PROTOCOL TITLE: ONC201 in Newly Diagnosed Diffuse Intrinsic Pontine Glioma and Recurrent/Refractory Pediatric H3 K27M Gliomas

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Oncoceutics, Inc.
3675 Market Street
Suite 200
Philadelphia, PA 19104

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Study Summary

Title	ONC201 in Newly Diagnosed Diffuse Intrinsic Pontine Glioma and Recurrent Pediatric H3 K27M Gliomas
Short Title	ONC201 in Pediatric H3 K27M glioma
Oncoceutics Protocol Number	ONC014
IND	136090
Regulatory Sponsor	Oncoceutics, Inc.
Phase	Phase 1
Methodology	Multicenter, open-label, seven arm, dose escalation and dose expansion, phase I study
Study Duration	Approximately 24 months from start of screening to last subject processed and finishing the study
Objectives	**Primary Objective**: • To determine the recommended Phase II dose of ONC201 in pediatric glioma patients as a single agent and in combination with radiation **Secondary Objectives:** • Assess the safety, toxicity and tolerability of ONC201 in pediatric patients with recurrent H3 K27M Gliomas • Assess the pharmacokinetics associated with administration of oral capsules or liquid formulation. • Estimate the median PFS, PFS at 6 months (PFS6), median OS, OS12, overall response rate, median duration of response
Exploratory Objectives	• Assess the association of clinical outcomes with tumor markers including: location (e.g. thalamus, brainstem, spinal cord); Histone H3 mutation (*H3F3A* / H3.3 vs *HIST1H3B* / H3.1); dopamine receptor D2 (DRD2) and dopamine receptor D5 (DRD5) expression • Assess the association of clinical outcomes with circulating markers including: Induction of serum prolactin; immune cytokines and effectors in the serial serum samples by ELISA • Correlation between H3 K27M mutation detected in tumor and cerebrospinal fluid specimens; • Changes in cranial nerve palsy scoring. • Determine intratumoral and CSF ONC201 concentrations.

Arms	Arm A & G: Pediatric patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) and have completed at least one line of prior therapy. Evidence of progression is not required so that ONC201 may be administered to patients in the maintenance setting or to patients with recurrent/refractory disease. If H3 K27M status of tumor is unknown or archival tumor tissue is not available, then patients must agree to submit a post-mortem biopsy specimen. Arm B: Pediatric patients with newly diagnosed diffuse intrinsic pontine glioma (DIPG), defined as tumors with a pontine epicenter and diffuse involvement of the pons, are eligible with or without histologic confirmation. If H3 K27M status of tumor is unknown or archival tumor tissue is not available, then patients must agree to submit a post-mortem biopsy specimen. Arm C: Pediatric patients with midline gliomas are eligible with or without histologic confirmation and must be eligible for tumor biopsy as deemed by the site Investigator. Arm D: Pediatric patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) and have completed at least one line of prior therapy and must be willing to undergo serial lumbar puncture to obtain cerebrospinal fluid (CSF). These patients will undergo lumbar puncture during sedation (as required) for MRI. Local anesthesia for spinal tap is also allowed. Arm E: Pediatric patients with glioma who are positive for the H3 K27M mutation and/or who have DIPG and who are 2-12 weeks from completion of first-line radiation. Arm F: Pediatric patients with previously-treated, histologically confirmed high-grade glioma with a known H3 K27M mutation, evidence of progressive disease contrast-enhanced brain MRI as defined by RANO-HGG criteria. Prior therapy with at least radiotherapy (RT) is required.
Major Eligibility	• 2 to less than 19 years of age. • Evaluable disease by RANO. • Karnofsky ≥ 50 for patients ≥ 16 years of age, and Lansky ≥ 50 for patients < 16 years of age.

Anticipated Number of Subjects	Arm A: 21 patients (9 dose escalation; 12 dose expansion) Arm B: 24 patients (12 dose escalation; 12 dose expansion) Arm C: 12 patients Arm D: 24 patients Arm E: 24 patients Arm F: 12 patients Arm G: 12 patients
Study Drug	Oral ONC201 provided as 125 mg capsules in Arms A, B, C, D, and F. For Arm E, oral ONC201 will be administered as a liquid formulation in Ora-Sweet. Subjects will be treated with oral ONC201 once every week. For Arm G, oral ONC201 will be administered on two consecutive days of each week. Dosing will be based on body weight and according to the dose cohort the patient is assigned to.
Duration of administration	ONC201 treatment will continue until confirmation of both radiographic and clinical disease progression, unacceptable toxicity, or withdrawal of consent, whichever comes first.
Statistical Methodology	Dose escalation will proceed according to a standard 3 + 3 design to define a recommended phase 2 dose (RP2D) for Arms A, B, E and G. RP2D will be defined as the maximum tolerated dose (MTD) or maximum administered dose (MAD). Following RP2D definition, a 12 patient dose-expansion will be initiated in Arms A, B, and E. Arms C, D and F will use the RP2D defined in Arms A or B, as appropriate. One cycle will be defined as 21 days.

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1. OBJECTIVES

The primary objective of this Phase I trial is to determine the recommended phase 2 dose (RP2D) of ONC201, an oral small molecule imipridone DRD2 antagonist, in pediatric patients with H3 K27M glioma as a single agent or newly diagnosed DIPG in combination with radiation.

1.1 General Study Design

This is a multicenter, open-label, seven arm, dose escalation and dose expansion, phase I study.

Arm A will define the RP2D for single agent ONC201 in pediatric patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) and have completed at least one line of prior therapy. This will allow for recurrent patients and also patients who have not yet recurred, but have completed radiation and will inevitably recur based on prior clinical experience and the literature.

Arm B will define the RP2D for ONC201 in combination with radiation in pediatric patients with newly diagnosed DIPG. If H3 K27M status of tumor is unknown or archival tumor tissue is not available, then patients must agree to submit a post-mortem biopsy specimen to participate in either arm.

Arm C will determine intratumoral drug concentrations and biomarker expression in pediatric patients with midline gliomas.

Arm D will determine H3 K27M DNA levels and drug concentrations in the CSF of pediatric H3 K27M-mutant glioma patients.

Arm E will determine the RP2D for single agent ONC201 administered as a liquid formulation in Ora-Sweet to patients with DIPG and/or H3 K27M glioma. All patients must be 2-12 weeks from completion of first-line radiation.

Arm F is a dose expansion cohort to confirm the safety and estimate the efficacy in a recurrent H3 K27M-mutant glioma population at the RP2D.

Arm G will define the RP2D for single agent ONC201 given on two consecutive days of each week in pediatric patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) and have completed at least one line of prior therapy. This will allow for recurrent patients and also patients who have not yet recurred, but have completed radiation and will inevitably recur based on prior clinical experience and the literature.

Arm A, B, and E will involve a 3 + 3 dose escalation design followed by a 12-patient dose expansion cohort. Arm G will involve a 3 + 3 dose escalation design.

Once Arm A has completed its first dose escalation, Arm B, E and G may initiate after completion of the first cohort in Arm A and can subsequently dose escalation or de-escalate independently. Arms C, D and F will use the RP2D defined in Arms A or B for previously treated or newly diagnosed patients, respectively.

After screening procedures and registration, subjects in Arms A-F will be treated with oral ONC201 once every week at a dose determined by their body weight and dose cohort. Arm G subjects will be treated with oral ONC201 twice per week on two consecutive days at a dose determined by their body weight and dose cohort. All subjects will remain on study until confirmed radiographic and clinical progression, unacceptable toxicity, death, withdrawal of consent, or another protocol criterion for subject withdrawal is met, whichever comes first. One treatment cycle will be defined as 21 days (3 weeks), corresponding to 3 doses of ONC201 for Arms A-F and 6 doses of ONC201 for Arm G.

Subjects will be evaluated for safety if they have received any study drug. Toxicity assessments will be continuous during the treatment phase. Adverse events will be monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Subjects will be evaluated for efficacy every 8 weeks with neuroimaging and clinical evaluation. Neuroimaging studies with contrast-enhanced brain MRI will be performed at baseline and every 8 weeks after treatment initiation. Tumor response will be evaluated by the Response Assessment in Neuro-Oncology (RANO) criteria for both HGG and low-grade glioma (LGG) for each patient.

End of treatment assessments will be performed within 30 days after last drug administration, unless the subject is unable to travel due to deteriorating medical condition. Post-treatment, all participants will be followed until resolution or stabilization of any serious or reportable adverse events occurring during treatment or starting within 30 days of last study drug. Assessments may continue for ongoing reportable adverse events.

Subjects who discontinue study drug must continue to be followed for collection of outcome and/or survival follow-up data until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up. Following the end of treatment assessments, all subjects will be contacted every 30 days (±7 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail.

1.2 Primary Objective

To determine the RP2D of ONC201 in pediatric glioma patients as a single agent and in combination with radiation.

1.3 Secondary Objectives

- Assess the safety, toxicity and tolerability of ONC201 in pediatric patients with H3 K27M gliomas.
- Assess pharmacokinetics associated with administration of oral capsules or liquid formulation.
- Estimate the median PFS, PFS at 6 months (progression-free survival at 6 months), median OS, OS12, overall response rate, median duration of response.

1.4 Exploratory Objectives

- Assess the association of clinical outcomes with tumor markers including: location (e.g. thalamus, brainstem, spinal cord); Histone H3 mutation (H3F3A / H3.3 vs HIST1H3B / H3.1); DRD2 and DRD5 expression;
- Assess the association of clinical outcomes with circulating markers including: Induction of serum prolactin; immune cytokines and effectors in the serial serum samples by ELISA;
- Correlation between H3 K27M mutation detected in tumor and cerebrospinal fluid specimens;
- Correlate changes in cranial nerve palsy scoring with clinical outcomes.
- Determine intratumoral and CSF ONC201 concentrations.

1.5 Endpoints

Safety
- Dose Limiting Toxicities
- Adverse events
- Laboratory evaluations
- KPS status
- Vital signs
- Physical examinations

Efficacy
- Objective response rate by RANO-HGG
- Objective response rate by RANO-LGG
- Duration of Response by either RANO-HGG or RANO-LGG
- Progression-free survival by RANO-HGG
- Progression-free survival by RANO-LGG
- Overall survival

Other
- Pharmacokinetic parameters
- Cranial nerve palsy score

2. BACKGROUND

2.1 Study Disease

ONC201 inhibits dopamine receptor D2 (DRD2) to cause downstream activation of ATF4, which causes induction of genes that lead to apoptosis. DRD2 antagonism also downregulates Akt and ERK activity to cooperatively induce complementary downstream apoptotic effects. These major oncogenic signaling pathways and key molecules are known to drive tumor genesis in pediatric high-grade glioma and diffuse intrinsic pontine glioma (DIPG). Clinical responses seen in tumors with H3.3 K27M mutation with ONC201, also rationalizes the use of this drug in tumors which harbor the mutation. It is also well known now that somatic mutations of the H3F3A and HIST1H3B genes encoding the histone H3 variants, H3.3 and H3.1, were recently identified in high-grade gliomas arising in the thalamus, pons and spinal cord of children and young adults (Solomon DA et al Brain Pathol 2016). Almost all DIPG tumors are now known to have this mutation (Castel D et al Acta Neuropathol 2015). Based on various mechanisms of ONC201 anti-tumor effects in malignancies harboring these genetic aberrations (including tumors in specific location like mid-line glioma and brain stem gliomas known to have these aberrations), suggests that use of ONC201 could be an attractive therapeutic strategy for these tumors that have failed standard treatments. Histone H3 K27M mutant gliomas have recently been recognized in the WHO as a distinct disease entity with a poor prognosis and are graded IV of IV (Louis et al, 2016).

Histone H3 is one of the proteins that comprise the octameric nucleosome and there are 3 variants: H3.1, 3.2, and 3.3. H3.3 is a replication-dependent H3 variant that represents only a small portion of the total cellular histone H3 pool (Bush et al, 2013). Recently, recurring heterozygous hotspot mutations in *H3F3A* and *H3F3B*, the only two genes in mammals that encode H3.3, were discovered in pediatric and young-adult high-grade astrocytomas. These mutations include K27M (lysine 27 to methionine amino acid substitution), as well as other recurrent amino acid substitutions at positions G34 and K36, of which the predominant mutations include G34R/V and K36M. Although both *H3F3A* and *H3F3B* encode H3.3 with identical amino acid sequences, the H3.3 K36M mutation occurs predominantly in *H3F3B* whereas the other mutations are almost exclusive to *H3F3A* (Behjati et al, 2013).

Different mutations in H3.3 segregate by distinct types of tumors. The K27M mutation is prevalent in pediatric diffuse intrinsic pontine glioma and high-grade astrocytomas primarily restricted to midline locations (spinal cord, thalamus, pons, brainstem) in children and younger adults (Schwartzentruber et al, 2012; Sturm et

al, 2012; Khuong-Quang et al, 2012; Aihara et al, 2014). The G34R/V mutations predominantly associate with pediatric glioblastoma in the cerebral hemispheres (Schwartzentruber et al, 2012). The mechanisms by which histone H3 alterations mediate their oncogenic effects are still poorly understood, although there is some evidence that H3 K27M mutations alter global epigenetic states, including lower overall histone H3 K27M trimethyl (H3K27me3) levels and reduced polycomb repressive complex 2 (PRC2) activity.

Midline gliomas harboring histone H3 mutations are considered to have a dismal prognosis with a designation of WHO grade IV. No prospective trials exclusive to histone H3 K27M mutant gliomas have yet been conducted and therefore prospectively collected outcome data are lacking. However, survival data are well published for pediatric diffuse intrinsic pontine glioma (DIPG), the vast majority of which contain histone H3.3 or H3.1 K27M mutations (Schwartzentruber et al, 2012; Sturm et al, 2012; Wu et al, 2012). In pediatric DIPG, from the time of diagnosis, median overall survival is 9 months, and no chemotherapy has proven efficacy in this disease and no therapy has proven effective at progression after radiotherapy (Cohen et al, 2011; Bailey et al, 2013; Rizzo et al, 2015; Chassot et a, 2012). Novel therapies are desperately needed for treatment of H3 K37M mutant gliomas.

2.2 IND Agent

ONC201 is a first-in-class small molecule (imipridone) selective DRD2 antagonist that functions by activating the integrated stress response (ISR) in tumors cells, leading to downstream anticancer effects that include inactivation of prosurvival Akt and ERK signaling along with induction and activation of the TRAIL apoptosis pathway (Allen et al., 2013). ONC201 has demonstrated broad spectrum antitumor efficacy in numerous solid and liquid tumor preclinical models, including cell lines and patient sample that are refractory to chemotherapy and targeted therapies, independent of mutations such in genes such as p53, *KRAS*, Raf, EGFR. ONC201 is orally available, has demonstrated a wide therapeutic window preclinically, is highly stable and water soluble, and is able to penetrate the blood-brain barrier. ONC201 does not induce cell death in normal cells. *In vivo* studies indicate that the safety margin (ratio of therapeutic dose to lowest dose with a mild adverse event) of ONC201 is at least 10-fold in rats and dogs in GLP toxicology studies. ONC201 has demonstrated antitumor activity in high grade gliomas, as demonstrated using *in vitro*, *ex vivo*, and *in vivo* models. In early clinical studies ONC201 has been well tolerated. The safety profile along with its mechanism of action makes ONC201 suited to address gliomas by potentially circumventing limitations of available therapies.

For further background information related to ONC201, please refer to the Investigator's Brochure.

2.2.1 Preclinical Efficacy in High Grade Glioma

ONC201 induces cell death in a broad spectrum of tumor types harboring diverse mutations in genes such as p53, KRAS, Raf, EGFR and others that results in resistance to chemotherapies and targeted agents. ONC201 induces caspase-mediated apoptosis in cancer cell lines and exhibits broad-spectrum cytotoxicity in vitro. ONC201 exhibits promising anticancer activity that has been demonstrated in multiple malignancies in preclinical models that include subcutaneous, orthotopic, and transgenic models in addition to a large body of in vitro data that demonstrate its cytotoxic effects and its mechanism of action.ONC201 displays single agent anti-tumor effects in subcutaneous and orthotopic colon cancer, subcutaneous triple negative breast cancer, subcutaneous non-small cell lung cancer, subcutaneous and orthotopic intracranial glioblastoma, and immunocompetent lymphoma transgenic mouse models. ONC201 also cooperates extensively with paclitaxel, docetaxel, and bevacizumab. We have chosen to target GBM for ONC201 development given the wealth of positive preclinical information that was generated with the study drug.

In addition to potent in vitro activity, ONC201 shrinks temozolomide-resistant GBM xenografts (Figure 1A) and prolongs the survival of mice with orthotopic xenografts as a monoagent and in combination with bevacizumab (Figure 1B). Corroborating observations by other investigators have demonstrated the compelling monoagent efficacy of ONC201 in radio- and chemo-resistant GBM cell lines. Other studies have demonstrated highly potent cytotoxic activity with ONC201 in three-dimensional neurosphere cultures of newly diagnosed and recurrent GBM patient samples.

