A Phase 0 Trial of Ceritinib in Patients with Brain Metastases and Recurrent Glioblastoma

Shwetal Mehta[1], Roberto Fiorelli[1], Xun Bao[2], Chelsea Pennington-Krygier[1], Alanna Derogatis[1], Seongho Kim[2], Wonsuk Yoo[1], Jing Li[2], and Nader Sanai[1]

ABSTRACT

Purpose: Ceritinib is an orally bioavailable, small-molecule inhibitor of anaplastic lympoma kinase (ALK), insulin-like growth factor 1 receptor (IGFR1), and focal adhesion kinase (FAK), which are highly expressed in glioblastoma and many brain metastases. Preclinical and clinical studies indicate that ceritinib has antitumor activity in central nervous system (CNS) malignancies. This phase 0 trial measured the tumor pharmacokinetics (PK) and pharmacodynamics (PD) of ceritinib in patients with brain metastasis or recurrent glioblastoma.

Patients and Methods: Preoperative patients with brain tumors demonstrating high expression of pSTAT5b/pFAK/pIGFR1 were administered ceritinib for 10 days prior to tumor resection. Plasma, tumor, and cerebrospinal fluid (CSF) samples were collected at predefined timepoints following the final dose. Total and unbound drug concentrations were determined using LC-MS/MS. In treated tumor and matched archival tissues, tumor PD was quantified through IHC analysis of pALK, pSTAT5b, pFAK, pIGFR1, and pIRS1.

Results: Ten patients (3 brain metastasis, 7 glioblastoma) were enrolled and no dose-limiting toxicities were observed. Ceritinib was highly bound to human plasma protein [median fraction unbound (Fu), 1.4%] and to brain tumor tissue (median Fu, 0.051% and 0.045% in gadolinium-enhancing and -nonenhancing regions respectively). Median unbound concentrations in enhancing and nonenhancing tumor were 0.048 and 0.006 μmol/L, respectively. Median unbound tumor-to-plasma ratios were 2.86 and 0.33 in enhancing and nonenhancing tumor, respectively. No changes in PD biomarkers were observed in the treated tumor samples as compared to matched archival tumor tissue.

Conclusions: Ceritinib is highly bound to plasma proteins and tumor tissues. Unbound drug concentrations achieved in brain metastases and patients with recurrent glioblastoma were insufficient for target modulation.

Introduction

The prognosis for patients with nonbenign primary or secondary brain tumors is dismal. In metastatic brain disease, the most frequent tumors of origin are lung (36%–64%), breast (15%–25%), and skin (5%–20%; ref. 1). Metastatic brain tumor treatment typically involves repeated cycles of surgery plus radiotherapy, but is often incurable. Among primary brain tumors, glioblastoma is the most common and the most lethal, with a median survival of 16 months despite repeated cycles of surgery, radiotherapy, and chemotherapy. Effective, brain-penetrant adjuvant therapies are in short supply for patients with both primary and secondary brain tumors.

The blood–brain barrier (BBB) is a perennial problem for developing such new therapies (2) and, alongside the lack of targetable driver mutations, ranks among the most formidable obstacles to brain tumor drug discovery. The BBB is a protective lining that surrounds capillaries in the brain parenchyma and tightly controls the ingress of substances into the brain from the circulation. Although it is heterogeneous in its permeability and modestly compromised in the setting of an intracranial tumor, the net effect of the BBB is that it excludes most anticancer agents from the tumor, contributing to the poor performance of many new drugs.

Targeted drugs are only effective when directly inhibiting strong disease drivers, yet only a small fraction of brain tumors feature known, actionable drivers. In this regard, multi-targeted agents may be advantageous using a polypharmacology approach (3). Ceritinib is a second-generation, selective inhibitor of receptor tyrosine kinases (RTK) anaplastic lymphoma kinase (ALK), insulin growth factor 1 receptor (IGF1R), and focal adhesion kinase (FAK). It is FDA approved for treatment of the 5% of patients with non–small cell lung cancer (NSCLC) with tumors harboring a gene rearrangement between echinoderm microtubule-associated protein-like 4 and anaplastic lymphoma kinase (*EML4-ALK*) and who have failed crizotinib (4–7). The efficacy of ceritinib in crizotinib-resistant ALK(+) NSCLC tumors has been attributed to inhibition of IGF1R in addition to ALK signaling, since activation of IGF1R is an identified mechanism of resistance against ALK inhibitors (8).

IGF1R is an RTK that belongs to the insulin receptor family of kinases and promotes cancer cell proliferation and metastasis (9). In the central nervous system (CNS), IGF1R and its ligands (IGF1, IGF2, and insulin) not only play an important role during brain development, but are also implicated in brain tumor growth (10). IGF1R is overexpressed in both glioblastoma and several CNS metastases and is implicated in tumor progression (11, 12). Ligand binding activates IGF1R through autophosphorylation and results in recruitment and phosphorylation of adaptor protein insulin receptor substrate 1 (IRS1; ref. 9). Phosphorylated IRS1 then triggers downstream mitogenic signaling through the PI3K/mTOR pathway. Several small-molecule inhibitors and antibodies have been tested to block IGF1R signaling in cancer, although none have demonstrated clinical efficacy (13).

[1]Ivy Brain Tumor Center, Barrow Neurological Institute, Phoenix, Arizona. [2]Karmanos Cancer Institute, Cancer Biology Program, Wayne State University School of Medicine, Detroit, Michigan.

