A Phase 1, Dose-Escalation, Pharmacokinetic and Pharmacodynamic Study of BIIB021 Administered Orally in Patients with Advanced Solid Tumors

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Abstract

**Purpose:** BIIB021 is the first oral, synthetic, non-geldanamycin–based HSP90 inhibitor that showed activity in preclinical models at low nanomolar concentrations. We performed a phase 1 trial of BIIB021 administered to subjects with advanced solid tumors.

**Experimental Design:** Sixty patients received BIIB021 capsules orally on days 1, 4, 8, 11, 15, and 18 of each course in schedule 1, and on days 1, 4, 8, 11, 15, 18, 22, and 25 of each course in schedule 2. The treatment schedules were repeated every 28 days. In addition to determining the MTD, we evaluated pharmacokinetics of BIIB021 and pharmacodynamic effects of BIIB021 [Hsp70, HER2 extracellular domain (HER2-ECD)].

**Results:** The MTD was 700 mg twice weekly when BIIB021 was dosed for 3 weeks out of each 4-week course. The MTD for continuous dosing regimen was established at 600 mg twice weekly. Gastrointestinal (nausea, vomiting), hot flashes, and neurologic (dizziness) events characterize the safety profile of BIIB021 dosed twice weekly, with events mostly mild or moderate. Plasma exposure to BIIB021 was dose-dependent. Cmax occurred at approximately 90 minutes and t1/2 was approximately 1 hour across dosing cohorts of 25 to 800 mg BIIB021 twice weekly. The biologic activity of BIIB021 was demonstrated in serum, PBMCs, and tumor tissue. Hsp70 levels were increased (>150% from baseline) and serum HER2-ECD was significantly decreased (>15% inhibition from baseline).

**Conclusions:** BIIB021 twice weekly, given with or without the 1 of 4-week rest period was tolerated in subjects with advanced solid tumors at doses that are pharmacodynamically active.

**Introduction**

Heat shock protein 90 (Hsp90) is a widely expressed molecular chaperone that plays an important role in the regulation of several key oncogenic signaling (client) proteins (1). Ansamycin drugs bind to the atypical adenosine triphosphate (ATP) binding site in the N-terminus of Hsp90 and inhibit the chaperone activity of Hsp90, resulting in the proteasomal degradation of Hsp90 client proteins (2). Because tumor cells rely on the activity of client proteins for cell-cycle progression and antiapoptotic survival signaling, drug-induced client protein degradation leads to cytosis and/or selective cell killing of tumor cells in vitro and in vivo. Recent studies have shown that Hsp90 exists in a distinct molecular form in tumor cells and that this distinction is at least partially responsible for the selective activity of ansamycin drugs against malignant cells (2, 3). Drugs that can specifically inhibit Hsp90 are currently being targeted in anticancer research because of their ability to simultaneously stimulate depletion of multiple oncogenic proteins (4).

BIIB021 is the first oral, synthetic, non-geldanamycin–based HSP90 inhibitor that showed activity in preclinical models at low nanomolar concentrations. The active pharmaceutical ingredient of BIIB021, BIIB021 methanesulfonic acid salt (CF1983 mesylate; BIIB021 MAS), is a synthetic, new chemical entity designed to inhibit Hsp90. BIIB021 has been tested both in vitro and in vivo to show specific activity with respect to the target. In vitro treatment...
Translational Relevance

HSP90 is a molecular chaperone protein essential for cellular survival. While ubiquitously expressed in unstressed normal cells, the HSP90 complex assists in the folding and function of a variety of client proteins. This study evaluated the first oral, synthetic, non-geldanamycin–based HSP90 inhibitor, BIIB021. In addition to determining the MTD and DLTs, we also assessed the biologic activity of BIIB021 by analyzing Hsp70 protein levels in cell lysates from PBMCs, HER2, and IGFBP-2. In addition, pharmacodynamic tumor biopsy data was evaluated for the measurements of HER2, Hsp70, cyclin-dependent kinase 4 (CDK4), C-RAF, p-AKT, AKT, extracellular signal-related kinase (ERK), and p-ERK levels. Hsp70 levels were increased (>150% from baseline) and serum HER2-ECD was significantly decreased (>15% inhibition from baseline). In addition, pharmacodynamic analysis of tumor tissue showed changes in multiple Hsp90-dependent client proteins, thereby, validating biomarkers for these agents.

of cells with BIIB021 leads to degradation of Hsp90 client proteins and induction of heat shock proteins in a dose- and time-dependent manner. The active pharmaceutical ingredient of BIIB021, BIIB021 MAS binds to the adenosine triphosphate binding site of Hsp90. BIIB021 administered orally, has demonstrated antitumor effect in multiple xenograft tumor models including the U87 glioblastoma, N87 gastric carcinoma, and BT474 breast carcinoma models.

