Phase I safety, pharmacokinetic and pharmacodynamic trial of BMS-599626 (AC480), an oral pan-HER receptor tyrosine kinase inhibitor, in patients with advanced solid tumors

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Purpose: We studied the safety, tolerability, and recommended dose of BMS-599626, an orally bioavailable inhibitor of the human epidermal growth factor receptor (HER) family of receptor tyrosine kinases.

Patients and methods: Patients with advanced solid tumors that expressed epidermal growth factor receptor (EGFR) and/or HER-2 were recruited and enrolled in a phase I, open-label, dose escalation trial of oral BMS-599626 starting at 100 mg/day given once daily for at least 28 days.

Results: Forty-five patients received BMS-599626 (100–660 mg/day). Dose-limiting toxic effects were reported at 660 mg/day (grade 3 elevation of hepatic transaminases [two patients] and QTc interval prolongation [one patient]).
BMS-599626 is a novel pyrrolotriazine analogue that acts as a reversible inhibitor of EGFR, HER-2, and HER-4 (and to a lesser extent HER-3) receptor tyrosine kinases [12, 13]. In vitro, BMS-599626 suppresses the proliferation of tumor cell lines that express either EGFR or HER-2 or both, with a concentration that causes 50% inhibition of growth (IC50) between 0.2 and 1.5 μM, and in vivo, BMS-599626 has shown efficacy in a number of tumor xenograft models that depend on EGFR and/or HER-2 receptor signaling (colon, lung, breast, and colon cancer) [13].

On the basis of the promising preclinical antitumor and safety data in animals, the primary objective of this phase I trial was to determine the maximum tolerated dose (MTD) of BMS-599626, when administered as a daily uninterrupted oral dose (28-day courses), to subjects with EGFR- or HER-2-expressing metastatic solid tumors who had progressed on, or following standard therapy, or for whom no effective standard therapy existed. Secondary objectives were to assess the safety and tolerability of BMS-599626 when administered orally and to describe basic pharmacokinetics (PKs), pharmacodynamics, and any preliminary evidence of antitumor activity.

Conclusion: BMS-599626 was generally well tolerated, with disease stabilization across a range of tumor types and doses.

Key words: biomarker, clinical trial, pan-HER inhibitor, phase I study

patients and methods

patient selection

All the patients entered into this study had a histologically or cytologically documented advanced solid malignancy, refractory to conventional therapies, or for whom no standard therapy exists. Immunohistochemistry (IHC) and/or FISH analysis of the most recently available tumor biopsy demonstrating ≥3 HER-2 expression or evidence of EGFR expression were required based on standard EGFR and HER IHC tests.

Eligible patients were those with a life expectancy of ≥3 months; age ≥18 years; Eastern Cooperative Oncology Group performance status score 0–1; adequate bone marrow (absolute neutrophil ≥ count 1500 cells/mm³; platelet count ≥ 100 000 cells/mm³; hemoglobin ≥ 9.0 g/dl), hepatic (total bilirubin ≤1.5 times the upper limit of normal [ULN]; alanine aminotransferase (ALT)/aspartate aminotransferase (AST) ≤2.5 times ULN) and renal (serum creatinine ≤1.5 times ULN) function; prothrombin time international normalized ratio/partial thromboplastin time ≤1.5 times ULN; serum potassium, calcium and magnesium levels within normal limits. Patients with any of the following were excluded: pregnant or breast-feeding women; prior anticancer chemotherapy, radiotherapy, hormonal therapy or immunotherapy within 4 weeks of entering the study (6 weeks for nitrosoureas, mitomycin-C, and liposomal doxorubicin); toxicity preexisting from previous anticancer therapy unless deemed irreversible; brain metastasis (as determined by computed tomography [CT] or magnetic resonance imaging); serious uncontrolled medical condition; pregnancy; second- or third-degree heart block; prolonged QTc interval (>450 ms); heart rate <50/min; concurrent treatment with proton pump inhibitor, histamine H2 antagonists, and drugs (or medical condition) that are generally accepted to have a risk of causing torsade de pointes for at least five half-lives of the drug before the first dose of BMS-599626.

study design

This was a two-center, open-label, phase I dose escalation study carried out at Institut Gustave Roussy (Villejuif, France) and Val D’Hebron University Hospital (Barcelona, Spain). The study was approved by the institutional review boards and corresponding local research ethics committees at each of the participating institutions, and all patients gave written informed consent.