Corresponding Author: Nader Sanai, Ivy Brain Tumor Center, Barrow Neurological Institute, 2910 North Third Avenue, Phoenix, AZ 85013. Phone: 602-406-8889; E-mail: Nader.Sanai@ivybraintumorcenter.org

Clin Cancer Res 2022;28:289–97

doi: 10.1158/1078-0432.CCR-21-1096

Translational Relevance

Overactivation of tyrosine kinase pathways plays a key role in driving brain tumor proliferation. Ceritinib is an orally available, potent inhibitor of anaplastic lympoma kinase (ALK), insulin-like growth factor 1 receptor (IGFR1), and focal adhesion kinase (FAK). To provide the first comprehensive analysis of ceritinib's central nervous system tumor penetration profile, we completed a phase 0 clinical trial in preoperative patients with brain metastasis or recurrent glioblastoma. Ceritinib is highly bound to plasma proteins and brain tumor tissues and its unbound drug concentrations in brain tumor tissue appear not to be sufficient for target inhibition of pFAK, pIGFR1, and pIRS1.

Yet another target of ceritinib is FAK (3), which regulates expression of IRS1 (downstream target of IGF1R) and also activates PI3K pathway (14–16). Activation of both IGF1R and FAK results in phosphorylation of IRS1 which in turn promotes tumor proliferation through AKT/mTOR pathway. Importantly, IGF1R, and FAK overexpression and activation are widespread in both CNS metastases and glioblastoma, raising the possibility of a role for ceritinib as an adjuvant therapy targeting IGF1R(+) or pFAK(+) brain tumors (3, 5, 11, 13, 15, 17). The roles of these aberrant pathways as oncogenic drivers of these tumors remain unknown.

Ceritinib is associated with the control of intracranial disease in patients with ALK(+) NSCLC although, to date, no study has directly measured ceritinib drug concentrations in human tumor tissue. Of the 124 patients with brain metastases reported in the phase I ASCEND-1 trial, 94 [$n = 19$ ALK inhibitor (ALKi)-naïve and $n = 75$ ALKi-pretreated] were included in a retrospective analysis. The intracranial disease control rate was 78.9% [15/19; 95% confidence interval (CI), 54.4–93.9] in ALKi-naïve patients and 65.3% (49/75; 95% CI, 53.5–76.0) in ALKi-pretreated patients. Of the 94 patients included in the retrospective study, 11 had measurable brain lesions with no prior brain irradiation and 6 achieved a partial intracranial response (18). More recently, ASCEND-7 was a phase II study that evaluated the efficacy of ceritinib in ALK(+) NSCLC brain metastases and/or leptomeningeal disease (LMD; refs. 19, 20). Radiographic evidence of extracranial and intracranial response were detected across four treatment arms following ceritinib therapy (20, 21).

Phase 0 clinical trials are commonly defined as first-in-human studies with no therapeutic or diagnostic intent, a limited number of patients, and microdosing of the experimental agent (22, 23). These characteristics, however, are not essential for phase 0 studies (24), which were introduced as a means of identifying pharmacokinetic (PK) and pharmacodynamic (PD) features of a tumor in response to novel therapy. For patients with brain tumors, traditional phase 0 design elements must be adjusted to accommodate the BBB and the significant risks of tumor-tissue acquisition (25). In the reported study, we adapt the phase 0 strategy (26, 27) through subtherapeutic presurgical dosing instead of microdosing and through matched archival controls instead of pre- and posttreatment biopsies to assess PD effects. The study objectives were (i) to quantify the PK profile of unbound ceritinib within brain tumor tissue and (ii) to identify the downstream molecular effects of ceritinib in patients with brain metastasis or recurrent glioblastoma.

Table 1. Patient demographics and clinical characteristics.

Characteristics	$N = 10$
Sex (male/female)	5/5
Age (years)	61 (40–72)
Weight (kg)	175 (104–221)
Height (cm)	69 (62–79)
ECOG/Zubrod performance status, *n* (%)	
0	3 (30%)
1	5 (50%)
2	2 (20%)
Diagnosis, *n* (%)	
Brain metastases	3 (30%)
Glioblastoma	7 (70%)
Extent of resection, *n* (%)	
GTR	6 (60%)
STR	1 (10%)
Unknown	0 (0%)
Not applicable	3 (30%)
Prior temozolomide, *n* (%)	7 (70%)
Prior radiotherapy, *n* (%)	10 (100%)
Prior bevacizumab, *n* (%)	1 (10%)
Timing of ceritinib, *n* (%)	
At occurrence	2 (20%)
First progression	6 (60%)
Second progression	2 (20%)

Abbreviations: GTR, gross total resection; STR, short tandem repeat.

Patients and Methods

This open-label, nonrandomized phase 0 clinical trial (NCT02605746) was conducted by the Ivy Brain Tumor Center at the Barrow Neurological Institute in Phoenix, Arizona. The study was approved by the local institutional review board and conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonization. Written informed consent was obtained from all patients before screening.

Study population

All study patients were older than 18 years and presented with a brain metastasis or recurrent World Health Organization (WHO) grade IV glioma (i.e, glioblastoma) necessitating resection (**Table 1**). Using archival tissue from prior tumor resections, eligible patients with brain metastases had tumors with pALK or IGF1R expression while patients with glioblastoma had tumors positive for pFAK or IGF1R expression (minimum, >20% positive cells). Other inclusion criteria included an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, absolute neutrophil count (ANC) ≥ 1.5×10^9/L, hemoglobin (Hgb) ≥ 8g/dL, serum total bilirubin ≤ 1.5 × upper limit of normal (ULN), aspartate transaminase (AST) < 3.0 × ULN, and alanine transaminase (ALT) < 3.0 × ULN. Patients who were febrile, had prior ceritinib treatment, were hypersensitive to any ceritinib excipients, had a history of disseminated bilateral fibrosis or interstitial lung disease, had a history of uncontrolled heart disease, had impaired gastrointestinal (GI) function or disease, or were receiving strong inhibitors or inducers of CYP3A4/5 were excluded.

