CLINICAL STUDY PROTOCOL

Protocol Number:	**STX-101-02**
Protocol Title:	A Phase 1 Study of E6201 for the Treatment of Central Nervous System (CNS) Metastases From *BRAF* V600-Mutated Metastatic Melanoma
Sponsor Contact and Medical Monitor:	**Linda J. Paradiso, DVM, MBA** **Chief Development Officer** Spirita Oncology, LLC 27 Strathmore Road Natick, MA 01760 Telephone: 713-898-8965 Email: linda.paradiso@spiritaoncology.com
Original Protocol	**Date: 18 January 2018**
Amendment 1	**Date: 14 June 2018**
Amendment 2	**Date: 19 January 2019**
Amendment 3	**Date: 04 October 2019**
Amendment 4	**Date: 31 December 2019**

INVESTIGATOR SIGNATURE PAGE

I have reviewed the above-titled protocol and agree that it contains all the information necessary to conduct the study as required. I will conduct the trial in accordance with the principles of the International Conference on Harmonisation (ICH) Good Clinical Practice, the Declaration of Helsinki and the applicable U.S. Food and Drug Administration (FDA) regulations.

I will maintain as confidential all written and verbal information provided to me by the Sponsor, including but not limited to, the protocol, case report forms, investigator's brochure, material supplied at investigator meetings, minutes of teleconferences, etc. Such material will only be provided as necessary to site personnel involved in the conduct of the trial, the Institutional Review Board (IRB) or local regulatory authorities.

I will obtain written informed consent from each prospective trial subject or each prospective trial subject's legal representative prior to conducting any protocol-specified procedures. The Informed Consent Document (ICD) used will have the approval of the IRB.

I will maintain adequate source documents and record all observations, treatments and procedures pertinent to trial subjects in their medical records. I will accurately complete and submit the electronic case report forms supplied by the Sponsor in a timely manner. I will ensure that my facilities and records will be available for inspection by representatives of Spirita Oncology, the IRB or local regulatory authorities. I will ensure that I and my staff are available to meet with representatives of Spirita Oncology during regularly scheduled monitoring visits.

I will notify the Medical Monitor within 24 hours of any serious adverse events. Following this notification, a written report describing the serious adverse event will be provided to Spirita Oncology as soon as possible, but no later than 5 days following the initial notification.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

1. SYNOPSIS

Protocol Number: STX-101-02	
Name of Sponsor/Company: Spirita Oncology, LLC	
Name of Investigational Product: E6201	
Name of Active Ingredient: (3*S*,4*R*,5*Z*,8*S*,9*S*,11*E*)-14-(Ethylamino)-8,9,16-trihydroxy- 3,4-dimethyl-3,4,9,10-tetrahydro- 1*H*-2-benzoxacyclotetradecine-1,7(8*H*)-dione	
Title of Study: A Phase 1 Study of E6201 for the Treatment of Central Nervous System (CNS) Metastases From *BRAF* V600-Mutated Metastatic Melanoma	
Principal Investigator(s): Hani Babiker, MD (University of Arizona) (Study Chair), and additional study centers as needed to ensure timely study accrual.	
Study Period (30 months): **Date First Subject Enrolled:** November 16, 2018 **Estimated Date Last Subject Completed:** April 2021	**Phase of Development:** 1

Objectives:

***Primary*:**

- To determine the overall rate of response of brain metastases in subjects with CNS metastases due to metastatic melanoma with a *BRAF* V600 mutation who have relapsed or progressed from initial or systemic disease

***Secondary*:**

- To determine the intracranial disease duration of response, duration of stable disease (SD) and time to progression of CNS metastases
- To determine the objective response rate of systemic disease other than in the CNS
- To evaluate progression-free survival (PFS)
- To evaluate overall survival (OS)
- To evaluate the impact of the *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome
- To evaluate the safety of E6201as monotherapy and in combination with dabrafenib in this population

Methodology:

This is a Phase 1 study of E6201 for the treatment of CNS metastases in *BRAF* V600-mutated metastatic melanoma. A total of N = 28 – 34 subjects with melanoma metastasized to the CNS will be included.

Selected subjects will be: both males and females age ≥18 years; histologically confirmed melanoma with *BRAF* V600 mutation with CNS metastasis; archived tumor sample from the primary, recurrent or metastatic disease with documented *BRAF* mutation; no prior treatment with *BRAF* or MEK inhibitors for systemic disease; recovered from all acute toxicities (≥ Grade 1) due to prior immunotherapy; determined to have adequate renal and hepatic function; and no known history of significant cardiac disease.

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Monotherapy Safety Run-in Phase: Following screening, a total of 4 subjects were enrolled. E6201 was administered by intravenous (IV) infusion over a 2-hour period at a dose of 320 mg/m^2 twice weekly (Days 1, 4, 8, 11, 15 and 18) for 3 weeks, repeated every 28 days (1 cycle) until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevented further study participation.

Combination Safety Run-in Phase: Following screening, a total of 6 – 12 subjects are anticipated to establish the recommended doses of E6201 plus dabrafenib. E6201 will be administered by IV infusion over a 2-hour period twice weekly (Days 1, 4, 8, 11, 15 and 18) repeated every 28 days plus dabrafenib orally twice daily (=1 cycle), according to the schedule below.

Dose Level	E6201 (IV)	Dabrafenib (PO)
1	320 mg/m^2 (MTD monotherapy)	150 mg BID (market dose)
-1[a]	240 mg/m^2 (Dose Level -1)	150 mg BID
-2[a]	240 mg/m^2 (Dose Level -1)	100 mg BID (Dose Level -1)
-3[a]	160 mg/m^2 (Dose Level -2)	100 mg BID (Dose Level -1)
-4[a]	160 mg/m^2 (Dose Level -2)	75 mg BID (Dose Level -2)
-5[a]	160 mg/m^2 (Dose Level -2)	50 mg BID (Dose Level -3)

[a] If necessary based on DLT (≥ 1 of 3 or ≥ 2 of 6 subjects experience DLT at the previous dose level)

Subjects who demonstrate clinical benefit (objective response or stable disease) will be allowed to continue therapy with E6201 plus dabrafenib until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

Dose-limiting toxicity (DLT) is defined as any one of the following events:

- Grade 4 hematologic toxicity for > 1 day
- Grade 3 hematologic toxicity with complications (e.g., thrombocytopenia with bleeding)
- Failure of Grade 3 thrombocytopenia, absolute neutrophil count (ANC), hemoglobin to recover to Grade ≤ 1 within 4 weeks despite the use of platelet and red blood cell (RBC) transfusions and/or growth factors
- ≥ Grade 3 non-hematologic toxicity not due to disease progression (excluding Grade 3 nausea, vomiting or diarrhea that is adequately controlled with supportive care and resolves to ≤ Grade 2 within 48 hours, or Grade 3 electrolyte disturbances responsive to correction within 24 hours)
- ≥ Grade 3 liver function tests (LFTs) lasting > 72 hours
- Treatment interruption > 14 days due to toxicity
- Other important medical event

Subjects who require a dose reduction due to toxicity will be considered to have had a DLT.

No DLTs were experienced in the Monotherapy Safety Run-in Phase. If a DLT is experienced in ≥ 2 of 6 subjects in the Combination Safety Run-in Phase during Cycle 1, no further subjects will be enrolled at that dose level. Subjects who experience a DLT may continue treatment at the next lower dose level until disease progression or unacceptable toxicity. The maximum tolerated dose (MTD) is defined as the doses of E6201 plus dabrafenib at which ≤ 1 of 6 subjects experiences a DLT.

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Subjects who experience E6201- and/or dabrafenib-related toxicity requiring a dose reduction may continue treatment at the next lower dose level for either drug until disease progression or unacceptable toxicity.

E6201 dose reductions for suspected E6201-related toxicity will be 240 mg/m^2 twice weekly (Dose Level -1) and 160 mg/m^2 twice weekly (Dose Level -2), administered over the same schedule as above, Days 1, 4, 8, 11, 15 and 18, repeated every 28 days.

Dabrafenib dose reductions for suspected dabrafenib-related toxicity will be 100 mg (Dose Level -1), 75 mg (Dose Level -2) and 50 mg (Dose Level -3) orally twice daily, according to the U.S. Product Label.

A total of 6 subjects will be treated at the combined MTD doses for both drugs in the Combination Safety Run-in Phase before beginning the Expansion Phase.

Expansion Phase: Following screening, an additional cohort of up to N=18 subjects will be treated at the E6201 plus dabrafenib combined MTD. Subjects treated at the MTD in the Combination Safety Run-in Phase will count towards accrual in the Expansion Phase. E6201 will be administered by IV infusion over a 2-hour period at the MTD and schedule determined in the Combination Safety Run-in phase, repeated every 28 days (=1 cycle), and dabrafenib will be administered orally twice daily at the MTD determined in the Combined Safety Run-in phase. Both regimens will be subject to dose reductions for toxicity, as described. Subjects who demonstrate clinical benefit (objective response or stable disease) will be allowed to continue therapy with E6201 plus dabrafenib until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

During the study, a Safety Review Committee (SRC), consisting of the actively recruiting investigators, the Sponsor Medical Monitor and study staff will review data from the Monotherapy and Combination Safety Run-in Phases before beginning the Expansion Phase, and will review data from the Expansion Phase on an ongoing basis.

The statistical objective is the evaluation intracranial disease objective response (OR) rate, defined as complete response (CR) or partial response (PR), on treatment with E6201 plus dabrafenib. The sample size is based on a one-arm binomial design $H_0 \leq 0.05$ ($\leq$ 5% response rate for historical control group) versus $H_1 > 0.05$ (> 5% response rate for experimental group). An objective response (OR) rate of 25% or greater is deemed clinically important. A total of 3 or more responses in a sample size of 18 subjects indicates the true OR rate is $\geq$ 25%, with power = 80%, alpha = 0.05, and provides evidence of clinical utility to move forward.

CNS disease response will be assessed according to 2 methodologies: Response Evaluation Criteria in Solid Tumors (RECIST v. 1.1) and Response Assessment in Neuro-Oncology – Brain Metastases (RANO-BM) and. Clinical benefit is defined as best response of CR, PR or SD.

Non-CNS systemic disease will be assessed according to RECIST v. 1.1.

Blood for hematology and serum chemistry determinations will be collected within 28 days of Cycle 1 Day 1 and on Day 1 of each cycle and at the End of Treatment Visit.

Electrocardiograms (ECGs) will be taken within 28 days prior to Cycle 1 Day 1, pre-infusion and 5 minutes following the end of the E6201 2-hour infusion, on Day 1 of every cycle thereafter, and at the End of Treatment visit.

CNS and non-CNS systemic disease assessments will be based on magnetic resonance image (MRI) or computed tomography (CT) if unable to perform MRI for intracranial disease. Assessments will be obtained at Week 8 and every 8 weeks thereafter until documented progression of disease (PD). Subjects who demonstrate clinical benefit will be allowed to continue therapy until progression of

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disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

Number of Subjects and Centers (planned): A total of 4 subjects were enrolled in the Monotherapy Safety Run-in Phase. A total of 6 – 12 subjects are anticipated in the Combination Safety Run-in Phase. A total of up to 18 subjects will be enrolled in the Expansion Phase for a total N = 28 – 34 for the study. Study centers include University of Arizona (Hani Babiker, MD - Study Chair) and additional study centers as needed to ensure timely study accrual.

Duration of Study: Accrual is expected to be 24 months, with the last subject followed for up to 6 months. The total study duration is expected to be 30 months. The anticipated accrual rate is 1 – 2 subjects per month.

Inclusion Criteria:

- Males and females ≥ 18 years of age
- Histologically or cytologically confirmed Stage IV metastatic *BRAF* V600-mutated melanoma
- Documented metastasis of the primary tumor to the CNS
- *BRAF*-mutation melanoma tumor status will be established prior to entry based on previous *BRAF*-gene analysis reports from a CLIA qualified laboratory. If a report is not available, the mutation analysis will be performed at Screening on archival tissue.
- Other metastatic melanoma systemic disease allowed
- At least one measurable brain metastasis, 0.5 – 3.0 cm, as assessed by MRI ≤ 3 weeks prior to initiation of study treatment, and does not require immediate local intervention (surgery or radiosurgery)
- Prior stereotactic radiosurgery and/or excision of up to 3 brain metastases is allowed > 3 weeks before initiation of study treatment, provided neurological sequelae have resolved completely and at least one measurable metastasis with documented disease progression is present on MRI
- One prior line of immunotherapy for metastatic disease is allowed, if ≥ 2 weeks has elapsed between the end of therapy and initiation of study treatment
- Prior melanoma adjuvant immunotherapy is allowed, if ≥ 6 months has elapsed between the end of therapy and initiation of study treatment
- Prior melanoma adjuvant BRAF/MEK inhibitor therapy is allowed, if ≥ 12 months has elapsed between the end of therapy and initiation of study treatment
- Able to swallow and retain oral medication with no clinically significant gastrointestinal abnormalities that may alter absorption, such as malabsorption syndrome or major resection of the stomach or bowels (Combination Safety Run-in and Expansion Phases of the study only)
- Asymptomatic or symptomatic CNS metastasis is allowed
- Stable dose of corticosteroids for CNS metastasis for ≥ 7 days is allowed
- Patients with seizures due to CNS metastases must be controlled with stable anti-epileptic treatment for ≥ 14 days
- Bisphosphonates and/or denosumab are allowed
- Adequate performance status: Eastern Cooperative Oncology Group (ECOG) ≤ 2
- Life expectancy of ≥ 3 months

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- Adequate hematologic parameters without ongoing transfusional support:
  - Hemoglobin (Hb) ≥ 9 g/dL
  - Absolute neutrophil count (ANC) ≥ 1.0 x 10^9 cells/L
  - Platelets ≥ 75 x 10^9 cells/L
- Adequate renal and hepatic function:
  - Creatinine ≤ 1.5 x the upper limit of normal (ULN), or calculated creatinine clearance ≥ 50 mL/minute x 1.73 m^2 per the Cockcroft-Gault formula
  - Total bilirubin ≤ 2 times the upper limit of normal (ULN) unless due to Gilbert's disease
  - ALT/AST ≤ 2.5 times ULN, or < 5 times ULN for subjects with liver metastases
- Negative serum pregnancy test within 14 days prior to the first dose of study therapy for women of child-bearing potential (WCBP), defined as a sexually mature woman who has not undergone a hysterectomy or who has not been naturally post-menopausal for at least 24 consecutive months (i.e., who has had menses any time in the preceding 24 consecutive months). Sexually active WCBP and male subjects must agree to use adequate methods to avoid pregnancy (oral, injectable, or implantable hormonal contraceptive; tubal ligation; intra-uterine device; barrier contraceptive with spermicide; or vasectomized partner) throughout the study and for 28 days after the completion of study treatment.
- Ability to provide written informed consent

Exclusion Criteria:

- Urgent need of treatment to prevent acute neurologic deterioration, including urgent neurosurgery or radiotherapy
- Symptoms of uncontrolled intracranial pressure
- Symptomatic or untreated spinal cord compression
- Prior treatment with any chemotherapeutic or investigational agent
- Prior treatment with any BRAF and/or MEK inhibitor for metastatic disease
- Prior treatment with > 1 line of immunotherapy for metastatic disease
- Serious cardiac condition within the last 6 months, such as uncontrolled arrhythmia, myocardial infarction, unstable angina or heart disease defined by the New York Heart Association (NYHA) Class III or Class IV
- QT interval corrected for rate (QTc) > 480 msec on the ECG obtained at Screening using Fridericia method for QTc calculation
- Active infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV) requiring systemic antiviral treatment within the last week prior to study treatment
- Other active infection requiring IV antibiotic usage within the last week prior to study treatment
- Any other medical intervention or other condition which, in the opinion of the Principal Investigator, could compromise adherence to study requirements or confound the interpretation of study results
- Pregnant or breast-feeding

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Criteria for evaluation:

***Safety*:** Safety will be assessed through the monitoring of adverse events (AEs), clinical laboratory parameters (hematology, serum chemistry), vital sign measurements, ECGs and physical examinations. Adverse events will be classified according to the Medical Dictionary for Regulatory Affairs (MedDRA) and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

***Efficacy*:** Efficacy assessments will be determined on the basis of MRI and/or CT scans with best treatment response at any protocol-specified time point for intracranial disease, duration of response, duration of stable disease, and time to CNS metastasis disease progression. Response rates also will be determined for non-CNS systemic disease. Progression-free survival (PFS) and overall survival (OS) will be evaluated.