BMS-599626 was self-administered orally (fasted) on an uninterrupted daily basis for at least two cycles. A cycle was defined as 4 weeks (28 days) with no planned break between cycles. The standard, phase I 3 + 3 design (3–6 patients per cohort) with flexible dosing was used to establish the MTD by evaluating dose-limiting toxic effects (DLTs) during the first full treatment cycle.
definition of DLT and MTD

DLT was defined as any one of the following toxic effects: grade 3 or 4 nausea, vomiting, or diarrhea, despite the use of optimal medical intervention; any other grade 3 or greater nonhematologic toxicity; delayed recovery from toxicity related to treatment with BMS-599626, which delays scheduled retreatment for >14 days; prolongation of the QTc interval ≥500 ms; LVEF below LLN (if≥10% decline) or a 20% decline in LVEF from baseline; grade 4 neutropenia for ≥5 consecutive days, or grade 3 or 4 neutropenia of any duration with sepsis or a fever >38.5°C; grade 4 thrombocytopenia or bleeding requiring a platelet transfusion. MTD was defined as the highest dose with an observed incidence of DLT in <33% of the patients in the cohort. Once the MTD was defined, this dose level was expanded to include a maximum of patients to better characterize the safety profile. The biologically active dose was determined by evaluating biomarker information. The recommended phase II dose(s) would be based on the MTD and the biologically active doses.

safety

Before therapy, physical examination and evaluation of medical history were carried out. This included complete blood cell count (CBC), white blood cell (WBC) differential and serum biochemistry, in addition to urinalysis, electrocardiogram (ECG), and transthoracic echocardiography carried out before each cycle. Weekly evaluations included physical examination, adverse event (AE) assessment, CBC, WBC differential and serum chemistry, including renal function. ECG was repeated before every cycle. AEs were recorded, graded using the National Cancer Institute—Common Toxicity Criteria version 3.0 and assessed by the investigator for any relationship with BMS-599626 treatment. Patients experiencing a DLT could be retreated with BMS-599626 at a dose reduced by one level, provided that all toxic effects considered related to BMS-599626 had recovered to baseline or grade 1 severity.

tumor assessments

Objective measurement of tumor mass was assessed in accordance with the modified World Health Organization criteria within 28 days before the start of treatment, every 8 weeks, and at the end of treatment. Patients demonstrating benefit from treatment with BMS-599626 continued therapy until progression or withdrawal criteria were met.

PK analysis

BMS-599626 was administered orally to patients on an uninterrupted once-daily schedule at dose levels of 100, 200, 320, 480, 600, and 660 mg. Plasma samples were collected for PK analysis on days 1, 8 and 29 pre-dose, and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 24 h post-dose. In addition, PK parameters for BMS-599626 were calculated in a subset of patients at the 600 mg dose level (MTD) on days 29, 30, 31, and 32, following concomitant administration with the following treatments: (i) Tango (an acidic buffered drink taken with BMS-599626), (ii) famotidine (40 mg 2 h before BMS-599626), and (iii) Tango and famotidine.

Bioanalytical analysis was carried out at BMS using a validated liquid chromatography-mass spectrometry method and stable isotopically labeled BMS-599626 as the internal standard. BMS-599626 plasma PK parameters were calculated after both single and repeated oral administration (days 1, 8, and 29) in cancer patients by Pharsight Corporation’s Reporting and Analysis Services group, using noncompartmental analysis with a validated version of WinNonlin® (V5.2) (Pharsight, Mountain View, CA).

pharmacodynamic assessments

fresh skin and tumor biopsies. Skin biopsies (required) from a clinically normal skin area and tumor biopsies (optional) were collected within 2 weeks before the first BMS-599626 dose and at day 8 of treatment. IHC analysis of EGFR, HER-2, and proteins involved in downstream signaling pathways, including STAT3, MAPK, P27, Ki-67, AKT were investigated using paraffin-embedded sections taken from paired biopsies.

fluorodeoxyglucose positron emission tomography imaging. Once the MTD was determined, tumor metabolism was assessed using 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) before treatment and twice during treatment (on day 1 of the second and third cycles of treatment) for 10 patients enrolled at that dose level. FDG PET scan was carried out using a Biograph LSO PET/CT (Siemens Medical System, Malvern, PA).

results

patient characteristics

Forty-five patients with advanced solid refractory tumors were enrolled between May 2004 and September 2005, 44 of whom received trial medication and were assessable for toxicity (one patient was not eligible because of exclusion criteria between consent and starting the trial and therefore did not receive study drug). For all patients, it was confirmed that the tumor expressed either EGFR (24/45), HER-2 (8/45), or both (12/45). The characteristics of the treated patients are listed in Table 1. A majority of subjects (77%) had received ≤1 prior chemotherapy regimens in the neoadjuvant/adjuvant setting. Most subjects (80%) had received ≥2 prior chemotherapy regimens in the metastatic setting. In total, 90 BMS-599626 treatment periods/cycles were administered, with a maximum treatment duration of six cycles being sustained by two patients (Table 1).