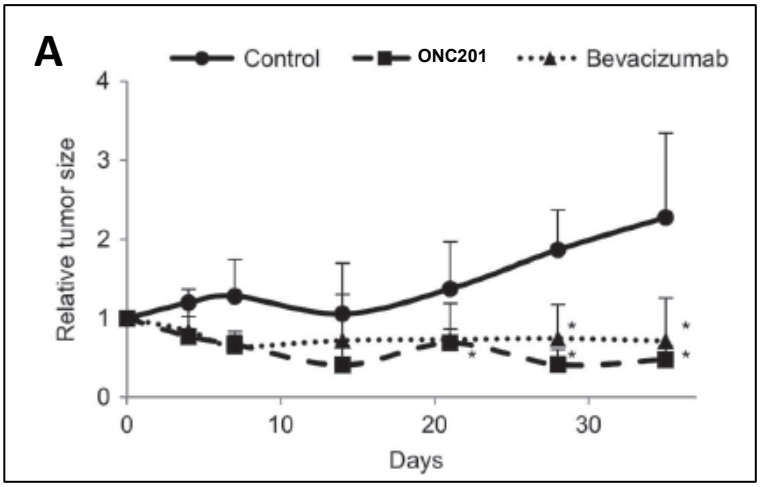


Figure 1 (A) Relative tumor size (compared to day 0) in a subcutaneous xenograft of T98G in mice treated with a single dose of vehicle, ONC201 (30 mg/kg, orally), or bevacizumab (10 mg/kg, iv) on day 0 (n = 8). (B) Overall survival of mice harboring SF767 intracranial tumors treated with a single oral dose of vehicle (n = 8), ONC201 (25 mg/kg, n = 7), bevacizumab (bev) (10 mg/kg, iv, n = 6), or ONC201and bevacizumab (n = 7) at 2 weeks after implantation.

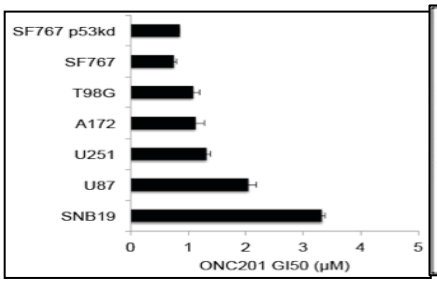


Figure 2 GI50 GBM cell lines at 72 hours after treatment with ONC201 or DMSO (n = 3).

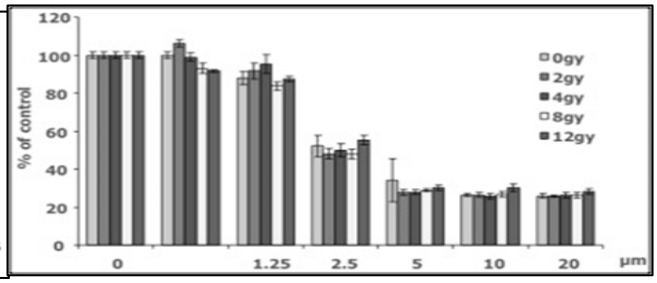


Figure 3 T98G TMZ-resistant and radio-resistant GBM cells following treatment with ONC201 at indicated concentrations (72 hr).

ONC201 demonstrates p53-independent activity in human GBM cell lines in the low micromolar range (Figure 2). ONC201 exert a strong cytotoxic effect, unlike temozolomide, against tumor cells isolated from a freshly resected GBM with an oligodendroglial component that was previously resected and irradiated. Observations by external investigators demonstrate the compelling monoagent efficacy of ONC201 in radio- and chemo-resistant GBM cell lines (Figure 3) and 3D neurosphere cultures (Figure 4).

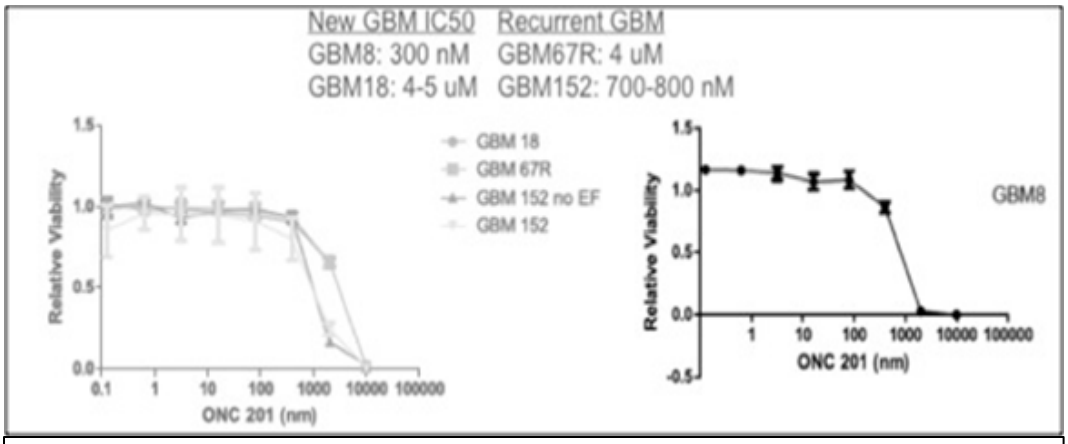


Figure 4 ONC201 kills newly diagnosed and recurrent GBM neurospheres. Cell viability of neurosphere GBM cultures following ONC201 treatment (72 hr).

2.2.2 Mechanism of Action

ONC201 is a selective antagonist of the G protein-coupled receptor DRD2 that was identified through a phenotypic screen as a p53-independent small molecule inducer of TRAIL gene transcription in tumor cells. A series of gene expression profiling and cell signaling investigations have unraveled signaling pathways that are engaged in tumor cells following ONC201 treatment.

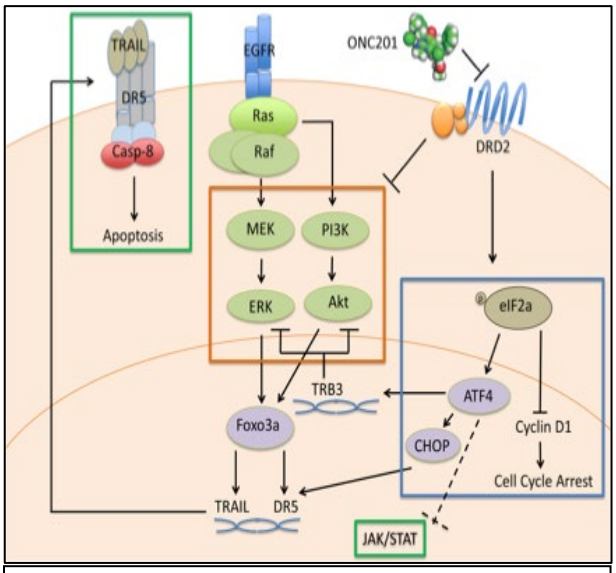


Figure 5 Proposed model of ONC201 mechanism of action in tumor cells.

Downstream of target engagement, ONC201 activates the integrated stress response (ISR), which is the same signaling pathway activated by ER stress-inducing compounds such as proteasome inhibitors (e.g. bortezomib). When the ISR is activated by ER stress-inducing compounds, the pathway is often referred to as the ER stress response. ONC201 causes an early-stage increase in the phosphorylation of eIF2-alpha at serine 51, which results in attenuation of protein translation and upregulation of the transcription factor ATF4 (Figure 5). ATF4 upregulates CHOP, which is also a transcription factor that regulates several apoptosis-related genes such as the TRAIL-receptor DR5. ATF4 and CHOP upregulate expression of TRB3, which interacts directly with Akt to decrease its kinase activity. TRB3 also serves as a scaffold protein in the MAPK signaling pathway that can negatively regulate this pathway. Decreased levels of phospho-MEK, -ERK, and –Akt have been documented in response to ONC201. The decreased ERK and Akt kinase activity results in less phosphorylated Foxo3a,

which is a transcription factor that regulates both the TRAIL and DR5 genes. Dephosphorylated Foxo3a undergoes nuclear translocation and activation in response to ONC201.

In summary, ONC201 inhibits DRD2 to cause downstream activation of ATF4, which causes induction of genes that lead to apoptosis. DRD2 antagonism also downregulates Akt and ERK activity to cooperatively induce complementary downstream apoptotic effects. ONC201 may not activate eIF2-alpha through PERK. This distinct mechanism may explain the lack of cross-resistance between ONC201 and other ER stress-inducing agents such as bortezomib. In addition, ONC201 has enhanced antitumor efficacy in combination with bortezomib that may be explained by engaging parallel stimuli that lead to an enhanced activation of the ISR in tumor cells.

2.2.3 Nonclinical Safety/Toxicology Studies in Animals

In rats and dogs, ONC201 was better tolerated when administered orally compared to intravenously. In rat non-GLP studies, the No-Observed Adverse Effect Level (NOAEL) was 225 mg/kg with oral administration compared to 100 mg/kg with a 2 hour infusion and 50 mg/kg with a 30 minutes infusion. Non-GLP clinical observations in rodents included decreased activity, altered gait, and mortality. In dog non-GLP studies, the NOAEL dose was at least 120 mg/kg with oral administration and clinical observations were limited to emesis and changes in fecal consistency. The non-GLP studies only evaluated at clinical observations, weight gain, food consumption and gross findings at necropsy. In general the toxicology/safety studies indicate that the acute toxicities associated with ONC201 are limited to the day of administration and are reversible.

In dog GLP studies, the NOAEL was at least 42 mg/kg. Observations were limited to decreased activity, decreased food consumption, emesis, salivation, and/or soft, loose or mucous feces. In rat GLP studies, the NOAEL was at least 125 mg/kg. Observations included decreased activity, decreased food consumption, decreased body weight, and abnormal stance and gait. Minor changes in serum chemistries were noted, largely in the 225 mg/kg rats that included slight increases in cholesterol and chloride. The significance of these findings is unknown as other clinical chemistry and histology did not corroborate this observation (e.g. liver findings). Rats receiving 125 or 225 mg/kg ONC201 had mild edema and inflammation that was primarily submucosal in the stomach and was completely resolved by day 19.

2.2.3.1 *Non-GLP Safety Studies*

Non-GLP studies were conducted in rats and dogs to assess clinical observations and body weight with ONC201.

Non-GLP toxicology studies in rats

The ability of rats to tolerate ONC201 by intravenous administration was explored as a function of infusion time. Clinical observations with intravenous administration included decreased activity, salivation, abnormal gait and stance, labored respiration, pale skin, nasal discharge, prostration during the dose, mild body twitching, and red discharge from the mouth.

The NOAEL following administration of ONC201 to Sprague-Dawley rats by a 30-minute intravenous infusion was 50 mg/kg. The NOAEL following administration of ONC201 to Sprague-Dawley rats by a 2-hour intravenous infusion was 100 mg/kg. Administration of 100 mg/kg ONC201 by intravenous infusion over 30 minutes resulted in the death of one male rat. Administration of 200 mg/kg ONC201 by a 2-hour infusion resulted in the death of both animals during the infusion period. Following these observations, the tolerance of oral ONC201 was explored given the potential to lower acute toxicity by lowering Cmax. Clinical observations with oral exaggerated doses of ONC201 included decreased activity, abnormal gait and stance, prostration, irregular respiration, moderate twitching, red discharge on the muzzle, scant feces, hunched posture, not eating, pilerection, and skin cold to touch. The NOAEL following administration of ONC201 to Sprague-Dawley rats by oral gavage was 225 mg/kg.

Non-GLP toxicology studies in dogs

In parallel to non-GLP toxicology studies in rats, the ability of beagle dogs to tolerate ONC201 was explored. No deaths were observed at any doses in dogs. The NOAEL following the 30-minute intravenous infusion of ONC201 to beagle dogs is considered to be at least 33.3 mg/kg. The 2 hour-intravenous infusion of 16.7 mg/kg ONC201 was not associated with any clinical signs of toxicity. Therefore the NOAEL following a 2 hour-intravenous infusion of ONC201 to beagle dogs is considered to be greater than 16.7 mg/kg, though higher doses were not explored as the oral route was selected for further development based on observations in rats. The NOAEL with oral ONC201 was considered to be at least 120 mg/kg in dogs. Clinical observations at doses of 66.7 to 120 mg/kg were limited to emesis and changes in fecal consistency.

2.2.3.2 *GLP Toxicology and Safety Studies*

Single Dose Oral Toxicity Study in Dogs (GLP)

A GLP study was performed to evaluate the toxicity and toxicokinetics of ONC201 following a single oral dose to Beagle dogs followed by a 2-day or an 18-day recovery period. Dogs received a single dose of 0, 4.2, 42, or 120 mg/kg by oral gavage. There was no mortality observed in this study. There were no definitive ONC201-related effects on group mean body weight or body weight gain, ECG rhythm or morphology, mean heart rate or arterial blood pressure, urinalysis, hematology parameters, coagulation parameters, clinical chemistry parameters, erythrocyte morphology, gross findings on necropsy, changes in absolute or organ to body or organ to brain weights.

Although not statistically significant, there were some dose-related decreases in group mean food consumption for the first week following dosing. The 120 mg/kg females had statistically significantly decreased group mean food consumption compared to the vehicle control group on Days 14, 15 and 18. There were no clinical signs of toxicity noted following a single dose of 4.2 mg/kg ONC201. At a dose of 42 mg/kg and 120 mg/kg, some dogs had clinical observations at approximately 1 hour post-dose including decreased activity, emesis, salivation, and/or soft, loose or mucous feces. Of uncertain relationship to ONC201 administration was the unusual finding of mononuclear cell inflammation in the blood vessels of the brain, which was multifocal and mild in one high dose female at Day 3, multifocal and minimal in one high dose female at Day 19, and minimal and focal in one control female at Day 19. Similar findings were not noted in any male animal.

Based on the results of this study, the NOAEL following oral administration of ONC201 at single doses of 4.2, 42 or 120 mg/kg to Beagle dogs is considered to be at least 42 mg/kg.

Single Dose Oral Toxicity and Toxicokinetic Study in Rats with a 19-Day Recovery and a 30-Minute Intravenous Infusion Toxicokinetic Arm (GLP)
A GLP study was performed to evaluate the toxicity of ONC201 following a single oral dose in Sprague-Dawley rats with necropsy after a 2-day or an 18-day recovery period. Rats received 0, 12.5, 125, or 125 mg/kg ONC201 by oral gavage.

There was no mortality observed in this study. There were no definitive ONC201-related effects on coagulation parameters, clinical chemistry parameters, gross findings at necropsy. There were no clinical signs of toxicity noted at single doses up to 125 mg/kg ONC201. There were no ONC201-related statistically significant changes in hematology parameters or clinical chemistries outside of the historical control range for these values.

At a dose of 225 mg/kg, clinical signs of toxicity were limited on the day of dose administration to one out of twenty males and one out of twenty females that showed signs of decreased activity and abnormal gait and stance. The male was also noted to have increased respiration. All were normal by Day 2. No ONC201 related changes were noted during the functional observational battery (CNS activity) performed on Day 1 between 1 and 2 hr post-dose with the exception of one 225 mg/kg female noted as having decreased activity. A statistically significant decrease in group mean body weight gain was noted on Day 7 for the 225 mg/kg males. A statistically significant decrease in group mean food consumption was noted on Day 7 for the 225 mg/kg males.

On Day 3 the 225 mg/kg females also had increased glucose, cholesterol, sodium and chloride. Sodium and chloride were statistically significantly increased for the 125 mg/kg females. Only cholesterol and chloride were outside historical control

ranges for this laboratory on day 19. As the increase in cholesterol was only noted for the females and no corresponding liver findings were observed, the significance of this finding is unknown. Though within normal historical control values for these laboratories, chloride remained increased for the 225 mg/kg males while cholesterol remained increased for the 225 mg/kg females.

Changes in brain and liver weights were noted but did not occur in a dose-dependent manner and no microscopic changes were noted for in these organs for the high dose females. These changes were considered incidental and unrelated to treatment. At the Day 3 necropsy, ONC201-related minimal to mild edema and/or mixed cell inflammation was present in the non-glandular stomach of 225 mg/kg males and females. This edema and inflammation was primarily submucosal, although in some animals the inflammation involved the serosa or mesentery. Two males and one female had minimal focal ulceration of the overlying squamous epithelium. Similar stomach findings were seen in 125 mg/kg animals, with a lower incidence than in 225 mg/kg. There was complete resolution of all stomach lesions at the Day 19 necropsy, indicating full recovery.

Based on the results of this study, the NOAEL following oral administration of ONC201 at single doses of 12.5, 125 or 225 mg/kg to Sprague-Dawley rats is considered to be at least 125 mg/kg.

Evaluation of the Effect of ONC201 Dihydrochloride on Respiratory Function Following Single-Dose Administration in Rats (GLP)
A GLP study was performed to determine the potential effects of ONC201 dihydrochloride on respiratory function in rats following a single oral gavage administration. Twenty four (6/group) male rats received 0, 12.5, 125, or 225 mg/kg ONC201 by oral gavage and were monitored in plethysmographic chambers. The oral administration of ONC201 dihydrochloride at 12.5 and 125 mg/kg did not induce any biologically relevant effects on respiratory rate, tidal volume or minute volume in conscious male rats. A marginal to moderate transient decrease in respiratory rate and minute volume was observed following the oral administration of ONC201 dihydrochloride at 225 mg/kg, which resolved by 2 hours.