***Pharmacodynamics*:** A correlation will be made between *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome.

Investigational Product, Dosage and Mode of Administration: E6201 for Injection is a natural product analog and inhibitor of mitogen-activated protein kinase/extracellular-signal regulated kinase kinase-1 (MEK1). It is a sterile white lyophilized powder containing 60 mg of E6201 formulated in cyclodextrin to improve E6201 solubility in water. E6201 for Injection is packaged in a 20-mL capacity Type I glass vial with a bromobutyl rubber stopper and an aluminum cap. Reconstituted and diluted E6201 solutions should be stored refrigerated (2 to 8°C) and protected from light before administration.

Reference Therapy: None

Statistical Methods:

***Efficacy Endpoints and Analyses*:** Intracranial response rates will be summarized using number and percentage of subjects with a best response of CR, PR, SD or PD assessed by RECIST v 1.1 and RANO-BM, along with 2-sided, 95% confidence intervals for the proportions. Non-CNS systemic disease response rates will be determined by RECIST v 1.1. For determination of intracranial disease duration of response, duration of stable disease, and time to progression of CNS metastases, and for PFS and overall survival, the Kaplan-Meier product- limit method will be used to estimate the median survival. Subjects who do not have disease progression will be censored at the last follow-up time.

Duration of intracranial disease response or stable disease will be calculated from the date of first response or stable disease to the date of progression or death.

Time to progression of CNS metastases will be calculated from the date of first treatment to the date of first evidence of progression of CNS disease.

Progression-free survival will be calculated from the date of first treatment to the date of first evidence of progression or death.

Overall survival will be calculated from the date of first treatment to the date of death from any cause; subjects who do not experience death will be censored at the last follow-up time.

All efficacy endpoints will be summarized separately for monotherapy and combination therapy phases of the study.

SAS Version 9.3 for Windows (SAS Institute, Cary, NC) or higher will be used for all analyses.

***Pharmacodynamic Endpoint Analyses*:** A correlation will be made between *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome for monotherapy and combination therapy study phases.

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***Safety Endpoints and Analyses*:** Safety endpoints for AEs include the following: incidences of all treatment-emergent adverse events (TEAEs) and all serious adverse events (SAEs); incidences of TEAEs and SAEs by severity; incidences of TEAEs and SAEs by relationship to study medications; incidences of all Grade 3 and 4 TEAEs and by severity and relationship to study medications; and discontinuation of subjects from the study due to AEs or death. Safety endpoints for AEs, clinical laboratory tests, vital signs, ECGs and physical examinations will be specified in the statistical analysis plan. All safety endpoints will be summarized separately for monotherapy and combination therapy phases of the study using descriptive statistics.

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2. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 1: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
A	Adenosine receptor
Abl	Abelson kinase
AE	Adverse event
ALT (SGPT)	Alanine transaminase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase
BBB	Blood brain barrier
BRAF, B-Raf	*BRAF* is the human proto-oncogene that encodes the serine/threonine-protein kinase B-Raf
BUN	Blood urea nitrogen
BZDp	Benzodiazepine receptor
C	Centigrade
CB2	Cannabinoid receptor
CCKA	Cholecystokinin A
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary cells
C_{max}	Peak concentration
CNS	Central nervous system
CR	Complete response
CRF	Case report form
CT	Computed tomography
CTC	Circulating tumor cell
CTCAE	Common terminology criteria for adverse events
CYP	Cytochrome P450
dL	Decaliter
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid

Abbreviation or Specialist Term	Explanation
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal growth factor
EphA2	Ephrin type A receptor
FAS	Full analysis set
FDA	Food and Drug Administration
FLT3	FMS-like tyrosine kinase-3
G1	Gap-1 cell cycle phase
GCP	Good Clinical Practice
G-CSF	Granulocyte colony-stimulating factor
GI	Gastro-intestinal
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GSH	Glutathione
GST	Glutathione-S-transferase
HBV	Hepatitis B virus
HCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
hERG	Human ether a-go-go
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
IC_{50}	Concentration of an inhibitor to reduce the response by one-half
ICD	Informed consent document
ICH	International Conference on Harmonisation
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
IWG	International Working Group
Kg	Kilogram
L	Liter
LDH	Lactate dehydrogenase
LFT	Liver function test

Abbreviation or Specialist Term	Explanation
LTB4	Leukotriene B4 receptor
m^2	Meters squared
MAPK	Mitogen-activated protein kinase
MedDRA	Medical Dictionary for Regulatory Activities
Mg	Milligram
mL	Milliliter
µ	Micro
M	Molar
MEK1	Mitogen-activated protein kinase/extracellular-signal regulated kinase kinase-1
MEKK1	Mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase-1
MRI	Magnetic resonance imaging
MT1	Melatonin ML1A receptor
MTD	Maximum tolerated dose
N	Number
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NK	Neurokinin receptor
NOAEL	No adverse effect level
NYHA	New York Heart Association
OR	Objective response
OS	Overall survival
PD	Pharmacodynamic, progressive disease
PDE	Phosphodiesterase
PFS	Progression-free survival
PK	Pharmacokinetic
PPS	Per protocol set
PR	Partial response
QTc	QT interval corrected for rate
RANO-BM	Response Assessment in Neuro-Oncology – Brain Metastases
RAS/RAF	RAS: family of GTPases that act as molecular switches, to turn on downstream RAF protein kinases (e.g., BRAF).

Abbreviation or Specialist Term	Explanation
RBC	Red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious adverse event
SD	Stable disease
SRC	Safety Review Committee
Src	Family of proto-oncogenic tyrosine kinases
TEAE	Treatment-emergent adverse event
TrkB	Tropomyosin-related kinase
TXA2/PGH2	Thromboxane A2/prostaglandin H2 receptor
U	Units
UDS	Unscheduled DNA synthesis
ULN	Upper limit of normal
UT II	Urotensin II receptor
V	Vasopressin receptor
V600E	*BRAF* gene mutation in which valine (V) is substituted by glutamic acid (E) at amino acid 600
V600K	*BRAF* gene mutation in which valine (V) is substituted by lysine (K) at amino acid 600
V600D	*BRAF* gene mutation in which valine (V) is substituted by aspartic acid (D) at amino acid 600
WBC	White blood cell
WCBP	Woman of child-bearing potential
WT	Wild-type

3. INTRODUCTION

3.1. E6201 Summary

E6201, a synthetic analog of a natural product, is a potent inhibitor of mitogen-activated protein kinase/extracellular-signal regulated kinase kinase-1 (MEK1) and mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase-1 (MEKK1). The RAS/RAF/MEK/ERK signaling pathway has long been viewed as a promising target in the development of novel anticancer therapies, based upon its central role in regulating the growth and survival of cells from a broad spectrum of human tumors.[1] Importantly, MEK1 is downstream of GTPase RAS and serine/threonine kinase RAF proteins, which are often mutated and abnormally active in cancer. Davies et al identified B-type RAF (BRAF) kinase, an upstream kinase of MEK1 in the signal transduction cascade, with somatic missense mutations in 67% of malignant melanomas and 12% of colorectal cancers.[2] All mutations were within the kinase domain with a predominant single substitution such as the valine-to-glutamic acid substitution at amino acid residue 600 (V600E). Mutated BRAF proteins have elevated kinase activity, leading to activation of MEK1, which then triggers extracellular signal-related kinase (ERK) phosphorylation, and activates downstream pathways.[2],[3],[4] These reports strongly suggest that cancers with elevated MEK1 activity may be promising therapeutic targets for E6201.[5] In addition to strong MEK1 inhibition, E6201 inhibits cancer-relevant kinases including the Src family tyrosine kinases (Lyn, Fyn, Lck, Yes, cSrc), tropomyosin-related kinase B (TrkB), FMS- related tyrosine kinase 3 (Flt3), Abelson kinase (Abl), and ephrin type-A receptor 2 (EphA2) kinase activities.[6,7] The results of in vitro and in vivo pharmacology studies show that E6201 may be useful for the treatment of cancers associated with the elevation of MEK1 kinase activity such as the *BRAF* mutation. In cell-systems cultured in vitro, E6201 demonstrated cytostatic growth inhibition of *BRAF*-mutated human cancer cells. In vivo, intermittent intravenous (IV) administration of E6201 caused statistically significant anticancer activity in *BRAF*-mutated human cancer xenografts. Changes in expression levels of phospho-ERK, cyclin D1, and phosphorylated pRb (p-Rb) in cancer cells or tumors may serve as pharmacodynamic (PD) biomarkers for MEK1 signaling pathway inhibition and efficacy of E6201. In addition to changes in these proteins, E6201 inhibited the spontaneous secretion of interleukin-6 (IL-6) and IL-8 in LOX melanoma cell lines. IL-8 has been identified as a transcriptional target of RAS/RAF/MEK signaling,[8] suggesting that its expression might be affected by *BRAF* mutation. Therefore, measurement of these cytokines in plasma and their messenger RNA (mRNA) in tumor tissue may also serve as PD markers to measure biological response to E6201.

The following information is taken from the E6201 for Injection Investigator's Brochure.[9]

3.1.1. Preclinical Pharmacology

E6201 is a potent MEK1 inhibitor with a half-maximal inhibitory concentration (IC_{50}) value of46.3 nmol/L. In addition to strong MEK1 inhibition, E6201 also inhibits the Src family tyrosine kinases (Lyn, Fyn, Lck, Yes, cSrc), TrkB, Flt3, Abl, and EphA2 and MEKK1 kinase activities with IC_{50} values ranging from 15.0 to 163.0 nmol/L. E6201 inhibited growth of 5 human cancer cell lines (SK-MEL-28 and LOX melanomas, MDA-MB-435 breast cancer, DBTRG-05MG glioblastoma, and COLO 205 colon cancer) which carry *BRAF* activating mutation. The IC_{50} values for E6201

in these 5 cell lines ranged from 43.7-463.6 nmol/L. AU-565 human breast cancer cells, without a *BRAF* mutation, were resistant to E6201 (IC_{50} >10 μmol/L). E6201 also inhibited human keratinocyte proliferation stimulated by epidermal growth factor (EGF) which activates the RAS/RAF/MEK/ERK pathway with an IC_{50} value of 160 nmol/L. E6201 was shown to completely block the cell cycle in the gap-1 phase (G1) of the cell cycle in SK-MEL-28 cells with no evidence of a hypodiploid cell population (apoptosis), indicating the cytostatic nature of E6201. The anticancer activity of E6201 was evaluated in vivo following IV administration on a Q4Dx3 schedule in 3 *BRAF*-mutated human cancer xenograft models (LOX melanoma, COLO 205 colon cancer, and DBTRG-05MG glioblastoma). Administration of E6201 at doses of 5, 10, 20, and 40 mg/kg resulted in statistically significant anticancer activity in these 3 models.

In a secondary PD study, E6201 or E6201 Captisol® formulation was examined against a panel of 126 receptors, channels, transporters, and enzymes of important physiological function. In these studies, E6201 showed significant binding or inhibition (>50% at 1 μmol/L) to human adenosine A1 receptor (A1) and enzyme activity of phosphodiesterase, PDE4.

E6201 inhibited IL-2 production by phytohemagglutinin-P-stimulated human lymphocytes with an IC_{50} value of 18 nmol/L.

In vitro studies of E6201 at 10 μmol/L and above caused a statistically significant inhibition of potassium tail current in human ether a-go-go (hERG) complementary deoxyribonucleic acid (cDNA)-transfected Chinese hamster ovary (CHO) cells; the IC_{50} value was 10.6 μmol/L (4128 ng/mL). E6201 at 1 and 10 μmol/L applied by perfusion for 30 minutes to isolated papillary muscles of guinea pigs showed no effects on the action potential parameters.

In vivo studies of E6201 at doses up to 60 mg/kg administered as a 30-minute IV infusion, showed no effects on central nervous system or respiratory function in rats. In dogs, E6201 at doses up to 30 mg/kg administered as a 30-minute IV infusion showed no effects on heart rate (HR), blood pressure (BP), or electrocardiogram (ECG) parameters.

Additional in vivo safety pharmacology studies in anesthetized dogs revealed that both vehicle (30% Captisol) and E6201 (6-30 mg/kg in 30% Captisol) administered as a 5-minute IV bolus injection exhibited effects on cardiopulmonary function, including increased mean BP, systolic left ventricular pressure, left ventricular end-diastolic pressure, mean right atrial pressure, mean pulmonary artery pressure, pulmonary artery wedge pressure, and cardiac output. These effects were possibly due to the physiochemical properties of Captisol (high osmolality and high viscosity). Similar effects were not apparent when E6201 was administered as a 30-minute IV infusion, except for a 36.5% increase in cardiac output observed when E6201 was administered at a dose of 30 mg/kg in 30% Captisol solution compared with the vehicle alone (30% Captisol). E6201 at doses up to 18 mg/kg in 18% Captisol solution administered as a 30-minute IV infusion had no effects on cardiopulmonary function.

3.1.2. Preclinical Pharmacokinetics

Pharmacokinetic (PK) studies were performed in Sprague-Dawley rats and dogs administered E6201 as 30-minute IV infusions on Days 1, 8, and 15 in which E6201 and ER-813010 (the much less active E isomer, trans enone of E6201) were measured. In rats administered doses ranging from 6 to 60 mg/kg, the exposure of E6201 and ER-813010 was proportional to dose increases

with no differences observed between males and females and were similar on Days 1 and 15, thereby suggesting no drug accumulation. In dogs administered doses ranging from 6 to 30 mg/kg, the exposure of E6201 and ER-813010 was roughly proportional to dose increases with slightly higher exposure observed in females than males and were similar on Days 1 and 15, thereby suggesting no drug accumulation. The PK profile of E6201 in rats was characterized by moderately extensive distribution and fast clearance and elimination, while in dogs the distribution was moderately small. The elimination half-life of E6201 averaged < 1.5 hours in rats and dogs.

In vivo studies of E6201 at doses up to 60 mg/kg administered as a 30-minute IV infusion resulted in estimated C_{max} values of 16.2 µg/mL in the male rat and 9.1 to 15.3 µg/mL in the dog. Additionally, E6201 18 mg/kg in 18% Captisol solution administered as a 30-minute IV infusion resulted in an estimated C_{max} value of 7.4 µg/mL and 30 mg/kg in 30% Captisol administered as a 5-minute IV bolus injection resulted in an estimated C_{max} value of 11.6 µg/mL.

In vitro, the major metabolic pathway of E6201 appears to be zusammen/entgegen (Z/E) isomerization mediated by glutathione-S-transferase (GST). Cytochrome P450 (CYP) enzymes CYP1A2, CYP2D6, and CYP3A4/5 appear to convert E6201 into N-de-ethylated and mono-oxygenated metabolites that are then conjugated by GSTs with glutathione (GSH). Additional metabolism of E6201 appears to be through NADPH-dependent reduction and glucuronidation. The Z/E- and NADPH-dependent metabolisms demonstrated species specificity; humans were the moderately active species. Therefore, E6201 metabolism is expected to be catalyzed by CYPs, GSTs, UDP-glucuronosyltransferases (UGTs), and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent reductases.

E6201 did not demonstrate any inhibitory effects on CYP enzymes in vitro. Protein binding in vitro in mouse, rat, dog, and human plasma was greater than 97%, suggesting that a majority of the drug will likely be in a protein-bound state in human studies.

Profile studies of E6201 showed significant binding of E6201 at concentrations of 1 and 50 µmol/L in a variety of targets: adenosine receptors A1, A2, and A3, angiotensin II type 1 receptor (AT1), peripheral benzodiazepine receptor (BZDp), peripheral cannabinoid receptor (CB2), cholecystokinin A receptor (CCKA), dopamine D1 receptor (D1), leukotriene B4 receptor (LTB4), melatonin ML1A receptor (MT1), neurokinin receptors NK1 and NK2, κ-opioid receptor (κ[KOP]), thromboxaneA2/prostaglandin H2 receptor (TXA2/PGH2), urotensin-II receptor (UT-II), vasopressin V2 receptor (V2), calcium and chloride channels, and norepinephrine and dopamine transporters. Significant enzyme inhibition was observed with E6201 at the same concentrations in phosphodiesterases PDE1, PDE2, PDE4, and PDE6.