dose escalation, DLT, and MTD

The dose of BMS-599626 was escalated from 100 mg/day to 660 mg/day in successive cohorts (Table 2). No DLT occurred in the 100 mg once-daily (n = 3), 200 mg once-daily (n = 3), 320 mg once-daily (n = 3), or 480 mg once-daily (n = 3) cohorts. DLTs were observed in three of six patients enrolled at the BMS-599626 with a dose level of 660 mg/day. DLTs consisted of transient grade 3 elevation of hepatic transaminases (two patients), and QTc(B) interval prolongation (one patient). The dose of 660 mg/day of BMS-599626 was therefore the maximum administered dose. Consequently, six patients were treated at the intermediate dose of 600 mg/day with no DLTs being observed. The dose of 600 mg/day was then expanded to a total of 26 patients. Of the 26 patients treated in this level, 4 experienced DLTs with 1 transient grade 4 and 3 transient grade 3 hepatic transaminase elevations. As <33% of patients receiving 600 mg/day experienced DLTs during cycle 1, this dose was defined as the MTD.

safety

All the 44 treated patients were assessable for toxicity. Treatment toxic effects are listed in Tables 2 and 3. At least one treatment-related AE was reported by 36 of the 44 untreated subjects. Most treatment-related AEs were mild (grade 1) to moderate (grade 2) in severity. The main toxic effects observed were diarrhea (30% of patients), anorexia (13%), asthenia (30%), and cutaneous toxic effects (30%). The most frequent cutaneous toxic effects were acneiform dermatitis, skin rash, and skin changes described mostly as periorificial and fissural pulpitis. There was no apparent cumulative toxicity, and there were no deaths due to drug-related toxicity.
Table 1. Patient characteristics

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<th>Performance status(^a)</th>
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\(^a\)Eastern Cooperative Oncology Group.

Table 2. DLT during the first cycle of treatment with BMS-599626

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<th>Dose levels (mg/day)</th>
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<th>Number of patients assessable at cycle 1</th>
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<th>Type of DLTs</th>
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<td>Four grade 3 transaminits</td>
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DLT, dose-limiting toxicity.

PK analysis

Complete plasma concentration–time profiles were obtained for 39 patients, including 22 patients at the MTD of 600 mg/day (Figure 1). A total of 18 patients also had additional samples collected for the analysis of the effect of famotidine and/or Tango on BMS-599626 exposure. The \(C_{\text{max}}\) and exposure of BMS-599626 in patients increased with dose. The increases were approximately dose proportional over the dose range at steady state, but variability was high within and between dose cohorts. Moreover, significant interindividual variability, especially at high dose (e.g. 58-fold variability between the highest and the lowest BMS-599626 concentration levels in day 29 of 600 mg cohort) was observed.

The mean plasma concentration–time profiles of BMS-599626 are presented in Figure 1 as both semilog and linear scale plots. On day 1, the elimination half-life \((t_{\text{1/2}})\) of BMS-599626 was relatively constant across the dose range studied, with mean values ranging from 15.0 to 17.7 h. Mean \(t_{\text{1/2}}\) under steady-state conditions (day 29) was slightly longer, ranging from 18.7 to 21.7 h. Peak plasma concentrations of BMS-599626 were observed at ~2–4 h following administration and then declined in a multieponential manner. Collectively, these results suggest that BMS-599626 reached steady-state conditions by day 8, as concentration levels on day 29 were not consistently higher.

To evaluate the impact of gastric acidity on absorption, an analysis of variance model was used to compare ln-transformed PK parameters of BMS-599626 (\(\text{AUC}_{\text{last}}\) and \(C_{\text{max}}\)) after repeated oral administration of 600 mg BMS-599626 alone and in combination with the following treatments: (i) Tango, (ii) famotidine, and (iii) Tango and famotidine. Co-administration of Tango marginally increased the \(\text{AUC}_{\text{last}}\) and \(C_{\text{max}}\) of BMS-599626 by ~18% and 6%, respectively. Co-administration of famotidine decreased the mean \(\text{AUC}_{\text{last}}\) and \(C_{\text{max}}\) of BMS-599626 by ~25% and 35%, respectively. When both famotidine and Tango were co-administered with BMS-599626, the \(\text{AUC}_{\text{last}}\) and \(C_{\text{max}}\) of BMS-599626 remained within 10% of those observed when BMS-599626 is administered alone, consistent with famotidine and Tango offsetting the other agent’s effect. In summary, it appears that decreased gastric pH results in enhanced BMS-599626 bioavailability, while increased pH reduces it.