Good oral absorption is suggested by, in vitro results from solubility assessment in simulated intestinal fluid, Caco-2 data on permeability/efflux and the relative cell growth inhibitory activity against tumor cells expressing low or high levels of P-glycoprotein (P-gp; ref.5). CF1983 base and its salts are readily absorbed from solutions in mice, rats (rodent toxicology species), and beagle dogs (nonrodent toxicology species). Absolute bioavailability ranged from 12% to 23% in the mouse, 43% to 82% in the rat and 24% to 28% in the dog. Upon administration of CF1983 capsules to dogs, F ranged from 11% to 26%. Bioavailability is limited by first-pass metabolism. This is consistent with the high intravenous clearance values observed.

On the basis of nonclinical toxicology results in mice, rats, and dogs, BIIB021 toxicities were anticipated to be dose-dependent and reversible with the possible exception of protracted hypospermia. In repeated dose toxicity studies, target organs of toxicity in rats and dogs included the gastrointestinal system, adrenals, thymus, spleen, and testes. Species-specific target organs were the pancreas and gallbladder in dogs and bone marrow in rats. Moreover, BIIB021 is not a P-gp substrate thus it is not affected by MDR and it has metabolic and toxicology profiles that are distinct from 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG).

This study was planned to determine the MTD and to evaluate the safety, pharmacokinetics of BIIB021 and its active metabolite (CNF1983 5-OH), and pharmacodynamic effects (Hsp70, HER2-ECD) of BIIB021 administered to subjects with advanced solid tumors.

Patients and Methods

Patient eligibility

Eligible patients included those ≥18 years of age with pathologically confirmed, advanced solid tumor that was refractory to conventional treatment or for which no standard therapy existed, ECOG performance status of 0 to 2, life expectancy of more than 12 weeks, absolute granulocyte count of ≥1,500/µL, platelet count ≥100,000/µL, a hemoglobin value of ≥9.0 g/dL, bilirubin ≤1.5 mg/dL, transaminases AST (SGOT) and ALT (SGPT) ≤2 times the upper limit of normal (ULN), serum creatinine <2 mg/dL or creatinine clearance >60 mL/min, prothrombin time <1.5 times normal and measurable or evaluable disease. Pregnant and breast-feeding women, patients with Class III or IV cardiac disease defined by the New York Heart Association Functional Classification, left ventricular ejection fraction <40%, acute myocardial infarction within 6 months, arrhythmia, poorly controlled angina, congenital long QT syndrome or first-degree relative with unexplained sudden death under 40 years of age, severe debilitating pulmonary disease, and untreated brain metastases or epidural tumors were also excluded. Written informed consent was obtained from all patients before any study-related procedure was performed, and approvals from the institutional medical ethical review boards were obtained.

Study design and treatments

This was an open-label, dose-escalation phase I study with subjects enrolled into 1 of 2 treatment schedules. There was no randomization between the 2 schedules as schedule 1 was only open for enrollment at U.S. sites and schedule 2 was only open for enrollment at a single UK site. The starting dose of 25 mg in schedule 1 was selected on the basis of the preclinical toxicology findings, calculated as one-tenth of the severely toxic dose in the rat, which was the most sensitive species to the toxic effects of BIIB021. The starting dose of 600 mg in schedule 2 was selected on the basis of the clinical information collected in schedule 1. At the time of enrollment opening in schedule 2, 600 mg was the highest dose declared well tolerated in schedule 1.