Study design

This study's primary objective was to determine the tumor concentration of unbound ceritinib following 10 oral doses of 750 mg in

patients with brain metastases or glioblastoma. A secondary objective was to evaluate tumor PD biomarkers corresponding to ALK/IGF1R/FAK pathway activity. The 10-day interval was selected based upon estimates of duration to steady-state, as well as the number of days a preoperative patient with brain tumor could safely delay a planned operation.

Enrolled patients in phase 0 were administered 750 mg per day (fasted) or 450 mg per day (with food) of ceritinib for 10 days prior to planned brain tumor resection. Patients were assigned to 1 of 2 time-escalation cohorts in which tumor resection was performed at either 4 or 24 hours following the final dose of ceritinib. During tumor resection, blood, cerebrospinal fluid (CSF), and tumor samples from gadolinium-enhancing (brain metastasis and glioblastoma) and -nonenhancing (glioblastoma) regions (based on preoperative MRI and intraoperative neuronavigation) were collected for PK and PD analyses.

The first three study patients (all with brain metastases) were presurgically administered ceritinib 750 mg orally every day under fasting. With the emergence of new data on the steady-state PKs of ceritinib (4), this regimen was reduced to 450 mg ceratinib orally, every day with food for all subsequent study patients (all with glioblastoma). The primary objective of the study protocol—tumor PK quantification—was not changed despite this modification. Patients were assigned to two time-escalation arms in which tumor resection was performed at 4 or 24 hours, respectively, following their final dose of ceritinib.

Statistical analysis

This is an exploratory study designed to evaluate a primary PK endpoint and secondary PD endpoints. No formal statistical hypothesis tests were performed and the sample size was justified based on feasibility. Descriptive statistics were performed to evaluate all tumor PK and PD measurements as well as patients' demographics and clinical characteristics. All continuous variables were summarized with means, SDs, coefficient of variation (CV), and ranges, and frequencies and proportions were used for all discrete data. In addition to these statistics, we calculated medians and geometric means due to the small samples and dependencies and exponential phenomena of PK parameters. Graphpad and SAS V9.4 were used to generate the data and plots.

Study clinical assessments

Adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf). Demographic data and medical history were recorded for all study patients. Physical examination, vital signs, organ functions, and other safety assessments (ECOG performance status, registration of concomitant medication, hematology, biochemistry, and urine analysis) were performed at baseline. Common Toxicity Criteria for Adverse Events 4.0 criteria were used to document adverse events.

Enrollment criteria

For patients with brain metastases, samples from prior tumor resections were examined with IHC staining for pSTAT5b and pJAK2, factors downstream of ALK signaling, as well as IGF1R staining. IHC staining for all samples was completed on the Leica Bond RX, a fully automated platform using validated assays that were optimized with breast, lung, and melanoma tissue samples from the Biobank. Briefly, archival formalin-fixed, paraffin-embedded (FFPE) slides were stained with anti-pSTAT5b (Abcam, catalog #ab52211; 1:50), anti-pJAK2 (Abcam, catalog #ab32101; 1:50), or anti-IGF1R (Cell Signaling Technology, catalog #3027; 1:100) for assessing percentage of pSTAT5b(+), pJAK2(+), or IGF1R(+) cells.

For patients with glioblastoma, samples from prior tumor resections were examined with IHC staining for IGF1R and pFAK. IHC staining for all samples was completed on the Leica Bond RX, using validated assays that were standardized with archival glioblastoma tissue. Briefly, archival FFPE slides was stained with anti-IGF1R (Cell Signaling Technology, catalog #3027; 1:100) and anti-pFAK pTyr397 (ThermoFisher, catalog #44–624G; 1:100) for assessing percentage of IGF1R(+) and pFAK(+) cells. Patients with tumor samples with more than 20% positive cells were deemed eligible for the trial. The stained slides were imaged using Aperio Versa System (Leica) and analyzed using ImageScope software. In parallel, the slides were also analyzed by a board-certified neuropathologist.

PK evaluation

Blood PK samples were collected from each patient at predosing and 0.5, 1, 2, 4, 6, 8, and 24 hours after the administration of the ninth presurgical dose of ceritinib. This day was chosen to avoid any confounding effects of brain surgery on day 10. Plasma was separated from whole blood by centrifugation (at 4°C, 1,500 g for 10 minutes), and plasma samples were stored at −80°C until analysis. Surgical resection of tumors was performed at predefined time points following the administration of the 10^{th} dose. Blood, tumor (including contrast-enhancing and -nonenhancing regions for patients with glioblastoma), and CSF samples were collected intraoperatively at 2 to 4, 6 to 8, or 23 to 25 hours after the administration of the 10^{th} dose. Tumor specimen locations were recorded with operating room MRI neuronavigation system, a standard surgical adjunct that registers preoperative MRI to the patient's cranium. Each tumor sample was immediately rinsed with ice-cold PBS to remove residual blood, blot-dried, and snap-frozen in liquid nitrogen.

The total concentrations of ceritinib in plasma, tumor, and CSF samples were determined using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method (28). The fraction unbound of ceritinib in plasma and tumor tissues were determined by equilibrium dialysis, and unbound drug concentration was calculated as the product of total concentration and fraction unbound (28).