3.1.3. Preclinical Toxicology

When E6201 was administered as a single IV bolus injection to rats using a Captisol solution (up to 60 mg/kg), pulmonary toxicity was observed. Most of the animals administered either 20 or 60 mg/kg of E6201 showed decreased activity and/or ptosis, which disappeared within 1 day. Some animals also showed severe clinical signs such as bradypnea, resulting in death in some cases; the incidence of mortality was 1/40 (2.5%) and 4/76 (5.3%) at 20 and 60 mg/kg, respectively. In animals with severe clinical signs, especially decedent animals, histopathological findings of perivascular edema/hemorrhage and alveolar edema were observed. These changes suggested that the most probable cause of death was acute pulmonary edema. As they were also

observed in the 30% Captisol/water solution group, the causes of the pulmonary edema were possibly related to the E6201 formulation (30% Captisol solution) rather than E6201 per se (ie, the high osmolality and high viscosity of Captisol). The osmolality of 30% Captisol solution was almost the same as that of 6 mg/mL E6201 in 30% Captisol solution. There was no evidence of degenerative change in the alveoli or any cardiac histopathological changes. These effects were obviated by changing administration from an IV bolus to a 30-minute infusion. These observations suggest that E6201 has little, if any, direct effect on the lung.

In a single IV bolus toxicity study in dogs (6, 18, or 30 mg/kg doses), E6201-related clinical signs were limited to transient decreased activity and abnormal gait at 30 mg/kg. In a PK study, gastrointestinal (GI) toxicity and deteriorating physical condition were observed at 33.22 mg/kg.

Based on the single-dose toxicity studies, repeat-dose IV toxicity studies in rats and dogs (Q7Dx3) were performed using 30-minute infusions to reduce formulation-related pulmonary toxicity. E6201 was administered to rats (6, 20, or 60 mg/kg) and dogs (6, 18, or 30 mg/kg) using a 30% Captisol solution; these doses were the maximum deemed feasible based on the solubility limitations (6 mg/mL) and the maximum dose volume in these animals (10 and 5 mL/kg in rats and dogs, respectively). There were no E6201-related effects observed at any dose in rats, while limited clinical signs of transient tremors, salivation and emesis were observed in dogs at doses ≥18 mg/kg without any clinical pathological or histopathological correlates. Consequently, the no observed adverse effect levels (NOAELs) were determined to be 60 and 6 mg/kg in rats and dogs, respectively, and the maximum tolerated dose (MTD) was ≥30 mg/kg in dogs.

In a 4-week study in rats, E6201 at doses up to 10 mg/kg/day were administered by bolus injection using another prototype formulation (20% hydroxypropyl-β-cyclodextrin solution) with no toxicity (including pulmonary toxicity) induced.

E6201 was negative in the reverse mutation assay in bacteria (Ames test), with or without metabolic activation, and the in vivo rat micronucleus and unscheduled DNA synthesis (UDS) tests. In the mouse lymphoma tk assay using the 3-hour treatment method, E6201 was negative in the absence of S9 mix, but positive in the presence of S9 mix. E6201 was characterized as clastogenic in this assay. However, in both the rat micronucleus assay and the UDS test, results were negative even at very high doses that would have generated sufficient amounts of metabolite(s) with high systemic exposures. Thus, the evidence suggests that risk of E6201 genotoxicity in humans is low.

3.1.4. Phase 1 Solid Tumor Study

This Phase 1 first-in-human study was designed to determine the MTD, dose-limiting toxicities (DLTs) and safety, and establish a recommended E6201 dose in patients with advanced solid tumors, expanded to patients with advanced melanoma. Pharmacokinetics (PK) and preliminary efficacy also were evaluated.[10,11]

Part A (dose escalation): Sequential cohorts received E6201 IV over 30 minutes once-weekly (D1, 8, 15 of a 28-day cycle), starting at 20 mg/m^2 and increased up to 720 mg/m^2.

Part B (expansion): Patients with *BRAF*-mutated or wild-type (WT) melanoma received E6201 320 mg/m^2 IV over 60 minutes once-weekly (D1, 8, and 15 of a 28-day cycle) or 160 mg/m^2 IV twice-weekly (D1, 4, 8, 11, 15, and 18 of a 28-day cycle; *BRAF*-mutated only).

In Part A (n=25), the MTD of E6201 was determined to be 320 mg/m^2 when administered as a 30-minute IV infusion once-weekly for the first 3 weeks of a 28-day cycle. DLTs included QTc prolongation (ie, QTcF prolongation >500 ms or QTcF increase from screening >60 ms) and CNS toxicity (ie, confusion). In Part B (n=30), the E6201 infusion time was increased from 30 to 60 minutes to decrease C_{max} and reduce the potential for QTc prolongation. E6201 320 mg/m^2 once weekly was confirmed as the MTD in Part B. At the time of data cutoff for Part B, no DLTs had been observed.

All subjects in all dose groups reported at least one treatment-emergent adverse event (TEAE). The most common TEAEs were nausea, fatigue, constipation, vomiting, hypokalemia, abdominal pain, and decreased appetite. Eleven (44.0%) subjects in Part A and 19 (63.3%) subjects in Part B had at least one TEAE assessed by the investigator as treatment-related (possibly or probably related to study medication or missing a causality assessment). The most common treatment-related AEs (possibly or probably related) in Part A were nausea (3 [12.0%] subjects), ECG QT prolonged (3 [12.0%] subjects), and hypokalemia (3 [12.0%] subjects). The most common treatment-related AEs (possibly or probably related) in Part B were nausea (8 [26.7%] subjects), vomiting (5 [16.7%] subjects), fatigue (4 [13.3%] subjects), hypokalemia (4 [13.3%] subjects), and decreased appetite (3 [10.0%] subjects). Grade 3 or 4 TEAEs were reported in 11 (44.0%) subjects in Part A and 15 (50.0%) subjects in Part B. The most common Grade 3/4 AEs in Part A were abdominal pain (2 [8.0%] subjects), hyperbilirubinemia (2 [8.0%] subjects), ECG QT prolonged (2 [8.0%] subjects), and syncope (2 [8.0%] subjects). Of these common Grade 3/4 AEs, only one was Grade 4 (abdominal pain). The most common Grade 3/4 AEs were abdominal pain (2 [6.7%] subjects) and dyspnea (2 [6.7%] subjects). No Grade 4 AE occurred in more than one subject. Overall, the incidence of TEAEs across treatment groups was small and variable. No dose-related or mutation-status-related trends were observed.

During Part A of the study when E6201 was administered as a 30-minute IV infusion once-weekly, postbaseline QTcB or QTcF prolongation of 450 to 500 ms was recorded in 0 to 7 (28.0%) subjects at selected assessment time points. Postbaseline QTcB/QTcF >500 ms was recorded in 1 subject in the 480 mg/m^2 dose group. QTcB or QTcF increases from baseline of 30 to 60 ms were recorded in 0 to 5 (20.0%) subjects and QTcB or QTcF increases from baseline of >60 ms were recorded in 0 to 2 (8.0%) subjects at selected assessment time points in Part A. ECG QT prolongation was reported as an AE in 3 (12.0%) subjects in Part A: 1 subject in the 320 mg/m^2 dose group and 2 subjects in the 480 mg/m^2 dose group. All 3 ECG QT prolongation AEs met criteria for DLT. The frequency of QTc prolongation events appeared to be higher in the 320 to 480 mg/m^2 dose groups.

During Part B when E6201 was administered as a ≥60-minute IV infusion once-weekly, postbaseline QTcB or QTcF prolongation of 450 to 500 ms was recorded in 0 to 9 (30.0%) subjects at selected assessment time points. Postbaseline QTcB >500 ms was recorded in 2 subjects in the *BRAF*-WT 320 mg/m^2 once-weekly treatment group. QTcB or QTcF increases from baseline of 30 to 60 ms were recorded in 0 to 7 (23.3%) subjects at selected assessment time points in Part B. A QTc increase from baseline of ≥60 ms was recorded in 1 subject in the *BRAF*-mutated 320 mg/m^2 once-weekly treatment group. ECG QT prolongation was reported as an AE in 1 (3.3%) subject in Part B: 320 mg/m^2 once-weekly *BRAF*-WT dose group. None of the QTc events

in Part B met protocol specified DLT criteria (QTcF prolongation >500 ms or QTcF increase from screening >60 ms). No dose-related or mutation-status-related trends were observed.

In Part A, there was one PR, in a patient with V600E *BRAF*-mutated papillary thyroid cancer (KRAS-WT, PTEN weakly reactive) receiving E6201 480 mg/m^2, who had a response for 4 cycles. In Part B, there were two PRs in patients with V600E *BRAF*-mutated melanoma (primary tumor on right leg, KRAS-WT, AKT-positive, PTEN-positive; primary tumor on right inguinal node, other mutation status unknown due to lack of tissue for testing), who had responses for >40 cycles (36.8 months), and one PR in a patient with *BRAF* WT, KRAS-WT melanoma (primary tumor on nose) who had a response for more than 2 cycles (3.0 months); all 3 patients were receiving 320 mg/m^2 once-weekly. Of particular note, one of the patients with stage IV malignant melanoma (MM) *BRAF*-mutated (V600E) with metastases to the bladder, liver, spleen, left adrenal gland, and brain (right thalamic and left occipital) achieved a durable PR beginning at the end of Cycle 2 and remains on E6201 therapy for > 8 years. The metastases to the liver, spleen, and left adrenal gland in this patient have completely resolved and are not visible on scans. Her brain lesions are currently stable for several years with the thalamic lesion currently barely discernible. This exceptional durable response led the team to conduct a complete exome and transcriptome sequencing analysis of the patient's archival tissue genome that revealed homozygous *BRAF* V600E and homozygous CDKN2A deletion (>90% DNA and RNA alteration frequency).[12]

E6201 exposure was dose-related, with PK characterized by extensive distribution and fast elimination.

A decrease in at least one of the following PD markers was observed in 9 of the 10 subjects with evaluable tumor tissue: p-ERK, Ki67, Cyclin D1, p-Rb. There were no evident trends in any of the circulating tumor cell (CTC) biomarkers with respect to initial E6201 treatment group (320 mg/m^2 once weekly or 160 mg/m^2 twice weekly) or mutation status (*BRAF*-mutated or WT). Although IL-6 and IL-8 derived from subjects could be detected, baseline measurements varied widely from individual to individual. There were no consistent trends in any of IL-6 and IL-8 biomarkers with respect to treatment groups.

In conclusion, an intermittent regimen of E6201 320 mg/m^2 IV once-weekly for the first 3 weeks of a 28-day cycle was reasonably well tolerated in patients with advanced solid tumors with evidence of clinical efficacy, including patients with metastatic melanoma with extension to the brain.

3.2. Rationale for CNS Metastases Indication

3.2.1. Clinical Unmet Need

Brain metastases arise in 10-20% of patients with solid tumors, most commonly in lung cancer, breast cancer and melanoma. Among patients with solid tumor brain metastases, approximately 75% have 1 to 3 lesions.[13] The incidence of brain metastases is expected to increase due to longer survival of patients on improved systemic therapies and increased surveillance for metastases. Prognostic scoring has been developed for patients with brain metastases for specific tumors, so therapy can be individualized to minimize treatment complications.[13] Improved treatment of metastases has been hampered because these patients have often been excluded from clinical trials.

The expanding mass of a brain metastasis underlies the clinical signs and symptoms they cause. Headache is the most common but focal neurologic deficits, changes in cognition, seizures and stroke all occur. Patients who receive only supportive care or steroids have a median survival of only one to 3 months.[14]

Besides systemic therapy against the specific malignancy, treatment for brain metastases consists of whole brain and targeted radiotherapy and surgery. Supportive care consists primarily of glucocorticoids and antiepileptics. Directed systemic therapy is hampered by the blood brain barrier (BBB). Brain metastases have variable BBB protection leading to inconsistencies in responses to systemic therapy.[13] Once brain metastases are established, targeted and immuno-oncology therapies appear to have mixed responses.[13],[15] Delivery of a drug to a brain metastasis would depend on physicochemical variables such as permeability, rate of flow to the metastases and distribution values. The first two would be expected to be increased with small molecules such as tyrosine kinase inhibitors. Micrometastases may be even more protected by the BBB.[16] If a drug cannot access the CNS or brain metastases, the patient may receive little benefit regardless of a systemic response. A drug which is an effective agent against brain metastases is particularly valuable. There are several features about melanoma brain metastases worth noting. A high percentage of patients develop them: up to 75% of melanoma patients will develop a brain metastasis.[17] It is not clear that brain metastases and melanoma are correlated with specific mutations, although patients with NRAS mutations may be at higher risk.[18] Melanoma is generally regarded as radio-resistant and brain metastases appear to respond at roughly the same rate as lesions elsewhere if combined with directed therapy such as vemurafenib.[19] Melanoma brain metastases have shown initial responses to molecularly targeted therapies, e.g., mutant BRAF inhibitors such as vemurafenib and dabrafenib.[20],[21],[22],[23],[24] Patients with melanoma NRAS mutations have responded to MEK1 inhibition.[18] However, initial impressive response rates have been followed by eventual relapse associated with resistance, mainly occurring due to paradoxical activation of the MAPK pathway via heterodimerization of RAF and hyperactivated downstream MEK signaling. Combination therapy with BRAF and MEK inhibitors provides improved responses compared to single-agent therapy.[25],[26],[27] Interestingly, both types of agents are not be expected to cross the BBB, thus, suggesting a disrupted BBB in CNS melanoma, and other brain metastases Thus, the importance of drug penetration through the intact BBB in patients with brain metastases is uncertain.[28] Matched analysis of brain metastases and systemic melanoma tumors suggests the PI-3K/AKT pathway is activated in brain metastases.[17]

While NRAS mutations in melanoma may be associated with increased risk for brain metastases, their occurrence is not absolutely specific and thus any melanoma patient with brain metastases could have any of the mutations associated with melanoma including *BRAF* V600 mutations. The presence of a *BRAF* mutation would need to be assured for patients receiving *BRAF* targeted therapy. This is true for non-melanoma patients as well. *BRAF* mutations have been found in up to 10% of colon carcinomas, papillary thyroid carcinoma's and a variety of lung cancers. Both lung and colon cancer as noted earlier can frequently have brain metastases. There are some suggestive data that over-expression/representation of mutant *BRAF* through a variety of molecular means (e.g. gene amplification, polysomy) are associated with enhanced sensitivity to BRAF inhibitors. Such over-representation appears to be limited to melanomas as opposed to other malignancies based on a review of the literature.[29] However, other variables may be important, such as the extent

of blood brain barrier disruption, presence of mutations in cell transporters leading to changes in drug concentration and other factors.

3.2.2. CNS Penetration – Preclinical Study

A blood brain barrier penetration study of E6201 was conducted in FVBn mice.[30],[31] E6201, given its target activity, may have a role in treating brain metastases of several cancers, especially melanoma. Resistance in peripheral tumor sites may be overcome with the appropriate targeted therapy, however, for the subsequent brain metastases, targeted therapies must reach the metastatic sites that can be behind an intact BBB. Many targeted therapies have been shown to be substrates of efflux transport proteins (p-glycoprotein, mdr1, and breast cancer resistance protein, bcrp) that are expressed on the luminal surface of the BBB capillary endothelial cells. In developing a rational dosing regimen for brain metastases, it is necessary to know the rate, extent, and mechanism of BBB penetration. To date, studies using BRAF/MEK inhibitors have shown low brain penetration relative to plasma.[28],[32],[33],[34]

FVBn mice were administered E6201 as a single dose, and brain-to-plasma E6201 concentration ratios were determined at different time points post-dose. The objective was to evaluate the brain distribution of E6201 in mouse models and examine the role of efflux transporters at the BBB on the CNS exposure of E6201.

Four "BBB genotype" mice were used; FVBn wild-type (control), mdr1a/b (-/-) (p-glycoprotein deficient), bcrp1 (-/-) (brcp deficient), and mdr1a/b(-/-):bcrp1(-/-) (dual knockout of both efflux proteins). LC-MS assays for both plasma and brain concentrations of E6201 were developed and used to determine the concentration-time profile (AUC) in both brain and plasma. Rapid equilibrium dialysis was used to determine unbound fractions of E6201 in brain and plasma, and a brain "partition coefficient" were determined for both total and unbound drug.

