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PRECLINICAL STUDIES

E6201, an intravenous MEK1 inhibitor, achieves an exceptional response in BRAF V600E-mutated metastatic malignant melanoma with brain metastases

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Summary

Malignant melanoma (MM) exhibits a high propensity for central nervous system dissemination with ~50% of metastatic MM patients developing brain metastases (BM). Targeted therapies and immune checkpoint inhibitors have improved overall survival for MM patients with BM. However, responses are usually of short duration and new agents that effectively penetrate the blood brain barrier (BBB) are needed. Here, we report a MM patient with BM who experienced an exceptional response to E6201, an ATP-competitive MEK1 inhibitor, on a Phase 1 study, with ongoing near-complete response and overall survival extending beyond 8 years. Whole exome and transcriptome sequencing revealed a high mutational burden tumor (22 mutations/Megabase) with homozygous BRAF V600E mutation. Correlative preclinical studies demonstrated broad activity for E6201 across BRAF V600E mutant melanoma cell lines and effective BBB penetration in vivo. Together, these results suggest that E6201 may represent a potential new treatment option for BRAF-mutant MM patients with BM.

Keywords MEK · BRAF · Inhibitor · Melanoma · Brain

Background

MM is an aggressive form of skin cancer projected in 2018 to have an estimated 91,270 new cases and 9320 deaths in the United States [1]. MM is the third leading cause of BM, behind only lung and breast cancer. The median overall survival for melanoma patients following diagnosis of BM is less than 6 months [2].

Approximately 30–50% of cutaneous MM harbor constitutively activating V600E mutations in BRAF, a key upstream signaling activator in the mitogen-activated protein kinase and extracellular signal-regulated kinase

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(MAPK-ERK) pathway [3]. Small molecule kinase inhibitors that target BRAF driver mutations (e.g., vemurafenib, dabrafenib) or the downstream MAPK signaling partner, MEK, (e.g. trametinib, cobimetinib), either alone or in combination, are more effective than chemotherapy in the treatment of BRAF V600E-mutated MM [4].

For patients with BRAF V600E-mutant MM with BM, the BRAF inhibitors dabrafenib and vemurafenib achieved median intracranial progression free survival (PFS) of 2.0–4.4 months, and median overall survival (OS) of 4.0–9.6 months [5–7]. The combination of dabrafenib and trametinib produced intracranial response rates of 44–59% [8]. Despite these promising results, most patients treated with BRAF and MEK inhibitors develop relatively rapid progressive disease due to the development of acquired drug resistance, including hyperactivated downstream MEK signaling [9, 10]. Recently, immunotherapies (ipilimumab, nivolumab, pembrolizumab) reported intracranial response rates of 10–55%, median intracranial PFS 2.5–4.8 months and median OS 3.7–12.7 months in patients with MM and BM [11–14].

Current therapeutic challenges in treating MM with BM with small molecule targeted agents or immunotherapeutic drugs include the inability to penetrate the intact BBB and to circumvent active drug efflux by proteins such as p-glycoprotein (P-gp) and breast cancer resistance protein (Bcrp) [15]. Although the BBB can be compromised in areas of large metastatic disease, small volume metastases and micro-metastases can maintain an intact BBB. Thus, targeted therapies that can more effectively penetrate an intact BBB and circumvent efflux mechanisms are needed.

E6201 is an ATP-competitive MEK1 inhibitor that has demonstrated preclinical activity in BRAF V600E mutant melanoma cell lines [16] and shown brain distribution characteristics that were minimally affected by P-gp and Bcrp efflux transport at the BBB [17]. A Phase 1 trial (NCT00794781) evaluating E6201 in 55 patients with advanced solid tumors confirmed a maximum tolerated dose (MTD) of 320 mg/m^2 once weekly and demonstrated activity in MM patients, including two patients with BM [18]. These data support the potential clinical utility for E6201 against MM with BM.

Herein, we report an exceptional response to E6201 in a patient with metastatic MM with BM. Potential molecular underpinnings of this exceptional response were investigated using tumor-normal paired exome-sequencing and tumor RNA-sequencing, which revealed a homozygous *BRAF* V600E mutation. Preclinical drug sensitivity studies demonstrated pan-susceptibility of *BRAF* V600E mutant melanoma cancer cell lines to E6201, irrespective of *BRAF* V600E zygosity. This report highlights a potentially promising role for E6201 in patients with *BRAF* V600E mutant MM-associated brain metastases.