PK analysis

Plasma PK parameters for total and unbound ceritinib were estimated based on the observed plasma concentration time profiles using the noncompartmental analysis. These included the steady-state peak plasma concentration ($C_{ss,max}$), time to reach the $C_{ss,max}$ ($T_{ss,max}$), steady-state trough plasma concentration ($C_{ss,min}$), steady-state area under the plasma concentration – time curve during one dosing interval (AUC_τ), apparent clearance for the total drug (CL/F), and unbound-to-total drug AUC ratio (AUC_u/AUC_t). The elimination rate constant (K) was estimated based on $C_{SS,min} = C_{SS,max} \times e^{-K\tau}$, where τ is dosing interval (24 hours). The elimination half-life is estimated as 0.693/K.

The extent of ceritinib penetration into the central nervous system (CNS) was assessed by the total drug tumor-to-plasma concentration ratio (K_p), unbound drug tumor-to-plasma concentration ratio ($K_{p,uu}$), and unbound drug CSF-to-plasma concentration ratio at the steady-state.

PDs analysis

To test the stability of proposed PD biomarkers in glioblastoma tissues (pFAK, IGF1R, pIGF1R, pIRS1, cleaved caspase-3, Ki67), we analyzed a historical cohort of four patients with matched primary and

recurrent glioblastoma who received standard-of-care Stupp regimen and were not enrolled in the study. FFPE tissues were stained with anti-STAT5B (Abcam, catalog #ab178941; 1:1000), anti-pSTAT5B (Abcam, catalog #ab52211; 1:50), anti-pJAK2 (ab32101; 1:50), anti-FAK (Cell Signaling Technology, catalog #3285; 1:100), anti-pFAKTyr397 (ThermoFisher, catalog #44–624G; 1:100), anti-IGF1R (Cell Signaling Technology, catalog #3027; 1:100), anti-pIGF1R (Abcam, catalog #ab39398; 1:50), anti-pIRS1 (ThermoFisher, catalog #44–816G; 1:100), anti-histone H3 (Cell Signaling Technology, catalog #9701; 1:200), and cleaved caspase-3 (Cell Signaling Technology, catalog #9661; 1:300) using our standardized IHC protocol with the BOND RX automated system (Leica Biosystems). The stained slides were imaged and quantified using the Aperio Image analysis software (Leica Biosystems) to assess differences in positivity for the above antibodies. The slides and images were analyzed by a board-certified neuropathologist.

PD assessment of the tumor tissue post–ceritinib treatment was conducted by comparing changes in biomarker levels in FFPE tissue from the patient's first tumor resection (at the time of initial diagnosis) and tumor tissue resected after presurgical drug exposure. To assess the PD effects of ceritinib in CNS metastases, phosphorylation of ALK, STAT5b and JAK2 were selected as primary determinants. For glioblastoma, we compared changes in IGF1R, FAK, and IRS1 phosphorylation in pre- and post–ceritinib-treated tissue. Other biomarkers assessed included the mitotic marker phosphohistone-3 and the apoptosis marker cleaved caspase-3. As a control, matched primary and recurrent glioblastoma tissues were used to assess changes in biomarker levels between primary and recurrent tumors.

Both archival FFPE tumor tissue and study specimens collected at the time of resection were assayed simultaneously using our standardized IHC protocol with the Leica BOND RX automated system. For each run, we included positive (historical glioblastoma tissue) and negative controls (no primary antibody). Stained FFPE slides were imaged using a Leica DM55500 microscope and analyzed using Aperio Image analysis software.

Results

Patient population and safety

Three patients with brain metastases were accrued and their primary tumor sites were breast, head and neck, and melanoma, respectively. Prior treatments for these patients included adriamycin and cyclophosphamide (4 cycles) plus taxol (1 cycle) and whole-brain radiation for patient 1 (breast); navelbine and cisplatin for patient 2 (head and neck); and nivolumab and ipilimumab plus radiosurgery treatment for patient 3 (melanoma). All patients had prior tumor specimens (from systemic disease sites) demonstrating IGF1R and/or ALK expression.

Seven patients with recurrent glioblastoma were accrued, with all 7 demonstrating WHO grade IV histology and FAK expression. All patients with glioblastoma had completed the Stupp regimen (29) prior to tumor recurrence and one patient with glioblastoma had received a single treatment of bevacizumab 2 months prior to surgical resection. No patients with glioblastoma received any other adjuvant chemotherapies or were treated with tumor-treating fields prior to enrollment.

Study patient demographics and clinical characteristics are described in **Table 1**. Three patients with CNS metastases received daily dose of 750 mg ceritinib, which is the maximally tolerated dose (MTD; ref. 4), for 10 days prior to tumor resection. The subsequent 7 patients with recurrent glioblastoma received a daily dose of 450 mg ceritinib with low-fat diet which has been shown to lower the drug-associated GI toxicities and grade 3 or 4 adverse events (30). Presurgical ceritinib was well tolerated and there were no dose-limiting toxicities. All observed toxicities at least possibly related to ceritinib were minor (CTCAE 4.0 grades 1 and 2), including diarrhea (20%), nausea (10%), vomiting (10%), and lymphopenia/thrombocytopenia (10%). All planned surgical resections occurred within the protocol-designated time interval following the last presurgical dose of ceritinib (median error, $\pm$ 120 minutes). All 10 evaluable study patients completed a 10-day course of ceritinib immediately prior to scheduled surgery.

Plasma and CNS PKs

Table 2 summarizes the steady-state plasma PK parameters of total and unbound ceritinib in 10 patients. Following daily oral administration of ceritinib at 750 mg or 450 mg for 9 days, the geometric mean $C_{ss,max}$ of total and unbound ceritinib were 1.230 and 0.015 μmol/L, respectively; the geometric mean $C_{ss,min}$ of total and unbound ceritinib were 0.881 and 0.010 μmol/L, respectively. The fluctuation between steady-state peak and trough plasma concentrations of total ceritinib was 1.4-fold (geometric mean). The geometric mean elimination half-life ($T_{1/2}$) was estimated to be 60 hours, and the geometric mean CL/F of total ceritinib was 41.2 L per hour (range, 29.8–66.9 L/hour) in 10 patients. Overall, the plasma PK parameters of ceritinib observed in our study were in line with those estimated from the population PK analysis involved a large population of cancer patients (31).