2.2.4 Pharmacokinetic Studies

2.2.4.1 *Pharmacokinetic Studies in Animals*

The measured half-life of ONC201 in mice is ~6 hours with intravenous administration as measured by an HPLC-UV assay.

In rats, exposure to ONC201 was dose-dependent and approximately dose-proportional. Exposure to ONC201 was slightly greater in female rats after a single oral gavage dose. Plasma $T_{1/2,e}$ ranged from 2.3 to 8.4 hours in 7 of 8 profiles.

Clearance ranged from 7.5 to 23.5 L/hr/kg in 7 of 8 profiles. Volume of distribution ranged from ~49 to ~103 L/kg in 6 of 8 profiles.

In dogs, exposure to ONC201 following oral gavage dosing at 4.2, 42, and 120 mg/kg ONC201 was dose-dependent and increased with greater ONC201 dose levels. Exposure to ONC201 was similar in male and female dogs with the observation that all mean male Cmax and AUC values were slightly greater than those corresponding female values. Elimination of ONC201 from plasma was similar between the mid and high dose levels; mean $T_{1/2,e}$ ranged from 4.6 to 7.8 hours. Mean T1/2,e following the low dose of 4.2 mg/kg was ~1 hour [the half-life determined for dogs in the low dose group may represent more of a distribution phase half-life rather than the terminal plasma elimination half-life]. Overall elimination of ONC201 was greater following the low dose.

2.2.4.2 *Pharmacokinetic Studies in Humans*

In a Phase I dose escalation clinical trial of ONC201 in adults with advanced solid tumors, the pharmacokinetics of single agent ONC201 administered once every one or three weeks was determined by LC-MS-MS analysis of plasma collected in the first cycle of therapy within 21 days of drug administration (Figure 6; Table 1). Trends of increasing exposure with dose were consistent with dose proportionality. Patients receiving 625mg ONC201 exhibited a mean half-life of 11.3 hours and achieved a Cmax of 3.6 ug/mL (~9.3 uM), which occurred at 1.8 hours following administration (Tmax). The mean volume of distribution was 369 L, consistent with a large distributive volume.

Mean AUC was 37.7 h.µg/mL and mean CL/F was 25.2 L/h. Generally, CL/F was observed to be variable but consistent across all dose groups. There were no apparent relationships between drug CL/F and patient sex and age. Noticeable, shallow trends were observed with patient weight and BSA. An overall increase in CL/F was observed as weight and BSA increased. Although a slight upward trend was observed, there was no strong correlation between CL/F and CLCR.

Stronger correlations were observed with the distributive volume estimate and patient weight and BSA. An increase in volume of distribution was observed with increasing patient weight or BSA, as expected. Trends of decreasing exposure with increasing weight were observed in plots of Cmax/Dose and AUC/Dose versus patient weight. Weight normalized CL/F was plotted versus Dose, showing a similar trend to un-normalized CL/F.

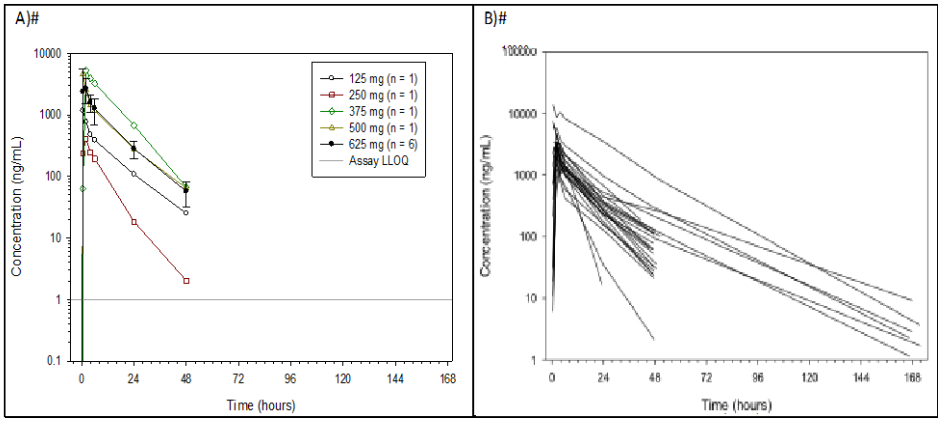


Figure 6 Mean ONC201 plasma concentrations verses time following the first dose of ONC201. Concentrations are shown as (A) a mean for each cohort, or (B) for individuals treated at 625mg.

Table 1 ONC201 pharmacokinetic parameters determined in patients receiving 625 mg ONC201 (n=39).

	C_{max} (ug/mL)	T_{max} (h)	T_{lag} (h)	AUC_{last} (h.ug/mL)	λ_z (h^{-1})	$t_{1/2}$ (h)	AUC (h.ng/mL)	V_z/F (L)	CL/F (L/h)
Mean	3.6	1.8	0.02	37.0	0.076	11.3	37.7	369	25.19
SD	2.6	0.9	0.08	41.6	0.046	5.2	41.6	193	14.22

In a Phase I/II clinical trial of ONC201 in adults with acute leukemias or high-risk myelodysplastic syndromes (NCT02392572), the pharmacokinetics of single agent ONC201 was determined by LC-MS-MS analysis of plasma collected in the first cycle of therapy within 21 days of drug administration. Oral doses of ONC201 were given at 125, 250, 375, 500mg and 625mg, twice each week on two consecutive days (e.g. Monday and Tuesday of each week). This dosing schedule maintained systemic concentrations that exceeded 1,000ng/mL therapeutic thresholds of ONC201 for >72 hours in patients who received 375mg or 625mg. In contrast, exposure with weekly dosing generally maintained >1,000 ng/mL concentrations for ≤24 hours (Figure 7).

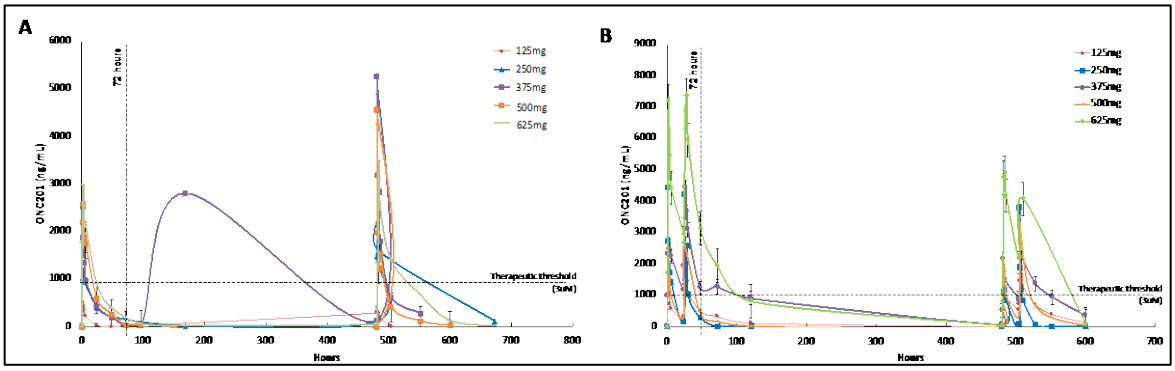


Figure 7 Plasma concentrations of ONC201 administered (A) once per week or (B) twice per week on two consecutive days to adults with relapsed/refractory acute leukemias or high-risk myelodysplastic syndromes. Hour 0 is the baseline sampled before the first dose on ONC201. Error bars reflect reflect SEM of samples from patients enrolled to receive the same dose.

2.3 Clinical Studies

2.3.1 Adult Experience

The clinical safety of ONC201 has been evaluated in a Phase I clinical trial that has established the recommended Phase II dose of 625mg once every one week. The design was an open-label, dose-escalation Phase I trial of monoagent ONC201 in patients with advanced, refractory tumors who had exhausted or refused standard treatment options for their respective indications. The primary objective of this study was to determine the recommended Phase II dose (RP2D) of ONC201 administered orally in patients with advanced cancers, as well as to evaluate the safety and tolerability of the drug. Secondary objectives included pharmacokinetics and pharmacodynamics evaluation of ONC201 and preliminary assessment of anti-tumor efficacy.

An accelerated dose escalation design was employed to reduce the number of patients treated at potentially sub-therapeutic dose and to accelerate the determination of the recommended Phase II dose. Ten evaluable (aged 47-80 years) received oral ONC201 once every 3 weeks at five dose levels ranging from 125 to 625 mg. The study design included one patient per cohort until any patient experiences a grade 2 adverse event during the first cycle of treatment, defined as 21 days. Five dose levels (125 mg, 250 mg, 375 mg, 500 mg, 625 mg) were selected for the study. Enrollment at each subsequent dose level required that all patients enrolled at the prior dose level completed Cycle 1 dosing and were evaluated 21 days later to assess safety.

On average, patients received 3.1 doses of ONC201. Nine out of ten patients completed at least 2 cycles, 4 patients completed at least four cycles, and one patient received six cycles and remains on therapy. 625 mg was the highest dose administered and was determined to be the RP2D. The only adverse event during the dose escalation phase that was attributed as possibly related to ONC201 was a low grade fever. No drug-related toxicities Grade >1 were observed in any patients

in this study. Explorative laboratory studies and physical exams did not reveal any drug-related abnormalities. Similarly, cardiovascular assessments revealed no drug-related effects.

Clinical and laboratory results indicated that the drug possessed biological activity in the treated patients. A 72-year-old with advanced clear cell endometrial (uterine) cancer had a mixed objective response with >50% decrease in lymphadenopathy (10/11 afflicted lymph nodes responded) after 2 doses. A 62-year-old male with renal cancer and bone metastasis with debilitating pain in the clavicle experienced relief from his clavicular pain. A 47-year-old male with appendiceal cancer had CA27.29 tumor biomarker of 30 units that was in the abnormal range, which decreased to 20 units (normal range) after 4 doses of ONC201.

Given the heterogeneity of the tumor types in the enrolled patients, no widely used biomarker was available to uniformly assay all patient samples. Since most solid tumors express cytokeratin-18, the serum M30 assay was selected to detect a caspase-cleaved form of cytokeratin-18 that occurs during apoptosis. Clinical studies have demonstrated the M30 assay to be predictive of clinical response (Demiray et al; 2006) in solid tumors. Induction of the M30 assay for apoptosis was noted in 67% of patients treated at the RP2D with a range of 1.25- to 4-fold increase.

An expansion cohort of this Phase I trial with ONC201 enrolled 18 additional patients with advanced solid tumors to confirm the tolerability of the 625mg ONC201 RP2D. The only adverse events among the 18 patients enrolled in the expansion cohort that were attributed as possibly-related to ONC201 were: nausea (1 patient), emesis (2 patients), and increased level of serum amylase (2 patients). All of these adverse events were Grade 1 and reversed rapidly. Laboratory studies and physical exams did not reveal any drug-related abnormalities. Similarly, cardiovascular assessments revealed no drug-related effects.

Another arm of this study has evaluated weekly dosing of ONC201. Three patients have been treated with 375mg ONC201 on a weekly basis and seventeen patients have been treated with 625mg on a weekly basis. There have been no reports to the sponsor of any drug-related adverse events in any of these patients. All three 375mg and seventeen of the 625mg patients have successfully completed the DLT window (21 days).

Based on the clinical experience described above, the recommended administration schedule of ONC201 in adults is 625mg once every week.

Ongoing clinical trials are evaluating ONC201 in: acute leukemias and myelodysplastic syndrome (NCT02392572), non-Hodgkin’s Lymphoma (NCT02420795); solid tumors (NCT02609230); multiple myeloma (NCT02863991); endometrial cancer (NCT03099499); glioblastoma (NCT02525692).

The first cohort has been enrolled in a study of single agent ONC201 in recurrent adult glioblastoma (NCT02525692) who received ONC201 every three weeks. For the every three week cohort, prior therapy included radiation, surgery, temozolomide, and others except for bevacizumab. Two of 17 patients had methylated MGMT; two of 17 patients had no measurable disease at enrollment due to salvage surgery. PFS6 was 11.8% with one confirmed response by RANO. This response occurred in a 22-year-old patient with a secondary glioblastoma possessing a H3.3 K27M mutation. Both lesions regressed, overall by 92% and the patient remains on study after >15 months. Six of 17 patients stable disease or a partial response as their best overall response by RANO. One patient enrolled after salvage surgery is disease-free after > 15 months and remains on therapy. Median OS was 9.7 months. ONC201 was very well tolerated with no drug-related SAEs or discontinuation due to toxicity. Two possibly-related AEs occurred: one grade 3 neutropenia that did not recur upon rechallenge and one grade 2 allergic reaction that was manageable with anti-histamines. Plasma PK at 2 hours post-dose was median 2,586 ng/mL (range 1,320-3,660), serum prolactin induction was observed as a surrogate marker of target engagement, and DRD2 was expressed in all evaluated archival tumor specimens. Additional cohorts are now enrolling on a once every week schedule.

2.3.2 **Pediatric Experience**

Clinical data continues to be collected for patients accrued to clinical trials and compassionate use patients in the pediatric setting. As of August 6, 2018, 28 pediatric patients ranging from age 3-19 have been treated with ONC201 by converting the adult RP2D of 625mg on a body weight basis. A total of 10 pediatric patients with H3 K27 mutant gliomas have received ONC201 on a Compassionate Use basis and 18 patients pediatric received ONC201 while enrolled on a clinical trial. The baseline patient characteristics were a median age of 9 years (range: 3 to 19) and 16 patients (57%) were female and 12 patients (43%) were male. All 28 patients (100%) were younger than 19 years of age at the time of consent. Patients received ONC201 once every one week at various doses ranging from 125mg-625mg. No treatment-related SAEs have been reported by the investigators to Oncoceutics as of the August 06, 2018. One patient, an 8-year-old male weighing 26kg received 375mg ONC201 orally had limited pharmacokinetic sampling. Sample analysis indicated a pharmacokinetic profile that was consistent with the adult experience at the RP2D, including a Cmax of 9uM, which occurred at 2 hours following administration.

2.4 Rationale for ONC201 in High Grade Glioma

2.4.1 Rationale for ONC201 in glioma

The first public disclosure of ONC201 is an expired Boehringer Ingelheim patent that described ONC201 (and a series of other unrelated small molecules) as having potential CNS activity, e.g. as an anticonvulsant (Stähle et al., 1971). An in vitro efficacy screen indicated that GBM is more sensitive to ONC201 relative to most other types of solid tumors (Figure 8). One of the key features in the selection process that identified ONC201 as an anticancer agent was its ability to penetrate the blood-brain barrier to address tumors residing in the CNS, unlike many available therapies. Ensuing animal studies revealed that ONC201 rapidly traverses the blood-brain barrier, is highly bioactive in the brain, does not appear to be neurotoxic, and is potently cytotoxic to all tested GBM tumors *in vitro*, *ex vivo*, and *in vivo* (Allen et al., 2013b). ONC201 has p53-indepenent activity against GBM cell lines, including those with resistance to chemotherapy and radiotherapy. In addition to cell lines, ONC201 exerts potent anticancer activity in recurrent GBM samples resistant to all standard-of-care therapies.

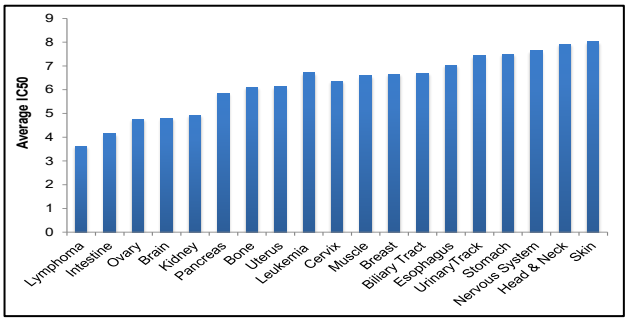


Figure 8 In vitro efficacy screen indicating the high sensitivity of GBM (brain cancer) to ONC201.

Using the regimen established in the first-in-human Phase I trial, a Phase II clinical trial is now assessing ONC201 in recurrent, bevacizumab-naïve, IDH1/2 wild-type (WT) GBM (Arrillaga-Romany et al, 2017). The first cohort of 17 patients has been completed: median overall survival (OS) was 41.6 weeks (Arrillaga-Romany et al, 2017), with survival at 6, 9, and 12 months of 71%, 53%, and 35%, respectively. Four of the 17 patients are alive (as of June 2017). Progression-free survival at 6 months (PFS6) was 11.8%. Two patients continued to receive ONC201 for > 14 months, one with a durable objective response (Revised Assessment in Neuro-Oncology [RANO] criteria) and another who remains disease-free after salvage surgery. The objective response was observed in a secondary GBM patient possessing a H3.3 K27M mutation (Figure 9), with a 92% regression that has remained durable.

There were no drug-related serious adverse events or treatment discontinuations due to toxicity. A Grade II allergic reaction occurred in one patient, but was manageable with antihistamines.