Pharmacokinetic data showed brain penetration in all four mouse types with AUC∞ ratios ranging from 2.66 to 5.39. The distribution advantages in the mutated mice versus the wile type (control) were 1.64, 1.39, and 2.03 respectively for mdr1a/b (-/-) (p-glycoprotein deficient), bcrp1 (-/-) (brcp deficient), and mdr1a/b(-/-):bcrp1(-/-) (dual knockout of both efflux proteins). The brain penetration of E6201 in each of the four strains of mice ranged from 11% (wild type) to 22% (dual knock out), where 10%-20% was considered good penetration. Compared to vemurafenib, dabrafenib, trametinib and cobimetinib, E6201 brain penetration was shown to be superior (see Table 2).

Table 2: Brain Penetration of Known BRAF/MEK Inhibitors

BRAF/MEK Drug	Brain Penetration
Vemurafenib[32]	0.4%
Dabrafenib[33]	2.3%
Cobimetinib[34]	2.7%
Trametinib[28]	15%
E6201[30],[31]	266%

3.3. Preclinical Evaluation of E6201 Plus Dabrafenib

Dabrafenib is a 4-(3-aminosulfonylphenyl)-5-(pyrimidin-3-yl) thiazole, and a potent and selective inhibitor of B-RAF kinase activity with a mode of action consistent with adenosine triphosphate (ATP)-competitive inhibition.

In a *BRAF* V600E+ metastatic melanoma patient-derived xenograft (PDX) mouse model, E6201 plus dabrafenib demonstrated increased tumor growth suppression relative to trametinib plus dabrafenib with 2-fold higher E6201 concentrations in tumor versus plasma (Figure 1, Table 3).[35]

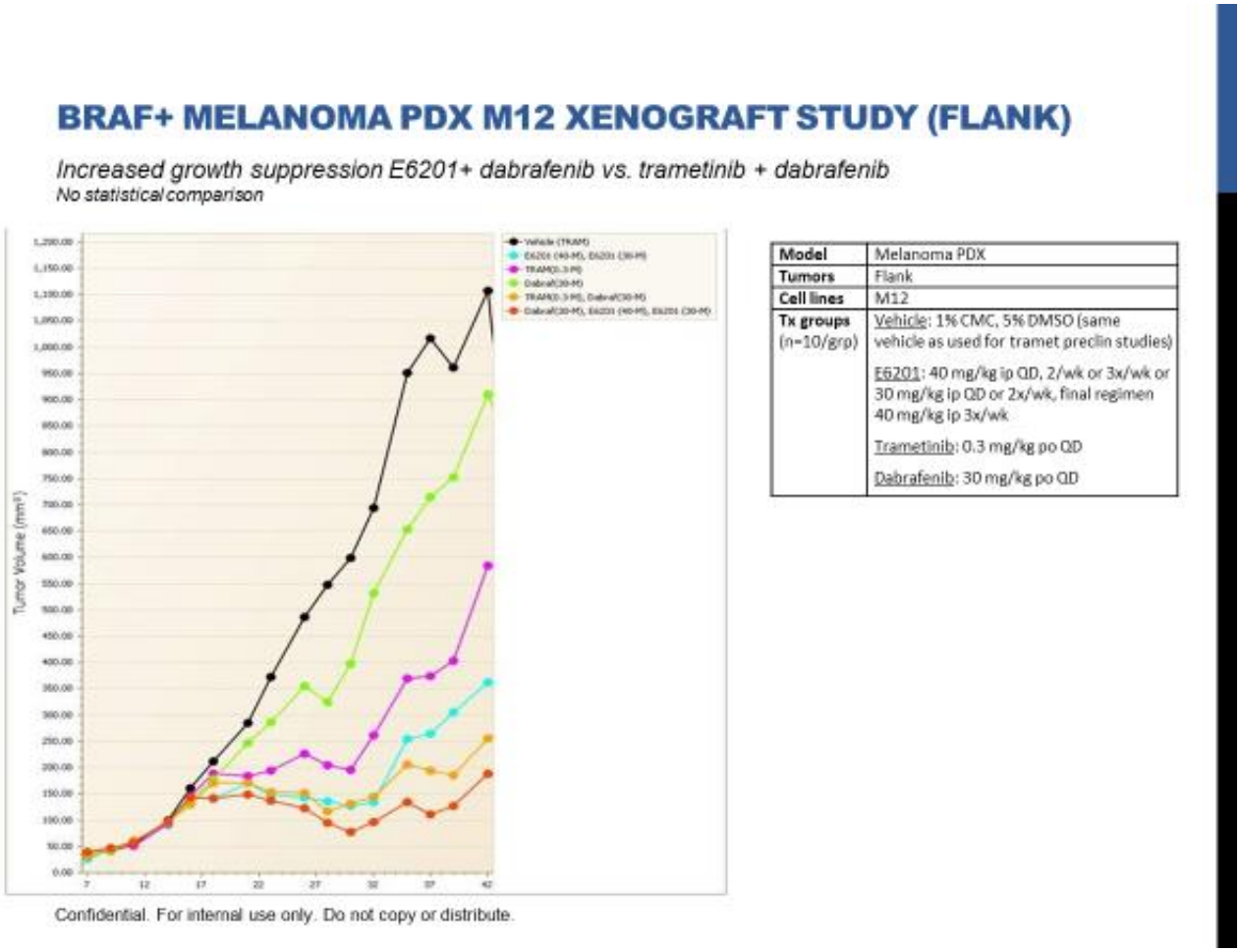

Figure 1: E6201 Plus Dabrafenib *BRAF* V600E+ PDX Metastatic Melanoma Mouse Model

Table 3: *BRAF* V600E+ PDX Metastatic Melanoma Mouse Model, E6201 Tumor:Plasma Concentrations

Group	E6201 Dose (mg/kg)	Mean Plasma Conc. (SD) (µg/mL)	Mean Flank Tumor Conc. (SD) µg/g	T:P Ratio (SD)
N=7	40	3.81 (1.52)	7.08 (1.28)	2.21 (1.34)
N=3	30	0.96 (0.15)	1.57 (0.21)	1.63 (0.05)

3.4. Dose Rationale

3.4.1. E6201

E6201 is a multi-targeted kinase inhibitor that targets both the FLT3 and MAPK pathways. In a Phase 1/2a study in FLT3+ or Ras+ acute myeloid malignancies, a total of 27 subjects were treated with E6201 weekly at doses of 240 and 320 mg/m^2 for 4 weeks repeated q28 days, and twice weekly at doses of 160, 240 and 320 mg/m^2 4 weeks, repeated q28 days. E6201 was well-tolerated at all doses and both weekly and twice weekly schedules of administration. Drug-related adverse events on the twice weekly schedule included increased ALT, increased AST, neutropenia, confusion, delirium and capillary leak syndrome in 1 patient each receiving 160 mg/m^2; thrombocytopenia and nausea in 1 patient each receiving 240 mg/m^2; abnormal liver function test (undefined) and decreased appetite in 1 patient each, and fatigue in 2 patients receiving 320 mg/m^2. Grade 3 or 4 drug-related adverse events on the twice weekly schedule included neutropenia in 1 patient receiving 160 mg/m^2; thrombocytopenia in 1 patient receiving 240 mg/m^2 and fatigue in 1 patient receiving 320 mg/m^2. No drug-related serious adverse events were observed on the twice weekly schedule. [37] However, no objective responses were seen. Biomarkers of FLT3 and MAPK kinase pathway inhibition were evaluated in peripheral blood and bone marrow following dosing. Higher and more durable inhibition of phospho-ERK was observed with 320 mg/m^2 twice weekly versus once weekly dosing.

E6201 has been well tolerated in the current study Monotherapy Safety Run-in Phase at a dose of 320 mg/m^2 twice weekly Days 1, 4, 8, 11, 15 and 18 for 3 weeks, repeated every 28 days. No DLTs were noted and no dose reductions for toxicity. Only 1 E6201-related AE was reported, Grade 2 thrombocytopenia in a patient whose medical history included thrombocytopenia. Two of the 4 subjects achieved stable disease of both intracranial and extracranial disease through C2 and C4, respectively. All 4 subjects have discontinued study due to progressive disease (3) or adverse event (1) (worsening mentation), not related to E6201 study drug.

3.4.2. Dabrafenib

Dabrafenib will be evaluated at the approved dose and schedule for treatment of metastatic melanoma, 150 mg orally twice daily. This dose also was found to be effective and safe for treatment of metastatic melanoma in combination with trametinib in the COMBI-V and COMBI-D studies and in the BREAK-MB study of melanoma metastasized to the brain.[36]

Therefore, to maximize the potential for activity in subjects with CNS metastases from *BRAF*- or MEK-mutated melanoma, under Amendment 3 of the study, E6201 will be evaluated at a dose of 320 mg/m^2 twice weekly Days 1, 4, 8, 11, 15 and 18, repeated every 28 days during the Combination Safety Run-In Phase, plus dabrafenib at a dose of 150 mg orally twice daily, with E6201 and dabrafenib dose reduction criteria for management of toxicity.

4. TRIAL OBJECTIVES AND PURPOSE

4.1. Primary Objective

To determine the overall rate of response of brain metastases in subjects with CNS metastases due to metastatic melanoma with a *BRAF* V600 mutation who have relapsed or progressed from initial or systemic disease

4.2. Secondary Objectives

- To determine the intracranial disease duration of response, duration of stable disease (SD) and time to progression of CNS metastases
- To determine the objective response rate of systemic disease other than in the CNS
- To evaluate progression-free survival (PFS)
- To evaluate overall survival (OS)
- To evaluate the impact of the *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome
- To evaluate the safety of E6201 as monotherapy and in combination with dabrafenib in this population

5. INVESTIGATIONAL PLAN

5.1. Overall Study Design

This is a Phase 1 study of E6201 for the treatment of CNS metastases in *BRAF* V600-mutated metastatic melanoma. A total of N = 28 – 34 subjects with melanoma metastasized to the CNS will be included.

Selected subjects will be: both males and females age ≥18 years; histologically confirmed melanoma with *BRAF* V600 mutation with CNS metastasis; archived tumor sample from the primary, recurrent or metastatic disease with documented *BRAF* mutation; no prior treatment with BRAF or MEK inhibitors for systemic disease; recovered from all acute toxicities (≥ Grade 1) due to prior immunotherapy; determined to have adequate renal and hepatic function, and no known history of significant cardiac disease.

Monotherapy Safety Run-in Phase: Following screening, a total of 4 subjects were enrolled. E6201 was administered by intravenous (IV) infusion over a 2-hour period at a dose of 320 mg/m^2 twice weekly (Days 1, 4, 8, 11, 15 and 18) for 3 weeks, repeated every 28 days (1 cycle), until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevented further study participation.

Combination Safety-Run-in Phase: Following screening, a total of 6 – 12 subjects are anticipated to establish the recommended doses of E6201 plus dabrafenib. E6201 will be administered by IV infusion over a 2-hour period twice weekly (Days 1, 4, 8, 11, 15 and 18) repeated every 28 days plus dabrafenib orally twice daily (=1 cycle), according to the schedule below.

Dose Level	E6201 (IV)	Dabrafenib (PO)
1	320 mg/m^2 (MTD monotherapy)	150 mg BID (market dose)
-1[a]	240 mg/m^2 (Dose Level -1)	150 mg BID
-2[a]	240 mg/m^2 (Dose Level -1)	100 mg BID (Dose Level -1)
-3[a]	160 mg/m^2 (Dose Level -2)	100 mg BID (Dose Level -1)
-4[a]	160 mg/m^2 (Dose Level -2)	75 mg BID (Dose Level -2)
-5[a]	160 mg/m^2 (Dose Level -2)	50 mg BID (Dose Level -3)

[a] If necessary based on DLT (≥ 1 of 3 or ≥ 2 of 6 subjects experience DLT at the previous dose level)

Subjects who demonstrate clinical benefit (objective response or stable disease) will be allowed to continue therapy with E6201 plus dabrafenib until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

Dose-limiting toxicity (DLT) is defined as any one of the following events:

- Grade 4 hematologic toxicity for > 1 day
- Grade 3 hematologic toxicity with complications (e.g., thrombocytopenia with bleeding)
- Failure of Grade 3 thrombocytopenia, absolute neutrophil count (ANC), hemoglobin to recover to Grade ≤ 1 within 4 weeks despite the use of platelet and red blood cell (RBC) transfusions and/or growth factors

- ≥ Grade 3 non-hematologic toxicity not due to disease progression (excluding Grade 3 nausea, vomiting or diarrhea that is adequately controlled with supportive care and resolves to ≤ Grade 2 within 48 hours, or Grade 3 electrolyte disturbances responsive to correction within 24 hours)
- ≥ Grade 3 liver function tests (LFTs) lasting > 72 hours
- Treatment interruption > 14 days due to toxicity
- Other important medical event

Subjects who require a dose reduction due to toxicity will be considered to have had a DLT.

If a DLT is experienced in ≥ 2 of 6 subjects in the Combination Safety Run-in Phase during Cycle 1, no further subjects will be enrolled at that dose level. Subjects who experience a DLT may continue treatment at the next lower dose level until disease progression or unacceptable toxicity. The MTD is defined as the doses of E6201 plus dabrafenib at which ≤ 1 of 6 subjects experiences a DLT.

E6201 dose reductions for suspected E6201-related toxicity will be 240 mg/m^2 twice weekly (Dose Level -1) and 160 mg/m^2 twice weekly (Dose Level -2), administered over the same schedule as above, Days 1, 4, 8, 11, 15 and 18, repeated every 28 days.

Dabrafenib dose reductions for suspected dabrafenib-related toxicity will be 100 mg (Dose Level -1), 75 mg (Dose Level -2) and 50 mg (Dose Level -3) orally twice daily, according to the U.S. Product Label.[36]

A total of 6 subjects will be treated at the combined MTD doses for both drugs established in the Combination Safety Run-in Phase before beginning the Expansion Phase.

Expansion Phase: Following screening, an additional cohort of up to N=18 subjects will be treated at the E6201 plus dabrafenib combined MTD. Subjects treated at the MTD in the Combination Safety Run-in Phase will count towards accrual in the Expansion Phase. E6201 will be administered by IV infusion over a 2-hour period at the MTD and schedule determined in the Combination Safety Run-in phase, repeated every 28 days (=1 cycle), and dabrafenib will be administered orally twice daily at the MTD determined in the Combined Safety Run-in phase. Both regimens will be subject to dose reductions for toxicity, as described. Subjects who demonstrate clinical benefit (objective response or stable disease) will be allowed to continue therapy with E6201 plus dabrafenib until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

During the study, a Safety Review Committee (SRC), consisting of the actively recruiting investigators, the Sponsor Medical Monitor and study staff will review data from the Monotherapy and Combination Safety Run-in Phases before beginning the Expansion Phase, and will review data from the Expansion Phase on an ongoing basis.

The statistical objective is the evaluation of the intracranial disease objective response (OR) rate, defined as complete response (CR) or partial response (PR), on treatment with E6201 plus dabrafenib. The sample size is based on a one-arm binomial design $H_0 \leq 0.05$ (≤ 5% response rate for historical control group) versus $H_1 > 0.05$ (> 5% response rate for experimental group). An objective response (OR) rate of 25% or greater is deemed clinically important. A total of 3 or more

responses in a sample size of 18 subjects indicates the true OR rate is ≥ 25%, with power of = 80%, alpha = 0.05, and provides evidence of clinical utility to move forward.

CNS disease response will be assessed according to 2 methodologies: Response Evaluation Criteria in Solid Tumors (RECIST v. 1.1).[38] and Response Assessment in Neuro-Oncology – Brain Metastases (RANO-BM).[39] Clinical benefit is defined as best response of CR), (PR) or stable disease (SD).

Non-CNS systemic disease will be assessed according to RECIST v. 1.1.

Blood for hematology and serum chemistry determinations will be collected within 28 days of Cycle 1 Day 1 and on Day 1 of each cycle and at the End of Treatment Visit.

ECGs will be taken within 28 days prior to Cycle 1 Day 1, pre-infusion and 5 minutes following the end of the E6201 2-hour infusion, on Day 1 of every cycle thereafter, and at the End-of-Study visit.