BIIB021 capsules were administered on days 1, 4, 8, 11, 15, and 18 of each course in schedule 1, and on days 1, 4, 8, 11, 15, 18, 22, and 25 of each course in schedule 2. The treatment schedules were repeated every 28 days (1 course) in the absence of disease progression or unacceptable toxicity. Dose escalation proceeded according to a predetermined scheme. Once the MTD was established, up to 10 additional subjects were enrolled at the MTD for each schedule. The 3 + 3 algorithm that was followed is...
described below: Three subjects were enrolled at the 25 mg dose. Subjects were treated at the next dose in cohorts of 3 until at least 1 subject experienced a DLT. If only 1 of 3 subjects experienced a DLT at a given dose, 3 additional subjects were enrolled at the current dose. If only 1 of 6 subjects experienced a DLT at a given dose, 3 additional subjects were enrolled at the next higher dose. If at least 2 of 3 or 2 of 6 subjects experienced a DLT at a given dose, then the MTD had been exceeded (stopping dose). Once the MTD had been exceeded, another 3 subjects were treated at the previous dose if only 3 subjects had been treated at that dose. The MTD was defined as the highest dose at which 6 subjects were treated with at most 1 experiencing a DLT.

Prophylactic administration of antiemetics was not permitted during course 1. Optimal absorption of BIIB021 is believed to require normal stomach pH and motility; therefore, any medications that affected the latter were prohibited. Proton pump inhibitors and H2 blockers were prohibited during the study and for 1 week before the initiation of BIIB021 therapy. However, short-acting antacids, such as calcium carbonate and magnesium hydroxide, were allowed. If used, these antacids were to be taken either 8 hours before or 4 hours after BIIB021 dosing. BIIB021 is primarily metabolized by cytochrome P450 2C19, therefore, medications that would be expected to affect the metabolism of BIIB021 were prohibited for 2 weeks before the initiation of BIIB021 therapy and during the study.

The BIIB021 capsules were formulated for immediate release of BIIB021. BIIB021 was supplied as 25 and 100 mg capsules. All subjects were to take BIIB021 in the morning with at least 6 ounces (180 mL) of water after an overnight fast. Subjects with diabetes were permitted to have a morning snack before dosing. Other oral medications were not to be taken within 1 hour before or after ingesting the BIIB021 capsules. After BIIB021 administration, subjects were not to eat for 2 hours, but they could have water. Subjects came to the clinic for BIIB021 administration during courses 1 and 2. The subjects were to store the BIIB021 at room temperature. If emesis occurred after the administration of BIIB021 capsules, the symptoms were to be managed with standard antiemetic therapy; however, a make-up dose of BIIB021 capsules was not to be administered.

Toxicity was assessed per NCI-CTCAE v3.0 (6). If a subject developed a treatment-related, grade 4 hematologic or grade ≥3 nonhematologic toxicity, BIIB021 was withheld. If a subject developed a treatment-related grade 4 ANC or platelet count, study treatment was withheld until the toxicity resolved to ≤grade 1. If the toxicity resolved within 5 days, study treatment could be resumed at the same dose. If the toxicity resolved within 14 days, study treatment could be resumed at the next lower dose. If the toxicity did not resolve within 14 days, the investigator and sponsor were to discuss discontinuation of the subject from the study. If it was determined the subject could remain on the study, treatment was resumed at the next lower dose. If a subject developed a treatment-related, grade 4, nonhematologic toxicity involving major organs/organ systems (lung, cardiac, neurological, liver, renal), the subject was discontinued from the study. Subjects who developed other treatment-related, grade 4, nonhematologic toxicities could be allowed to resume study treatment at the next lower dose if the toxicity resolved to grade 2 within 5 days, but treatment was withheld until the toxicity had resolved to grade 0 or grade 1. If a subject developed a treatment-related, grade 3, non-hematologic toxicity, study treatment was withheld until the toxicity resolved to ≤grade 1. If the toxicity resolved within 5 days, study treatment could be resumed at the same dose. If the toxicity resolved within 14 days, study treatment could be resumed at the next lower dose. If the toxicity did not resolve within 14 days, the investigator and sponsor were to discuss discontinuation of the subject from the study. If it was determined the subject could remain on the study, treatment was resumed at the next lower dose.