The median plasma concentration at 2 hours post-dose was 2.6 µg/mL (range 1.3–3.7), surpassing therapeutic thresholds. Serum prolactin induction was observed as a surrogate marker of target engagement and DRD2 was expressed in all evaluated archival tumor specimens.

On the basis of an OS improvement of 5–6 months relative to historical controls [8, 9] and the objective response observed in the H3.3 mutant GBM patient, the study was expanded to evaluate an additional 36 patients. This also includes a 6 patient window cohort, using a once weekly ONC201 dosing schedule (deemed equivalent in a separate Phase I study) after the first 17 patients were treated.

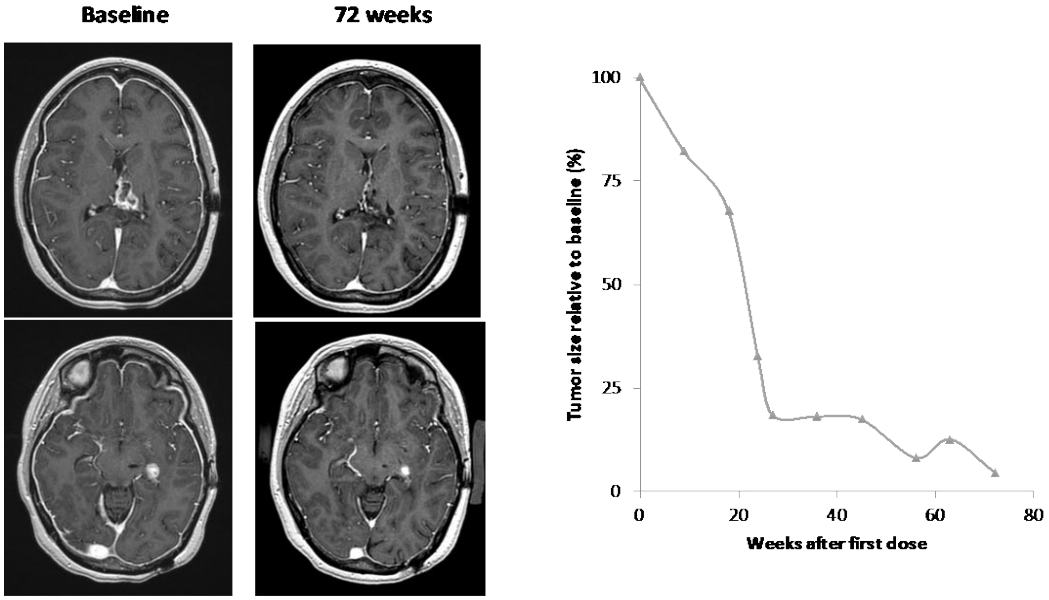


Figure 9. Responding GBM patient with H3F3A K27M mutation. Contrast-enhanced MRI: Left image shows baseline and right image shows after 72 weeks of ONC201 (625 mg, every 3 weeks). Overall tumor burden by RANO over time plotted at the right.

2.4.2 Rationale for ONC201 in Histone H3 K27M glioma

In the Phase II trial of ONC201 in adult recurrent GBM, a dramatic response observed to ONC201 (Figure 9) that was exclusive to the lone subject with histone H3 K27M mutation in the study (Arrillaga-Romany et al, 2017). Based on this exceptional response, we explored mechanistic connections between histone H3 K27M mutation and the mechanism of action of ONC201.

The expression of dopamine receptor D2 (DRD2), the target of ONC201 relative to the dopamine receptor D5 (DRD5), a D1-like dopamine receptor that opposes DRD2 signaling, may be predictive biomarkers for ONC201 efficacy (see Investigator's Brochure). Tumor cell lines that exhibit a high DRD2 expression and concomitant low DRD5- expression signature are more sensitive to ONC201 in preclinical studies.

In publicly available ChIP-Seq databases, we found that H3.3 and components of the PRC2 methyl transferase complex (which is inhibited by the K27M mutation) each mark both the *DRD2* and *DRD5* gene in DIPG and isogenic models (Funato et al, 2014; Mohammad et al, 2017; Piunti et al, 2017). While the precise epigenetic mechanisms regulating the balance of the DRD2:DRD5 expression is an active area of investigation, we hypothesized that H3.3 K27M gliomas foster a chromatin landscape that leads to high DRD2 expression and suppression of DRD5 expression, which in turn may make these tumor cells more sensitive to ONC201. In support of this hypothesis, DRD5 expression was absent by immunohistochemistry (IHC) analysis in the H3.3 K27M responding subject. Our hypothesis that DRD5 is an inversely correlated biomarker for ONC201 efficacy is strengthened by the observation that two other glioma subjects who remained progression-free on study at 5 months also did not exhibit intratumoral expression of DRD5. Similar trends were observed with OS.

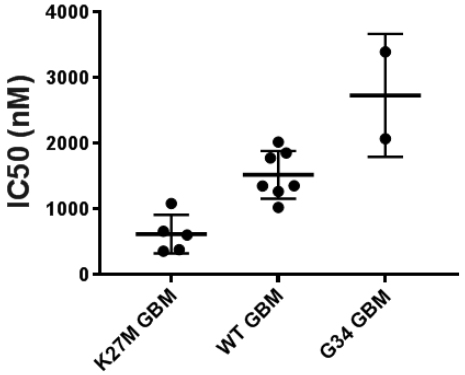
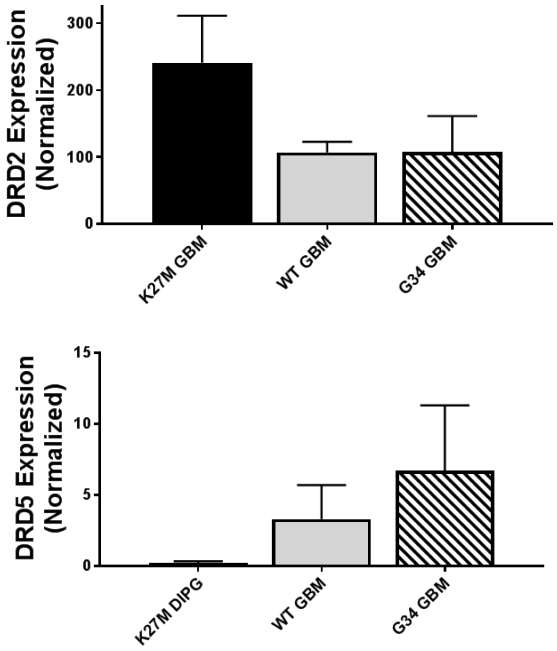


Figure 10. Patient-derived glioma cell lines are differentially sensitive to ONC201 depending on their histone mutation status. Primary glioma cell lines derived from individual patients were treated for 5 days with ONC201 and cell viability was measured using CellTiter-Glo.

In further support of our biomarker hypothesis, Dr. Andrew Chi's laboratory at NYU School of Medicine tested the efficacy of ONC201 against a panel of patient-derived glioma tumorsphere cultures grown in serum-free neural stem cell media. We found that ONC201 was more potently cytotoxic to histone H3 K27M mutant lines (median IC_{50} ~0.6 uM, n=5 lines) compared to histone H3 wildtype glioma lines (median IC_{50} ~1.5 uM, n=7 lines; p<.01). Patient-derived lines included five histone H3 K27M mutant DIPG lines (two *HIST1H3B* and three *H3F3A* mutant), two *H3F3A* G34 mutant pediatric GBMs (one G34V, one G34R), and 7 H3 K27M/G34R wildtype (3 pediatric, 4 adult) GBMs. (Figure 10)

In addition, expression of DRD2 and DRD5 in untreated patient glioma samples from NYU Langone Health was analyzed by RNASeq. Tumors included H3K27M bearing glioma (K27M GBM, n=8), wild-type pediatric and adult glioma (pGBM n=3 and aGBM n=25, respectively), and H3G34R bearing glioma (G34 GBM, n=3). DRD2 expression was significantly increased in histone H3 K27M mutant glioma tumors compared to adult and pediatric H3 K27M wildtype tumors (mean normalized counts; H3 K27M = 194, median for H3 WT GBM = 107, p=.02) (Figure 11). In contrast, DRD5 expression in all glioma tumors tested were low, however DRD5 expression in histone H3 K27M mutant glioma tumors showed a trend towards lower expression in K27M wildtype glioma (mean normalized counts; H3 K27M = 0.2, median for H3 WT GBM = 3.3) (Figure 11). Therefore, the DRD2 and DRD5 expression profiles of H3 K27M mutant patient gliomas appear consistent with an expression signature in preclinical models that predicts sensitivity to ONC201.

Figure 11. DRD2 and DRD5 expression in patient glioma tumors



Abbreviations: K27M GBM, Histone H3 K27M mutant patient-derived primary glioma cell lines; WT GBM=Histone H3 K27M wildtype pediatric and adult glioblastoma; G34 GBM= histone H3 G34R/V mutant pediatric GBM.

2.4.3 Rationale for dose regimen

ONC201 will be administered once per week that has been established in Phase I in adult solid tumors (Stein et al, 2017; NCT02250781). Preclinical and clinical studies have shown that ONC201 exhibits sustained pharmacodynamic effects that outlive its pharmacokinetics. Preclinical and clinical studies with ONC201 suggests saturation of efficacy at a human dose of approximately 125mg. A dose of 625 mg is expected to exceed the target dose with maximal efficacy by 5-fold, which is supported by pharmacokinetic and pharmacodynamic studies. This has been sufficient to achieve therapeutic intratumoral drug concentrations and pharmacodynamics in adult patients with recurrent glioblastoma (Figure 12). The adult dose of 625mg weekly will be the targeted RP2D in the pediatric population, which will be dosed on a body weight basis.

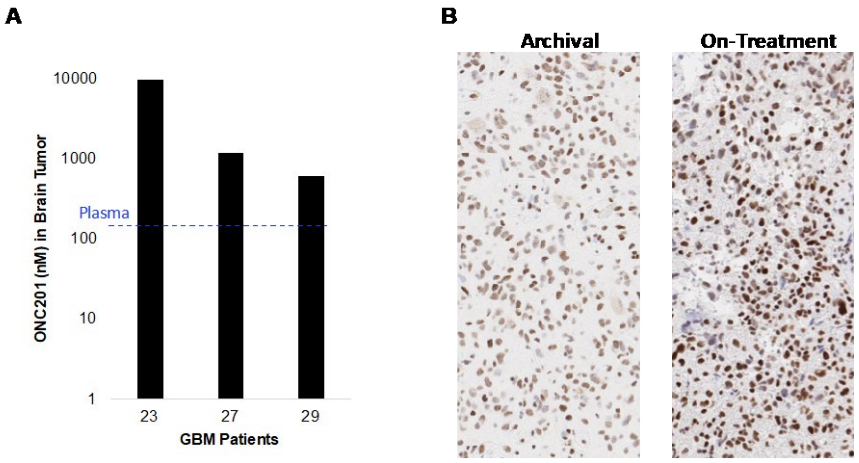


Figure 12. (A) Intratumoral ONC201 concentrations and (B) ATF4 IHC analysis of archival tumor specimens or re-resection samples taken 1 day after the second dose of 625mg ONC201 PO from adult patients with recurrent glioblastoma.

Systemic exposure with once per week dosing in patients results in concentrations that exceed the 1,000ng/mL threshold for ~24 hours. In vitro studies indicate that maximum antiproliferative and pro-apoptotic effects of ONC201 can require continuous incubation for at least 48 hours in some cancer cell lines. This generated the hypothesis that consecutive day dosing could maintain therapeutic concentrations for >48 hours. As summarized in Section 2.2.4.2, PK results from a Phase I/II indicate that twice per week of ONC201 on two consecutive days maintains >1,000ng/mL systemic concentrations for >72 hours in patients who received 375mg or 625mg. Given the safety profile of ONC201 and these findings, the safety twice per week dosing on two consecutive days will be evaluated in this clinical trial in addition to weekly dosing.

2.5 Correlative Studies Background

The mechanism of action of ONC201 involves DRD2 antagonism, which results in a number of effects that involve changes in signaling pathways in tumor cells and stimulation of the immune system. Furthermore, the sensitivity of tumor cells to ONC201 has been founded to be associated with predictive biomarkers.

Downstream of target engagement in tumor cells, ONC201 causes activation of the integrated stress response and dual inactivation of Akt and ERK, the latter of which leads to TRAIL and DR5 induction that induce tumor cell death. There are a series of proteins that participate in these signaling pathways that are associated with tumor cell response to ONC201 and have been shown to contribute to its activity in preclinical models: ATF4, CHOP, DR5, TRAIL. Immunohistochemical assays for FFPE tumor tissues have been developed for these pharmacodynamic markers. In addition, serum prolactin induction serves as a surrogate biomarker of DRD2 antagonism that can be assessed by ELISA.

DRD2 is expressed on the surface on immune cell, such as NK cells, and its antagonism results in immune cell activation (Figure 13). Activation of NK and CD8+ T cells have been observed in response to ONC201 in preclinical studies and in biopsies taken from ONC201-treated advanced cancer patients. This activation is observed in circulation (among peripheral blood mononuclear cells) as well as in the tumor and is associated with a both an increase in the number of cells as well as their activation (e.g. Granzyme B expression).

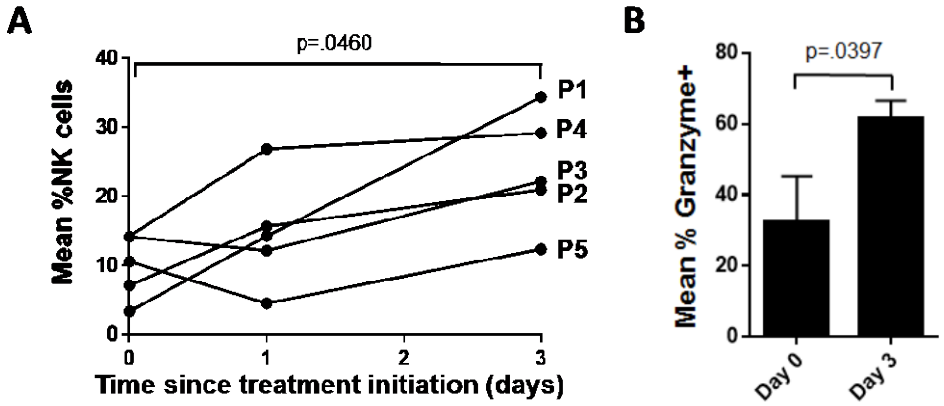


Figure 13. (A) Circulating NK cells among PBMCs and (B) percentage of Granzyme B+ circulating NK cells in ONC201-treated advanced solid tumors patients (625mg, PO)

In addition to the above pharmacodynamic biomarkers, the expression of DRD2 and DRD5, a D1-like dopamine receptor that oppose DRD2 signaling, in tumor cells may be predictive biomarkers. Tumor cell lines that exhibit a DRD2+DRD5- expression signature are more sensitive to ONC201 in preclinical studies. Furthermore, the same expression signature in archival tumor specimens from the every three week schedule cohort in the recurrent GBM study (ONC006) was associated with a superior progression-free survival and overall survival.

Preclinical studies also indicate that histone H3 K27M mutant gliomas may be more sensitive to ONC201 than other gliomas. We will therefore sequence histone H3 at the K27 hotspot location in all archival tumor samples using a central, CLIA-approved sequencing method post-hoc and evaluation mutation status with response to ONC201.

A novel CSF tDNA assay for H3.3 and H3.1 K27M has been developed and validated. CSF samples obtained at baseline and throughout treatment will be analyzed using a droplet digital PCR machine to provide minimal residual disease estimates and to predict treatment response and recurrence.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

1. 2 to less than 19 years of age.

2. Patient body weight must be above the minimum necessary for the patient to receive the ONC201 dose indicated for the currently enrolling dose level. The minimum body weight ranges from 10-27.5kg depending on the dose level.

3. Arm A and G: Patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) and have completed at least one line of prior therapy. Evidence of progression is not required so that ONC201 may be administered to patients in the maintenance setting or to patients with recurrent disease. No more than two episodes of recurrence from radiotherapy and/or chemotherapy are allowed. Use of bevacizumab solely for treatment of radiation necrosis, pseudoprogression, or treatment effect will not be considered a recurrence. Post-mortem biopsy is required if H3 K27M status of tumor is unknown and archival tumor tissue not available.

   Arm B: Patients with newly diagnosed diffuse intrinsic pontine glioma (DIPG), defined as tumors with a pontine epicenter and diffuse involvement of the pons, are eligible with or without histologic confirmation. Post-mortem biopsy is required if H3 K27M status of tumor is unknown and archival tumor tissue not available.

   Arm C: Patients with midline gliomas are eligible with or without histologic confirmation and must be eligible for tumor biopsy as deemed by the site Investigator.

   Arm D: Patients with recurrent glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory), have completed at least one line of prior therapy, must be willing to undergo serial lumbar puncture to obtain cerebrospinal fluid (CSF), and must be scheduled to undergo sedated MRIs. Local anesthesia for spinal tap is also allowed. Evidence of progression is not required so that ONC201 may be administered to patients in the maintenance setting or to patients with recurrent disease. No more than two prior episodes of recurrence from radiotherapy and/or chemotherapy are allowed. Use of bevacizumab solely for treatment of radiation necrosis, pseudoprogression, or treatment effect will not be considered a recurrence. Spinal tap should not be performed if treating clinician or lumbar puncture proceduralist has concern of signs of elevated intracranial pressure, including recent worsening in headache or somnolence.