CNS and non-CNS systemic disease assessments will be based on magnetic resonance image (MRI) or computed tomography (CT) if unable to perform MRI for intracranial disease. Assessments will be obtained at Week 8 and every 8 weeks thereafter until documented progression of disease (PD). Subjects who demonstrate clinical benefit will be allowed to continue therapy until progression of disease, observation of unacceptable adverse events, intercurrent illness or changes in the subject's condition that prevents further study participation.

Safety will be assessed through the monitoring of adverse events (AEs), clinical laboratory parameters (hematology and serum chemistry), vital sign measurements, ECGs and physical examinations. Adverse events will be classified according to the Medical Dictionary for Regulatory Affairs (MedDRA) and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

Efficacy assessments will be determined on the basis of MRI and/or CT scans with best treatment response at any protocol-specified time point for intracranial disease, duration of response, duration of stable disease, and time to CNS metastasis disease progression based on RECIST v.1.1 and RANO-BM. Response rates also will be determined for non-CNS systemic disease. Progression-free survival (PFS) and overall survival (OS) will be evaluated.

A correlation will be made between *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome.

5.2. Number of Subjects and Centers

A total of 4 subjects were enrolled in the Monotherapy Safety Run-in Phase. A total of 6 – 12 subjects are anticipated in the Combination Safety Run-in Phase. A total of up to 18 subjects will be enrolled in the Expansion Phase for a total of N = 28 – 34 for the study. Study centers include University of Arizona (Hani Babiker, MD), and additional study centers as needed to ensure timely accrual to the study.

5.3. Treatment Assignment

This is an open-label study. In the Monotherapy Safety Run-in Phase, subjects received E6201 by IV infusion over a 2-hour period at a dose of 320 mg/m^2 twice weekly (Day 1, 4, 8, 11,15 and 18)

for 3 weeks of a 28-day cycle. In the Combination Safety Run-in and Expansion Phases, subjects will receive E6201 by IV infusion over a 2-hour period at a dose of 320 mg/m^2 twice weekly (Day 1, 4, 8, 11, 15and 18) of a 28-day cycle, plus dabrafenib at a dose of 150 mg orally twice daily.

5.4. Duration of Study

Accrual is expected to be 24 months, with the last subject followed for up to 6 months. The total study duration is expected to be 30 months. The anticipated accrual rate is 1 – 2 subjects per month.

5.5. Dose Adjustment Criteria

Subjects who experience a toxicity requiring dose reduction may continue at the next lower dose level until disease progression or unacceptable toxicity. Adverse events considered for dose reduction should not include the events assessed by the investigator as exclusively related to underlying disease or other medical condition or concomitant treatment.

5.5.1. Safety Criteria for Adjustment or Stopping Doses

5.5.1.1. Toxicity Grading Criteria

Toxicity grading is based on NCI Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE v4.03); http://evs.nci.nih.gov/ftp1/CTCAE/About.html.

5.5.1.2. Dose Modifications and Dose Reductions

Dose-limiting toxicity (DLT) is defined as any one of the following events:

- Grade 4 hematologic toxicity for > 1 day
- Grade 3 hematologic toxicity with complications (e.g., thrombocytopenia with bleeding)
- Failure of Grade 3 thrombocytopenia, absolute neutrophil count (ANC), hemoglobin to recover to Grade ≤ 1 within 4 weeks despite the use of platelet and red blood cell (RBC) transfusions and/or growth factors
- ≥ Grade 3 non-hematologic toxicity not due to disease progression (excluding Grade 3 nausea, vomiting or diarrhea that is adequately controlled with supportive care and resolves to ≤ Grade 2 within 48 hours, or Grade 3 electrolyte disturbances responsive to correction within 24 hours)
- ≥ Grade 3 liver function tests (LFTs) lasting > 72 hours
- Treatment interruption > 14 days due to toxicity
- Other important medical event

Subjects who require a dose reduction due to toxicity will be considered to have had a DLT.

No DLTs were experienced in the Monotherapy Safety Run-in Phase and no dose reductions were required for toxicity. If a DLT is experienced in ≥ 2 of 6 subjects in the Combination Safety Run-in Phase during Cycle 1, no further subjects will be enrolled at that dose level. Subjects who experience a DLT may continue treatment at the next lower dose level until disease progression or

unacceptable toxicity. The MTD is defined as the doses of E6201 plus dabrafenib at which ≤ 1 of 6 subjects experiences a DLT.

Subjects who experience E6201- and/or dabrafenib-related toxicity requiring a dose reduction may continue treatment at the next lower dose level for either drug until disease progression or unacceptable toxicity. Adverse events considered for dose reduction should not include the events assessed by the investigator as exclusively related to underlying disease or other medical condition or concomitant treatment. If treatment must be delayed for reasons other than toxicity, contact the Medical Monitor to discuss the reasons for delay and plans for resuming study therapy.

The E6201 dose modification guidelines are outlined in Table 4. Provisions are not made for dose levels below 160 mg/m^2 twice weekly. If 160 mg/m^2 twice weekly is determined to be unsuitable based on toxicity as defined above, the subject will be permanently discontinued from E6201 treatment.

Table 4: E6201 Dose Modification Guidelines

E6201-Related Toxicity[a]	During a Cycle	Dose Adjustment for Next Treatment Day
Grade 1		
	Continue treatment	Maintain dose level
Grade 2		
First incidence	Interrupt until resolved to Grade 0-1 or baseline	Maintain dose level
Second incidence	Interrupt until resolved to Grade 0-1 or baseline	240 mg/m^2 twice weekly (75% of dose)
Third incidence despite dose reduction	Interrupt until resolved to Grade 0-1 or baseline	160 mg/m^2 twice weekly (50% of dose)
Fourth incidence despite dose reduction	Discontinue treatment permanently	NA
Grade 3		
First incidence	Interrupt until resolved to Grade 0-1 or baseline	240 mg/m^2 twice weekly (75% of dose)
Second incidence	Interrupt until resolved to Grade 0-1 or baseline	160 mg/m^2 twice weekly (50% of dose)
Third incidence despite dose reduction	Discontinue treatment permanently	NA
Grade 4		
First incidence[b]	Interrupt until resolved to Grade 0-1 or baseline	160 mg/m^2 twice weekly (50% of dose)
Second incidence despite dose reduction	Discontinue treatment permanently	NA

NA = not applicable.

a: Excluding Grade 3 nausea and vomiting not controlled by antiemetics, insomnia, obesity/weight gain, infertility, amenorrhea, galactorrhea, glucose intolerance due to dexamethasone used as an antiemetic, asymptomatic hypercholesterolemia or hypertriglyceridemia, and any ≥ Grade 3 nonhematological toxicities due to disease and disease progression.

b: Grade 4 toxicities judged to be E6201-related and considered life-threatening require discontinuation of study treatment. E6201 treatment following non-life-threatening Grade 4 toxicities could resume following discussion with sponsor.

E6201 Dose modifications for QTc prolongation are defined in Table 5 below.

Table 5: E6201 Dose Modification Guidelines for QTc Prolongation

QTc Criteria	E6201 Dose
QTc greater than 500 msec or ≥ 60 msec from baseline on at least 2 separate ECGs	Withhold until recovery to baseline, then resume at reduced unit dose (50%)
QTc interval prolongation with signs/symptoms of life-threatening arrhythmia	Permanently discontinue

Dabrafenib dose reduction guidelines are outlined in Table 6.

Table 6: Dabrafenib (TAFINLAR®) Dose Reduction Guidelines[36]

Action	Recommended Dose
First Dose Reduction	100 mg orally twice daily
Second Dose Reduction	75 mg orally twice daily
Third Dose Reduction	50 mg orally twice daily
Subsequent Modification	Permanently discontinue if unable to tolerate dabrafenib 50 mg orally twice daily

Recommended dabrafenib dose modification guidelines outlined in the U.S. Product Label are found in Table 7.

Table 7: Dabrafenib (TAFINLAR) Dose Modification Guidelines[36]

Severity of Adverse Reaction[a]	Dosage Modification for TAFINLAR[b]
New Primary Malignancies	
Non-Cutaneous RAS Mutation-positive Malignancies	Permanently discontinue TAFINLAR.
Cardiomyopathy	
• Symptomatic congestive heart failure • Absolute decrease in LVEF of greater than 20% from baseline that is below LLN	Withhold TAFINLAR until LVEF improves to at least the institutional LLN and absolute decrease to less than or equal to 10% compared to baseline, then resume at same dose.
Uveitis	
• Uveitis, including iritis and iridocyclitis	For mild or moderate uveitis that does not respond to ocular therapy, or for severe uveitis, withhold TAFINLAR for up to 6 weeks. • If improved to Grade 0-1, then resume TAFINLAR at same or lower dose. • If not improved, permanently discontinue TAFINLAR.
Febrile Reactions	
• Fever of 101.3°F to 104°F	Withhold TAFINLAR until fever resolves, then resume at same or lower dose.
• Fever higher than 104°F • Fever complicated by rigors, hypotension, dehydration, or renal failure	• Withhold TAFINLAR until fever resolves, then resume at lower dose. Or • Permanently discontinue TAFINLAR.
Skin Toxicity	
• Intolerable Grade 2 • Grade 3 or 4	Withhold TAFINLAR for up to 3 weeks. • If improved, resume TAFINLAR at lower dose. • If not improved, permanently discontinue TAFINLAR.
Other Adverse Reactions[c], including Hemorrhage	
• Intolerable Grade 2 • Any Grade 3	Withhold TAFINLAR. • If improved to Grade 0-1, resume TAFINLAR at lower dose. • If not improved, permanently discontinue TAFINLAR.
• First occurrence of any Grade 4	• Withhold TAFINLAR until improves to Grade 0-1, then resume at a lower dose. Or • Permanently discontinue TAFINLAR.
• Recurrent Grade 4	Permanently discontinue TAFINLAR.

[a]National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.0.
[b]See Table 6 for recommended dose reductions of TAFINLAR.
[c]Dose modification of TAFINLAR is not required for new primary cutaneous malignancies.

5.5.1.3. Schedule Adjustments for Toxicity

- Dosing delays are permitted for either E6201 or dabrafenib. Once a toxicity has resolved to ≤ Grade 1, the subject may resume treatment. The dose level must be reduced to the next lower dose level when treatment is re- initiated following a delay for drug-related toxicity unless otherwise specified.
- The maximum delay allowed between treatment cycles for either E6201 or dabrafenib is 2 weeks. If toxicity has not resolved after 2 weeks of delay, the Medical Monitor should be contacted to discuss permission for resuming treatment after a longer delay for subjects who are experiencing clinical benefit.

5.5.2. Supportive Care Guidelines

- Medications may be administered for the management of symptoms associated with the administration of E6201 or dabrafenib, as required.
- Prophylactic pre-medication will not be used routinely. Adequate treatment for nausea and/or vomiting and diarrhea is permitted during Cycle 1. After Cycle 1, prophylaxis of nausea and/or vomiting and diarrhea is permitted.
- Granulocyte stimulating growth factors (e.g., G-CSF or GM-CSF) are allowed according to standard ASCO guidelines.
- Erythropoiesis-stimulating agents, transfusions, etc. are permitted for management of hematologic toxicities.

5.6. Criteria for Study Termination

If the sponsor, investigators, study monitor or regulatory officials discover conditions arising during the study that indicate that the study should be halted or that the study site should be terminated, this action may be taken after appropriate consultation between the sponsor and investigators.

Conditions that may warrant termination include, but are not limited to, the following:

- The discovery of an unexpected, serious, or unacceptable risk to subjects enrolled in the study
- A decision on the part of the sponsor to suspend or discontinue testing, evaluation, or development of the product
- Failure of an investigator to enroll subjects into the study at an acceptable rate
- Failure of an investigator to comply with pertinent FDA regulations
- Submission of knowingly false information from the study site to the sponsor, study monitor or the FDA
- Insufficient adherence to protocol requirements
- Study termination and follow-up would be performed in compliance with the conditions set forth in 21 CFR 312.50 and 21 CFR 312.56.

6. STUDY POPULATION

6.1. Subject Inclusion Criteria

Subjects must meet all of the following criteria to participate in the study:

- Males and females ≥ 18 years of age
- Histologically or cytologically confirmed Stage IV metastatic *BRAF* V600-mutated melanoma
- Documented metastasis of the primary tumor to the CNS
- *BRAF*-mutation melanoma tumor status will be established prior to entry based on previous *BRAF*-gene analysis reports from a CLIA qualified laboratory. If a report is not available, the mutation analysis will be performed at Screening on archival tissue.
- Other metastatic melanoma systemic disease allowed
- At least one measurable brain metastasis 0.5 – 3.0 cm, as assessed by MRI ≤ 3 weeks prior to initiation of study treatment, and does not require immediate local intervention (surgery or radiosurgery)
- Prior stereotactic radiosurgery and/or excision of up to 3 brain metastases is allowed > 3 weeks before initiation of study treatment, provided neurological sequelae have resolved completely and at least one measurable metastasis with documented disease progression is present on MRI
- One prior line of immunotherapy for metastatic disease is allowed, if ≥ 2 weeks has elapsed between the end of therapy and initiation of study treatment
- Prior melanoma adjuvant immunotherapy is allowed, if ≥ 6 months has elapsed between the end of therapy and initiation of study treatment
- Prior melanoma adjuvant BRAF/MEK inhibitor treatment is allowed if ≥ 12 months has elapsed between the end of therapy and initiation of study treatment
- Able to swallow and retain oral medication with no clinically significant gastrointestinal abnormalities that may alter absorption, such as malabsorption syndrome or major resection of the stomach or bowels (Combination Safety Run-in and Expansion Phases of the study only)
- Asymptomatic or symptomatic CNS metastasis is allowed
- Stable dose of corticosteroids for CNS metastasis for ≥ 7 days is allowed
- Patients with seizures due to CNS metastases must be controlled with stable anti-epileptic treatment for ≥ 14 days
- Bisphosphonates and/or denosumab are allowed
- Adequate performance status: Eastern Cooperative Oncology Group (ECOG) ≤ 2 (APPENDIX A)
- Life expectancy of ≥ 3 months
- Adequate hematologic parameters without ongoing transfusional support:
    - Hemoglobin (Hb) ≥ 9 g/dL

  - Absolute neutrophil count (ANC) ≥ 1.0 x 10^9 cells/L
  - Platelets ≥ 75 x 10^9 cells/L
- Adequate renal and hepatic function:
  - Creatinine ≤ 1.5 x the upper limit of normal (ULN), or calculated creatinine clearance ≥ 50 mL/minute x 1.73 m^2 per the Cockcroft-Gault formula (APPENDIX B)
  - Total bilirubin ≤ 2 times the upper limit of normal (ULN) unless due to Gilbert's disease
  - ALT/AST ≤ 2.5 times ULN, or < 5 times ULN for subjects with liver metastases
- Negative serum pregnancy test within 14 days prior to the first dose of study therapy for women of child-bearing potential (WCBP), defined as a sexually mature woman who has not undergone a hysterectomy or who has not been naturally post-menopausal for at least 24 consecutive months (i.e., who has had menses any time in the preceding 24 consecutive months). Sexually active WCBP and male subjects must agree to use adequate methods to avoid pregnancy (oral, injectable, or implantable hormonal contraceptive; tubal ligation; intra-uterine device; barrier contraceptive with spermicide; or vasectomized partner) throughout the study and for 28 days after the completion of study treatment.
- Ability to provide written informed consent

6.2. Subject Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

- Urgent need of treatment to prevent acute neurologic deterioration, including urgent neurosurgery or radiotherapy
- Symptoms of uncontrolled intracranial pressure
- Symptomatic or untreated spinal cord compression
- Prior treatment with any chemotherapeutic or investigational agent
- Prior treatment with any BRAF and/or MEK inhibitor for metastatic disease
- Prior treatment with > 1 line of immunotherapy for metastatic disease
- Serious cardiac condition within the last 6 months, such as uncontrolled arrhythmia, myocardial infarction, unstable angina or heart disease defined by the New York Heart Association (NYHA) Class III or Class IV (APPENDIX C)
- QT interval corrected for rate (QTc) > 480 msec on the ECG obtained at Screening using Fridericia method for QTc calculation
- Active infection with human immunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV) requiring systemic antiviral treatment within the last week prior to study treatment

- Other active infection requiring IV antibiotic usage within the last week prior to study treatment
- Any other medical intervention or other condition which, in the opinion of the Principal Investigator, could compromise adherence to study requirements or confound the interpretation of study results
- Pregnant or breast-feeding

6.3. Discontinuation of Subjects

6.3.1. Procedures for Withdrawal

Any subject may be removed from study for the following reasons:

- Subject withdrawal of the informed consent
- Subject noncompliance
- An increasing or unexpected pattern of unacceptable toxicity
- Disease progression or confirmed loss of clinical response
- Investigator judgment when the well-being and best interest of the subject is compromised

Subjects experiencing unacceptable toxicity should be removed from the study once complete resolution of toxicity has been documented. Individual subjects may be discontinued from the study by the investigator or sponsor at any time if either determines that it is not in the best interest of the subject to continue.