Study assessments

Tumor response assessment was based on imaging or on direct tumor measurements of superficial lesions. Measurements were obtained at screening and after every 2 courses. Tumor responses were evaluated using the Response Evaluation Criteria in Solid Tumors Version 1.0 (RECIST) criteria (7). Any partial tumor response (PR) or complete tumor response (CR) was confirmed at least 4 weeks later using the same method of measurement as the baseline assessment. For subjects in the expanded cohorts, subjects with gastrointestinal stromal tumor (GIST) in schedule 1, and for all subjects in schedule 2, positron-emission tomography with 18fluorodeoxyglucose (18FDG-PET) scans were to be performed on day 1 of courses 2 and 3 (8). Patients with diabetes were excluded from participating in the expanded cohort phase of schedule 1 and from schedule 2 because of the potentially confounding effects of diabetes on 18FDG-PET.

Pharmacokinetic analyses

The pharmacokinetic profile of CF1983 (the active ingredient in BIIB021 capsules) and its metabolite were determined in all patients analyzed by methods previously published (9). Serial heparinized blood samples (12) were collected during course 1, on days 1 and 18 including a 24 hour post-dose sample collected on days 2 and 19. Twenty-four hour urine collection was obtained during course 1, day 1. Plasma concentration-time profiles determined for the first dose of BIIB021 were analyzed using noncompartmental methods. Estimates of the area under the concentration-time curve (AUC) and slope of the terminal decay phase were used to calculate values of the following pharmacokinetic parameters: terminal half life (t1/2), apparent total body clearance, time of maximum concentration, apparent volume of distribution, and maximum plasma concentration (Cmax). The plasma profiles were also fitted by nonlinear regression analysis whenever possible. Pharmacokinetic parameters were summarized by dose cohort and schedule using descriptive statistics for the evaluable population.
Pharmacodynamic analyses

The pharmacodynamic activity of BIIB021 was evaluated by analyzing Hsp70 protein levels in cell lysates from PBMCs of subjects dosed with 100 to 800 mg BIIB021 twice weekly. Pharmacodynamic activity was also measured by analyzing serum Hsp70 levels in subjects dosed at >600 mg in schedule 1 and all subjects in schedule 2, HER2 in all subjects dosed, and IGFBP-2 in all subjects dosed during the study. In addition, pharmacodynamic tumor biopsy data was evaluated for the measurements of HER2, Hsp70, cyclin-dependent kinase 4 (CDK4), C-RAF, p-AKT, AKT, extracellular signal-related kinase (ERK), and p-ERK levels (at pretreatment and at 24 hours after first dose was to be taken from 1 subject per dose and 5 subjects in the expanded cohort during the schedule 2; refs. 9–14).

Results

Patient demographics and clinical characteristics

A total of 60 subjects were enrolled in the study. In schedule 1, 54 subjects were enrolled and 50 subjects received at least 1 dose of study treatment. In schedule 2, 6 subjects were enrolled and all received at least 1 dose of study treatment. Of the 54 subjects enrolled in schedule 1, 50 were treated in dose cohorts of 25 (n = 6), 50 (n = 4), 100 (n = 4), 200 (n = 3), 300 (n = 3), 400 (n = 3), 500 (n = 3), 600 (n = 3), 700 (n = 17), and 800 mg (n = 4). Six subjects were enrolled in the schedule 2 in dose cohorts of 600 (n = 3) and 700 mg (n = 3). Thirty-six subjects (67%) withdrew due other causes, 5 subjects (9%) discontinued due to adverse events (AE), 5 subjects (9%) withdrew consent, and 2 subjects (4%) died of causes unrelated to the study treatment. Subject baseline disease characteristics were similar among schedule 1 and schedule 2 and across dosing cohorts as summarized in Supplementary Table S1.

Of the 50 subjects in the safety population treated in schedule 1, the highest cumulative exposure to BIIB021 was in the 600 mg dose cohort, in which 1 subject (GIST) received 59 doses for a total exposure of 35,400 mg. The median number of doses taken across all dose cohorts was 12 doses. Median time on study was 62.5 days (range: 17–319 days) for the 50 subjects treated in schedule 1. With the exception of the 600 mg dose cohort (in which 1 of the 3 subjects remained on study for 319 days), the median days on study was similar across dose cohorts and no dose-related differences were seen across schedule 1. Of the 6 subjects in the safety population treated in schedule 2, the highest exposure to BIIB021 was in the 600 mg dose cohort, in which 1 subject (colorectal cancer) received 46 doses for a total exposure of 27,600 mg. The median number of doses taken across the 2 dose cohorts in schedule 2 was 14.5 doses (14 doses in the 600 mg cohort and 15 doses in the 700 mg cohort). Median time on study was 78 days (range 63 to 173 days) for the 6 subjects treated in schedule 2. Median time on study was similar for the 600 and 700 mg dose cohorts within schedule 2, and slightly longer when compared to schedule 1.