   Arm E: Patients with glioma who are positive for the H3 K27M mutation (positive testing in CLIA laboratory) or have diagnosed diffuse intrinsic pontine glioma (DIPG), defined as tumors with a pontine epicenter and diffuse involvement of the pons, are eligible with or without histologic confirmation. Patients must be 2-12 weeks from completion of first-line radiation. Evidence of progression is not required so that ONC201 may be administered to patients in the maintenance setting or to patients with recurrent disease.

Arm F:

Pediatric patients with histologically confirmed diagnosis of high-grade glioma in any tumor sample with a known histone H3 K27M mutation identified by IHC or DNA sequencing test performed in a CLIA setting. Evidence of progressive disease on contrast-enhanced brain MRI as defined by RANO-HGG criteria is required. Patients must have had previous therapy with at least radiotherapy.

4. Karnofsky ≥ 50 for patients ≥ 16 years of age, and Lansky ≥ 50 for patients < 16 years of age. For Arm F, Karnofsky ≥ 60 for patients ≥ 16 years of age, and Lansky ≥ 60 for patients < 16 years of age

5. From the projected start of scheduled study treatment, the following time periods must have elapsed: 5 half-lives from any investigational agent, 4 weeks from cytotoxic therapy (except 23 days for temozolomide and 6 weeks from nitrosoureas), 6 weeks from antibodies, or 4 weeks (or 5 half-lives, whichever is shorter) from other anti-tumor therapies. For patients who have received radiotherapy, patients in any arm must be at least 2 weeks from the completion of local palliative radiotherapy (re-irradiation for progressive disease or upfront radiation at initial diagnosis). For Arm F, patients must be at least 90 days from prior radiation to the first dose of ONC201unless the progressive lesion is outside of the high-dose radiation target volume or there is unequivocal evidence of progressive tumor on a biopsy specimen.

6. Adequate organ function defined as:

   Bone Marrow:
   - Peripheral absolute neutrophil count (ANC) ≥ 1000/mm3 and
   - Platelet count ≥ 100,000/mm3 (transfusion independent, defined as not receiving platelet transfusions for at least 7 days prior to enrollment).

   Renal Function:
   - Creatinine clearance or radioisotope glomerular filtration rate (GFR) ≥ 70mL/min/1.73 m2 or normal serum creatinine based on age as shown below or GFR > 70ml/min/1.73m^2:

     Age < 5 years: 0.8 mg/dL maximum
     Age 5 to < 10 years: 1.0 mg/dL maximum
     Age 10 to < 15 years: 1.2 mg/dL maximum
     Age ≥ 15 years: 1.5 mg/dL maximum

   Liver Function:
   - Total Bilirubin (sum of conjugated + unconjugated) ≤ 1.5 x upper limit of institutional normal and
   - SGPT (ALT) ≤ 110 U/L and
   - Serum albumin ≥ 2 g/dL.

   Neurologic Function:

- Patients with seizure disorder may be enrolled if seizure disorder is well controlled.

7. Ability to understand a written informed consent document, and the willingness to sign it. Assent will be obtained when appropriate based on the subjects age.

8. All adverse events Grade > 1 related to prior therapies (chemotherapy, radiotherapy, and/or surgery) must be resolved to grade 1 or baseline, except for alopecia and sensory neuropathy Grade ≤ 2, or other Grade ≤ 2 not constituting a safety risk based on investigator's judgment, are acceptable.

9. For patients post pubertal: Female patients must agree to use effective contraception during the period of the trial and for at least 90 days after completion of treatment. Male patients must be surgically sterile or must agree to use effective contraception during the period of the trial and for at least 90 days after completion of treatment. The decision of effective contraception will be based on the judgment of the principal investigator.

10. Corticosteroid dose must be stable or decreasing for at least 3 days prior to the baseline CT or MRI scan.

11. MRI brain and entire spine MRI within 14 days prior to start of study drug for Arms A, B, C, E, F and G. Subjects undergoing screening for Arm D must have an MRI of brain and entire spine within 3 months prior to start of study drug. Subjects in Arm D will have a baseline MRI of brain and spine with lumbar puncture after study consent is signed and other eligibility criteria are fulfilled.
12. For Arms A, B, C, D, F and G: Ability to swallow and retain orally administered capsules.
13. Archival tumor specimen: Subjects in all arms must submit at least 5 unstained slides from a tumor specimen that harbors H3 K27M mutation if archival tissue is available. For subjects in Arms A, B, E or G, if no archival tumor tissue is available, or if H3 K27M status of tumor is unknown, then subjects must agree to submit a post-mortem biopsy specimen. Subjects in Arm C do not require prior tumor biopsy or confirmation of the presence of the H3 K27M mutation. Subjects in Arm D must have confirmation of the presence of the H3 K27M mutation in any glioma sample prior to enrollment. Subjects in Arm F must submit at least 5 unstained slides from a tumor specimen that harbors H3 K27M mutation. Note that the H3 K27M mutation is often reported as H3 K28M in gene sequencing assays.

3.2 Exclusion Criteria

1. For Arms A, B, D, E, F and G: Evidence of diffuse leptomeningeal disease or evidence of CSF dissemination.

2. Current or planned participation in a study of another investigational agent or using an investigational device.

3. History of allergic reactions attributed to compounds of similar chemical or biologic composition to ONC201 or its excipients.

4. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection or psychiatric illness/social situations that would limit compliance with study requirements.

5. Any known clinically significant active infection including bacterial, fungal or viral including hepatitis B (HBV), hepatitis C (HCV) or any underlying disease or in the recent past which could compromise enrollment and safety of the patient

6. Known history of cardiac arrhythmias including atrial fibrillation, tachyarrhythmias or bradycardia, unless arrhythmia is controlled and after Cardiology has cleared patient to receive ONC201. Receiving therapeutic agents known to prolong QT interval will be excluded, however the use of Zofran is permitted. History of CHF, or MI or stroke in the last 3 months will be excluded.

7. Active illicit drug use or diagnosis of alcoholism.

8. Known additional malignancy that is progressing or requires active treatment within 3 years of start of study drug.

9. Concomitant use of potent CYP3A4/5 inhibitors during the treatment phase of the study and within 72 hours prior to starting study drug administration.

10. Concomitant use of potent CYP3A4/5 inducers, which include enzyme inducing antiepileptic drugs (EIAEDs) (see Appendix B), during the treatment phase of the study and within 2 weeks prior to starting treatment. Concurrent dexamethasone is allowed.

11. For Arm F: Exclusively non-contrast-enhancing disease or primary malignant lesion located in the pons or spinal cord.

12. For Arm F: Atypical non-astrocytic histologies such as ependymoma, ganglioma and pleomorphic xanthoastrocytoma, pilocytic astrocytoma, or pilocytic astrocytoma
    and subependymal giant cell astrocytoma (SEGA).

13. Prior treatment with ONC201.

3.3 Inclusion of Women and Minorities

Both males and females of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

The investigator is responsible for enrolling only those patients who have met all eligibility criteria. The investigator is required to register each patient with the Study sponsor (Oncoceutics) prior to enrollment. After a patient signs the informed consent form, the investigator notifies the medical monitor. The investigator must provide de-identified patient source documentation and a registration form prior to the initiating study treatment.

A patient identifier will be assigned at the time of approval. Screening and eligibility *MUST* be entered into Redcap EDC within 7 business days of screening visit.

5. TREATMENT PLAN

5.1 Treatment Regimen

After screening procedures and registration, all subjects will be treated with study treatment, which should begin as close as possible to the date on which the participant is registered. ONC201 will be administered in an outpatient setting.

ONC201 will be administered orally once every week for Arms A-F or twice every week on two consecutive days for Arm G at the designated dose level (see below) that will be converted from the adult equivalent dose based on body weight using the scheme below. C1D1 dose will be taken in clinic. For doses that occur on days where a clinic visit is not scheduled, subjects may take the medication at home.

One treatment cycle is defined as three weeks (21 days).

The target adult dose of 625mg will be allometrically scaled as described below and rounded to 125mg (the strength of one capsule). The specific dose of ONC201 will depend on the body weight of the patient and the dose level they are designated to received. The body weight of patients will be rounded to the near 5kg interval to use the dose chart below. Patients who are <10kg are not eligible to receive the drug at any dose level. If body weight changes occur while on treatment, the number of capsules per dose may be reassessed at beginning of a given treatment cycle.

*NE= not eligible due to body weight and capsule size.

		ONC201 Dose Level		
		2	1	-1
Patient body weight (kg)	ONC201 (mg) equivalent of 625mg adult dose	ONC201 (mg) to be administered*; rounded to capsule strength		
10	145	125	NE	NE
15	197	250	125	NE
20	250	250	125	NE
25	289	250	125	NE
30	331	375	250	125
35	372	375	250	125
40	410	375	250	125
45	450	500	375	250
50	486	500	375	250
>55		625	500	375

***For Arm G: The dose is the amount of ONC201 to be administered each day of the Day 1 & 2 every week dosing schedule.**

For Arms A, B, C, D, F and G ONC201 will be provided as an oral capsule at a strength of 125mg per capsule (alternative strengths may be manufactured). For doses that occur on days where a clinic visit is not scheduled, subjects may take the medication at home.

ONC201 should be taken with a glass of water and consumed over as short a time as possible. Food should not be consumed 2 hours before or for 2 hours after administration of ONC201. Subjects should swallow the capsules as a whole and not chew them. Do not crush or empty the capsule. If vomiting occurs during the course of treatment, no re-dosing of the subject is allowed before the next scheduled dose.

Subjects should be instructed NOT to drink or eat any grapefruit juice and/or grapefruit-related citrus fruits (e.g., Seville orange, pomelos) during the study.

For Arm E, the investigational pharmacy will dissolve the number of ONC201 capsules indicated in the dosing table above into Ora-Sweet at a concentration of 4-40mg/ml with vortexing. Capsules of ONC201 can be opened and the contents of the capsule transferred to amber plastic oval prescription bottles with child-resistant caps. Solutions should be prepared so that only one dose of ONC201 is contained per bottle (i.e. one bottle per week). After administration of the entire contents of the bottle, 1 tablespoon of water or Ora-Sweet should be added to the container, shaken for 10 seconds, and the entire contents of the bottle should be consumed by the patient. The solution may be dispensed for use within 8 weeks from preparation.

Dose Escalation Procedures

Dose escalation will be performed according to a standard 3 + 3 design, as outlined below. Dose levels will be defined by cohorts that are specific to each arm. A **dose limiting toxicity (DLT)** is defined as a drug-related adverse event (AE) or abnormal laboratory value that occurs in the first cycle of treatment, meets **criteria for DLT in section 11.1** and is assessed as unrelated to disease, disease progression, inter-current illness, or concomitant medications, and is judged by the investigator to be "possibly related", "probably related" or "definitely related" to ONC201.

Number of Patients with DLT at a Given Dose Level	**Escalation Decision Rule**
0 out of 6	Enter 3-6 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). A total of six patients will be entered at the next lowest dose level.
1 out of 6	Enter a total of 6 patients at this dose level. • If 1 of these 6 patients experience DLT at this dose level, proceed to the next dose level. • If 2 or more of the 6 patient cohort at this level suffers DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. A total of six patients will be entered at the next lowest dose level.
≤1 out of 6 at highest dose level below the maximally administered dose	This is the maximum tolerated dose and is generally the recommended Phase II dose (RP2D).

Arm A: Single agent dose escalation will proceed according to a standard 3 + 3 design to define a recommended phase 2 dose (RP2D). RP2D will be defined as the maximum tolerated dose (MTD) or maximum administered dose (MAD) that is targeted to achieve the equivalent of the adult 625mg dose (dose level 2) as the MAD. Dose escalation will begin at dose level 1 and will be calculated using the table above. Dose escalation or de-escalation using the outlined dose levels by 125mg increments due to the capsule size. Following RP2D definition, a 12 patient dose-expansion will be initiated.

Arm B: Dose escalation of ONC201 in combination with radiation will begin once dose level 2 of Arm A is open for enrollment. Focal radiotherapy of 54 Gy given in 30 fractions over a 6-week period will be given in combination with ONC201 at the dose level described below.

Dose escalation will begin at dose level -1 as defined in the table above (two capsules less than the adult equivalent of 625mg). Dose escalation or de-escalation

will proceed by 125mg increments due to the capsule size. Following RP2D definition, a 12 patient dose-expansion will be initiated.

The first dose of ONC201 should be administered on Cycle 1 Day 1 at least 2 hours after the first dose of radiation occurs. Radiation is given as indicated above and ONC201 is given until confirmed radiographic progression and clinical progression on 2 consecutive clinic visits within 4-8 weeks. Patients will continue to receive the same dose of ONC201 during and after radiotherapy.

3D conformal or intensity-modulated radiation therapy (IMRT) techniques are allowed. IMRT is encouraged for increased dose conformality. Simulation for radiation planning may be performed with CT using a thermoplastic mask for positioning. Daily sedation for radiation simulation and treatment may be used if necessary. Radiation will be prescribed to the planned target volume (PTV), as defined below, to a dose of 54 Gy in 30 once-daily fractions given over six weeks. Ideally 95% of the PTV will receive at least 95% of the prescription dose with no more than a 107% hotspot.

Tumor volumes will be defined as:
GTV = gross tumor volume as defined on MRI T1+contrast, T2, or FLAIR sequences. If MRI is unavailable, planning may be based on CT.

Clinical target volume (CTV) = GTV + 1-2 cm anatomically constrained expansion, recognizing barriers to tumor spread such as bone.

PTV = 3-5 mm volumetric expansion on the CTV, according to local practice based on available image guidance.

Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Arm C: ONC201 will be administered as a single agent for 1 dose approximately 24 hours prior to planned biopsy or resection. Following the surgery, subjects will continue to take the ONC201 on a weekly basis. Subjects who have previously received radiation therapy will receive the ONC201 as a single agent as per its RP2D definition in Arm A. Subjects who are newly-diagnosed, will receive the ONC201 in combination with radiation and following radiation as per the RP2D definition in Arm B or the current dose level if the RP2D has not yet been established.

Arm D: ONC201 will be administered as a single agent as per its RP2D definition in Arm A or Arm B for previously treated or newly diagnosed patients, respectively. If the RP2D has not yet been established, the current dose in Arm A or B will be used as appropriate.

Arm E: Dose escalation will begin at dose level -1 as defined in the table above (two capsules less than the adult equivalent of 625mg). Dose escalation or de-escalation will proceed by 125mg increments due to the capsule size that will be dissolved in the liquid formulation. Following RP2D definition for the liquid formulation, a 12 patient dose-expansion will be initiated.

Arm F: ONC201 will be administered as a single agent oral capsule as per its RP2D definition in Arm A.

Arm G: Dosing for Arm G is twice per week on two consecutive days. Dose escalation will begin at dose level -1 as defined in the table above (two capsules less than the adult equivalent of 625mg each day). Dose escalation will proceed by 125mg increments each day due to the capsule size to dose level 1 (3 patients) and dose level 2 (6 patients). A total of 12 patients are expected to accrue to this arm in the absence of DLTs.

5.2 Screening Procedures

After providing informed consent, subjects will undergo screening for eligibility to participate in the study. Screening will start within 14 days prior to the first dose of ONC201. Subjects in Arms A, B, C, E, F and G who have had an adequate MRI performed as part of routine care prior to informed consent but within 14 days of the first dose of ONC201 will not have to repeat the baseline MRI. Subjects in Arm D must have had an adequate MRI performed within 3 months of study enrollment. Subjects on Arm D will undergo an MRI of the brain and spine with a lumbar puncture after informed consent and before receiving study drug. At least 3D FLAIR and post contrast T1 sequence should be obtained with thin cut orthogonal planes in order to gauge the degree of brainstem expansion, cisternal effacement, and craniocaudal extent. Best efforts should be made to keep the MRI technique constant for each patient from baseline and throughout treatment. Refer also to the Schedule of Events for details of study procedures.
The following procedures will be performed or obtained at screening for the purpose of determining study eligibility. Screening studies, if performed within 2 weeks prior to start of treatment, are acceptable for use as baseline (pre-Cycle 1, Day 1 assessments).
Screening Assessments:

- Pathology report confirming the diagnosis of any glioma, including but not limited to glioblastoma, astrocytoma, oligodendroglioma, high-grade glioma, malignant glioma, etc. For Arm F, histology must be a high-grade glioma.
- Documentation confirming Histone H3 K27M mutation from a CLIA-certified laboratory with an approved test (e.g. immunohistochemistry, DNA sequencing) on any glioma tumor sample for Arm D and Arm F (if available for Arms A, B, C, E and G).
- Collection of archived tumor material for research: The subject will be asked to arrange to provide archival tumor tissue from a surgical resection as

specified in the Subject Selection Criteria (Section 3). This is required for Arm F.