Any subject who becomes pregnant during the study must be discontinued from the study immediately but should be followed through delivery or termination of the pregnancy. Subjects should also notify the investigator if they become pregnant within 28 days following the last dose of study drug. Spirita Oncology also must be notified if a subject becomes pregnant on study.

If a subject is discontinued from the study before completing the specified duration of treatment, they should be encouraged to complete the end-of-study assessments and to agree to report any serious adverse events for 28 days following the last dose of study drug. The date the subject is withdrawn and the primary reason for discontinuation will be recorded on the case report form (CRF).

6.3.2. Replacement of Study Subjects

Subjects who are screened but do not receive E6201 or dabrafenib in Combination Safety Run-in and Expansion Phases, and subjects treated in Cycle 1 who do not complete 4 of 6 doses (e.g., D1, 4, 8, 11, 15 and18) of their prescribed E6201 study drug treatment, or do not complete 67% of their daily dabrafenib doses, or do not complete the C1D28 evaluation due to progression of disease, withdrawal of consent or non-drug-related adverse events, will be replaced. Subjects who meet one of the above criteria but also experience a DLT will not be replaced with respect to assessment of an MTD for the combination treatment regimen of E6201 plus dabrafenib.

7. TREATMENT OF SUBJECTS

7.1. Description of Treatment Plan

7.1.1. Treatment Duration

Treatment will continue until confirmation of disease progression, unacceptable toxicity, or subject decision to discontinue therapy.

7.2. Concomitant Medications

7.2.1. Permitted Medications

All medications and other treatments taken by subjects 4 weeks before and throughout the study period will be recorded in the CRF module. Any changes in documented, permitted concomitant medications being taken at the beginning of the clinical trial or added during the time the subject is participating in this study (through the End of Treatment Visit) must be recorded in the CRF module.

7.2.2. Prohibited Medications

Concurrent anti-tumor therapy of any kind or any other investigational agent is prohibited. Follow dabrafenib (TAFINLAR) U.S. Product Label recommendations for management of concomitant medications that may cause drug interactions.[36]

7.3. Treatment Compliance

E6201 will be administered by IV infusion in clinic by the clinic staff and dose recorded.

Dabrafenib (TAFINLAR) will be supplied by prescription to the subject by the investigator or trained designee for the subject to take on an outpatient basis between visits. Subjects will be supplied with a Patient Diary to record their daily dabrafenib treatment and any missed doses. The Diary will be reviewed by the clinic staff at each patient visit to assess treatment compliance.

7.4. Randomization and Blinding

Not applicable. This study is a non-randomized, sequential-arm study.

8. STUDY DRUG MATERIALS AND MANAGEMENT

8.1. E6201

8.2. Study Drug Description

E6201 for Injection is a natural product analog and inhibitor of mitogen-activated protein kinase/extracellular-signal regulated kinase kinase-1 (MEK1). It is a sterile white lyophilized powder containing 60 mg of E6201 formulated in cyclodextrin to improve E6201 solubility in water. See Table 8 for a description of the drug product. The final study drug product will be administered at 320 mg/m^2 in a total volume of 250 mL intravenously over a 2-hour period twice weekly.

Table 8: E6201 Investigational Product

	Investigational Product
Product Name:	E6201
Dosage Form:	E6201 is a lyophilized powder reconstituted in water followed by final dilution into 250 mL normal saline
Unit Dose	320 mg/m^2
Route of Administration	Intravenous
Physical Description	White lyophilized powder
Manufacturer	Eisai, Inc.

8.3. Study Drug Packaging and Labeling

E6201 is packaged in a 20-mL Type I glass vial with a bromobutyl rubber stopper and an aluminum cap for intravenous administration. The study drug vials will be labeled with strength and other information as per local regulatory requirements.

8.4. Study Drug Storage

E6201 is demonstrated to be stable when stored in the defined container closure system. E6201 should be stored at 2-8°C and protected from light. Reconstituted and diluted E6201 solutions should be stored refrigerated (2 to 8°C) and protected from light before administration.

8.5. Study Drug Preparation

E6201 will be provided directly from the Spirita Oncology drug distribution center.

Each vial contains 60 mg of E6201 and will be reconstituted with 8.5 mL Sterile Water to provide a solution of 10 mL at a concentration of 6.0 mg/mL and pH of 4.5. Once the Sterile Water is added, gently swirl to mix and let sit until totally dissolved. This may take up to 30 minutes to go into solution. Each reconstituted vial will contain 10 mL of E6201 solution. After reconstitution, the appropriate number of vials may be combined as needed per dose calculation. The resulting

solution will be diluted to 250 mL in 0.9% Sodium Chloride. Reconstituted and diluted E6201 solutions can be stored at 2-8 °C and for up to 24 hours.

8.6. Administration

Subjects should be scheduled to begin study therapy following completion of Screening. E6201 will be administered at the dose level prescribed per protocol by IV infusion over 2 hours (± 10 minutes). In the Monotherapy Safety Run-in Phase, subjects received E6201 twice weekly on days 1, 4, 8, 11, 15 and 18 of a 28-day schedule. In the Combination Safety Run-in and Expansion Phases, subjects will receive E6201 twice weekly on days 1, 4, 8, 11, 15 and 18 of a 28-day schedule.

In the absence of lengthy clinical experience, E6201 should be considered an irritant and precautions should be taken to avoid extravasation. With IV administration of E6201, extravasation may occur with, or without an accompanying stinging or burning sensation (even if blood returns on aspiration of the infusion needle). If any signs or symptoms of extravasation occur, the infusion should be immediately terminated and restarted in another vein. The application of ice over the site of extravasation for approximately 30 minutes may be helpful in alleviating the local reaction.

E6201 must not be given by intramuscular or subcutaneous route.

8.7. Dabrafenib

8.7.1. Description

Dabrafenib mesylate (TAFINLAR) is an inhibitor of some mutated forms of BRAF kinases, including BRAF V600E, V600K, V600D. Dabrafenib also inhibits wild-type *BRAF* and CRAF kinases. Dabrafenib inhibits cell growth of various *BRAF* V600 mutation-positive tumors *in vitro* and *in vivo*.

Dabrafenib is supplied as 50 mg and 75 mg capsules for oral administration. Each 50 mg capsule contains 59.25 mg dabrafenib mesylate equivalent to 50 mg of dabrafenib free base. Each 75 mg capsule contains 88.88 mg dabrafenib mesylate equivalent to 75 mg of dabrafenib free base.

See the dabrafenib (TAFINLAR) U.S. Product Label for additional information on study drug description.[36]

8.7.2. Packaging and Labeling

See the dabrafenib (TAFINLAR) U.S. Product Label.[36]

8.7.3. Storage

See the dabrafenib (TAFINLAR) U.S. Product Label.[36]

8.7.4. Administration

Subjects should be instructed to take dabrafenib at least 1 hour before or at least 2 hours after a meal. Doses should be approximately 12 hours apart. Subjects should be advised to avoid concurrent administration of strong inhibitors of CYP3A4 or CYP2C8. The subject may be advised

to read the FDA-approved patient labeling (Medication Guide) found in the TAFINLAR U.S. Product Label. See TAFINLAR prescribing information for additional information.[36]

8.8. Study Drug Accountability

The investigator must maintain accurate records of receipt of E6201 study drug, dispensing information, and the prompt return or destruction of unused supplies. A drug accountability log will be supplied to each clinical site for purposes of recording E6201 study drug dispensation for the study and will be monitored by Sponsor personnel. If the site has an electronic study drug accountability form that is in keeping with institutional practice and the form collects the same information as the form supplied by the Sponsor, this form may be substituted for the Sponsor's drug accountability form.

8.9. Study Drug Handling and Disposal

Unused or expired E6201 will be destroyed per institutional policy. Unused dabrafenib will be handled according to institutional policy.

9. STUDY PROCEDURES

See Schedule of Study Procedures in APPENDIX D.

9.1. Screening Procedures

The following evaluations are to be performed within 28 days of study treatment to determine subject eligibility:

- Administration of informed consent
- Medical history, physical examination and vital signs
- ECOG Performance Status
- Height and weight
- The following laboratory tests:
  - Hematology
  - Serum Chemistry
- Beta HCG pregnancy test for WCBP
- 12-lead ECG
- Review of concomitant medications
- Baseline CT or MRI of systemic and intracranial disease

9.2. Requirements During Treatment Cycle 1

9.2.1. Cycle 1, Day 1

- Abbreviated physical examination
- Vital signs
- ECOG Performance Status
- Weight
- The following lab tests
  - Hematology, if not performed within previous 24 hours
  - Serum chemistry, if not performed within previous 24 hours
- E6201 study drug administration (Day 1 and Day 4)
- Begin twice daily dabrafenib treatment and provide Dabrafenib Patient Medication Diary to subject (applicable to Combination Phases of study only)
- 12-lead ECG, pre-dose, at 5 minutes after the infusion ends (± 5 minutes)
- Review of concomitant medications

- Assessment of adverse events
- Assessment for survival

9.2.2. Cycle 1, Day 8 (± 1 day)

- Vital signs
- ECOG Performance Status
- E6201 study drug administration (Day 8 and Day 11)
- Continue dabrafenib twice daily treatment
- Review Dabrafenib Patient Medication Diary with subject (applicable to Combination Phases of study only)
- Review of concomitant medications
- Assessment of adverse events
- Assessment for survival

9.2.3. Cycle 1, Day 15 (± 1 day)

- Vital signs
- ECOG Performance Status
- E6201 study drug administration (Day 15 and Day 18)
- Continue dabrafenib twice daily treatment
- Review Dabrafenib Patient Medication Diary with subject (applicable to Combination Phases of study only)
- Review of concomitant medications
- Assessment of adverse events
- Assessment for survival

9.3. Requirements During Treatment Cycles After Cycle 1 (Cycle 2 and beyond) [± 3 days]

- Abbreviated physical examination (Day 1 only)
- Vital signs
- ECOG Performance Status
- Weight (Day 1)
- The following lab tests:
  - Hematology, if not performed within previous 24 hours

  - Serum chemistry, if not performed within previous 24 hours
- E6201 study drug administration (Days 1, 4, 8, 11, 15 and18)
- Continue dabrafenib twice daily treatment
- Review Dabrafenib Patient Medication Diary with subject (applicable to Combination Phases of study only)
- 12-lead ECG pre-dose, at 5 minutes after the infusion ends (± 5 minutes)
- Review of concomitant medications
- Assessment of adverse events
- Assessment for survival
- CT or MRI of systemic and intracranial disease for re-staging (Day 28 of even numbered cycles, ± 7 days)

9.4. At Relapse or Progression of Disease

- Disease assessment for response
- Review of concomitant medications
- Assessment of adverse events
- Assessment for survival

9.5. End of Treatment (28 Days After Last Dose of E6201 and/or Dabrafenib Study Medication ± 5 days)

- Physical examination
- Vital signs
- ECOG Performance Status
- Weight
- The following lab tests
  - Hematology
  - Serum chemistry
- 12-lead ECG
- Review of concomitant medications
- Assessment of adverse events
- Review Dabrafenib Patient Medication Diary (applicable to Combination Phases of study only)
- Assessment for survival

9.6. Long-Term Follow-Up

Long-term follow-up will consist of a clinic visit or telephone call to assess survival every 3 months for up to 6 months.

10. DESCRIPTION OF ASSESSMENTS

10.1. Safety Assessments

Safety will be assessed through the monitoring of adverse events (AEs), clinical laboratory parameters (hematology, serum chemistry), vital sign measurements, ECGs and physical examinations. Adverse events will be classified according to the Medical Dictionary for Regulatory Affairs (MedDRA) and graded according to the National Cancer Institute Common Terminology Adverse Event (NCI CTCAE) version 4.03.

10.2. Safety Parameters

10.2.1. Vital Signs

Vital sign measurements include temperature, blood pressure and pulse rate. Additional measurements may be obtained if clinically indicated. Any value considered clinically significant by the investigator will be recorded as an AE on the CRF. Clinically significant changes compared to baseline values should be followed until clinical resolution.

10.2.2. Weight and Height

Weight will be measured at Screening, Day 1 of each treatment cycle, and the End of Treatment Visit. Height will be measured at screening.

10.2.3. Physical Examination

Complete physical examinations include the following body system evaluations: General Appearance, Skin, Musculo-skeletal, Eyes, Ears, Nose, Throat, Cardiovascular, Chest, Abdomen, Lymph Nodes, and Neurological. Symptom-oriented (abbreviated) evaluations will be performed at study visits where indicated, and otherwise when clinically indicated.

10.2.4. Electrocardiogram (ECG)

ECGs will be taken within 28 days prior to Cycle 1 Day 1, pre-infusion, 5 minutes following the end of the E6201 2-hour infusion, Day 1 of every cycle thereafter (pre-infusion and 5 minutes following the end of the infusion), and at the End of Treatment visit.

10.2.5. Laboratory Assessments

Clinical laboratory tests include hematology and serum chemistry (Table 9).

Table 9: Clinical Laboratory Parameters

Hematology	Serum Chemistry
Red blood cell count	Serum creatinine
Hemoglobin	BUN
Hematocrit	Glucose (non-fasting)
White blood cell count	Albumin
Differential:	AST
Neutrophils	ALT
ANC	LDH
Lymphocytes	Total bilirubin
Monocytes	Total protein
Eosinophils	Alkaline phosphatase
Basophils	Calcium
Platelets	Phosphorus
	Magnesium
	Sodium
	Potassium
	Chloride
	Bicarbonate

10.3. Adverse and Serious Adverse Events

10.3.1. Definition of Adverse Events

10.3.1.1. Adverse Event (AE)

An adverse event (AE) includes any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions as indicated by physical signs, symptoms, and/or laboratory changes occurring whether or not temporally associated with study drug administration and whether or not considered related to study drug. This definition includes an exacerbation of pre-existing medical conditions or events, intercurrent illnesses, hypersensitivity reactions, drug interactions, or clinically significant laboratory findings.

An AE does not include the following:

- Medical or surgical procedures, e.g., tooth extraction, transfusion, surgery (The medical condition that leads to the procedure is to be recorded as an AE.)
- Pre-existing conditions or procedures present or detected at the start of the study that do not worsen

- Hospitalization for elective surgeries or for other situations in which an untoward medical event has not occurred
- Abnormal laboratory value, unless it is clinically significant
- Overdose of study drug or concomitant medication unaccompanied by signs/symptoms (If sign/symptoms occur, the final diagnosis should be recorded as an AE.)
- Pregnancy by itself, unless a complication occurs during pregnancy leading to hospitalization; in this case (The medical condition that leads to the hospitalization is to be recorded as the AE.)
- A significant worsening of the disease under investigation which is captured as an efficacy parameter in this study and, thus, is not to be recorded as an AE.

10.3.1.2. Serious Adverse Event (SAE)

A serious adverse event (SAE) is defined as an adverse event that results in any of the following outcomes:

- Death
- Life-threatening, i.e., immediate risk of death from the event as it occurred; (This does not include an adverse event that, had it occurred in a more serious form, might have caused death.)
- Persistent or substantial disability/incapacitation
- Results in or prolongs an existing inpatient hospitalization
- Congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based on medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

10.3.1.3. Unexpected Adverse Event

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed; or, is not consistent with the risk information described in the protocol or elsewhere. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator's Brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator's Brochure listed only cerebral vascular accidents.

"Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the investigational therapy but are not specifically mentioned as occurring with the investigational therapy.

10.3.1.4. Adverse Event Reporting Period

The adverse event reporting period begins from the date of the first dose of study drug(s) to 28 days following the last dose of study drug(s).