Ceritinib was highly bound to human plasma proteins, with the median fraction unbound of 1.4% (range, 0.6%–2.6%). The drug showed variable and extremely high binding to brain tumor tissues, with the median fraction unbound of 0.051% (range, 0.006%–1.6%) and 0.045% (range, 0.006%–0.21%) in enhancing and nonenhancing tumor regions, respectively (**Table 3**). The penetration of ceritinib into brain tumors and CSF was summarized in **Table 3** and **Fig. 1**. Across 2 to 24 hours after the administration of the 10th dose, the median total ceritinib concentrations in enhanced and nonenhanced tumors were 36.10 nmol/g (or μmol/L; range, 2.023–139.4) and 2.77 nmol/g (range, 1.259–36.35), respectively, whereas the median unbound ceritinib concentrations in enhanced and nonenhanced tumors were 0.048 nmol/g [range, below the lower limit of quantitation (BLQ)–0.87] and 0.006 nmol/g (range, BLQ–0.027), respectively. Ceritinib CSF concentrations (median, 0.012; range, 0.001–0.103 μmol/L) were similar to unbound drug concentrations in nonenhancing tumor regions (**Table 3** and **Fig. 1**). The extent of CNS penetration is often assessed by K_p and $K_{p,uu}$, while $K_{p,uu}$ is more pharmacologically relevant. Ceritinib exhibited the median K_p of 33.14 (range, 2.49–95.86) and 3.49 (range, 1.55–37.14) in enhanced and nonenhanced tumors, respectively, whereas it showed the median $K_{p,uu}$ of 2.86 (range, 0.01–40.6) and 0.33 (0.01–2.71) in enhanced and nonenhanced tumors, respectively. Notably, in 1 patient (patient 1) with breast cancer brain metastasis, the ceritinib fraction unbound in the tumor was approximately 30-fold higher than the median value. As a result, the unbound ceritinib concentrations in both tumor and CSF were more than 10-fold higher than the median levels of 10 patients and the K_p was unusually high (40.6; **Table 2**).

PD analyses

Among the 3 patients with CNS metastases, PD analyses were performed on tissue samples from 2 patients (patients 1 and 3: breast

Table 2. Steady-state plasma PK parameters of total and unbound ceritinib in patients.

	Total ceritinib							
Patient	Dose (mg)	$T_{ss,max}$ (h)	$C_{ss,max}$ (μmol/L)	$T_{ss,min}$ (h)	$C_{ss,min}$ (μmol/L)	AUC_τ (μmol/L·h)	CL/F (L/h)	$T_{1/2}$ (h)
1	750	8.1	2.234	24.0	2.096	45.052	29.8	260.3
2	750	6.0	1.230	24.0	0.766	20.097	66.9	35.2
3	750	6.0	1.834	24.1	0.920	24.633	54.6	24.2
5	450	7.9	1.020	21.6	0.935	19.748	40.8	172.0
7	450	4.4	1.088	24.6	0.790	21.321	37.8	53.1
8	450	6.0	0.909	23.1	0.580	16.081	50.1	35.6
9	450	8.0	1.048	24.3	0.779	22.451	35.9	56.7
11	450	4.0	1.121	24.0	0.800	20.257	39.8	49.2
13	450	6.2	1.162	24.0	0.900	23.613	34.1	65.1
14	450	4.0	1.143	23.9	0.795	23.266	34.7	45.5
Geometric mean		5.9	1.230	23.7	0.881	22.786	41.2	59.9
Arithmetic mean		6.1	1.279	23.7	0.936	23.652	42.5	79.7
SD		1.6	0.418	0.8	0.420	7.907	11.4	75.8
CV, %		26.2	32.7	3.5	44.9	33.4	26.8	95.1

	Unbound ceritinib					
Patient	$T_{ss,max}$ (h)	$C_{ss,max}$ (μmol/L)	$T_{ss,min}$ (h)	$C_{ss,min}$ (μmol/L)	AUC_τ (μmol/L·h)	AUC_u/AUC_t
1	8.1	0.042	24.0	0.020	0.602	0.013
2	6.0	0.021	24.0	0.014	0.335	0.017
3	6.0	0.025	24.1	0.014	0.360	0.015
5	21.6	0.010	21.6	0.010	0.190	0.010
7	24.6	0.011	24.6	0.011	0.175	0.008
8	6.0	0.015	23.1	0.011	0.263	0.016
9	1.0	0.002	24.3	0.001	0.020	0.001
11	6.0	0.017	24.0	0.011	0.265	0.013
13	6.2	0.024	24.0	0.014	0.364	0.015
14	7.9	0.025	23.9	0.018	0.500	0.022
Geometric mean	7.0	0.015	23.7	0.010	0.239	0.010
Arithmetic mean	9.3	0.019	23.7	0.012	0.307	0.013
SD	7.5	0.011	0.8	0.005	0.166	0.006
CV, %	80.5	57.2	3.5	42.4	54.1	43.5

Note: Plasma PK parameters were estimated using the noncompartmental analysis.
Abbreviation: $T_{ss,min,}$ trough sampling time.

and melanoma; **Fig. 2**). Compared with the archival pretreatment tissue from the primary tumor (breast and melanoma) the ceritinib-treated metastatic tissues had increased expression of pALK, pSTAT5b, and pJAK2, and no significant difference in the mitotic marker pH3 and apoptosis marker cleaved caspase-3 (**Fig. 2**). Among the 7 patients with recurrent glioblastoma, 1 patient was excluded from PD analyses due to pseudoprogression evident in the acquired tissue (patient 9). No significant changes in expression were observed in the tested biomarkers (pFAK, pIRS1, pIGF1R, cleaved caspase-3, and pH3) amongst the remaining 6 recurrent glioblastoma patient tumors after treatment with ceritinib (**Fig. 3**).