- A contrast-enhanced brain and entire spine MRI must be obtained within 14 days of the first dose of study treatment.
- Medical history, eligibility, and concomitant medications: The subject must be eligible by all of the Subject Selection Criteria per Section 3. Concomitant medications will be reviewed for allowed or prohibited medications. In addition, for Arm F, provide imaging assessments from initial diagnosis, surgery planning, post-surgical, post chemoradiation, scheduled and unscheduled MRIs.
- 12-Lead ECG: A standard 12-lead ECG will be required only at screening. The ECG strips or reports will be retained with the source. The ECG will be reviewed by the investigator (paper or electronic tracing) and will be available for comparison with subsequent ECGs by the investigator if needed. Any ECG finding performed during the study period after screening that is judged by the investigator as a clinically significant change (worsening) compared to the baseline value will be considered an AE, recorded, and monitored. The following will be recorded on the CRF:
  - PR Interval (msec)
  - QRS Interval (msec)
  - QT Interval (msec)

- Heart Rate (BPM; recorded from the ventricular rate).
- Physical exam and laboratories: For complete details see the Schedule of Events.
- Complete physical exam, including neurological exam, and KPS or Lansky assessment
- Vital signs: height (height is only required at screening), weight, temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), amylase, lactate dehydrogenase (LDH)
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Coagulation test (required at screening only): PT/INR, , PTT
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential

5.3 Baseline Procedures

One treatment cycle will be defined as 21 days, corresponding to 3 doses of ONC201 (i.e., one cycle is 3 weeks). If ONC201 dosing is delayed, the cycle count should be interrupted.

The subject will have all of the C1D1 (i.e., baseline) procedures detailed below performed prior to initiating study treatment (first dose of ONC201), within 14 days of treatment initiation. Evaluations performed at screening that fall within 14 days of treatment initiation will not need to be repeated.

- Medical history, eligibility, and concomitant medications: The subject must be eligible by all of the Subject Selection Criteria per Section 3. Concomitant medications will be reviewed for allowed or prohibited medications.
- Physical exam and laboratories: For complete details see the Schedule of Events.
- Complete physical exam, including neurological exam, KPS or Lansky assessment, and cranial nerve palsy scoring (Appendix C).
- Vital signs: height (height is only required at screening), weight, temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), amylase, lactate dehydrogenase (LDH).
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Coagulation test (required at screening only): PT/INR, PT, PTT
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential, if applicable.

Research Studies

- Research blood samples: All subjects will have the below research blood samples collected at baseline (prior to the first dose of ONC201). Details of these procedures are provided below. Preparation and shipping instructions are provided in the lab manual.
- >2 mL of blood will be drawn into one red-top BD Vacutainer tube without anti-coagulant) for measurement of serum prolactin and other proteins and cytokines as pharmacodynamic biomarkers.
- >2 mL of blood will be drawn into one purple-top EDTA BD Vacutainer tube for measurement of ONC201 plasma concentrations.
- At discretion of physician, ≥4 mL of cerebrospinal fluid prior to the C1D1 dose may be collected to assess correlation between H3 K27M mutation detected in tumor and cerebrospinal fluid specimens. For patients in Arm D: CSF will be obtained via lumbar puncture during a MRI procedure with sedation, as required. Local anesthesia for spinal tap is also allowed. At least 4 mL of CSF should be collected for patients <5 years of age and 8-10 mL should be collected for patients ≥5 years of age.
- **ONC201 Administration:** Subjects will take one dose of oral ONC201 in the clinic at the dose specified in Section 5.1. Subjects will be dispensed a supply of ONC201 to be taken weekly at home that is sufficient for dosing until the next treatment cycle or scheduled visit. For patients in Arm C, the first dose of ONC201 should be administered one day (~ 24 hours) prior to a scheduled

tumor biopsy. For patients on Arm G patients, Day 1 of Cycle 1 is to be taken in the clinic and the patient will be dispensed a supply of ONC201 to be taken the next day, as well as twice per week on consecutive days, that is sufficient for dosing until the next treatment cycle or scheduled visit.

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5.4 On-Treatment Procedures

For the first 6 months of treatment (9 cycles), at the beginning of each 21-day treatment cycle (i.e., at Day 1 of Cycle 2 and Day 1 of each cycle thereafter), subjects will have the following procedures. After 6 months of treatment (9 cycles), patients will have the option to return to the study center at the beginning of every third cycle (every 63 days (±10 days)), subjects will have the following procedures. Patients returning to the study center every 3 cycles are required to have serum chemistry and hematology performed locally every three weeks and the results provided to the Investigator. After Cycle 9, research blood collection will be performed only at the MRI visits. If ONC201 dosing is delayed, the cycle count should be interrupted. NOTE: Patients on the OraSweet formulation must return to the clinic at less than 8-week intervals. See below.

- Concomitant medications
- Adverse event assessment
- Physical exam and laboratories: For complete details see the Schedule of Events.
- Complete physical exam, including neurological exam, KPS or Lansky assessment, and cranial nerve palsy scoring (Appendix C).
- Vital signs: weight, temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), amylase, lactate dehydrogenase (LDH).
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- **Research Blood Collection**: Research blood samples will be collected at 0.5 hours (C1D1), 2 hours (C1D1), 4 hours (C1D1), 24 hours (C1D2), and 48 hours (C1D3) after the first dose of ONC201, and then 7 days (C1D8), 14 days (C1D15), and 21 days (C2D1 pre-dose) following the first dose of ONC201. Subjects in Arm G will have additional samples drawn on C1D2 at 0.5, 2, 4 hours following the second dose. For all arms, research blood samples will also be collected pre-dose on Day 1 of every even cycle thereafter (ie, C2D1, C4D1, C6D1, until C8, and then on the MRI visits thereafter). Details of these procedures are provided below. Preparation and shipping instructions are provided in the lab manual. Note: For subjects who live outside of the United States or live a significant distance from the study site, the Cycle 1 Day 8 and Cycle 1 Day 15 research blood specimens may be

omitted with prior approval from the Study Sponsor. However, all efforts must be made to obtain these blood specimens if feasible.

- >2 mL of blood will be drawn into one red-top BD Vacutainer tube without anti-coagulant) for measurement of serum prolactin and other proteins and cytokines as pharmacodynamic biomarkers
- >2 mL mL of blood will be drawn into one purple-top EDTA BD Vacutainer tube for and measurement of ONC201 plasma concentrations.
- **ONC201 Administration:** Subjects will be dispensed a supply of ONC201 to be taken weekly (for Arm G: twice per week on consecutive days) at home that is sufficient for dosing until the next treatment cycle or scheduled visit. If the patient has completed nine cycles without major (grade 3-4) ongoing toxicity or protocol non-compliance, the patient may be dispensed an 9-week supply. However, if the patient is taking the OraSweet formulation, do not dispense a 9-week supply. Patients on the OraSweet formulation must return the clinic before 8 weeks to obtain ONC201 as the stability of ONC201 is 8 weeks.
- **Collection of Study Drug diary:** Subjects will be provided at the beginning of each cycle (or every third cycle after Cycle 9) a Study Drug diary to track drug administration and side effects while receiving therapy.
-

5.5 Efficacy Procedures

- Patients will have tumor (disease) response assessments by contrast-enhanced MRI performed at 8 weeks (+/- 10 days) after initiation of study drug and then every 8 weeks (+/- 10 days) thereafter. Disease assessments should occur every 8 weeks after initiation of study drug regardless of the timing of treatment cycles. After Cycle 9, this may result in a cumulative change in the visit schedule, which is acceptable. However, it is important that the interval since the last MRI/CT assessment is approximately 8 weeks, be no shorter than 46 days and no longer that 66 days (8 weeks +/- 10 days).
- For patients in Arm D: CSF will be obtained via lumbar puncture during the MRI procedure with sedation (as required) every 8 weeks. Local anesthesia for spinal tap is also allowed. At least 4 mL of CSF should be collected for patients <5 years of age and 8-10 mL should be collected for patients ≥5 years of age.
- Efficacy will be assessed by RANO response criteria (Wen PY, et al. 2010) as per Section 11.
- In addition to reassessment scans, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response (OR). To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met.
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5.6 End of Treatment Visit

If subjects come off treatment for progression of disease, intolerance, or patient withdrawal, or other study criterion, they will be seen in the clinic for assessments of efficacy and safety 30 days (+/- 7 days) after the last dose of study drug, unless the subject's medical condition limits their ability present for a clinical evaluation. End of treatment evaluations will include:

- Concomitant medications
- Adverse event assessment
- Physical exam and laboratories: For complete details see the Schedule of Events.
- Complete physical exam, including neurological exam, KPS or Lansky assessment, and cranial nerve palsy scoring (Appendix C).
- Vital signs: weight, temperature, resting blood pressure, pulse and respiration rate.
- Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH).
- Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count
- Pregnancy test (urine or serum β-HCG) for women of child-bearing potential
- If tumor assessments have not been done within the prior 4 weeks of coming off study, these will be obtained within the next 2 weeks after the last dose of study drug. For patients who discontinue for toxicity or withdrawal, tumor imaging is at the judgment of the investigator, suggested to be every 8-12 weeks in appropriate setting.
- Collection of Study Drug diary: Subjects Study Drug diary at the last cycle will be collected and reviewed by clinical staff.

After the end of treatment, each subject will be followed for 30 days for adverse event monitoring and 90 days for serious adverse event reporting.

Subjects removed from protocol therapy for unacceptable adverse events (Section 7) will be followed until resolution or stabilization of the adverse event.

Subjects who discontinue treatment will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up (see section 5.9 below).

5.7 General Concomitant Medication and Supportive Care Guidelines

In vitro cytochrome P450 assays were conducted in human hepatocytes. In these studies, ONC201 is not an inducer of the CYP450 system (CYP 1A2, 2B6 and 3A4). ONC201 was observed to be a mild inhibitor of the CYP450 enzymes (1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) at 35-429uM, i.e. at least 3.5-fold above the Cmax observed in the first-in-human trial.

Any medication taken by a subject during the course of the study and the reason for its use will be documented in the clinical source documentation.

5.8 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. Treatment will be discontinued for confirmed progression of disease, intolerance (defined as NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 Grade 4, Grade 3, or intolerable Grade 2 toxicity that does not return to Grade 1 or baseline after 4 week interruption of treatment or reduction of dose) despite dose modification, or patient withdrawal. A patient who experiences an adverse event is able to remain on study at a reduced dose (see section 6), if deemed by the physician to be safe and beneficial for the patient to continue.

In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Confirmed radiographic disease progression. In the absence of clinical progression and occurrence of radiographic progression, patients will have the option to remain on study.
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Treatment interruption for more than 4 consecutive weeks due to intolerance despite appropriate dose modification,
- Participant decides to withdraw from the study
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the opinion of the treating investigator,
- Global deterioration of health-related symptoms,
- Protocol non-compliance,
- Pregnancy,
- Lost to follow-up, or
- Study termination by Sponsor.

If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document patient outcome, if possible. The Investigator should inquire about the reason for withdrawal, request that the patient to return all unused investigational product(s), request the patient to return for a final visit, if applicable, and follow-up with the patient regarding any unresolved adverse events.

If the patient withdraws from the trial and also withdraws consent for disclosure of future information, then no further evaluations should be performed and no

additional data should be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent.

5.9 Duration of Follow Up

All subjects will be followed post-treatment until death, withdrawal of permission to record at least survival data, or when the subject is lost to follow-up. When available, tumor imaging will be followed for 6 months after coming off study. All subjects will be contacted every 30 days (+/- 7 days) to assess for study-treatment related toxicities, initiation of any new anti-cancer treatments, and survival status. Contact may be performed by a site visit, telephone contact, e-mail, or mail. The date of death, initiation of any new anti-cancer treatments and date of last contact should be documented if this information is available.

Post-mortem biopsy is required if H3 K27M status of tumor is unknown and archival tumor tissue not available to determine the H3 mutation status.

Participants will be removed from study when any of the following criteria apply: lost to follow-up, withdrawal of consent, death. The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

If a subject withdraws permission to record at least survival data after coming off treatment, this must be documented along with the date the subject withdraws permission. Subjects will be considered lost to follow up if no medical records are available to be reviewed and two phone calls each to the subject and then the subject's next-of-kin (if the subject does not respond) are not returned over two consecutive 30 day periods. All attempts should be documented in the subject’s medical records. If it is determined that the subject has died, the site will use permissible local methods to obtain the date and cause of death. The site staff will consult publicly available sources, such as public health registries and databases, in order to obtain updated contact information or final survival data. If after all attempts, the subject remains lost to follow-up, then the last known alive date as determined by the investigator should be reported and documented in the subject’s medical records.

5.10 Taking a Participant Off Study

Patients will be removed from study when any of the criteria listed in Section 5.8 applies. The reason for study removal and the date the patient was removed must be documented in the clinical source documentation.

Severe adverse events, availability of new adverse toxicology in animals, and financial difficulties due to withdrawal of funds may result in stopping the trial. An investigator, Sponsor, or IRB may take such actions. If the trial is terminated for safety reasons, subjects will be notified immediately and assured that

appropriate treatment and follow-up will be available. If an investigator terminates the trial the investigator will inform the Sponsor, subjects, and IRB about the reason for such action. Similarly, if the Sponsor terminates the trial, it will inform the investigators, the IRB, and the subjects of the reason for such an action. Similar notifications will be sent by the IRB if it takes such an action.

The reason for taking a participant off study, and the date the participant was removed, must be documented in the clinical source documentation.

6. DOSING DELAYS/DOSE MODIFICATIONS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications

Below are dose modifications (Table 6.1 and 6.2) for adverse events that are attributable to study drug, including: nausea, vomiting, diarrhea, neutropenia, and thrombocytopenia. If a subject experiences other adverse events, or several adverse events and there are conflicting recommendations based on Grade, the investigator will use the recommended dose adjustment that reduces the dose. Dose modifications will be made based on an adverse event that occurs during any time during a cycle.

Criteria for disrupting treatment, dose modification, or discontinuation are listed in Table 6.1. Dose modifications are per the clinical judgment of the investigator and agreement of the subject, the treatment will be resumed according to the table below. Dose modification may not be required for adverse events that are clinically manageable.

Table 6.1 Dose adjustment rules for Adverse Events (AEs), including Nausea, Diarrhea, Neutropenia, Thrombocytopenia, and Other AEs. Alopecia does not require dose adjustments.

CTCAE Grade	Management/Next Dose for ONC201
≤ Grade 2	No change in dose
Grade 3 or 4	Hold until ≤ Grade 2. If resolved to ≤ Grade 2 within 7 days, resume dosing at dose level lower (one less capsule per dose). No more than two dose reductions per patient are permitted. Patients requiring a delay of >6 weeks or a dose of <125mg should go off protocol therapy. Patients with grade 3 or greater neutropenia associated with fever must also go off therapy.

Participants who experience an adverse event that requires a treatment delay or dose reduction should be monitored with appropriate laboratory testing or other clinical evaluation at least weekly until resolution; if the adverse event does not resolve within

4 weeks, the interval for testing may be reduced after consultation and written approval by Sponsor.

For holds for reasons other than treatment related toxicities, if the participant does not meet criteria to resume treatment within 6 weeks of the event precipitating the hold, the study agent(s) may be restarted with approval from the Sponsor, as long as there has been no significant evidence of disease progression (e.g., by clinical findings, symptoms, tumor markers) during the treatment interruption.

7. ADVERSE EVENTS AND REPORTING REQUIREMENTS

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol- specified procedure, whether or not considered related to the medicinal product or protocol- specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the ONC201, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to onset of menses or menopause occurring at a physiologically appropriate time.

Adverse events may occur during the course of the use of ONC201 product in clinical trials or within the follow-up period specified by the protocol, from overdose (whether accidental or intentional), from abuse and from withdrawal. Adverse events may also occur in screened subjects during any pre-allocation baseline period as a result of a protocol-specified intervention, including washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Progression of the cancer under study is not considered an adverse event.

All adverse events will be recorded from the time the date of initiation of study therapy through 30 days following cessation of treatment and at each examination. Serious Adverse Events will be followed through 90 days following cessation of treatment. Both Adverse events and Serious Adverse Events will not be collected for subjects during the pre-screening period (for determination of archival tissue status) as long as that subject has not undergone any protocol- specified procedure or intervention. If the subject requires a blood draw, fresh tumor biopsy etc., the subject is first required to provide consent to the main study and AEs will be captured according to guidelines for standard AE reporting.

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. All Adverse Events regardless of seriousness or relationship to the Investigational Product will be recorded in the Case Report Forms. Serious Adverse Events should be reported per the requirements described in Section 7.1.

7.1 Reporting of Serious Adverse Events

Serious Adverse Events: A serious adverse event is any adverse event occurring at any dose or during any use of ONC201 that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- The subject becomes pregnant. If subject becomes pregnant, the investigator must immediately (within 24 hours of awareness of the pregnancy) notify the Sponsor or designee of this event.
- Is another important medical event

Progression of the cancer under study is not considered an adverse event.

Inpatient hospitalization for events due to progression of the cancer will not be considered a serious adverse event.