BMS-599626 dose proportionality was assessed using a power model approach. Linear, quadratic, and cubic fits were applied to the ln-transformed dose versus ln-transformed \(\text{AUC}_{\text{all}}\) at steady state. The slope for the linear fit was 1.19 with 95% confidence interval (CI) of 0.85–1.54 \((P < 0.0001)\). Furthermore, quadratic and cubic fits were not statistically significant \((P > 0.05)\). Collectively, these results support BMS-599626 dose proportionality over the dose range tested (100–600 mg daily), however, the high variability in the data resulted in wide CIs.

**tumor response**

There were no objective responses. Eleven patients (with colorectal \([4]\), non-small-cell lung (NSCLC) \([3]\), pancreas \([1]\), prostate \([1]\), maxillary sinus \([1]\), and liver \([1]\) cancer) appeared to benefit from treatment with prolonged stabilization of the disease received BMS-599626 for ≥4 months; two of these patients remained on study for 26 months (Figure 2). Forty-three patients withdrew because of disease progression and one because of AEs.
pharmacodynamic assays
fresh skin and tumor biopsies. In this trial, 44 paired skin samples and 18 tumor samples were collected. Five subjects had tumor present in both pre- and post-dose samples, however no clear trends were identified in changes. Two of these subjects showed expected changes for most markers. This lack of clear trends may be due in part to the fact that the samples were small and tumors are generally heterogeneous for the expression of many markers. Skin was used as a surrogate tissue to assess putative pharmacodynamic markers Ki-67, P27, p-STAT3, p-ERK, and p-AKT. No significant changes in the markers were seen until the markers reached the 600 mg dose, indicating that at this level the EGFR and HER-2 pathways were affected. At the 600 mg dose, Ki-67, p-ERK, and p-AKT levels decreased between pre- and post-exposure biopsies, in parallel P27 and p-STAT3 levels increased upon treatment (Figure 3A and B). These changes reflect effective down-regulation of EGFR and HER-2 pathways.

18F-FDG PET imaging. Once the MTD was determined, 18F-FDG PET data at baseline and day 1 of the second and third cycles of treatment were available for 10 patients and showed a metabolic response in two patients (20%), a progressive disease in six patients (60%) (Figure 4).

discussion
The results of this phase I trial show that oral BMS-599626 was generally well tolerated at doses up to 660 mg daily, as supported by the AE profile and duration on study of some patients. Stable disease was the best response in 11 heavily pretreated patients with EGFR-expressing and/or HER-2-overexpressing metastatic solid tumors. In this patient population, there was evidence of clinical benefit despite the absence of partial response as defined by the modified RECIST criteria. Two patients had disease stabilization for >6 months.

Most of the treatment-related AEs were consistent with those previously observed for anti-EGFR therapy, including diarrhea and skin rash [14, 15]. Drug-related toxicity was primarily restricted to grade 1 and 2 diarrhea, anorexia, and skin rash. The DLTs were prolonged QTc(B) interval and hepatic toxicity (ALT and AST). No grade 4 toxicity was reported. No HER-2 induced cardiotoxicity was observed nor other QTc findings. Treatment with trastuzumab has been reported to be associated

Table 3. Incidence of drug-related adverse events (all grades)

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ALT, alanine aminotранфераза; AST, аспартат aminotransferase; NCI–CTC, National Cancer Institute—Common Toxicity Criteria.
with an increased incidence of reversible cardiomyopathy [16]. However, it is not known whether this effect is specific to trastuzumab or a class effect associated with HER-2 inhibition [17]. In our study, no evidence of drug-related cardiac dysfunction was observed, except QTc prolongation in one patient. However, recent demonstrations of cardiotoxicity with a number of targeted therapies make careful monitoring of left ventricular function necessary in daily practice and in clinical trials during early drug development [17, 18].