Methods

Phase 1 study

Results from a Phase 1 study of E6201 were recently reported [18]. In brief, patients were enrolled in this Phase 1 study to determine the MTD, dose-limiting toxicities (DLTs), pharmacokinetic (PK) profile, safety, and preliminary efficacy of the MEK inhibitor, E6201. Inclusion criteria included age $\geq$ 18-years, refractory solid tumors (Part A) or BRAF mutated and wild-type MM with progression on standard therapy (Part B). Part A (dose-escalation) enrolled 25 patients in sequential cohorts receiving E6201 IV at doses of 20–480 mg/m^2 over 30 min once-weekly (Days 1, 8, 15 of a 28-day cycle). The MTD was determined to be 320 mg/m^2 once weekly. Part B (expansion) enrolled 30 patients treated at the MTD of 320 mg/m^2 once weekly with the infusion time lengthened to 60 min due to QTc prolongation with the 30-min infusion during Part A. The trial was conducted with approval from the Institutional Review Board, in accordance with the Declaration of Helsinki and Good Clinical Practice and was registered with the NIH (NCT00794781). Here we provide additional correlative analysis for one of the patients described in the recent E6201 Phase 1 publication [18].

Whole exome and RNA-sequencing

Following patient consent, DNA and RNA were extracted from an archival FFPE block (metastatic urinary bladder biopsy in 2009, 1 year prior to E6201 treatment), and constitutional DNA was isolated from a fresh peripheral blood sample by the Translational Genomics Research Institute's Collaborative Sequencing Center. Paired tumor/normal whole exome sequencing and tumor RNA sequencing were performed as previously described [19]. Briefly, tumor/normal whole exome and tumor RNA libraries were sequenced on the Illumina HiSeq using V3 reagents. Genome-wide exome sequencing was performed for 1) identification of somatic coding point mutations and small insertions and deletions within exons for >20,000 genes, and 2) for regional whole genome analysis to detect copy number changes and structural events. RNA sequencing was performed for fusion detection and differential expression analysis. Quality filtered reads were aligned to the NCBI human reference genome (build 37), with >260 M aligned tumor exome reads, >170 M aligned normal exome reads, and > 140 M aligned RNA reads. The mean target coverage was 277X for the tumor exome and 233X for the normal exome. 94% of target bases had 50X coverage and 85% of target bases had 100X coverage for the tumor sample. Somatic variant calling (SNVs, CNVs, SVs) and fusion detection were performed as previously described [19]. Detection by two of three callers was required for

somatic mutations. Focal copy number events were defined as regions of copy gain or loss <25 Mb, with a log2fc of > = |2|.

Cancer cell line analysis

The potency (IC_{50}) of E6201 was determined via 14-point drug dose response viability assays for E6201, trametinib, cobimetinib, and vemurafenib. E6201 was provided by Spirita Oncology, LLC, and trametinib, cobimetinib, and vemurafenib were purchased from Selleck Chemicals (Houston, TX). Drug sensitivity was conducted as follows: 2000 cells were plated per well in a 96 well plate, 24 h later, drug treatments or a vehicle control were added, in triplicate, using a 2-fold dilution, 14-point drug dose response curve. Four days later, cell viability was assessed using a Cell-Titer Glo viability assay. Subsequent IC50 analyses were performed with PRISM 6, using a 3-variable, Hill-slope model.

Downstream pathway activation was evaluated using Western blot analysis. Briefly, melanoma cell lines were plated at a density of 200,000 cells per well in a 6-well plate. The next day, cells were washed in PBS and switched to 0.2% FBS containing media with DMSO vehicle control or E6201 treatment at the IC50 concentration for each cell line. Lysates were collected 1 h or 24 h after treatment. 20 μg of total protein were analyzed by SDS-PAGE. Phosphorylated and total Akt and ERK1/2 proteins and total GAPDH proteins were evaluated using total or phospho-specific antibodies from Cell Signaling Technology (Danvers, MA).

Brain penetration

Friend leukemia virus strain B (FVB) wild-type and *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice (Taconic Farms, Germantown, NY), balanced for sex, were used to determine the steady-state brain distribution of E6201. All mice used were 8–15 week-old adults at the time of the experiment. Animals were maintained in a 12-h light/dark cycle with unrestricted access to water and food. The studies were carried out in accordance with the guidelines set by the Principles of Laboratory Animal Care (National Institutes of Health, Bethesda, MD) and were approved by Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota.