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time of initiation of therapy through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to ONC201, must be reported within 24 hours to Oncoceutics.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to ONC201 that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to Oncoceutics.

Investigators **must** report to Oncoceutics any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 3 days of the last dose of treatment on the SAE form provided by Oncoceutics.

SAE reports and any other relevant safety information are to be forwarded to the pharmacovigilance@oncoceutics.com and the Oncoceutics facsimile number within 24 hours of learning of its occurrence: 1-844-245-7650

Oncoceutics will submit all required SAE Reports and Annual Progress Reports to the FDA as required by FDA or other local regulators.

Investigators **must** report SAEs to the local IRB following local IRB reporting requirements.

All subjects with serious adverse events must be followed up for outcome until resolution of the SAE.

For further information related to preclinical and clinical safety experience with ONC201, including expected adverse events, please refer to the Investigator's Brochure.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

8. PHARMACEUTICAL INFORMATION

The study drug ONC201 is provided as 125 mg free base (approximately 150 mg of dihydrochloride), that may include the following excipients: microcrystalline cellulose, sodium starch glycolate, and/or magnesium stearate, filled into hydroxypropyl methylcellulose (HPMC) capsule shells. Alternative strengths may be manufactured.

The ONC201 drug product capsules are intended for oral administration. The product is stored in a multi-dose container. The capsules are packaged in high-density polyethylene (HDPE) white opaque bottles, closed with an induction seal and capped with a white ribbed SecuRx® polypropylene (PPE) cap. The capsules are to be stored in the original closed container at room temperature (15 to 30°C).

The study drug bottle label bears the following information. If alternative strengths are manufactured, the dosage on the label will be inserted in place of '125 mg'.

ONC201 Capsules, 125 mg

For Oral Use Only

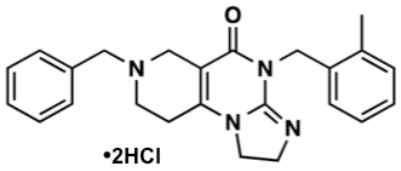
Caution: New Drug--Limited by Federal (or United States) law to investigational use.

Storage: Preserve in original tightly closed containers at room temperature (15 to 30°C)

Sponsor: Oncoceutics, Inc.

Batch # xxx-xxx-xxxx-xx **Mfg date: XX-XXXX**

8.1 Drug Substance Description

Compound Code(s)	ONC201•2HCl
Alternative Name(s)	ONC201 TIC10 NSC-350625
Chemical Name(s)	7-benzyl-4-(2-methylbenzyl)-1,2,6,7,8,9-hexahydroimidazo[1,2-a]pyrido[3,4-e]pyrimidin-5(4H)-one 2HCl
Molecular Formula	C24H26N4O (free base) C24H26N4O•2HCl (salt)
Molecular Weight	386.49 (free base) 459.41 (salt)
Molecular Structure	

8.2 Drug Product Description

8.2.1 Form

The drug product is comprised of ONC201 dihydrochloride (ONC201•2HCl) formulated with excipients that may include the following: microcrystalline cellulose (MCC, Avicel® PH-112), sodium starch glycolate, and/or magnesium and filled into Size 2, white, opaque, vegetable-based

hydroxypropylmethylcellulose (HPMC) capsule shells intended for oral administration. The ONC201•2HCl drug substance is a white to off-white solid. The drug product will be packaged as 10 or 25 capsules per bottle. The capsules are filled into a 30cc high-density polyethylene (HDPE) white opaque bottle sealed with a 28cc child-resistant SecuRx polypropylene (PPE) cap with a heat induction seal.

For Arm E, the investigational pharmacy may dissolve ONC201 into Ora-Sweet at a concentration of 4 to 40mg/ml with vortexing. Capsules of ONC201 can be opened and the contents of the capsule transferred to amber plastic oval prescription bottles with child-resistant caps. Solutions should be prepared so that only one dose of ONC201 is contained per bottle (i.e. one bottle per week). After administration of the entire contents of the bottle, 1 tablespoon of water or Ora-Sweet should be added to the container, shaken for 10 seconds, and the entire contents of the bottle should be consumed by the patient. The solution may be dispensed for use within 8 weeks from preparation.

8.2.2 Storage and Stability

Based on the current stability data at 40°C/75%RH room temperature (25°C/60%RH) will be used for the drug product storage. Drug product stability studies found no change after 1 month at 40°C/75%RH when stored with or without desiccant. Similarly, no changes have been observed when stored at room temperature for 1 year. Clinical trial batches will be produced without desiccant. No shelf-life has been established for this product at this point. However, representative clinical trial batches have been placed on stability. Any batches that are out of specifications will be removed from the trial.

For the ONC201 drug substance, stability results show little to no change in assay, impurities or appearance. The only changes observed under the accelerated conditions (40°C/75%RH) where a slight increase in moisture content was observed, from 1.2% at time 0, to 6.5% at 2 months, and to 6.2% at 3 months. The moisture content plateaued at approximately the monohydrate. The increase in moisture content did not result in increased impurity levels or decreased potency. These results suggest robust stability of the drug substance when stored at room temperature and accelerated conditions.

8.3 Drug Product Supply, Administration and Inventory

8.3.1 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

For the liquid formulation, the investigational pharmacy will dissolve the number of ONC201 capsules indicated in the dosing table above into Ora-Sweet at a concentration of 4 to 40mg/ml with vortexing. Capsules of ONC201 can be opened and the contents of the capsule transferred to amber plastic oval prescription bottles with child-resistant caps. Solutions should be prepared so that only one dose of ONC201 is contained per bottle (i.e. one bottle per week). After administration of the entire contents of the bottle, 1 tablespoon of water or Ora-Sweet should be added to the container, shaken for 10 seconds, and the entire contents of the bottle should be consumed by the patient. The solution may be dispensed for use within 8 weeks from preparation.

8.3.2 Availability

ONC201 will be provided by the Sponsor, Oncoceutics, Inc.

8.3.3 Administration

Patient will take the first dose of ONC201 in the clinic. All subsequent doses are to be taken at home. Patients will receive a supply of ONC201 while in the clinic that is sufficient for dosing until the next treatment cycle or scheduled visit. They will take ONC201 weekly at home as specified by their cohort. A Study Drug diary will be provided to the patient.

Patients should take the dose of ONC201 specified by their physician 2 hours prior and 2 hours following food or a meal at approximately the same time of day every 7 days. For patients receiving ONC201 in combination with radiation, ONC201 should be taken on Mondays at least 2 hours post radiation assuming radiation is administered on a Monday through Friday schedule. For Arm G, patients take ONC201 on two consecutive days (e.g. Monday and Tuesday) at approximately the same time of day every 7 days.

ONC201 should be taken with a glass of water and consumed over as short a time as possible. Patients should swallow the capsules as a whole and not chew them. Do not crush or empty the capsule. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. The occurrence and frequency of any vomiting during a treatment cycle must be noted as an adverse event. Missed doses will not be made up, if more than 3 days from the intended day of administration.

8.3.4 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage).

8.3.5 Destruction and Return

Unused supplies of the agent should be returned to the Sponsor within 60 days of the completion of the study. Unused supplies of the agent may also be destroyed on-site upon completion of the study, after accountability has been audited by the Sponsor or designated representative.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Detailed procedures and supplies for biospecimens are described in the **laboratory manual** associated with this protocol.

9.1 Pharmacokinetics

Plasma will be used for evaluation of ONC201 concentration, as well as identification and quantification ONC201 metabolites. Samples of peripheral blood will be collected in K2EDTA tubes with a minimum of 2mL collected per time point for PK analyses at the following time points: C1D1: 0 (pre-dose), 0.5, 2, 4, 24 (pre-dose C1D2), 48 hours (C1D3), 7 (C1D8 pre-dose), 14 (C1D15 pre-dose), and 21 days (C2D1 pre-dose) following the first dose of ONC201; pre-dose on Day 1 of every even cycle. Subjects in Arm G will have additional samples drawn on C1D2 at 0.5, 2, 4 hours following the second dose. Subjects in Arm C will have pharmacokinetics sampled with the first dose of ONC201 following surgery. Pharmacokinetics will not be sampled on the single dose prior to surgery.

9.2 Predictive Tumor Biomarker Studies

If available, archival tumor tissue samples will be obtained at the time of enrollment for subjects enrolled in this study to assess predictive biomarkers, including histone H3 mutation and expression of DRD2 and DRD5. A minimum of 5 FFPE tissue slides will be obtained if available as described in Section 3. Tumor tissue slides may also be requested from biopsies obtained after enrollment.

9.3 Circulating Pharmacodynamic Analyses

Prolactin and other proteins will be measured in serum as pharmacodynamic markers. **One red-top tube (without anti-coagulant) of blood** will be collected at the following time points for all arms: C1D1: 0 (pre-dose), 0.5, 2, 4, 24 (C1D2), 48 hours (C1D3), 7 (C1D8 pre-dose), 14 (C1D15 pre-dose), and 21 days (C2D1 pre-dose) following the first dose of ONC201; pre-dose on Day 1 of every even cycle up to Cycle 8. After Cycle 8 blood for pharmacodynamic markers will be collected every third cycle, beginning with Cycle 12.

9.4 Immune Studies

Immune cytokines and effectors such as IL-2, IL-4, IL-10, IL-17A, TNF-alpha, IFN-gama, sFasL, sFas, Granzyme A/B, perforin, granulysin will be assessed on serum samples obtained for pharmacodynamic analyses. No additional blood samples will be collected from the patient for these studies.

9.5 Imaging submission

Each contrast-enhanced MRI (or CT if MRI is contraindicated) must be submitted for sponsor review. For Arm F, this includes: MRI scans prior to current study (Initial diagnosis, surgery planning, post-surgical, post chemoradiation, scheduled and unscheduled MRIs); Baseline scan, disease assessment scans performed every 8 weeks (+/- 7 days), and End of Treatment scan done 30 +/- 7 days post last dose.

The complete MRI imaging data sets must be submitted to Oncoceutics Inc. in digital DICOM format within 14 days (+/- 7 days) from each MRI acquisition.

Each submission should be de-identified, labeled with the patient study ID number, the time point of the scan (e.g. initial diagnosis,; Baseline, Week 8, etc.), and the industry protocol number. To submit you can use any of these methods:

1) **FTP Transfer:** Each site should coordinate with Oncoceutics Inc. to set up FTP transmission of images. An imaging manual will be provided under separate cover.

2) **Shipment/Mail:** If the above electronic data transfer cannot be achieved, the de-identified images can be burned to a CD in DICOM format and labeled with the patient study ID number, the time point of the scan (e.g. Baseline, Week 8, etc.), the date of the scan, and the industry protocol number listed on the CD cover and mailed to Oncoceutics Inc. via FedEx Standard overnight at:

Oncoceutics Inc.
3675 Market Street
Suite #200
Philadelphia, PA 19104
#215-966-6115
clinicaloperations@oncoceutics.com

10. STUDY CALENDAR

Study visits and procedures may be scheduled with a +/- 3 business day window except for Screening procedures (-14 days) and neuroimaging (MRI) (+/- 10 days). **One treatment Cycle is defined as 3 weeks (21 days).** One **Disease Assessment Cycle is defined as 8 weeks** (56 days +/- 10 days).

	Screening / Baseline	**Treatment Cycles**	**Disease Assessment Cycles**	**End of Treatment / Follow-up**	**Survival Follow Up**
	Within 14 days of Treatment	**Every 3-9 weeks (21 +/- 3/63 +/-7 days)**	**Every 8 weeks (56 +/- 10 days)**	**(30 +/- 7 days post last dose)**	**(30 +/- 7 days of EOT/Follow Up Visit)**
Informed Consent	X				
Demographics	X				
Inclusion/Exclusion Criteria	X				
Medical/Disease history, including prior MRI/CT scans	X				
Histone K3 K27M mutation[1]	X				
KPS or Lansky assessment	X	X			
Cranial nerve palsy scoring	X	X		X	
Physical exam, Neurologic exam	X	X		X	
Vital signs[2]	X	X		X	
Hematology[3]	X	X		X	
Serum chemistries[4]	X	X		X	
12 Lead ECG	X				
Coagulation tests[5]	X				
Pregnancy test[6]	X			X	
Disease assessment[7]	X		X	X	
Adverse Event Assessment[12]		→	→	→	→
Concomitant medications		→	→	→	→
Research Blood samples: NOTE footnote 8 for all collection timepoints		X[8]		X	
ONC201 Dispensation[9]	X	X			
Disease status and survival[10]				X	X
Archival tumor tissue[11]	X				
CSF sample[13]	X		X		

1. Documentation confirming Histone H3 K27M mutation from a CLIA-certified laboratory with an approved test (e.g. immunohistochemistry, DNA sequencing) on any tumor sample is required for subjects on Arm D and F and is recommended for subjects on Arms A, B and C. Presence of this mutation in circulating DNA obtained from cerebrospinal fluid will be assessed (if obtained). Post-mortem biopsy is required if H3 K27M status of tumor is unknown and archival tumor tissue not available.
2. Vital signs: Height (required only at screening), weight, blood pressure, respiration, pulse, oral temperature. Determine if significant weight loss or gain (±10%).
3. Hematology: WBC count, 3-cell differential (ANC, monocyte count, lymphocyte count), hemoglobin, hematocrit, and platelet count. Hematology is required for every cycle (every 3 weeks), may be performed locally, if no study center visit is performed.

4. Serum chemistry: sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorous, random glucose, albumin, total protein, creatinine, blood urea nitrogen (BUN), uric acid, ALT, AST, total bilirubin, direct and/or indirect bilirubin, alkaline phosphatase (ALP), amylase, lactate dehydrogenase (LDH). Serum chemistry is required for every cycle (every 3 weeks), may be performed locally, if no study center visit is performed.
5. Coagulation tests (required at screening only): PT/INR, PT, PTT
6. Pregnancy Test – baseline within 14 days of start of treatment and at end of study - urine or serum β-HCG in women of child bearing potential.
7. Neuroimaging (disease assessments). Contrast-enhanced MRI disease assessments will be performed at 8 weeks and then every 8 weeks (+/- 10 days) thereafter until patients come off study. In addition, for Arm F, provide imaging assessments from initial diagnosis, surgery planning, post-surgical, post chemoradiation, scheduled and unscheduled MRIs
8. **Research Blood samples**: Blood for PK/PD evaluations will be collected at the following timepoints: pre-dose, 0.5, 2, 4, 24 (C1D2), 48 hours (C1D3), 7 (C1D8 pre-dose), 14 (C1D15 pre-dose), and 21 (C2D1 pre-dose) days following the first dose of ONC201; pre-dose on Day 1 of every even cycle through Cycle 8. Subjects in Arm G will have additional PK samples drawn on C1D2 at 0.5, 2, 4 hours following the second dose; no PD samples are required at these times. For all arms, after Cycle 8 blood for pharmacodynamic markers will be collected every third cycle, beginning with Cycle 12. Please refer to the laboratory manual for complete details on timing, collection, handling, and shipping of research blood specimens. Note: For subjects that live outside of the United States or live a significant distance from the study site, the Cycle 1 Day 8 and Cycle 1 Day 15 research blood specimens may be omitted with prior approval from the Study Sponsor. However, all efforts must be made to obtain these blood specimens if feasible.
9. ONC201 Dispensation. At the C1D1 visit and at each treatment cycle (every 3 weeks), subjects will be given a supply of ONC201 for administration at home until the next treatment or scheduled visit. After 9 cycles on therapy, patient may be dispensed 9 weeks of capsules. Patients on OraSweet formulation must return before 8 weeks for a resulpply of ONC201. For patients also receiving radiation, ONC201 should be taken on Mondays assuming that radiation is administered on a Monday through Friday schedule.
10. All subjects will be followed post-treatment until death, withdrawal of permission to record at least survival data, or subject is lost to follow-up every 30 days (±7 days) after coming off study.
11. Obtain archival tumor tissue at the time of enrollment, in accordance with the laboratory manual. Subjects are required to submit archival tumor samples as specified in Section 3.
12. All adverse events will be recorded from the time the date of initiation of study therapy through 30 days following cessation of treatment and at each examination. Serious Adverse Events will be followed through 90 days following cessation of treatment.
13. For patients in Arm D: CSF will be obtained via lumbar puncture during the every 8-week MRI procedure with sedation, as required. Local anesthesia for spinal tap is also allowed. At least 4 mL of CSF should be collected for patients <5 years of age and 8-10 mL should be collected for patients >5 years of age. These patients will only undergo lumbar puncture during sedation for MRI.
14. If a patient completes 6 months of treatment (9 cycles), the patient may be seen every 9 weeks for routine assessment of physical exam, laboratory assessments, including serum chemistries, hematology, research blood sampling and AE/concomitant medication assessment.

11. MEASUREMENT OF EFFECT

After signing informed consent patients will undergo screening procedures including baseline radiologic imaging by contrast-enhanced MRI within 14 days prior to initiating study drugs.

Patients who remain on study will have tumor assessments performed at 8 weeks (+/- 7 days) after initiation of therapy and then every 8 weeks (+/- 7 days) thereafter. In addition to reassessment scans, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response. To be assigned a status of PR or CR changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met.

