6.25+0.125	79	20	0.2945	1.2	0

NOTE: See Materials and Methods for experimental details.
*P values were for probability for delay of tumor growth (in days) to the target size of 500 mg.

(Table 2; Fig. 6). These results suggest that the combination of these two agents produced optimal tumor response not only at the MTD but also at dose-level below MTD.

Discussion

Dysregulation of various signaling pathway components is involved in the development and progression of cancer including, breast, prostate, lung, pancreatic, liver, and sarcoma. BMS-754807, a small molecule inhibitor of IGF-1R/IR, is a very effective inhibitor of cellular growth in many of these tumor types both *in vitro* and *in vivo*, suggesting it may have applicability in the treatment of a broad range of human cancers. Due to the importance of IGF-1R in tumorigenesis, a number of pharmaceutical companies have developed antagonists to IGF-1R and those studies have been recently reviewed (33). Due to the high homology of IGF-1R and IR, many of the antagonists being developed are mAbs that offer the possibility of selectively targeting IGF-1R and avoiding inhibition of IR signaling, which could lead to dysregulation of glucose homeostasis. According to first reports on clinical studies, therapy with IGF-1R antibodies was well tolerated and responses were observed alone (18, 34) and in combination with chemotherapy (35) in solid tumors. Interestingly, hyperglycemia was among the observed toxicities of IGF-1R antibody treatment. The fact that hyperglycemia was demonstrated in patients receiving antibody therapy may indicate that inhibition of molecules downstream of IGF-1R/IR such as Akt may on its own induce both hyperglycemia/hyperinsulinemia and glucose intolerance as was shown in Akt2 knockout mice (36). Alternatively, inhibition of IGF-1R/IR hybrid receptors by antibody binding might also contribute to these observed effects. Both IGF-1R and IR receptors are widely expressed in mammalian cells and there is significant overlap in their function. Although IGF-1 and IGF-II both interact with IGF-1R, IGF-II can also bind with high affinity to the isoform A of IR, which is also thought to have both antiproliferative and antiapoptotic effects in cells, whereas the classic IR mediates glucose uptake. Because the antibodies bind specifically to IGF-1R and not to IR, this could potentially be an escape mechanism for IGF-II and insulin signaling. Results of a phase I single-ascending dose study indicate that BMS-754807 given at single doses leads to exposures above the expected preclinical therapeutic exposures.[5]

In addition to IR activity, BMS-754807 showed potency against Met TrkA/B, AurA, and AurB. Functional assays in cells driven by IGF-1R, Met, or TrkA, however, showed a markedly higher selectivity for IGF-1R than for Met, and the cell cycle and apoptotic effects and profiles of BMS-754807 in Rh41 were very different from an Aurora kinase inhibitor, VX-680. Finally, in a phase I single-agent multiple-dose study, BMS-754807 has been administered chronically. To date, no dose-limiting toxicities have been observed and dose escalation is ongoing.[5] These chronic exposures would not be anticipated to be safely achieved with potent Aurora inhibitor molecules from previously reported data for this class of mitotic inhibitor (37). Thus, there is an apparent disconnect between the biochemical data of BMS-754807 for Aurora kinase activity.

We have shown *in vitro* that BMS-754807 has significant opportunity to synergize with cytotoxics and targeted agents; *in vivo*, BMS-754807 achieved greater therapeutic benefit in combination with cetuximab. It is anticipated that BMS-754807 will improve the efficacy of ErbB family (EGFR and Her-2/neu) inhibitors and of chemotherapy by increasing and broadening the sensitivity and by preventing or reversing molecular resistance to these agents (29, 38–40). The synergistic effects that have been observed when BMS-754807 was combined with other targeted agents, even at lower than the MTD for this compound, and lower than the optimal doses of cetuximab, indicate that BMS-754807 has the potential to be used in combination in clinical studies.

In summary, BMS-754807 is a highly potent, small molecule inhibitor of IGF-1R/IR that has entered into clinical development. Although the equipotent activity against IR may

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[5] F. G. Finckenstein, M.D., unpublished data.

be viewed as a risk for this compound, given the involvement of IR in signaling pathways in oncogenesis, including the existence of hybrid receptors, a small molecule that inhibits both IGF-1R and IR may have a distinct advantage over an IGF-1R selective agent including mAbs.

Disclosure of Potential Conflicts of Interest

Acknowledgments

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