The determination of steady-state plasma and brain concentrations of E6201 was accomplished by implanting Alzet osmotic mini pumps (model 1003D; Durect Corporation, Cupertino, CA) loaded with 6 mg/mL E6201 in the peritoneal cavity of wild-type and *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice to deliver the drug at a constant rate of 6 μg/h. The E6201 formulation was prepared by reconstituting lyophilized powder in single-use vials (received from Spirita Oncology, LLC), containing 60 mg E6201 and 3 g Captisol, with 8.5 mL sterile water for injection, which yielded a final concentration of 6 mg/mL E6201. The drug formulation was loaded into minipumps and primed overnight in sterile PBS at 37 °C on the day before the experiment. The pumps were implanted into the peritoneal cavity as described previously [20]. Briefly, mice were anaesthetized using isoflurane, and the hair over the skin on the abdominal cavity was removed. A small incision was made in the skin on the lower right abdomen, followed by an incision in the exposed peritoneal membrane under the cutaneous opening, and the primed pump was inserted into the peritoneal cavity. The peritoneal membrane was sutured with absorbable sutures, and the opening in the skin was sealed with surgical clips. The whole procedure was performed on a heating pad until the animals fully recovered. The half-life for E6201 in mice is approximately 45 min [17] and so an infusion lasting for 7 h was considered sufficient to attain steady-state E6201 levels in both plasma and brain. The mice were sacrificed, and blood and brain samples were isolated 7 h (steady state) following the implantation of osmotic minipumps. Plasma was obtained by centrifugation of blood samples at 3500 rpm for 15 min. The plasma and brain samples were stored at −80 °C until analysis by LC-MS/MS, using the method previously described [17].

Data availability Sequencing files can be accessed through the European Bioinformatics Institute's EGA (European Genome-phenome Archive), accession number is pending (https://www.ebi.ac.uk/ega/home).

Results

Case presentation

Results from the phase 1 clinical trial for the investigational MEK inhibitor, E6201, were recently published [18]. Here we provide a detailed case report and correlative analysis for one of the patients enrolled on this trial that experienced an exceptional response on E6201. This patient is an 84-year-old white female with metastatic MM who was initially diagnosed with cutaneous MM of the right calf in 1958 at age 25. She underwent a wide local excision at that time. An in-transit recurrence in the right inguinal area developed approximately 16 months later. A lymph node (LN) dissection was performed, and pathological evaluation indicated metastasis to a single inguinal LN. She did not receive any adjuvant therapy. She remained in complete remission until approximately 50 years after her LN dissection, when in 2009 at the age of 75, she presented with hematuria. A cystoscopic biopsy performed on a urinary bladder lesion confirmed MM. CT and MRI scans demonstrated metastases involving the bladder, left adrenal gland, liver, spleen, abdominal lymph nodes and brain (left parietal and right thalamic metastasis). She received stereotactic radiation therapy (SRT) to the left parietal and right posterior thalamic brain lesions, then dacarbazine from

November 2009 to March 2010. In April 2010 CT imaging revealed progression of disease in the liver, left adrenal gland and other metastatic foci including recurrence of the right thalamic brain metastasis. BRAF inhibitors were not FDA-approved in 2010, and BRAF status was not evaluated at that time. She was referred to our Phase 1 clinical trials program. Screening CT and MRI scans showed bulky metastatic disease in the porta-hepatis (due to nodal disease), spleen, left adrenal gland and a single recurrent right thalamic lesion (6 mm) (Fig. 1 Panels A–E). She was enrolled in Part B of a Phase 1 trial of E6201, an IV MEK1 inhibitor with Cycle 1, Day 1 (C1D1) delivered on May 17, 2010. The patient received E6201 at the MTD of 320 mg/m^2 IV once weekly on Days 1, 8 and 15 of a 28-day cycle. After C3D1, CT revealed significant reduction in the porta-hepatic adenopathy, which ultimately resolved into only a calcified cystic remnant, and resolution of the splenic lesion (Fig. 1: C27D1 Panel I and C94D1 Panel J). The thalamic brain metastasis has remained stable for over 8 years. At C67D1 a new minute left parietal lobe abnormality developed (Fig. 1 Panel C, lower image) which was deemed to be non-specific, most likely not malignant, and has remained unchanged for more than 36 months (Fig. 1 Panels C–E). From a safety perspective, the patient developed QT prolongation (>500 ms) that resolved after increasing the infusion time from 60 to 120 min in association with a dose reduction to 240 mg/m^2, then to 160 mg/m^2. She currently maintains an ECOG of 0 and is tolerating treatment well and is beyond cycle 104 (> 8 years).

Whole exome and transcriptome sequencing analysis

To investigate potential molecular mechanisms associated with this patient's exceptional response to E6201, paired tumor/normal whole exome sequencing and tumor RNA sequencing were performed from archival formalin fixed paraffin embedded (FFPE) tissue from a pre-E6201 treatment metastatic urinary bladder sample. Copy number analysis identified focal events, such as focal deletion of *CDKN2A* on chromosome 9, as well as widespread aneuploidy (Fig. 2a). Somatic mutational analysis revealed a high mutational burden of 22 mutations per megabase (Mut/Mb), suggesting this tumor is hypermutated. A total of 222 somatic protein coding point mutations were identified, with two of these mutations previously identified as recurrent, hotspot mutations in cancer: *BRAF* V600E and *CTNNB1* N387 K [21]. As shown in Fig. 2b, the *BRAF* V600E mutation was detected at a DNA allele frequency of 90%, and an RNA allele frequency of 100%, suggesting overrepresentation of the mutant *BRAF* V600E allele (hereafter referred to as homozygous *BRAF* V600E).