Safety and tolerability

An MTD of 700 mg twice weekly was established for BIIB021 when subjects were dosed for 3 weeks out of each 4-week course (schedule 1). The dose of 800 mg twice weekly (schedule 1) was not tolerated based on 2 subjects in that cohort experiencing DLTs: syncope and dizziness. In these two patients, there were no associated cardiac symptoms or ECG changes. Enhanced parasympathetic nervous system activity was also ruled out. An MTD was not formally established for the 4 out of 4-week dosing regimen (schedule 2), but 700 mg twice weekly was tolerated in the 3 subjects treated at this dose. Overall, the most common AEs, those that occurred in >20% of subjects (both schedules) included nausea, vomiting, hot flashes, dizziness, diarrhea, fatigue, anorexia, constipation, headache, hyperhidrosis, and pyrexia. Gastrointestinal, hot flashes, and neurologic events characterize the safety profile of BIIB021 dosed twice weekly. Gastrointestinal events were mostly mild to moderate nausea and vomiting. Vomiting accounted for the temporary suspension of BIIB021 in 2 subjects. Hot flashes were mostly mild and accounted for 1 discontinuation of BIIB021. Neurologic events were mostly mild to moderate dizziness and accounted for 2 discontinuations of BIIB021. The safety and tolerability of continuous twice weekly dosing of BIIB021 appeared similar to that observed with twice-weekly dosing for 3 weeks followed by a 1-week rest period. No ocular or pulmonary toxicities, as seen with other HSP inhibitors, were noticed in this study.

Pharmacokinetics

Plasma concentrations of BIIB021 and its primary metabolite, BIIB021 5-Ch2OH, were consistent from day 1 to day 18 (schedule 1) or day 25 (schedule 2), suggesting no time-dependent changes occurred (i.e., no self-induction or
inhibition of clearance). Figure 1 depicts individual $C_{\text{max}}$ and AUC as function of dose. Within dose cohorts there was moderate intersubject variability in plasma exposure to BIIB021. Comparing dose cohorts, plasma exposure to BIIB021 was dose-dependent, as indicated by $C_{\text{max}}$ and the AUC from time equals zero to the last measurement (AUCall). Absorption of BIIB021 was fairly rapid, with $C_{\text{max}}$ occurring within approximately 90 minutes after administration in all dose cohorts. The $t_{1/2}$ of BIIB021 was approximately 1 to 2 hours, with residual BIIB021 concentration dropping to near or below the limit of quantitation (BLQ; 1 ng/mL) by 6 to 8 hours after administration. There was no evidence of BIIB021 accumulation upon repeated administration.

**Pharmacodynamic results**

**Intracellular Hsp70 PBMC.** Mean and SD of intracellular Hsp70 PBMC maximum percent change from baseline for schedule 1 is shown in Fig. 2. Hsp70 levels were increased >150% above baseline (with baseline values represented as 100%) in subjects dosed at ≥100 mg BIIB021, suggesting that BIIB021 is biologically active in these subjects treated at ≥100 mg twice weekly.

**Serum Hsp70.** Mean and SD of serum Hsp70 maximum percent change from baseline for schedule 1 is shown in Fig. 3. There was significant change in serum Hsp70 levels at all doses >600 mg, demonstrating the biologic activity of BIIB021.

**Serum HER2-ECD.** Mean and SD of serum HER2-ECD maximum percent change from baseline for schedule 1 is shown in Fig. 4. There was significant reduction (>15% change below baseline) of serum HER2-ECD for most dose cohorts >200 mg in schedule 1 and schedule 2, suggesting biologic activity of BIIB021 at doses >200 mg twice weekly. There was no obvious distinction between the 2 dosing schedules.

**Serum IGFBP-2.** Mean and SD of serum IGFBP-2 maximum percent change from baseline for schedule 1 is shown in Fig. 5. No significant changes (i.e., >150% change from baseline) in mean serum IGFBP-2 levels were observed after treatment with BIIB021.