Discussion

In this brain tumor phase 0 study, we elucidate the PK and PD profile of ceritinib in plasma and tumor tissues of patients with CNS metastases and recurrent glioblastoma. These data represent the first-ever analysis of ceritinib drug concentrations in human brain tumor tissue. Our findings indicate high binding of ceritinib to plasma proteins and brain tumor tissues and limited unbound drug exposure in contrast to enhancing and nonenhancing brain tumors.

Poor blood–brain penetration is a barrier to brain tumor drug development and adjuvant therapy efficacy (2). Although many non-randomized studies of new CNS oncology agents use radiographic and clinical endpoints to assess drug effect, these efforts depend on preclinical and *in silico* analyses to predict the CNS penetrance of the agent. This approach has obvious limitations and can lead to assumptions of CNS penetrance that may confound assessments of clinical efficacy in metastatic disease, where brain tumor formation and progression is influenced by systemic disease status. In this study, we measure, for the first time, the brain tumor penetration and pharmacodynamic effects of ceritinib as it relates to the FAK and IGF1R signaling pathways in patients with brain tumor. Our findings demonstrate that free drug levels are well below the biochemical IC_{50} of ceritinib for IGF1R and FAK (8 nM and 30 nM, respectively; refs. 3, 32). Thus, ceritinib may not achieve pharmacologically-relevant drug concentrations in patients with recurrent glioblastoma or select patients with brain metastasis where these pathways are therapeutically relevant.

Table 3. The concentrations of total and unbound ceritinib in enhancing tumors, nonenhancing brain tumors, and CSF, as well as the extent of penetration (K_p and $K_{p,uu}$) in patients.

Patient	Time	Total drug tumor conc. (nmol/mg)		Unbound drug tumor conc. (nmol/mg)		CSF (μM)	Fraction unbound (%)		K_p		$K_{p,uu}$	
		EN	NE	EN	NE		EN	NE	EN	NE	EN	NE
1	2–4 h	53.775	—	0.870	—	0.103	1.618	—	30.43	—	40.64	—
2	2–4 h	13.275	—	0.005	—	0.001	0.039	—	14.22	—	0.34	—
3	2–4 h	139.425	—	0.065	—	0.008	0.047	—	68.88	—	2.17	—
5	2–4 h	93.836	36.351	0.053	0.017	0.009	0.056	0.045	95.86	37.14	4.11	1.29
11	2–4 h	2.023	1.259	BLQ	BLQ	0.017	0.003	0.006	2.49	1.55	0.01	0.01
7	6–8 h	58.893	1.741	0.018	0.001	0.015	0.030	0.030	64.25	1.90	1.55	0.05
9	6–8 h	17.770	1.490	0.001	BLQ	—	0.006	0.006	22.90	1.92	1.32	0.11
13	6–8 h	28.484	16.263	0.043	0.027	0.003	0.153	0.163	34.66	19.79	4.43	2.71
8	23–25 h	18.340	7.840	0.055	0.007	—	0.298	0.090	31.62	13.52	4.99	0.65
14	23–25 h	43.715	2.771	0.063	0.006	0.015	0.144	0.213	54.99	3.49	3.54	0.33
Median		36.100	2.771	0.048	0.006	0.012	0.051	0.045	33.14	3.49	2.86	0.33
Mean		46.954	9.674	0.117	0.008	0.022	0.239	0.079	42.03	11.33	6.31	0.73
SD		42.312	12.954	0.266	0.010	0.034	0.493	0.081	28.44	13.40	12.19	0.98
CV, %		90	134	227	124	156	206	102	68	118	193	133

Abbreviations: conc., concentration; EN, enhancing tumor; NE, nonenhancing tumor.

Nevertheless, interindividual variability in ceritinib concentrations was observed among patients and, in a case of a patient with breast cancer metastasis, resulted in significantly higher measured levels of unbound ceritinib. Drug nonspecific binding to tissues is largely driven by nonspecific binding to tissue phospholipids. The significantly lower binding of ceritinib in the breast cancer metastasis as compared with glioblastoma may be due to different tissue compositions, especially in relative amounts of phospholipids. Further study with a larger sample size of patients with breast cancer brain metastasis is needed to confirm our observation and elucidate the underlying mechanism. Regardless, our data suggests that the limited unbound (i.e., pharmacologically active) drug exposure in studied brain tumors was mainly attributable to the high binding of ceritinib to plasma proteins and brain/tumor tissues.