Preclinical evaluation of homozygous *BRAF* V600E mutation as a surrogate for response to E6201

BRAF V600E homozygosity has been reported in a subset of malignant melanomas, typically occurring due to chromosome 7 aneuploidy or *BRAF* gene amplification [22] and has been associated with increased vemurafenib sensitivity in retrospective clinical analyses [23, 24]. To investigate the influence of *BRAF* V600E zygosity on E6201 response, we evaluated the efficacy of E6201 across a panel of eight *BRAF* V600E mutant melanoma cancer cell lines. Six cell lines were homozygous for *BRAF* V600E (M-262, M-229, M-321, SKMEL28, UACC2641, and UACC2404) and two were heterozygous (UACC903 and UACC558) [16, 25]. *BRAF* mutation status was validated by Sanger sequencing. All eight cell lines also showed *CDKN2A* deletion (Supplemental Fig. 1). As shown in Fig. 3, seven of eight *BRAF* V600E-mutant melanoma cell lines were sensitive to E6201, with IC_{50} values <600 nM, which is below the achievable C_{max} at the recommended human dose of 320 mg/m^2 [18]. Four cell lines, three of which were homozygous for the *BRAF* V600E mutation, were hypersensitive to E6201, with IC_{50} values <100 nM. However, there was no significant difference in E6201 sensitivity based on *BRAF* V600E zygosity in this cell line panel. We also evaluated sensitivity of these cell lines to other MAPK pathway inhibitors, including MEK inhibitors trametinib and cobimetinib and the BRAF inhibitor vemurafenib. *BRAF* V600E zygosity was also not associated with differential response to these other MAPK pathway inhibitors (Supplemental Fig. 1). Together, these data suggest that *BRAF* V600E homozygosity alone may not explain the exceptional response to E6201 seen in this patient.

Effect of E6201 on MAPK pathway activation

To evaluate the impact of E6201 treatment on downstream MAPK pathway activation in *BRAF* V600E homozygous and heterozygous melanoma cell lines, Western blot analysis was performed using four melanoma cell lines: UACC-2641 and SKMEL-28 (*BRAF* V600E homozygous), and UACC-558 and UACC-903 (*BRAF* V600E heterozygous). Cells were either serum-starved or treated with IC_{50} doses of E6201 or vehicle control for 1 h or 24 h. Phospho-ERK levels were high in all cell lines even in the absence of serum (Supplemental Fig. 2), consistent with constitutive activation of MAPK driven by BRAF mutation. Moreover, phospho-ERK levels were reduced in response to E6201 treatment, perhaps more so for *BRAF* V600E homozygous cell lines. Phospho-Akt levels were low-to-moderate in all lines and did not change during treatment (Supplemental Fig. 2).