**Tumor biopsy.** Pharmacodynamic analysis was performed on tumor tissue obtained from two patients enrolled in schedule 2 (600 mg and 700 mg dose cohorts, respectively). Results from patient 1 suggest that at 24 hours posttreatment, there were changes in multiple Hsp90-dependent client proteins, including degradation of HER2, p-AKT, AKT, p-ERK, CRAF, and CDK4 when normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (Fig. 6). Induction of Hsp70 was also observed in the tumor tissue sample, consistent with the results obtained in PBMCs. No notable changes in AKT, p-ERK, and CDK4 were obtained in the tumor biopsy from patient 2, and other client proteins including HER2 and CRAF were not determinable in the tumor biopsy that was obtained from this subject.

**Antitumor activity**

Of the 46 subjects in schedule 1 with measurable tumors at baseline, 9 subjects (20%) had stable disease, 32 subjects...
had progressive disease, and for 5 subjects (11%) response was unknown. These 5 subjects discontinued from the study before the first scheduled posttreatment computed tomography scan. There were two subjects who each had an individual target lesion with at least 30% decrease in lesion diameter compared with baseline (adrenocortical carcinoma and metastatic colorectal carcinoma; Supplementary Fig. S1).

18FDG-PET imaging
Eight subjects from the 700 mg dose cohort were available for evaluation of antitumor activity, as assessed by 18FDG-PET imaging. The results are summarized in Supplementary Fig. S2. One subject (schedule 2, 700 mg dose cohort) experienced metabolic PR (>25% decrease in SUVmax of target lesions), 3 subjects (2 from schedule 1, 700 mg cohort; 1 from schedule 2, 700 mg cohort) each experienced >25% decrease in SUVmax and SUVmean of individual lesions, and 2 subjects (1 from schedule 1, 700 mg cohort; 1 from schedule 1, 100 mg cohort) each experienced >30% decrease in the longest diameter of an individual lesion.

Discussion
Testing the drug dose and schedule of drug delivery is essential to achieve sufficient intratumoral HSP90 inhibition to influence the growth of the tumor. Therefore, our study evaluated the safety, pharmacokinetic, and pharmacodynamic effects of escalating doses of BIIB021 administered on two schedules: twice weekly for 3 weeks out of each 4-week course (schedule 1) or twice weekly for 4 weeks out of each 4-week course (schedule 2) in subjects with advanced solid tumors and hematologic malignancies. It is believed that a more prolonged suppression of tumor HSP90 activity is critical to determine whether significant clinical responses are attainable. One such example is the disappointment with melanoma study in which 17-AAG showed no objective clinical responses despite a strong preclinical rationale (15). The pharmacodynamic assessment of tumor specimens pre- and post-17-AAG showed minimal HSP90 inhibition.

The dose range for our study was 25 mg to 800 mg twice weekly. An MTD of 700 mg twice weekly was established for the 3 out of 4-week dosing schedule. The 800 mg twice-weekly dose was not tolerated based on 2 DLTs: syncope and dizziness. An MTD was not formally established for the 4 out of 4-week dosing schedule, but 6 subjects received continuous dosing of BIIB021 at 600 mg and 700 mg twice weekly without experiencing a grade 3 or grade 4 AE or DLT. No clear relationship was observed between BIIB021 dose or dosing schedule and the results of chemistry and hematology laboratory tests or other clinical measurements evaluated during the study. No clear relationship was observed between BIIB021 dose or dosing schedule and ACTH/cortisol abnormalities or positive stool occult blood. The most common BIIB021-related AEs included nausea, hot flashes,
vomiting, and dizziness. Most of these events were mild or moderate. No ocular or pulmonary toxicities were noted in our study. Preclinical data have demonstrated that geldanamycin and 17-AAG downregulate AKT and ERK1/2-mediated signaling that subsequently leads to cytotoxicity in cultured human retinal pigment epithelial cells which are essential for physiological function of adjacent photoreceptors (16). Findings from other HSP inhibitors’ studies, such as AUY922 have shown visual symptoms as DLTs (17). During the conduct of this study, a parallel study of BIIB021 in breast cancer, Study 120BC101, reported DLT of grade 3 aphasia in a subject taking 700 mg BIIB021 twice weekly. The investigators assessed this event as compatible with complex partial seizure and decided that dosing of BIIB021 across all studies would not exceed 600 mg per dose when administered twice weekly. Neurological toxicity is a class-effect of HSP inhibitors. Geldanamycin itself cannot cross the blood–brain barrier while 17-AAG and 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG) are blood–brain barrier permeable (18). Because of this permeability to CNS, investigators are exploring this pathway in many neurodegenerative diseases such as Parkinson’s disease, dementia with Levy bodies, Alzheimer’s disease, etc. as these diseases are thought to be caused by misfolding and subsequent accumulation of toxic proteins (19, 20).