10.3.1.5. Recording of Adverse Events

Each AE should be recorded in standard medical terminology on the AE CRF module. Whenever possible, the AE should be evaluated and reported as a diagnosis rather than as individual signs or symptoms. For example, cough, runny nose, sneezing, sore throat, and head congestion should be reported as 'upper respiratory infection'. If a definitive diagnosis is not possible, the individual signs and symptoms should be recorded. Dates of start (onset) and stop (recovery), action taken, and outcome will be recorded in the AE CRF module.

All clinically significant abnormal changes in laboratory parameters will be recorded as an AE on the AE module, with the following exceptions: clinically significant abnormal laboratory changes determined to be related to the study condition and concomitant conditions, e.g., diabetes, of which the investigator was previously aware and that have not worsened.

The investigator will evaluate all AEs with regard to maximum intensity and relationship to study drug, as follows.

10.3.1.5.1. Maximum Intensity

Maximum intensity should be assigned using one of the severity grades as outlined in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v4.03); if the AE is not specifically listed in CTCAE v4.03, use the following grades:

- Grade 1: mild
- Grade 2: moderate
- Grade 3: severe
- Grade 4: life-threatening or disabling
- Grade 5: death

10.3.1.5.2. Relationship to Study Drug

The degree of certainty with which an AE is attributed to study drug (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of known pharmacology of the study drug and/or reactions of similar nature previously observed with study drug. Each AE will be assigned one of the following five categories:

Not related: There is not a temporal relationship to the study drug (e.g., too early, too late), or there is a reasonable causal relationship to another drug, concurrent illness, or circumstance.

- *Unlikely related*: There is a temporal relationship to study drug, but there is not a reasonable causal relationship between the time of study drug administration and the AE (i.e., it is doubtful the AE is related to the study drug); could be reasonably explained by other

factors, including underlying disease, complications, concomitant drugs, or concurrent treatment.

- *Possibly related*: There is a reasonable temporal sequence from time of study drug administration (e.g., occurred in a time frame relevant to study drug dose); or for which the possibility of the study drug being the causative factor (e.g., existence of similar reports attributed to the study drug; reactions attributable to the pharmacological effect) could not be excluded, although other factors such as underlying disease, complications, concomitant drugs, or concurrent treatment are presumable.
- *Probably related*: There is a reasonable temporal sequence from time of study drug administration; and for which the possibility of factors other than the study drug administration, such as underlying disease, complications, concomitant drugs, or concurrent treatment, could not be excluded as the cause.
- *Definitely related*: Follows a clear temporal sequence from time of study drug administration; could not be possibly explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; follows a response pattern known to be associated with study drug administration.

10.3.1.6. Adverse Event Reporting

Each AE is to be reported by the investigator as serious or non-serious according to the definitions above. This classification determines the regulatory reporting procedures to be followed as described in Table 10.

Table 10: Reporting Guidelines for Adverse Events

Gravity of AE	Reporting Time to Spirita Oncology	Type of Report
Serious	Within 24 hours after the site becomes aware of the event	Initial SAE Report
Non-Serious	Per AE CRF module	Completed AE CRF Module

Any SAE, regardless of relationship to investigational therapy that occurs within 28 days following the last dose of study drug must be reported to the Medical Monitor within 24 hours after the site becomes aware of the event. The investigator is encouraged to discuss with the Medical Monitor any adverse experiences for which the issue of reportability is unclear or questioned. The initial report should be followed by submission of a more detailed SAE Report when follow-up information is available.

If the SAE occurs more than 28 days after the last dose of study drug, SAEs should be reported only if considered related to E6201 or dabrafenib. In the event of subject death, the reason for death should be recorded as the SAE, with 'death' recorded as the outcome on the SAE CRF module.

The SAE also will be recorded as an AE on the AE CRF module. Note: the SAE Report is different from the AE CRF. In areas of both forms where the same data are reported, the forms will be completed in a consistent manner. For example, the same term should be used for the AE on both forms, with the same start and stop dates, action taken, outcome, etc. A checkbox on the AE CRF module for whether the AE resulted in an SAE, will link the two types of report for a given event.

An SAE Report should be prepared with as much available information concerning the event as possible so that a written report can be filed with the appropriate regulatory authorities. If causality cannot be determined definitively at the time of the SAE occurrence, it is important to notify Spirita Oncology within the timeline stated above, and to attribute the relationship as 'Not Assessable' (only applicable for the initial SAE Report). When new significant information is obtained, and the outcome and attribution of the event is known, the investigator will communicate this in a follow-up SAE Report. This relevant information will be provided in a timely manner to allow reporting to regulatory authorities within the required reporting period. Any SAE follow-up information requested by Spirita Oncology should be provided in a timely manner.

As necessary, the SAE Report should be accompanied by relevant pages from the CRFs, e.g., medical history, AEs, concomitant medications. Additional information may be requested by Spirita Oncology in an expedited manner to ensure that the initial reporting of the SAE made to the regulatory authorities complies with the required time frame. Spirita Oncology may be required to collect and report additional information to the regulatory authorities in a follow-up report, containing a final evaluation of the event, including copies of hospital reports, autopsy reports, or other relevant information.

10.3.1.7. Adverse Event and Serious Adverse Event Follow-Up

All AEs and SAEs should be followed until resolution, return to baseline, or until the point it is deemed that further recovery is unlikely. All measures required for AE management and the ultimate outcome of the AE will be recorded in the source document and AE CRF module.

10.3.1.8. 7.1.8. Ongoing Safety Evaluation

A study safety evaluation will be conducted on a regular (monthly) basis by teleconference. Dose exposure, dose-limiting toxicity, AE/SAE profiles and clinical laboratory abnormalities, and other safety measures will be reviewed during each convened meeting. Subject accrual will not be interrupted during the regular scheduled safety evaluations. These discussions will be led by the Spirita Oncology Medical Monitor and Principal Investigator.

10.4. Efficacy Assessments

Efficacy assessments will be determined on the basis of MRI and/or CT scans with best treatment response at any protocol-specified time point for intracranial disease, duration of response, duration of stable disease, and time to intracranial lesion disease progression. Response rates also will be determined for non-CNS systemic disease. Progression-free survival (PFS) and overall survival (OS) will be evaluated.

10.4.1. Efficacy Endpoints

10.4.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects with intracranial OR following E6201 plus dabrafenib treatment. OR for subjects with CNS metastases from metastatic melanoma include a best response of CR or PR, as defined by RECIST v.1.1[38] (APPENDIX E) **and** RANO-BM[39] (APPENDIX F).

10.4.1.2. Secondary Efficacy Endpoints

To evaluate intracranial disease duration of response, duration of SD and time to progression of CNS metastases, progression-free survival and overall survival, as defined below:

- Duration of Response: length of time from the first evidence of objective response (CR, PR) of intracranial disease lesions to the first objective evidence of progression
- Duration of Stable Disease: length of time from first evidence of stable disease of intracranial disease lesions (CR, PR, SD) to for objective evidence of disease progression or death, whichever is earlier
- Time to Progression of CNS Metastases: length of time from date of first administration of study drug to first objective evidence of disease progression of intracranial lesions
- Progression-Free Survival: length of time from the date of first administration of study drug to the first objective evidence of disease progression or death, whichever is earlier
- Overall Survival: length of time from the date of first administration of study drug to the date of death from any cause.

10.4.2. Timing of Assessments to Determine Objective Response

Disease assessments will be made at the end of Cycle 2 every even numbered cycle thereafter. Disease assessments may be made at other time points at the discretion of the Investigator.

10.5. Pharmacodynamic Assessment

A correlation will be made between *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome.

11. STATISTICAL METHODOLOGY

11.1. Determination of Sample Size

The statistical objective is the evaluation of the intracranial disease OR rate, defined as CR or PR, on treatment with E6201 plus dabrafenib. The sample size is based on a one-arm binomial design $H_0 \leq 0.05$ ($\leq$ 5% response rate for historical control group) versus $H_1 > 0.05$ ($>$ 5% response rate for experimental group). An objective response rate (OR) of 25% or greater is deemed clinically important. A total of 3 or more responses in a sample size of 18 subjects indicates the true OR rate is $\geq$ 25%, with power = 80%, alpha = 0.05, and provides evidence of clinical utility to move forward.

11.2. Analysis Populations

The full analysis set (FAS) includes all subjects who are administered any fraction of a dose of either E6201 or dabrafenib study medication. For a particular measure, the per-protocol set (PPS) includes those subjects in the FAS who have a valid baseline and one or more post-treatment assessments for that measure of interest.

The PD population consists of all subjects in the FAS who complete a baseline and at least one follow-up PD assessment.

Efficacy analyses will be conducted separately for monotherapy and combination therapy phases of the study.

11.3. Statistical Analysis Methods

All data will be analyzed using Statistical Analysis System (SAS Version 9.3 or higher for Windows, SAS Institute, Cary, NC). Continuous variables will be summarized using number, mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized using number and frequencies.

11.3.1. Safety Analysis

11.3.1.1. Adverse Events

All safety endpoints will be summarized using descriptive statistics and will be based on the FAS dataset.

All AEs will be coded based on the Medical Dictionary for Regulatory Affairs (MedDRA; Version 17.0 or higher). An AE will be considered a treatment emergent adverse event (TEAE) if the onset is after the first dose of study drug or if the condition was present at baseline but worsened after the first dose.

All AEs for each subject will be listed, including intensity grading, relationship to study drug, action taken and outcome. Subject listings of deaths, SAEs, and AEs leading to treatment discontinuation will be provided. Subject narratives will be provided for deaths, SAEs and other significant AEs. Summary tables will be prepared to examine TEAE severity and relationship to study treatment.

AE summaries will be produced separately for each dose cohort and overall, each disease cohort by dose and overall, and by monotherapy combination phases of the study. All summaries will show, by subject group, dose cohort and overall, the number and percentage of subjects experiencing at least 1 TEAE of each preferred term, arranged by system organ class, and the number of occurrences of the event. Separate summaries will be produced by relationship to study medication, by severity, and for those events with an incidence rate of at least 2% in any group or overall.

SAEs will be summarized in a similar manner; overall, by relationship to study medication, and by severity.

In addition to the above, summaries of the number and percentage of subjects discontinuing the study due to AEs and, due to death, will be presented.

11.3.1.2. Laboratory Data

Laboratory data will be listed by subject. Values above and below normal ranges will be indicated, and whether statistically significant. All laboratory values will be graded according to the NCI-CTCAE version 4.03 criteria. Laboratory data will be summarized by actual value and change from baseline using number of non-missing observations, mean standard deviation, median, minimum and maximum. In addition, shift tables and the incidence of Grade 3 or 4 laboratory values will be presented.

11.3.1.3. Vital Signs

Vital signs will be listed by subject. Values above and below normal ranges will be indicated as will clinical significance. Vital sign data will be summarized by actual value and change from baseline using number of non-missing observations, mean, standard deviation, median, minimum and maximum.

11.3.1.4. Other Safety Data

Data collected for physical examinations, ECGs and related measures will be listed.

11.3.2. Efficacy Analysis

11.3.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects with intracranial OR following E6201 plus dabrafenib treatment. Intracranial response rates will be summarized using number and percentage of subjects with a best response of CR, PR, SD or PD assessed by RECIST v. 1.1 and RANO-BM, along with 2-sided, 95% confidence intervals for the proportions.

11.3.2.2. Secondary Efficacy Endpoints

Non-CNS systemic disease response rates will be determined by RECIST v 1.1. For determination of intracranial disease duration of response, duration of stable disease and time to progression of CNS metastases, and for PFS and overall survival, the Kaplan-Meier product- limit method will be used to estimate the median survival. Subjects who do not have disease progression will be censored at the last follow-up time.

Duration of intracranial disease response or stable disease will be calculated from the date of first response or stable disease to the date of progression or death.

Time to progression of CNS metastases will be calculated from the date of first treatment to the date of first evidence of progression of intracranial lesions.

Progression-free survival will be calculated from the date of first treatment to the date of first evidence of progression or death.

Overall survival will be calculated from the date of first treatment to the date of death from any cause; subjects who do not experience death will be censored at the last follow-up time.

SAS Version 9.3 for Windows (SAS Institute, Cary, NC) or higher will be used for all analyses.

11.3.3. Pharmacodynamic Endpoint Analysis

A correlation will be made between *BRAF* mutational status (e.g., type, heterozygosity or homozygosity) in archival tissue with clinical outcome.

12. STUDY MANAGEMENT

12.1. Data Management

The investigator is responsible for completing and maintaining adequate and accurate source documentation. Source documentation constitutes original records, which may include progress notes, medication administration records, laboratory reports, ECG tracings, discharge summaries, CRF worksheets, etc. Data for this study will be submitted electronically. Access to the database will be provided following a brief on-line training session. Each user will receive a unique username and password, which should not be shared. The investigator must sign the investigator's statement for each subject indicating that the data reported are accurate.

12.2. Monitoring

The sponsor, Spirita Oncology, is responsible for ensuring the proper conduct of the study with regard to ethics, protocol adherence, site procedures, integrity of the data, and applicable laws and/or regulations. At regular intervals during the study and following completion of the study, the sponsor's study monitors will contact the study site via visits to the site, telephone calls, and letters in order to review study progress, CRF completion, and address any concerns or questions regarding the study conduct. During monitoring visits, the following aspects of study conduct will be carefully reviewed: informed consent of subjects, subject recruitment, subject compliance with the study procedures, source data verification, drug accountability, use of concomitant therapy by subjects, AE and SAE documentation and reporting, and quality of data. Records pertaining to these aspects are expected to be kept current.

12.3. Audits and Inspections

Authorized representatives of Spirita Oncology, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of a Spirita Oncology audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization, and any applicable regulatory requirements. The investigator should contact Spirita Oncology immediately if contacted by a regulatory agency about an inspection.

12.4. Amendments

Any amendments to the protocol will be written and approved by Sponsor, Spirita Oncology. All amendments must be submitted to the IRB for approval prior to implementing the changes. In some instances, an amendment requires changes to the informed consent form, which also must be submitted for IRB approval prior to administration to subjects. If any changes to the CRF are required, Spirita Oncology will issue supplemental or revised CRF pages on behalf of the sponsor.

12.5. Institutional Review Board (IRB)

The Principal Investigator must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the subject consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

13. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with Good Clinical Practices and all applicable regulatory requirements, Spirita Oncology, LLC may conduct a quality assurance audit. See Section 12.3 for more details regarding the audit process.

14. ETHICS

14.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. The investigator must submit written approval to Spirita Oncology, LLC before he or she can enroll any subject into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. Spirita Oncology will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

14.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice, applicable regulatory requirements and corporate policy on Ethical Standards (APPENDIX G).

14.3. Written Informed Consent

The Principal Investigator(s) at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any study procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the subject.

15. DATA HANDLING AND RECORDKEEPING

15.1. Health Insurance Portability Accountability Act of 1996

The investigator agrees to comply with all applicable federal, state, and local laws and regulations relating to the privacy of subject health information, including, but not limited to, the Standards for Individually Identifiable Health Information, 45 CFR. Parts 160 and 164 (the Health Insurance Portability Accountability Act of 1996 [HIPAA] Privacy Regulation). The investigator shall ensure that study subjects authorize the use and disclosure of protected health information in accordance with HIPAA Privacy Regulation and in a form satisfactory to the sponsor.

15.2. Financial Disclosure

The investigator shall provide to the sponsor sufficient accurate financial information to allow Spirita Oncology to submit complete and accurate financial certification or disclosure statements to the FDA. The investigator shall promptly update this information yearly and for one year following completion of the study.

15.3. Inspection of Records

Spirita Oncology will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

15.4. Access to Original Records

It is an expectation of regulatory authorities that monitors, auditors, and representatives of national and international government regulatory agency bodies have access to original source documentation to ensure data integrity. “Original” in this context is defined as the first documentation of an observation and does not differentiate between hard copy and electronic records.

15.5. Retention of Records

Study-related records must be retained for at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by applicable regulatory requirements or by an agreement with the Sponsor.

The investigator must not destroy any study-related records without receiving approval from the sponsor. The investigator must notify the sponsor in the event of accidental loss or destruction of any study records. If the investigator leaves the institution where the study was conducted, the sponsor must be contacted to arrange alternative record storage options.