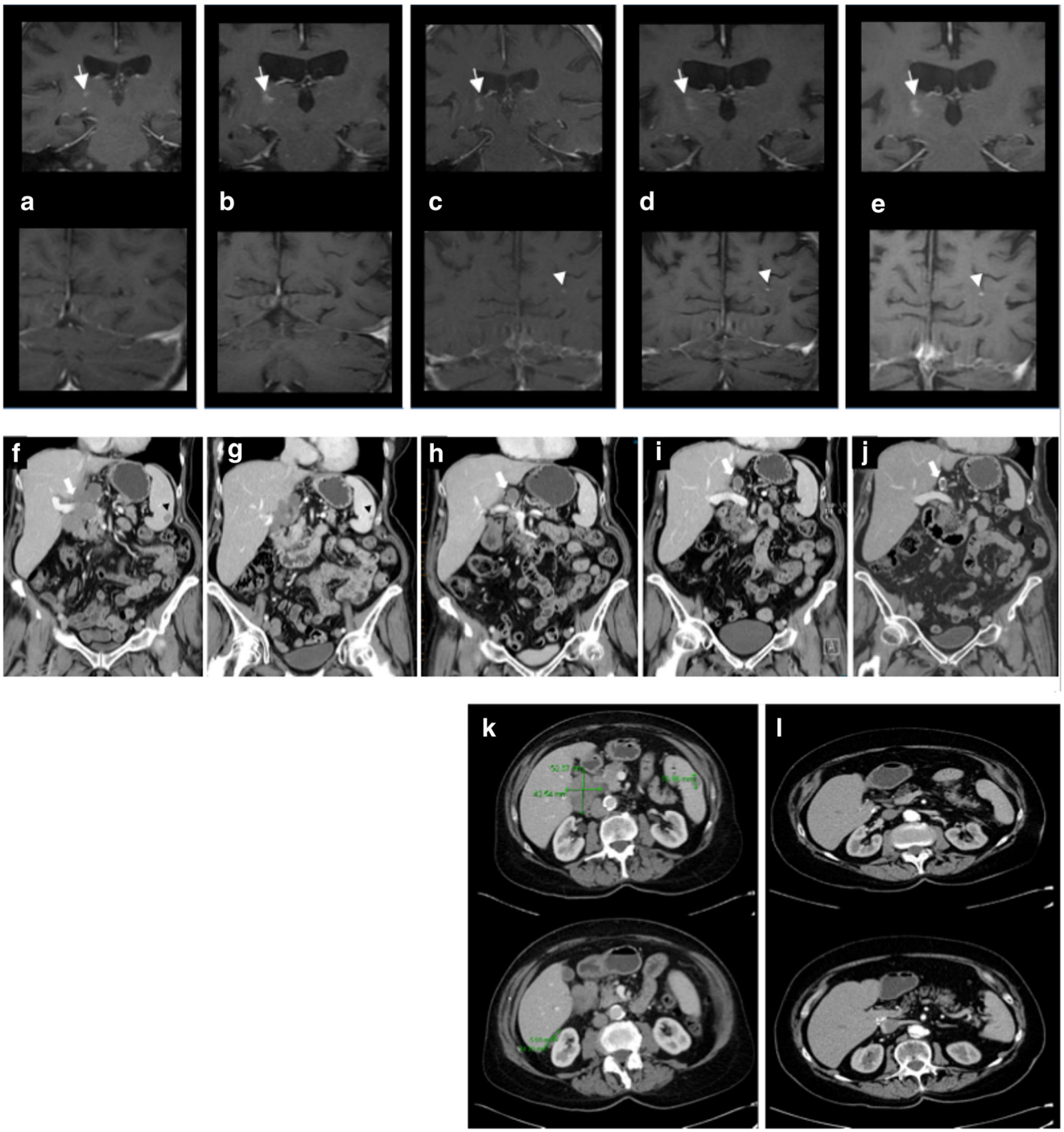

Fig. 1 Treatment Response. An 84-year old female with metastatic melanoma metastasized to the brain with >8-year response to E6201. Panel **a**) C5D1. Panel **b**) C56D1. Panel **c**) C67D1. Panel **d**) C89D1 and Panel **e**) C93D1. **a**–**e**): T1W MRI contrast enhanced (gadolinium) serial coronal images of the brain demonstrate a 6 mm metastasis in the right thalamus (arrows) that has remained stable over 8 years. At C67D1 (panel **c**), a new lesion developed in the left parietal lobe (arrowhead) which has remained stable over 36 treatment cycles. Panel **f**) baseline, Panel **g**) C3D1. Panel **h**) C15D1, Panel **i**) C27D1, and Panel **j**) C94D1. **f**–**j**). Contrast-enhanced serial CT coronal images of the abdomen and pelvis demonstrate resolution of metastatic disease located in the porto-hepatis region of the upper abdomen (arrows) and splenic metastasis (arrowhead). Note rapid reduction in tumor burden following end of C3 (panel **h**) which eventually resulted in a residual cystic remnant with a calcified rim (arrows)–a sign of healing in addition to resolution of the splenic lesion (panel **i**, **j**). Panel **k**) C3. Panel **l**) C76. Transverse images from serial contrast-enhanced CT scans of the abdomen demonstrates resolution of metastatic disease found in porto-hepatis region and the right supra-adrenal gland. C = cycle. D = day. T1W MRI = T1-weighted magnetic resonance imaging. CT = computed tomography