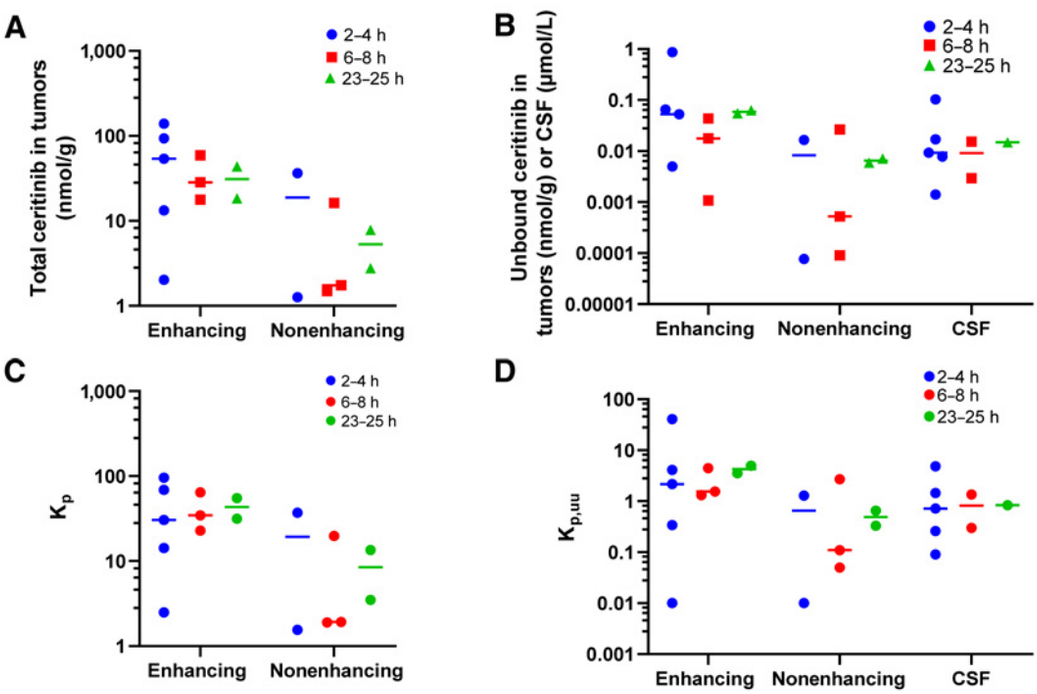

Figure 1.
The penetration of ceritinib into brain tumors and CSF in patients. **A,** The concentrations of total ceritinib in enhancing and nonenhancing tumors. **B,** The concentrations of unbound ceritinib in enhancing tumors, nonenhancing tumors, and CSF. **C,** The total K_p in enhancing and nonenhancing tumors. **D,** The $K_{p,uu}$ in enhancing and nonenhancing tumors as well as unbound drug plasma-to-CSF concentration ratio. Symbols (●, ■, ▲), observed values; short bars, median values at specific time points.

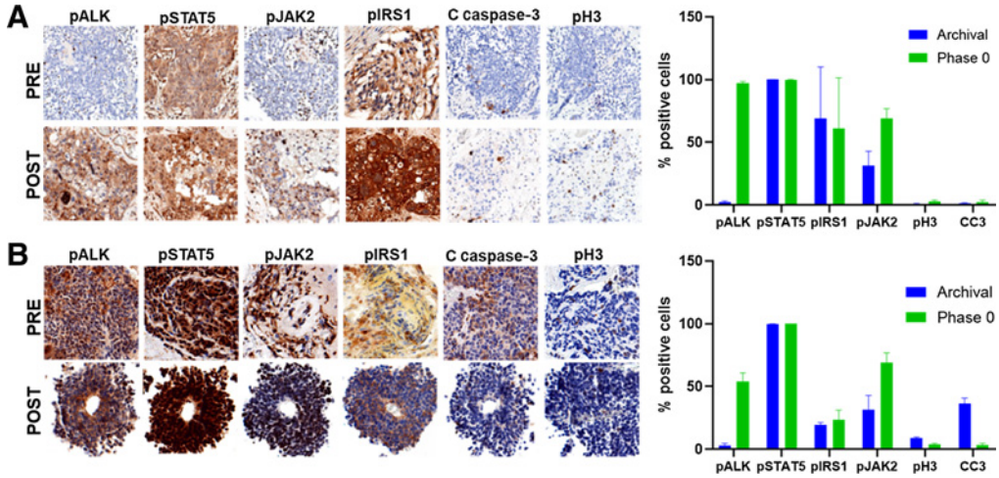

Figure 2.

PD analyses of CNS metastasis tumor tissues after ceritinib treatment. **A** and **B,** Representative IHC images and quantification of positive cells from archival and post–ceritinib-treated tumor tissue stained for pALK, pSTAT5, pJAK2, cleaved caspase-3, and phospho-histone H3 staining from patients 1 and 3. C, cleaved.

The ASCEND-7 phase II clinical trial in patients with ALK(+) NSCLC with brain metastases assessed the intracranial effects of ceritinib using the modified RECIST v1.1 guideline. High disease control rate (DCR) was observed across all four arms of the study, which included prior radiotherapy and ALKi treatment, no radiotherapy but prior ALKi, prior radiotherapy but no ALKi, and no prior radiotherapy or ALKi treatment. Patients in all four arms of this phase II study had high overall response rate and DCR in extracranial disease. These data strongly suggest that ceritinib is CNS penetrant. Several differences between ASCEND-7 and this phase 0 trial may explain the discrepancy between our observed low tumor drug concentrations and the clinical/radiographic responses reported in ASCEND-7: (i) unlike the patient population enrolled in ASCEND-7 study, none of the phase 0 patients in this study were ALK(+) NSCLC. It remains possible that ALK(+) NSCLC is uniquely permeable to ceritinib, as compared with the patients with brain tumor in this phase 0 study. (ii) BBB integrity is