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APPENDIX A. EASTERN COOPERATIVE GROUP (ECOG) PERFORMANCE STATUS SCALE

ECOG PERFORMANCE STATUS*	
Grad	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* From ECOG, Robert Comis, MD, Group Chair

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APPENDIX B. COCKCROFT-GAULT FORMULA FOR CALCULATION OF CREATININE CLEARANCE

Creatinine clearance must either be measured or estimated using the Cockroft-Gault formula, as outlined below.

$$\text{Creatinine clearance (mL/min)} = \frac{(140 - \text{age [years]}) \times \text{weight [kg]}}{\text{serum creatinine [µmol/L]}} \quad \text{(Females)}$$

$$\frac{(140 - \text{age [years]}) \times \text{weight [kg]} \times 1.2}{\text{serum creatinine [µmol /L]}} \quad \text{(Males)}$$

* Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

APPENDIX C. NEW YORK HEART ASSOCIATION (NYCA) CLASSIFICATION FOR HEART FAILURE NYHA CLASSIFICATION - THE STAGES OF HEART FAILURE

Class	Patient Symptoms
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

APPENDIX D. SCHEDULE OF STUDY PROCEDURES

Study Activity	Screening[a]	Treatment Cycle 1			Treatment Cycles after Cycle 1		Progression or Relapse	End of Treatment[l]	Long-Term Follow-Up[n]
		Day 1	Day 8[i]	Day 15[i]	Day 1	End of Cycle 2[j,k]			
Signed ICD	X								
Medical History	X								
Physical Examination	X	X			X[h]			X	
Vital Signs	X	X	X	X	X			X	
ECOG Perform. Status	X	X	X	X	X			X	
Height	X								
Weight	X	X			X			X	
Hematology[b]	X	X			X			X	
Serum Chemistry[c]	X	X			X			X	
Beta-hCG for WCBP	X								
12-lead ECG	X	X[d]			X[d]			X	
Concomitant Medications	X	Continuous							
AE Assessment		Continuous							
Disease Assessment[e]	X					X	X		
E6201 Administration[f]		X (+D4)	X (+D11)	X (+D18)	X (D 1, 4, 8, 11, 15, 18)				
Dabrafenib Dosing[g,m]		X	X	X	X			X[m]	
Assessment of Survival		X			X	X	X	X	X

[a] Screening to be performed within 28 days of Cycle 1, Day 1
[b] Hematology parameters collected at Screening, Cycle 1 Day 1 (only if not performed in the previous 24 hours), Day 1 of each subsequent cycle (within 3 days of Day 1 of each subsequent cycle) and End of Treatment. See Table 9 for tests to be conducted at each time point.
[c] Serum chemistry collected at Screening, Cycle 1 Day 1 (only if not performed within the previous 24 hours), Day 1 of each subsequent cycle (within 3 days of Day 1 of each subsequent cycle) and End of Treatment. See Table 9 for tests to be conducted at each time point.
[d] ECGs performed pre-dose and at the end of the E6201 2-hour infusion (± 5 minutes)
[e] Disease Assessment may include CT, MRI, and/or physical exam
[f] E6201 IV twice weekly Day 1,4, 8, 11, 15 and 18 of each 28-day cycle
[g] Dabrafenib dose orally twice daily, continuously. Provide Dabrafenib Patient Medication Diary.
[h] Abbreviated physical exam
[i] Day 8, 15 (±1 day)
[j] Day 28 (±7 days)
[k] Perform at the end of every even numbered cycle thereafter
[l] End of Treatment visit should be within 28 days from last dose of study medication (±5 days)
[m] Review Dabrafenib Patient Medication Diary (each clinic visit + End of Treatment)
[n] Long-term follow-up for 6 months consists of clinic visits or telephone calls every 3 months to assess survival status

APPENDIX E. RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST 1.1)[38]

1. Measurability of Tumor at Baseline

1.1 Definitions

At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

1.1.1. Measurable

Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable)
- 20mm by chest X-ray

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed. See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

1.1.2. Non-measurable

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥ 10 to < 15mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.1.3. Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

1.2. Specifications by methods of measurements

1.2.1. Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

1.2.2. Method of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and P10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). More

details concerning the use of both CT and MRI for assessment of objective tumour response evaluation are provided in Appendix II of the paper.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response. Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer), have been published. In addition, the Gynecologic Cancer Intergroup has developed CA125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

2. Tumor response evaluation

2.1. Assessment of overall tumor burden and measurable disease

To assess objective response or future progression, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 1). In studies where the primary endpoint is tumor progression (either time to progression or proportion with progression at a fixed date), the protocol must specify if entry is restricted to those with measurable disease or whether patients having non-measurable disease only are also eligible.

2.2. Baseline documentation of 'target' and 'non-target' lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded). For evidence to support the selection of only five target lesions, see analyses on a large prospective database in the article by Bogaerts J, et al, Eur Jour Cancer,2009;45:248-260.

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. As noted in Section 1, pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20mm· 30mm has a short axis of 20mm and qualifies as a malignant, measurable node. In this example, 20mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. In addition, it is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

2.3. Response criteria

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

2.3.1. Evaluation of target lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

2.3.2. Special notes on the assessment of target lesions

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report themas being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate,

however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm.

Lesions that split or coalesce on treatment. As noted in Appendix II of the paper, when non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

2.3.3. Evaluation of non-target lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only *qualitatively* at the time points specified in the protocol.

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

2.3.4. Special notes on assessment of progression of nontarget disease

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some Phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a

measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

2.3.5. New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET (=FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image) at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow- up:

   - If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD.

   - If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan).

   - If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

2.4. Evaluation of best overall response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement (See Section 1.6). Specifically, in non-randomized trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

2.4.1. Time point response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.

When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

Table 1: Criteria for Overall Response for Target (+/- Non-target) Disease

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD
CR = complete response, PR= partial response, SD=stable disease, PD= progressive disease, NE=inevaluable			

Table 2: Criteria for Overall Response for Non-target Disease Only

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	NE

Unequivocal PD	Yes or No	PD
Any	Yes	PD
CR = complete response, PR= partial response, SD=stable disease, PD= progressive disease, NE=inevaluable		

2.4.2. Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50mm with three measured lesions and at follow-up only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

2.4.3. Best overall response: all time points

The best overall response is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

Table 3: Best Overall Response When Confirmation of CR and PR is Required

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PD[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD

CR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise, NE
NE	NE	NE

CR = complete response, PR= partial response, SD=stable disease, PD= progressive disease, NE=inevaluable

[a]If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and, in fact, the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR, and the best response is PR.

2.4.4. Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in **Tables 1–3.**

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/ sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

2.5. Frequency of tumor re-evaluation

Frequency of tumor re-evaluation while on treatment should be protocol specific and adapted to the type and schedule of treatment. However, in the context of phase II studies where the beneficial effect of therapy is not known, follow-up every 6–8 weeks (timed to coincide with the end of a cycle) is reasonable. Smaller or greater time intervals than these could be justified in specific regimens or circumstances. The protocol should specify which organ sites are to be evaluated at baseline (usually those most likely to be involved with metastatic disease for the tumor type under study) and how often evaluations are repeated. Normally, all target and non-target sites are evaluated at each assessment. In selected circumstances, certain non-target organs may be evaluated less frequently. For example, bone scans may need to be repeated only when complete response is identified in target disease or when progression in bone is suspected.

After the end of the treatment, the need for repetitive tumor evaluations depends on whether the trial has as a goal the response rate or the time to an event (progression/death). If ‘time to an event’ (e.g. time to progression, disease-free survival, progression-free survival) is the main endpoint of the study, then routine scheduled re-evaluation of protocol specified sites of disease is warranted. In randomized comparative trials in particular, the scheduled assessments should be performed as identified on a calendar schedule (for example: every 6–8 weeks on treatment or every 3–4 months after treatment) and should not be affected by delays in therapy, drug holidays or any other events that might lead to imbalance in a treatment arm in the timing of disease assessment.

2.6. Confirmatory measurement/duration of response

2.6.1. Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials (see Bogaerts J, et al, Eur Jour Cancer,2009;45:248-260). However, in all other circumstances, i.e. in randomized trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation

of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

2.6.2. Duration of overall response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

2.6.3. Duration of stable disease

Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be taken into account if comparisons between trials are to be made.

2.7. Progression-free survival/proportion progression-free

2.7.1. Phase II trials

This guideline is focused primarily on the use of objective response endpoints for phase II trials. In some circumstances, ‘response rate’ may not be the optimal method to assess the potential anticancer activity of new agents/regimens. In such cases ‘progression-free survival’ (PFS) or the ‘proportion progression-free’ at landmark time points, might be considered appropriate alternatives to provide an initial signal of biologic effect of new agents. It is clear, however, that in an uncontrolled trial, these measures are subject to criticism since an apparently promising observation may be related to biological factors such as patient selection and not the impact of the intervention. Thus, phase II screening trials utilizing these endpoints are best designed with a randomized control. Exceptions may exist where the behavior patterns of certain cancers are so consistent (and usually consistently poor), that a non-randomized trial

is justifiable. However, in these cases it will be essential to document with care the basis for estimating the expected PFS or proportion progression-free in the absence of a treatment effect.

2.7.2. Phase III trials

Phase III trials in advanced cancers are increasingly designed to evaluate progression-free survival or time to progression as the primary outcome of interest. Assessment of progression is relatively straightforward if the protocol requires all patients to have measurable disease. However, restricting entry to this subset of patients is subject to criticism: it may result in a trial where the results are less likely to be generalizable if, in the disease under study, a substantial proportion of patients would be excluded. Moreover, the restriction to entry will slow recruitment to the study. Increasingly, therefore, trials allow entry of both patients with measurable disease as well as those with non-measurable disease only. In this circumstance, care must be taken to explicitly describe the findings which would qualify for progressive disease for those patients without measurable lesions. Furthermore, in this setting, protocols must indicate if the maximum number of recorded target lesions for those patients with measurable disease may be relaxed from five to three. As found in the 'special notes on assessment of progression', these guidelines offer recommendations for assessment of progression in this setting. Furthermore, if available, validated tumor marker measures of progression (as has been proposed for ovarian cancer) may be useful to integrate into the definition of progression. Centralized blinded review of imaging studies or of source imaging reports to verify 'unequivocal progression' may be needed if important drug development or drug approval decisions are to be based on the study outcome. Finally, as noted earlier, because the date of progression is subject to ascertainment bias, timing of investigations in study arms should be the same.

APPENDIX F. RESPONSE ASSESSMENT IN NEURO-ONCOLOGY BRAIN METASTASES (RANO-BM)[39]

Before the RANO brain metastases (RANO-BM) working group convened in 2011, clinical trials of patients with brain metastases were notable for their heterogeneity with respect to patient population, imaging modality, frequency of assessment, definitions of progression, and response (WHO versus modified Macdonald Criteria versus RECIST 1.1 versus ad hoc radiographic assessment criteria), among other features. Even though BMs are the most common malignant brain tumor, affecting up to one third of adults with cancer, the field lacked the common definitions and guidelines for response assessment and clinical trial design that are needed for quality and consistency in trial reporting. RANO-BM proposed response assessment on the basis of literature review and consensus opinion. For clinical trials of systemic agents, the group recommended the use of RECIST 1.1 for non-CNS response assessment and RANO-BM for CNS response assessment. With features that were adopted from RECIST and RANO-high grade gliomas (RANO-HGG) to meet the particular needs of patients with solid tumor brain metastases, RANO-BM response assessment is based on the sum diameter of one-dimensional measurements, corticosteroid dosing, and clinical status (see Table 1 below). Guidance is also provided for cases in which patients were treated with stereotactic radiosurgery or immunotherapy to avoid equating treatment effect with tumor progression.

Several controversies arose during the development of these criteria. As a result of concerns over reproducibility and the interpretation of changes in small lesions as well as to maintain consistency with RECIST 1.1, measurable disease was defined in RANO-BM as a contrast-enhancing lesion that can be accurately measured in at least one dimension with a minimum size of 10 mm. The working group acknowledged that many patients present with subcentimeter brain metastases and, therefore, RANO-BM provides guidance for investigators who choose to lower the minimum size limit of measurable disease to 5 mm. Similarly, the use of one-dimensional versus volumetric assessments was debated within the working group. Ultimately, one dimensional measurements formed the basis of RANO-BM criteria as the group felt that the existing data on volumetric assessments were not strong enough to justify the real-time cost and complexity as for RANO-HGG. However, RANO-BM does provide guidance for those investigators who wish to include volumetric assessment within their trials.

Table 1. Neuro-Oncology Response Criteria for Brain Metastases

Criterion	CR	PR	SD	PD
Target lesions	None	≥ 30% decrease in sum LD relative to baseline	< 30% decrease relative to baseline but < 20% increase in sum LD relative to nadir	≥ 20% increase in sum LD relative to nadir*
Nontarget lesions	None	Stable or improved	Stable or improved	Unequivocal PD*
New lesion(s)†	None	None	None	Present*
Corticosteroids	None	Stable or decreased	Stable or decreased	NA‡
Clinical status	Stable or improved	Stable or improved	Stable or improved	Worse*
Requirement for response	All	All	All	Any‡

NOTE. Reprinted from Lin et al, with permission from Elsevier. (Lin NU, Lee EQ, Aoyama H, et al: Response assessment criteria for brain metastases: Proposal from the RANO group. Lancet Oncol 16:e270-e278, 2015)

Abbreviations: CR, complete response; LD, longest dimension; NA, not applicable; PD, progressive disease; PR, partial response; SD, stable disease.

*Progression occurs when this criterion is met.

†New lesion = new lesion not present on prior scans and visible in at least two projections. If a new lesion is equivocal, for example, because of its small size, continued therapy may be considered and follow-up evaluation will clarify whether it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan showing the new lesion. For immunotherapy-based approaches, new lesions alone do not define progression.

‡Increase in corticosteroids alone will not be taken into account in determining progression in the absence of persistent clinical deterioration.

APPENDIX G. ETHICAL STANDARDS

Ethics and Regulatory Considerations

This study will be conducted according to Good Clinical Practice (GCP), US 21 Code of Federal Regulations (CFR) Part 50, (Protection of Human Subjects), US 21 CFR Part 56 (Institutional Review Boards), International Conference on Harmonisation Guidance for Industry, E6 Good Clinical Practice: Consolidated Guidance, the Nuremberg Code, and the Declaration of Helsinki.

General Instructions

The U.S. Food and Drug Administration (FDA) regulates studies of drugs, biologics, and medical devices. Consequently, these studies are subject to GCP and FDA regulations and guidance issued by the FDA and are included in, but not limited to, the following parts of the CFR and guideline document:

- 21 CFR Part 11 – Electronic Records; electronic signatures
- 21 CFR Part 50 – Protection of Human Subjects
- 21 CFR Part 54 – Financial Disclosure
- 21 CFR Part 56 – Institutional Review Boards
- 21 CFR Part 312 – Investigational New Drug Application
- FDA Guidance for Industry: Oversight of Clinical Investigations – A Risk-Based Approach to Monitoring, August 2013
- FDA Guidance for IRBs, Clinical Investigators, and Sponsors, June 2010
- FDA Guidance for Industry: Investigator Responsibilities – Protecting the Rights, Safety, and Welfare of Study Subjects, October 2009
- FDA Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE studies, December 2012
- Guidance for Industry E6 Good Clinical Practice: Consolidated Guidance, 1996

Copies of these materials are available from the sponsor upon request. The purpose of these regulations and legal obligations is to define the standards and principles for the proper conduct of clinical trials that have been developed by the medical, scientific, and regulatory communities. They are not intended to impede or restrict clinical research.

The ethical standards defined within GCP are intended to ensure that:

- Human subjects are provided with an adequate understanding of the possible risks of their participation in the study, and that they have a free choice to participate or not;
- The study is conducted with diligence and in conformance with the protocol in such a way as to insure the integrity of the findings;
- The potential benefits of the research justify the risks.
- Spirita Oncology, LLC is the Sponsor of the IND. The Sponsor is responsible for the following:

- Selecting qualified investigators,
- Providing investigators with the information they need to properly conduct an investigation,
- Ensuring proper monitoring of the investigation,
- Ensuring that the study is conducted according to the general investigational plan and protocols contained in the IND,
- Maintaining the IND, and
- Ensuring that FDA and all participating investigators are properly informed of significant new information regarding adverse effects or risks associated with the drug being studied.