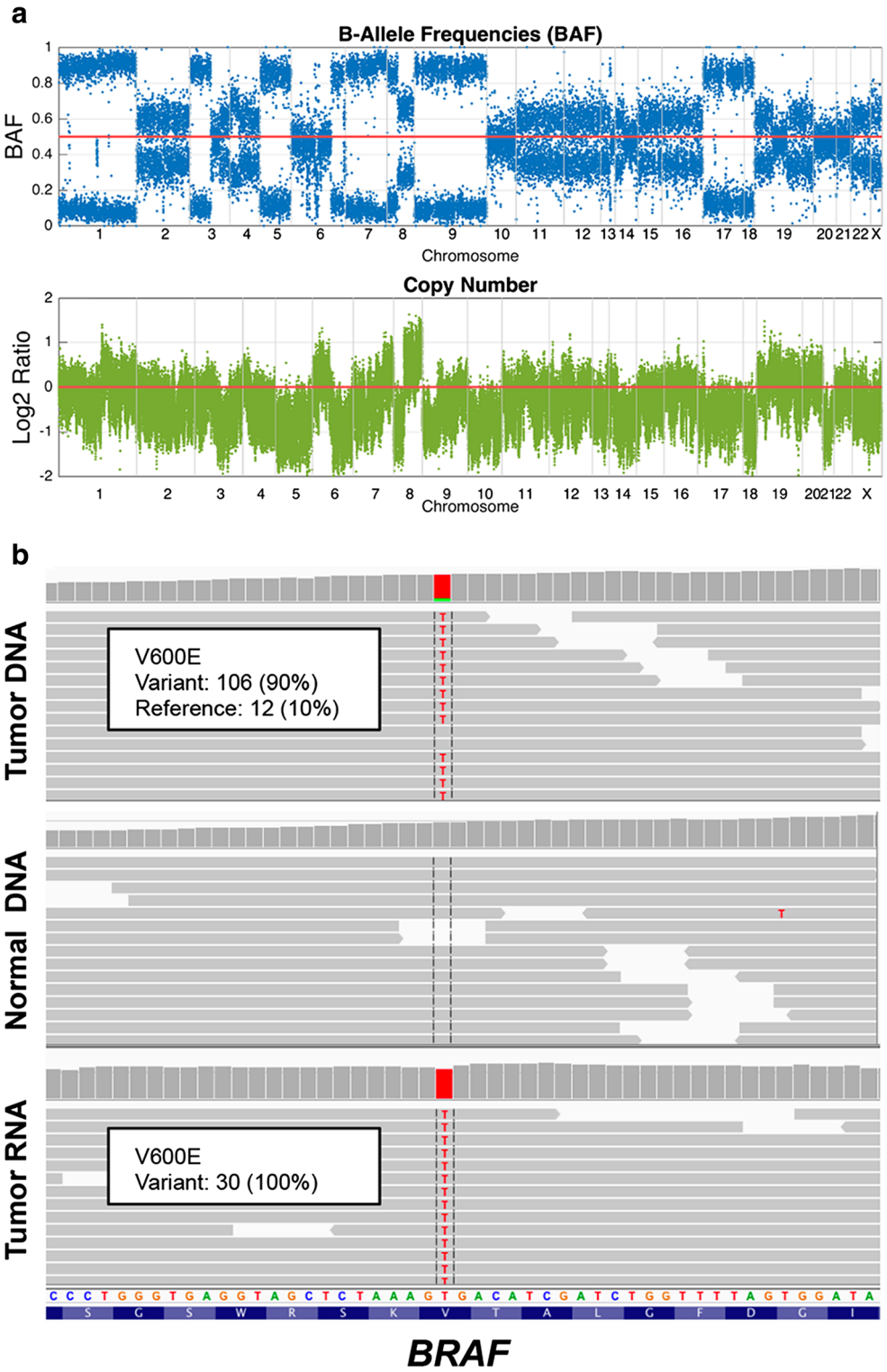

Fig. 2 Whole-exome and transcriptome sequencing reveals over-representation of BRAF V600E in a metastatic tumor sample prior to E6201 treatment. a Copy number plot and B-allele frequency plot reveal widespread tumor aneuploidy. **b** Representative images from the Integrated Genome Viewer (IGV) show detection of a somatic BRAF V600E mutation at a DNA allele ratio of 90% and a RNA allele ratio of 100%

Preclinical evaluation of E6201 brain distribution

E6201 brain distribution was evaluated in wild-type and *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice at steady-state. The half-life of E6201 in mice is approximately 45 min [17]. Animals were implanted with Alzet osmotic mini pumps to deliver 6 μg/h for seven hours to approximate the steady state condition. The steady-state concentrations of total E6201 in brain (48.22 ± 20.99 ng/g in wild-type mice; 82.32 ± 21.59 ng/g in *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice) were higher than that in plasma (26.54 ± 4.60 ng/mL in wild-type mice; 18.57 ± 2.46 ng/mL in *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice), for both wild-type and *Mdr1a/b*$^{-/}$