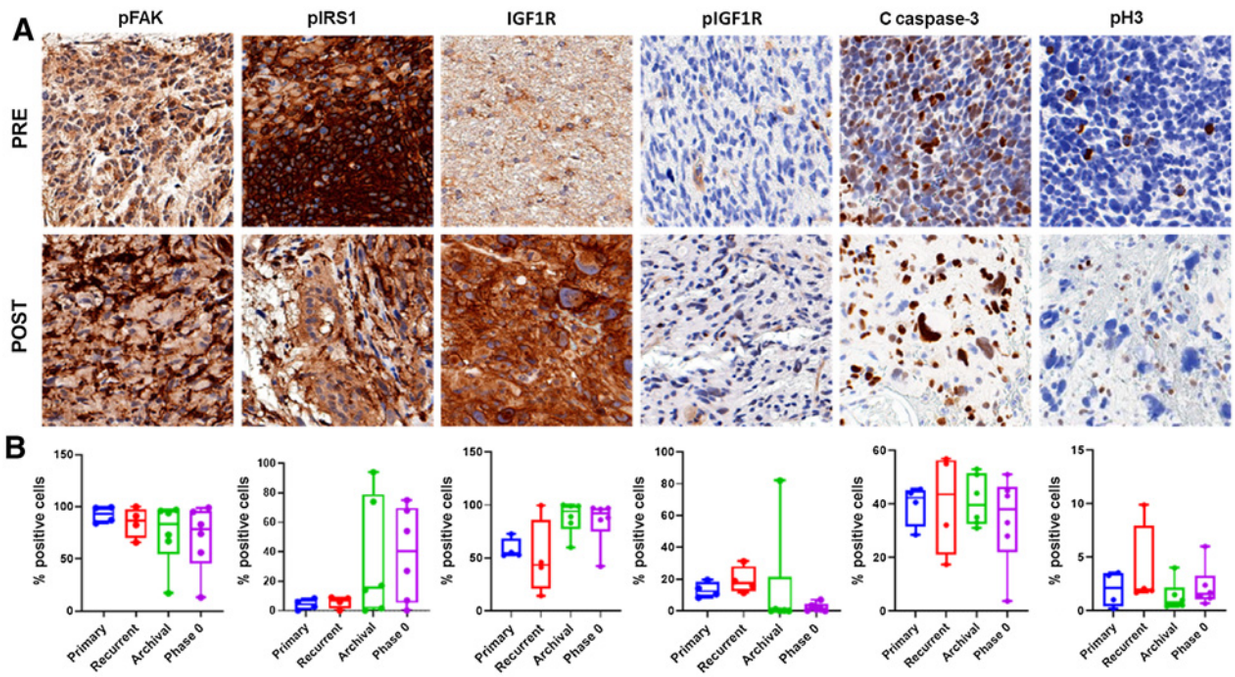

Figure 3.

PD analyses of glioblastoma tumor tissues after ceritinib treatment. **A,** Representative IHC images of archival and post–ceritinib-treated tumor tissue stained for pFAK, pIRS1, IGF1R, pIGF1R, cleaved caspase-3, and phospho-histone H3 staining from patients with glioblastoma. **B,** Quantification of percentage of positive cells in four control primary and recurrent glioblastoma tumor tissues and pretreatment archival versus post–ceritinib-treated (phase 0) tissues.

variable across different tumor types, owing to patient and tumor genetics, as well as the distinct cytoarchitectural features of each tumor type (33). This likely contributes to the interindividual variability observed in our study. (iii) Additionally, although our protocol used enough presurgical ceritinib to reach steady-state, the total drug exposure was only 10 days, in contrast to the months-long regimen prescribed to ASCEND-7 patients.

Across all patients with glioblastoma, we did not observe any PD effects on the downstream effectors of ALK, IGF1R, and FAK based on pSTAT5, pJAK2, or pIRS1 levels. There was also no significant change in proliferation or apoptosis markers in posttreatment samples. Lack of PD response could be attributed to the limited drug penetration, however, it is important to note that these PD analyses relied on control specimens from prior resections/biopsies (median interval, 12 months) that were not acquired immediately pretreatment. Nevertheless, our data suggest that 10 days of ceritinib does not lead to target modulation, consistent with its observed limited CNS penetration. Further studies are warranted to better understand ceritinib's CNS penetration capabilities in the setting of other disease, including ALK(+) NSCLC brain metastases.

Phase 0, window-of-opportunity, and other tissue-based PK/PD-driven clinical trial designs are starkly underrepresented in today's neuro-oncology and brain tumor drug development efforts. Over the past 50 years, only 22 such studies have been identified in the literature (25). Nevertheless, the value of such studies to guiding new therapeutic strategies for incurable brain tumors cannot be overstated. A positive result can provide the necessary justification to accelerate a drug's development. A negative result, such as in this study, sheds light on the limited potential of a new agent for CNS disease. Accordingly, our observations from this phase 0 study of ceritinib indicate it should not be pursued as an anticancer agent in glioblastoma and select brain metastases due to its extremely limited penetration of these tumors. Collectively, this study underscores the utility of phase 0 studies in precisely calculating brain tumor drug penetration, as well as in revealing the variability associated with tumor histologies in patients with brain tumor. Our experience also serves as a reminder that brain tumor phase 0 study results should be interpreted in context and not extrapolated beyond the tested circumstances.

Authors' Disclosures

N. Sanai reports grants from Ben & Catherine Ivy Foundation and Novartis during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

S. Mehta: Conceptualization, data curation, supervision, writing–original draft, writing–review and editing. **R. Fiorelli:** Data curation, formal analysis, methodology. **X. Bao:** Data curation, formal analysis, methodology. **C. Pennington-Krygier:** Methodology. **A. Derogatis:** Methodology. **S. Kim:** Formal analysis. **W. Yoo:** Methodology, writing–review and editing. **J. Li:** Formal analysis, methodology, writing–review and editing. **N. Sanai:** Conceptualization, supervision, funding acquisition, writing–review and editing.

Acknowledgments

We are grateful to the patients participating in this study. Results were presented at EANO 2019, ESMO Congress 2019, and SNO 2019. This work was supported by Novartis Pharmaceuticals and The Ben & Catherine Ivy Foundation.

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Received March 25, 2021; revised July 28, 2021; accepted October 20, 2021; published first October 26, 2021.

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