Seven subjects experienced treatment-emergent QT interval prolongation (QTcF or QTcB >450 msec or change from baseline >30 msec). An independent ECG review confirmed the QT intervals for all QTcF or QTcB >500 msec or change from baseline >30 msec. All 7 subjects had also taken concomitant medication that may have caused QT interval prolongation. The results of this study show no clear relationship between BIIB021 and QT interval prolongation. Cardiac, especially electrocardiac changes following HSP90 inhibitors have been reported before (21). Moreover, other studies have demonstrated overexpression of hsp70 in cardiac myocytes and endothelial cells and that it may protect them against heat or ischemia in vitro (22). The inhibition of HSP in cardium may be responsible for these abnormalities.

In our study, pharmacokinetic analysis did not reveal any drug accumulation after administration of multiple doses on both schedules. Pharmacodynamic analysis showed induction of Hsp70 in PBMCs. Hsp70 levels increase as a result of Hsp90 inhibitor-induced activation of HSF1, which then enters the nucleus and activates Hsp70 gene expression. Hsp70 levels were increased (>150% from baseline) in dose cohorts ≥100 mg BIIB021 twice weekly. The serum HER2-ECD was significantly decreased (>13% inhibition from baseline) in most dose cohorts ≥200 mg twice weekly.
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Most phase I trials carried out pharmacodynamic assays to assess HSP90 inhibition on PBMCs. Our study is one of the few that also evaluated these assays in tumor tissue in addition to PBMCs. We observed induction of Hsp70 in the tumor tissue sample, consistent with the results obtained in PBMCs (Fig. 5). These pharmacodynamic analyses provide evidence that BIIB021 is biologically active in both surrogate and tumor tissue. The data from preliminary studies suggested that monitoring of client protein responses in PBMCs is useful to demonstrate the biologic activity of HSP90 inhibitors but this method has shown to be of little benefit to predict clinical activity or biologic response to HSP90 inhibitors. This can be explained on the fact that HSP90 inhibitors preferentially accumulate in tumor cells instead of normal cells. Moreover, the client proteins to HSP90 inhibition (for example, HER2) are preferentially expressed in tumor cells. On the other hand, expression of PBMC HSP70 secondary to HSP90 inhibition seems useful in determining biologically active dose of the agent but not to predict clinical response. Preclinical data and our study suggest that serum HSP70 levels might be a useful pharmacodynamic marker of drug response.

Given the clear need for alternative pharmacodynamic approaches to monitor the clinical efficacy of HSP90 inhibitors, several noninvasive functional imaging techniques are being explored. We incorporated PET with [18F]fluorodeoxyglucose (FDG-PET) into our study. Anti-tumor activity, as assessed by 18F-FDG-PET imaging, began during the 600 mg dose cohort in Schedule 1, and was conducted for all subjects in Schedule 2. Of the 8 subjects in Schedule 1 who underwent 18F-FDG-PET evaluation, 2 sub-

- Her2-ECD reduction of >15% was seen in most dose groups >300mg, AUC >4 μg·h/mL and Cmax >2 μg/mL
- Changes in Her2-ECD indicate tissue penetration and effect
- Her2-ECD may be tumor derived, however, low levels of Her2-ECD can be obtained from normal epithelial cells

![Graph showing AUC vs. ΔHer2-ECD and Cmax vs. ΔHer2-ECD](image)

Figure 4. BIIB021 reduces serum HER2-ECD. Her2-ECD reduction of >15% was seen in most dose groups >300 mg, AUC >4 μg·h/mL and Cmax >2 μg/mL. Changes in Her2-ECD indicate tissue penetration and effect. Her2-ECD may be tumor derived, however, low levels of Her2-ECD can be obtained from normal epithelial cells.