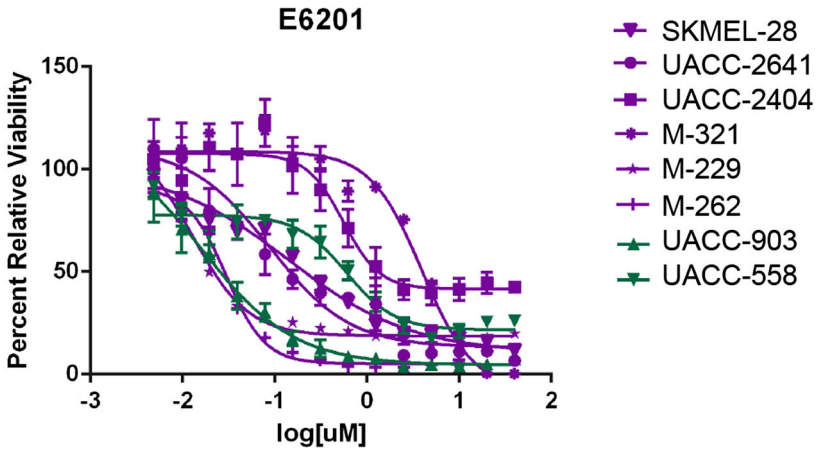

Fig. 3 BRAF V600E melanoma cell lines show broad sensitive to E6201 irrespective of BRAF zygosity. Melanoma cell lines bearing BRAF V600E homozygous (purple) or heterozygous (green) mutations were assessed for responses to the MEK inhibitor E6201. 14-point drug dose-response assays were performed in 96-well plates with CellTiterGlo 72-h viability endpoints. No significant differences in response were observed between homozygous and heterozygous cell lines

Cell line	*BRAF* Status	E6201 IC50 (nM)
SKMEL-28	V600E - homozygous	142
UACC-2641	V600E - homozygous	88
UACC-2404	V600E - homozygous	551
M-321	V600E - homozygous	3940
M-229	V600E - homozygous	13
M-262	V600E - homozygous	30
UACC-903	V600E - heterozygous	16
UACC-558	V600E - heterozygous	598

$^{-}Bcrp1^{-/-}$ mice (Fig. 4a). The corresponding steady-state brain-to-plasma ratios (Kp) in wild-type and $Mdr1a/b^{-/-}Bcrp1^{-/-}$ mice were 1.8 and 4.38, respectively (Fig. 4b), indicating that E6201 has a high partitioning into the brain.

Discussion

The treatment of cutaneous MM has witnessed a burgeoning era of efficacious drug development that has demonstrated improved OS for patients with metastatic disease. However, despite recent promising results, significant challenges remain in the treatment of patients with BM. First, the currently approved drugs achieve only limited responses, usually of short duration, in BM [5–8, 11–14]. Second, the BBB prevents effective drug delivery in most patients with MM and BM. The current BRAF and MEK inhibitors poorly cross the intact BBB and do not circumvent brain efflux proteins. Third, the development of resistance usually occurs via mechanisms that cause hyperactivated MEK downstream signaling [10]. One example is the development of a MEK1 activating point mutation, C121S, which confers resistance to vemurafenib and selumetinib [26].

Thus, it is critical to develop drugs that effectively penetrate the BBB, circumvent efflux proteins (P-gp and Bcrp1), overcome resistance in the brain microenvironment, and are effective against MAPK downstream signaling resistance mutations. E6201 has unique structural characteristics that show promise in achieving these goals. As an ATP-competitive MEK inhibitor, E6201 demonstrates higher preclinical in vitro cytotoxicity compared with allosteric inhibitors [26]. In addition, the macrocyclic structure of E6201, with a low-number of rotatable bonds and hydrogen-bonds donor, allows for improved BBB penetration and circumvents efflux proteins [27]. Total E6201 steady-state concentrations in the brain were higher than those in plasma, in both wild-type and $Mdr1a/b^{-/-}Bcrp1^{-/-}$ mice. The increase in Kp by about ~2.5 fold in mice lacking P-gp and Bcrp (Kp = 4.38) versus wild-type mice (Kp = 1.8), indicates that E6201 is influenced minimally by efflux transport at the BBB when compared to other small molecule MEK inhibitors, such as trametinib and cobimetinib [17, 28, 29]. The higher E6201 brain-to-plasma concentrations at steady-state reported here, together with a previous study on the detailed characterization of E6201 brain distribution [17], indicate that E6201 has promising brain distribution properties for the treatment of brain metastases. Finally, E6201 has demonstrated effectiveness in cell lines with known MAPK pathway resistance, including the MEK1-C121S resistance mutation [26].