The pharmacodynamic biomarker analysis provided evidence that the target was truly modulated in vivo. Different proteins in the HER signaling pathway are potential biomarkers of BMS-599626 therapy [4]. At the 600 mg dose, Ki-67, p-Erk, and p-Akt decreased upon treatment and p27 and p-Stat3 increased; such changes are in line with the expected mechanism of action [19]. No significant changes in these markers were seen until the patients were treated at the 600 mg dose; therefore, one could argue that a 600 mg dose of BMS-599626 is the minimum dose required for clinical efficacy. These biomarker assays have provided proof of the biological effect of this pan-HER inhibitor, most notably through Ki-67 modulation in paired skin biopsies.
EGFR and HER-2 signaling inhibition represent a highly promising arena for the application of molecularly targeted cancer therapies [20]. Different strategies of EGFR inhibitors from both the monoclonal antibody (mAb) and tyrosine kinase inhibitor (TKI) class have been developed and entered clinical practice [21]. Several EGFR inhibitors have recently gained US Food and Drug Administration approval for cancer therapy in the United States (and many other countries), including the mAbs cetuximab [22], trastuzumab [23], and panitumumab [24], and the small molecule TKIs erlotinib [15, 25], gefitinib [26, 27], and lapatinib [28]. A number of newer inhibitors are currently in clinical development with different spectra of activity or mechanisms of receptor inhibition. These include

![Figure 2. Tumor response; waterfall diagram showing changes in tumor size in target lesions in patients enrolled at 600 mg dose level.](image)

**Figure 2.** Tumor response; waterfall diagram showing changes in tumor size in target lesions in patients enrolled at 600 mg dose level.

**Figure 3.** A and B: Skin biopsies were collected from each patient, pre- and post-dose of the drug. Three fields at 40x magnification were taken and the percent of positive cells over the total number of cells was calculated for each marker. Comparisons were made between the pre- and post-drug samples. At the 600 mg dose, Ki-67, p-ERK, and p-AKT levels decreased between pre- and post-exposure biopsies, in parallel P27 and p-STAT3 levels increased upon treatment. The results are mean ± standard deviation.

![Image of Ki-67, pStat3, pERK, pAKT, p27 changes](image)
mAbs, such as pertuzumab [29] and matuzumab [30], dual inhibitors of EGFR and vascular endothelial growth factor receptor, such as ZD6474, AEE788, and BMS514, inhibitors of multiple EGFR family members, such as lapatinib [31–34], and irreversible inhibitors, such as canertinib and HKI-272 [20]. Compared with lapatinib, the main advantage of BMS-599626 is improved brain penetration observed in mice, which must be confirmed in phase II trials in brain tumor patients. BMS-599626 is currently under investigation in phase II trials and the dose schedule (QD versus BID) is being explored in a small exploratory GBM trial. HKI-272 (neratinib) is an irreversible EGFR/HER/ErbB inhibitor that has been shown to inhibit the growth of T790M mutant cells in vitro in human lung cancer cell lines and in murine cells transfected with sensitizing EGFR mutations [35–38]. A phase I HKI-272 monotherapy trial in patients with solid tumors is close to completion. Preliminary analyses of the trial showed that HKI-272 can achieve SD control for over 6 months in some patients with NSCLC that has progressed after treatment with gefitinib or erlotinib [39]. A recently reported phase II trial of HKI-272 in NSCLC patients demonstrated that HKI-272 had low activity both in patients with prior benefit from TKIs and in TKI-naive patients. This was potentially due to insufficient bioavailability caused by diarrhea-imposed dose limitation. However, responses were seen in patients with the rare G719X EGFR mutation, highlighting the importance of obtaining comprehensive genetic information on trials of targeted agents [40]. HKI-272 might also offer benefit to NSCLC patients who have relapsed after an initial response to erlotinib [40]. In our study, some patients with NSCLC, breast cancer, or colon cancer seem to have a clinical benefit with SD. BIBW 2992 is a potent irreversible inhibitor of EGFR and HER-2. Preclinical studies show that BIBW 2992 is a highly potent and selective EGFR/HER-2 inhibitor. Phase I trials showed objective response and SD in unselected patients with advanced NSCLC [41–43]. Findings from a single-arm phase II trial of BIBW 2992 in chemotherapy-treated EGFR TKI domain mutation-positive patients showed that 62% of 129 patients had a partial response with a disease control rate of 94% [44]. Phase III trials with BIBW 2992 are ongoing both in first-line treatment of NSCLC as well as in second- and third-line settings.

Future research will be needed to elucidate the role of these agents in patients with EGFR inhibitor naive and EGFR inhibitor refractory disease, to define the molecular characteristics that predict response, and to determine whether these drugs should be used in combination with other targeted agents, radiotherapy, or chemotherapy. Many questions yet remain to be answered, particularly with regard to how to best combine EGFR inhibitors with conventional cancer therapies, and how to select those tumors most likely to benefit from EGFR inhibition strategies. Molecular analysis has shown that mutations such as EGFR, BRAF, PI3K, PTEN and KRas could predict sensitivity to HER-targeted therapies and should be carried out in early clinical development [45]. As this phase I trial was carried out in 2004–2005, none of our patients’ tumors were analyzed for these mutations.

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**disclosure**

The authors declare no conflict of interest.
references

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