Figure 4. BIIB021 reduces serum HER2-ECD. Her2-ECD reduction of >15% was seen in most dose groups >300 mg, AUC >4 μg·h/mL and Cmax >2 μg/mL. Changes in Her2-ECD indicate tissue penetration and effect. Her2-ECD may be tumor derived, however, low levels of Her2-ECD can be obtained from normal epithelial cells.

[Graph showing AUC vs. ΔHer2-ECD and Cmax vs. ΔHer2-ECD](image)

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disease following trastuzumab (25). These results led investigators to further explore HSP inhibitors in HER2-positive breast cancer. Both preclinical and preliminary clinical data suggest a benefit of HSP90 inhibitors in acute myelogenous leukaemia (AML). FLT3, a tyrosine kinase is a HSP90 client which is commonly mutated and constitutively active in a subpopulation of patients with AML and is considered as a poor prognostic indicator, especially in older patients (26). Similarly, the dependence of chronic myelogenous leukaemia (CML) on BCR-ABL, another client to HSP90 inhibition suggests that CML is another disease where HSP inhibitors may prove to be of benefit (27). One patient, a 56-year-old male with CML, s/p fludarabine, rituximab/cytoscan/deca- 
don, cyclophospamide, and alemtuzumab received BIIB021, 25 mg QD showed clinical activity as shown in Supplementary Fig. S3 and Supplementary Table S2. Our patient and other’s data suggests that drug-resistant CML may be an appropriate indication for the use of HSP90 inhibitors, either alone or in conjunction with ABL tyrosine kinase inhibitors (TKI). Another malignancy where HSP90 inhibitors may be appropriate is non-small-cell lung cancer (NSCLC) based on the data documenting that 44% of 29 NSCLC tumors had a deletion on chromosome 14 that encompasses HSP90A associating with a survival benefit (28). ALK is another HSP90 client associated with NSCLC and hence HSP inhibition warrants further investigation.

Currently, there are more than 20 clinical trials listed evaluating HSP90 inhibitors (29). Though the approach in HSP90 inhibitors is encouraging, but many important questions remain. The foremost is which tumor type is best suited for study? While, HER-2 is the most sensitive client protein to HSP90 inhibition, several tumor types have shown promise in preclinical activity. One approach is to target malignancies that HSP90 client is the critical driver protein, such mutant EGFR in NSCLC, HER-2 in breast cancer, etc (30). Another approach is to target HSP90 cochaperones including HSP40, HSP70, or CDC37. Zhang and colleagues demonstrated that celastrol inhibits the HSP90-CDC37 complex formation (31). This HSP90 cochaperone complex is prudent in regulating various kinases involved in tumor formation and proliferation, including ERBB2, EGFR, and BRAF. The current drug development paradigm of identifying a MTD may not be applicable in this situation as target modulation is critical to the antitumor effects elicited with HSP90 inhibition. HSP inhibitors selectively accumulate in tumor tissue over normal tissue. This rapid clearance from normal tissues and the...
blood compartment may hinder traditional pharmacokinetic monitoring, dosing, and scheduling. That is why direct tumor assessment either by biopsy or noninvasive methods is crucial in testing their optimal clinical efficacy. Given the recent developments of novel synthetic analogs with improved chemical properties, the future is quite promising for this class of agents. Another important approach will include implementing HSP90 inhibition as an adjuvant to other chemotherapeutics, thereby shutting down the heat shock response to increase cellular stress and protein degradation.

In summary, this study demonstrated the biologic activity of BIIB021 in serum, PBMCs, and tumor tissue. Plasma exposure to BIIB021 was dose-dependent. $C_{\text{max}}$ occurred at approximately 90 minutes and $t_{1/2}$ was approximately 1 hour across dosing cohorts of 25 to 800 mg BIIB021 twice weekly. BIIB021 was not associated with ocular and pulmonary toxicities, which hindered the development of other agents in this class. The results of this study indicate that further study of BIIB021 for the treatment of patients with solid tumors is warranted.

**Disclosure of Potential Conflicts of Interest**

C. Takimoto has a commercial research grant from Conforma. U. Banerji is employed (other than primary affiliation; e.g., consulting) as a CR-UK clinical senior lecturer in The Institute of Cancer Research. S. O’Brien and M.W. Saif are employed (other than primary affiliation; e.g., consulting) as a CR-UK medical director in Biogen-Idec. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**


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