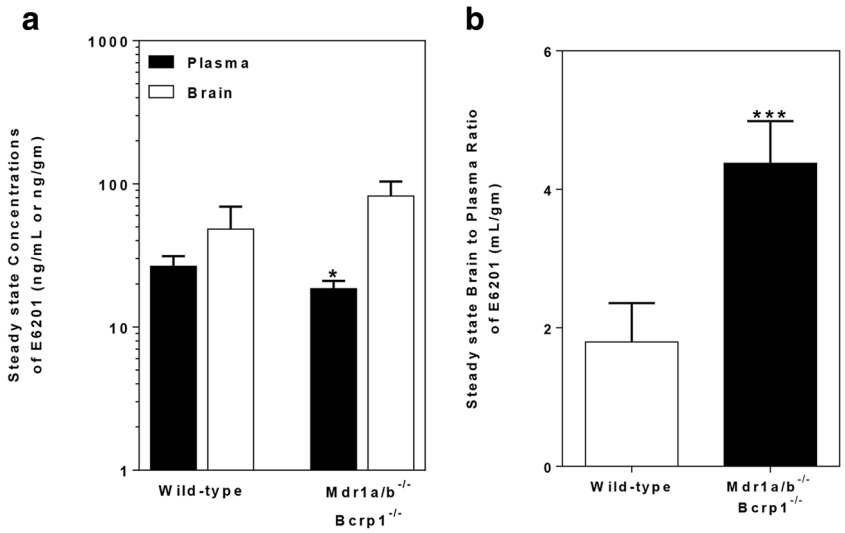

Fig. 4 Steady-state distribution of E6201 in FVB wild-type and *Mdr1a/b*$^{-/-}$*Bcrp1*$^{-/-}$ mice. a Steady-state plasma and brain concentrations, **b** brain-to-plasma ratios. *$P < 0.05$ and ***$P <$ 0.001, for statistical comparison by unpaired t-test. Data represent mean ± S.D., $n = 4$

Our patient with *BRAF* V600E-mutated metastatic melanoma achieved an exceptional response to E6201 in a Phase 1 trial. The patient has remained on E6201 therapy for over 104 months (>8 years), with stability of a right thalamic lesion on MRI, no signs of progression in the brain and resolution of intra-abdominal metastases. The prolonged response of the brain metastases without recurrence or progression to E6201 in this patient provides clinical support for the improved brain distribution seen in preclinical studies.

Genomic characterization of this patient's pre-treatment tumor identified a *BRAF* V600E homozygous mutation, a *CTTNB1* mutation and a focal *CDKN2A* deletion. We investigated the efficacy of E6201 in *BRAF* V600E homozygous and heterozygous melanoma cell lines but found no significant difference in E6201 sensitivity based on *BRAF* mutation zygosity. Instead, E6201 demonstrated activity across the *BRAF*-mutant melanoma cell lines, irrespective of *BRAF* mutation zygosity. In a prior preclinical study, E6201 sensitivity correlated with wildtype *PTEN* status, and hypersensitivity correlated with *BRAF* V600E mutation [16]. Alterations in *PTEN* were not identified in this patient's tumor, consistent with this preclinical study suggesting wildtype PTEN correlated with E6201 sensitivity. The patient's tumor also contained a *CTNNB1* N387 K hotspot mutation. *CTNNB1* alterations are rare in melanoma, typically occurring in melanoma tumors without *BRAF* mutations. The influence of *CTNNB1* mutation on MEK inhibitor response in melanoma remains unknown. However, a pan-cancer preclinical cell line study identified *CTNNB1* mutation as a molecular marker associated with sensitivity to MEK inhibitors [30]. In that study, MEK inhibitors (trametinib, selumetinib) showed 12 to 37 times greater activity in *CTNNB1* mutant cell lines, raising the possibility that *CTNNB1* mutation status may impact MEK inhibitor response. The interplay of this patient's genomic abnormalities may have influenced the exceptional response to E6201 therapy. Alternatively, other genomic and/or patient factors could have impacted the response and remain areas for future investigation. Notably, we did not biopsy the patient's brain metastasis, and thus our genomic analysis was limited to a metastatic bladder lesion. It is possible that the brain metastasis has distinct genomic characteristics not represented by other metastatic sites, and that these alterations could provide additional insight into this patient's treatment response.

In conclusion, this study reports genomic profiling and preclinical correlative studies for an exceptional responder to the investigational MEK inhibitor, E6201. Promising preclinical results with E6201, as well as clinical activity, effective BBB penetration, and an ability to overcome MAPK resistance mechanisms, suggest that E6201 may be a potential treatment for *BRAF* mutated melanoma patients with BM. These findings have led to the development of a Phase 1 trial in patients with BRAF- or MEK-mutated cutaneous melanoma with active BM (NCT03332589).

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflicts of interest Thomas J. Myers and Linda J. Paradiso are employees of Spirita Oncology, LLC. Daniel D. Von Hoff is a consultant for Spirita Oncology, LLC. Spirita Oncoloy, LLC provided funding for a portion of this study. The other authors declare no potential conflicts of interest.

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