Efficacy will be assessed by the RANO response criteria will be used as noted below.

11.1 Definitions

Dose Limiting Toxicity
Safety assessment will be performed using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The DLT window is the defined as the first cycle of ONC201, i.e. Cycle 1 Day 21. This will constitute the first 3 doses of ONC201 in Arm A, the first 4 doses of ONC201 in Arm B, and the first 6 doses of ONC201 in Arm G.

General:

- Patients who discontinue ONC201 treatment due to an Adverse Event (AE) at least possibly related to ONC201 during cycle 1

Non Hematologic DLT:

- ≥ Grade 3 non-hematological toxicity (excluding transient electrolyte abnormalities lasting less than 96)
- ≥ Grade 3 nausea, vomiting, or diarrhea that has persisted for > 72 hours despite optimal antiemetic or antidiarrheal therapy
- Grade 3-4 AST/ALT in combination with a Grade 2 elevation in bilirubin

Hematologic DLT:

• Grade 4 neutropenia lasting ≥ 7 days
• Grade 4 neutropenia and fever of > 38.5°C
• Grade 3 neutropenia with ≥ Grade 3 infection
• Thrombocytopenia of any grade if associated with clinically significant bleeding (Clinically significant as determined by the PI or resulting in a transfusion of RBCs.)
• Grade 4 thrombocytopenia
• Grade 4 anemia

11.2 Response Assessment Criteria

Response will be assessed according to the RANO criteria (2017) established by the Response Assessment in Neuro-Oncology working group. Each patient will be independently assessed according to RANO-HGG and RANO-LGG for the assessment of T1 contrast-enhancing lesions and contrast non-enhancing lesions respectively. (Wen YP, 2010)

Evaluable for Objective Response
Patients who have received at least one dose of therapy will be considered evaluable for response. These patients will have their response classified according to the definitions stated below.

Disease Parameters
For the purposes of this study, patients should be reevaluated for response at 8 weeks of therapy and then every 8 weeks thereafter. In addition to baseline scan(s), confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

ORR by RANO-HGG is defined as the proportion of subjects in the analysis population who have confirmed complete response (CR) or partial response (PR) using RANO-HGG criteria determined by the investigator and by independent assessment. Duration of response is defined as time from first RANO-HGG response (only if confirmed) to disease progression in subjects who achieve a PR or better.

ORR by RANO-LGG is defined as the proportion of subjects in the analysis population who have confirmed CR, PR, or minor response (MR) using RANO-LGG criteria determined by the investigator and by independent assessment. Duration of response is defined as time from first RANO-LGG response (only if confirmed) to disease progression in subjects who achieve a MR or better.

11.3 Disease Parameters

Measurable Disease
Bi-dimensionally, contrast-enhancing, measurable lesions with clearly defined margins by MRI scan, with a minimal diameter of 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0 mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are too many measurable lesions to measure at each evaluation, the investigator must choose the largest two to be followed before a participant is entered on study. The remaining lesions will be considered non-measureable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and non-measurable lesions are assessed.

Non-Measurable Evaluable Disease
Unidimensionally measurable lesions, masses with margins not clearly defined, lesions with maximal diameter < 1cm.

11.4 Response/Progression Categories

The modified RANO Response Criteria to be used in this study are summarized below.

11.4.1 Complete response (CR). All of the following criteria must be met:

a) Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. In the absence of a confirming scan at least 4 weeks later, this scan will be considered only stable disease.
b) No new lesions.
c) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
d) Participants must be on no steroids or on physiologic replacement doses only.
e) Stable or improved non-enhancing (T2/FLAIR) lesions.
f) Stable or improved clinically, for clinical signs and symptoms present at baseline and recorded to be disease related.

11.4.2 Partial response (PR). All of the following criteria must be met:

a) Greater than or equal to 50% decrease compared to baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks. In the absence of a confirming scan at least 4 weeks later, this scan will be considered only stable disease.
b) No progression of non-measurable disease.
c) No new lesions.
d) All measurable and non-measurable lesions must be assessed using the same techniques as baseline.
e) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.
f) Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared to baseline scan.
g) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

Participants with non-measurable disease cannot have a partial response. The best response possible is stable disease.

11.4.3 Progressive disease (PD). Any of the following criterion must be met:

a) > 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over best response or baseline if no decrease) on stable or increasing doses of corticosteroids.
b) Any new enhancing measurable lesion.
c) Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication side effects, complications of therapy, cerebrovascular events, infection, etc.). The definition of clinical deterioration is left to the discretion of the investigator but it is recommended that a decline in the Karnofsky Performance Score (KPS) from 100 or 90 to 70 or less, a decline in KPS of at least 20 from 80 or less, or a decline in KPS from any baseline to 50 or less, for at least 7 days, be considered neurologic deterioration, unless attributable to co-morbid events or changes in corticosteroid dose.
d) Failure to return for evaluation due to death or deteriorating condition.

11.4.4 Minor Response (MR). Apply to T2/FLAIR hyper-intense lesions only
All of the following criteria must be met:
a) 25–50% reduction in perpendicular diameters of lesion
b) No new lesions.
c) The steroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan.
d) Stable or improved, for clinical signs and symptoms present at baseline and recorded to be disease related clinically.

11.4.5 Stable disease (SD). All of the following criteria must be met:
a) Does not qualify for CR, PR, or progression.
b) All measurable and non-measurable sites must be assessed using the same techniques as baseline.
c) Stable clinically.

11.4.6 Unknown response status. Progressive disease has not been documented and one or more measurable or non-measurable lesions have not been assessed.

11.5 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 14 days before the beginning of the treatment.

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 is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

11.6 Evaluation of Best Response

The best overall response is the best response recorded from the start of the treatment until disease progression (taking as reference for progressive disease the smallest measurements recorded since the treatment started). If a response recorded at one scheduled MRI does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, i.e. still present on the subsequent MRI at least four weeks later, it will be recorded as a sustained response, lasting until the time of tumor progression. Participants without measurable disease may only achieve SD or PD as their best "response."

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for reporting adverse events can be found in Section 7.

12.1 Data Reporting

Investigative sites are responsible for completing and submitting data and/or data forms according to the instructions for the selected information management system.

12.2 Data Collection

12.2.1 Data Collection Forms

Qualified clinical trials study monitors that represent Oncoceutics will complete on-site monitoring. Oncoceutics will be responsible for all data management and statistical analysis.

Case Report Forms will be completed in a timely manner. Case Report Form completion may be formally delegated to other study personnel listed in the delegation of authority (DOA) form and signed by the PI.

The following steps will be taken to ensure accurate, consistent, complete and reliable data:

1. The Sponsor or designee will conduct an initiation meeting at the study site prior to the start of the study. The study protocol, procedures and CRFs will be reviewed in detail and the study personnel will be trained to carry out the procedures defined in the protocol.
2. The Investigator will be provided with a Study Site Binder for storing study related regulatory and study site documentation; e.g., study logs and forms.
3. All written study documentation entries must be made in blue or black ink. The investigator must review all entries for completeness and accuracy. When changes or corrections are made on any study documentation, the person

making the change must draw a single line through the error, then initial and date the correction.

4. Periodic monitoring visits will be conducted on a regular basis by the Sponsor or designee in order to verify the accuracy of data entered on each CRF against the raw data from source documents at the site. Items needing correction/clarification will be identified and brought to the attention of the study site personnel and Principal Investigator, and corrections will be made as appropriate.
5. The CRF will then be sent to the Sponsor or designee for final review and data management. The study database will be validated using appropriate validation processes.
6. The Sponsor or designee may perform a regulatory audit of the study site, and may include a complete review of the overall study conduct, regulatory documentation, and selected subject CRFs and source documents.

12.2.2 Registration and Eligibility

In order to begin the patient registration process, the site must obtain a slot for the candidate patient from the Sponsor (Oncoceutics). At the time of receipt of the signed consent form, the Sponsor (Oncoceutics) medical monitor must be notified via email to initiate eligibility review. The study staff must include de-identified patient source documentation, the completed New Patient Registration Form (provided by the Sponsor, Oncoceutics) and a tentative therapy start date. Correspondence from the Sponsor for patient registration approval will be granted within 48 hours of receipt of adequate documentation.

A patient identifier will be assigned at the time of approval. Screening and eligibility MUST be entered into Redcap EDC within 7 business days of screening visit.

12.2.3 Patient Consent Form

Informed consent must be obtained before protocol-specified procedures or interventions are carried out. The investigator will explain the nature of the study and will inform the subject that participation is voluntary and that they can withdraw at any time. A copy of the signed consent form will be given to every participant and the original will be maintained with the subject's records.

The consent form must be approved by the IRB and be acceptable to the Sponsor. Consent forms must be written so as to be understood by the prospective subject. The Informed Consent should be translated and certified into the local language of the respondent, as deemed necessary. Informed consent will be documented by the use of a written consent form approved by the IRB and signed and dated by the subject and by the person who conducted the informed consent discussion.

The signature confirms the consent is based on information that has been understood. Each signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or the Sponsor or its designee. The subject should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects, and should receive copies of any signed and dated consent form updates and any amendments to the written information provided to subjects.

12.2.4 Site Monitoring

To ensure compliance with current federal regulations and the ICH guidelines, data generated by this study must be available for inspection upon request by representatives of the FDA, national and local health authorities, the Sponsor and duly authorized representative of any entity providing support for this trial. Contractors of the Sponsor will conduct routine monitoring or audit activities for this study. The general scope of such visits would be to inspect study data (regulatory requirements), source documentation and CRF completion in accordance with current FDA Good Clinical Practices (GCP), the ICH guidelines and the respective local and national government regulations and guidelines.

12.2.5 Institutional Review Board Approval

This protocol, the informed consent document, relevant supporting information and all types of patient recruitment and advertisement information must be submitted to an IRB for review and must be approved before the study is initiated. An amendment to remove a life-threatening situation can by implemented by the Investigator prior to obtaining IRB approval by the site. In such situations, the IRB must be notified immediately and the amendment forwarded to the IRB for their consideration.

The investigator is responsible for keeping their local IRB informed of the progress with study renewal at least once a year. The investigator must also keep the local IRB informed of any significant adverse events, per local institutional guidelines.

Records Retention: FDA regulations (21 CFR 312.62) require clinical investigators to retain all study- related documentation, including source document and CRFs, long enough to allow the sponsor to use the data to support marketing applications. If this study is conducted under an IND, all records must be maintained for:

- Two years after the FDA approved the marketing application, or
- Two years after the FDA disapproves the application for the indication being studied, or
- Two years after the FDA is notified by the sponsor of the discontinuation of trials and that an application will not be submitted.

For all studies, including studies with FDA-IND exemption, the Investigator/Institution/Sponsor will take measures to prevent accidental or premature destruction of study documents. For such studies conducted under IND exemption, records will be retained for a minimum of seven (7) years past official study termination.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design

The primary endpoint is the RP2D, defined as the MTD or MAD (if not MTD is reached). The RP2D will be defined separately for Arm A (single agent weekly dosing), Arm B (weekly dosing in combination radiation), and Arm G (single agent twice per week dosing. The total number of patients enrolled in this study will depend on the incidence of DLTs during dose escalation.

Safety will be assessed by quantifying the toxicities and grades experienced by subjects who have received ONC201, including serious adverse events (SAEs). Other safety endpoints include laboratory safety assessments, KPS status, vital signs and physical examinations. Monitoring for toxicity will follow a Bayesian-based rule for the probability that the rate of DLT exceeds a maximal tolerated level of 33%. We will assume a *Beta*(1,2) prior, which is prior information equivalent to one DLT observed in three treated patients. This minimally informative prior is justified as there is some clinical experience with the combination therapy. Early termination for toxicity will be considered based on a posterior probability above 75% (in parentheses), that the toxicity rate exceeds 33%. Toxicity will be carefully monitored in each arm. Termination of an arm will be considered if DLT is observed in: 2 of 3 patients (0.84), 3 of 6 patients (0.81), 4 of 9 patients (0.79), 5 of 12 patients (0.58), 6 of 15 patients (0.78), 7 of 18 patients (0.77), 8 of 21 patients (0.77), 9 of 24 patients (0.77).

Secondary efficacy endpoints include changes in cranial nerve palsy scoring, overall response rate (ORR), median progression-free survival (PFS), defined as the time from first dose to the first documented disease progression according to RANO or death due to any cause, whichever occurs first; progression-free survival at 6 months; median overall survival (OS); median duration of response. Radiographic endpoints will be evaluated by RANO-HGG and RANO-LGG. For patients with DIPG in Arms A, B, E and G, PFS and OS will be compared to historical outcomes: PFS of 7 months and OS of 11 months from diagnosis (Cooney et al, 2017). These three arms will be analyzed individually, as well as aggregated by categorizing patients according to disease status/treating setting: newly diagnosed disease (Arm B), post-radiation adjuvant setting (Arm A E and G), and recurrent disease (Arm A and G).

Descriptive statistics will be provided for selected demographic, safety, PK, PD and biomarker data by dose, dose schedule and time, response rate, clinical benefit

rate and time to progression as appropriate. PK for patients in Arm E who received the liquid formulation will be analyzed/compared separately from patients in other Arms who received oral capsules. Descriptive statistics on continuous data will include means, medians, standard deviations, and ranges, while categorical data will be summarized using frequency counts and percentages.

All subjects who received at least 1 dose of study drug will be included in the primary endpoint analysis.

Intratumoral ONC201 will be summarized using descriptive statistics for all subjects in Arm C. A sample size of 12 achieves 95% power to detect a difference of -500nM between the null hypothesis mean of 100nM and the alternative hypothesis mean of 600nM with an estimated standard deviation of 500nM and with a significance level (alpha) of 0.05000 using a two-sided one-sample t-test. We are looking for an effect size of 600nM since the target threshold for intratumoral drug concentrations is 600nM representing the IC50 of ONC201 in H3 K27M-mutant glioma cells *in vitro*.

CSF ONC201 concentrations will be summarized using descriptive statistics for subjects in Arm D. A sample size of 12 achieves 95% power to detect a difference of -500nM between the null hypothesis mean of 100nM and the alternative hypothesis mean of 600nM with an estimated standard deviation of 500nM and with a significance level (alpha) of 0.05000 using a two-sided one-sample t-test. We are looking for an effect size of 600nM since the target threshold for intratumoral drug concentrations is 600nM representing the IC50 of ONC201 in H3 K27M-mutant glioma cells *in vitro*.

13.2 Molecular Correlative Studies

Pharmacodynamic effects will be evaluated with blood assays that include serum prolactin and immune cytokine analyses. A target of 50% induction over baseline of prolactin and a 2-fold increase in number or activation marker (e.g. granzyme B) are expected as pharmacodynamic effects of ONC201 based on preclinical and clinical studies.

In addition, the feasibility of detection of the H3 K27M mutation in cerebrospinal fluid will be evaluated in patients who have CSF sampling and are found to have the H3 K27M mutation in their tumor biopsy.

13.3 Efficacy Analyses

Separate efficacy analyses will be performed for Arms A, B, E, F and G individually, for all patients, and for patients with a known H3 K27M mutation. In Arm A, E, and G, efficacy analyses will be performed for the entire cohort in each arm and separately for patients with recurrent disease and patients with non-recurrent disease post-radiation.

Pooled analyses across arms will be performed for DIPG, as well as non-DIPG H3 K27M glioma, and separated by treatment setting: newly diagnosed, recurrent, post-radiation non-current.

Radiographic efficacy endpoints such as ORR and PFS will be assessed using RANO-HGG, as well as RANO-LGG.

ORR by RANO-HGG is defined as the proportion of subjects in the analysis population who have confirmed complete response (CR) or partial response (PR) using RANO-HGG criteria. Duration of response is defined as time from first RANO-HGG response (only if confirmed) to disease progression in subjects who achieve a PR or better.

ORR by RANO-LGG is defined as the proportion of subjects in the analysis population who have confirmed CR, PR, or minor response (MR) using RANO-LGG criteria. Duration of response is defined as time from first RANO-LGG response (only if confirmed) to disease progression in subjects who achieve a MR or better.

For PFS and OS secondary endpoints, Kaplan-Meier (KM) curves and median estimates from the KM curves will be provided as appropriate. Subjects without efficacy evaluation data or without survival data will be censored at Day 1.

13.4 Demographic and Baseline Characteristics

Baseline characteristics will be assessed by the use of tables and/or graphs for each cohort separately. No statistical hypothesis tests will be performed on these characteristics. The number and percentage of subjects screened, the primary reasons for screening failure, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, primary and secondary diagnoses, and prior and concomitant therapies will be summarized by treatment either by descriptive statistics or categorical tables.

14. PROTOCOL SIGNATURES

SPONSOR PROTOCOL SIGNATURES

Study Title:	ONC201 in Newly Diagnosed Diffuse Intrinsic Pontine Glioma and Recurrent/Refractory Pediatric H3 K27M Gliomas
Study Number:	ONC014
Version:	5
Date:	14 May 2020

This clinical trial protocol was prepared by:

DocuSigned by:
Michael Chiarella
Signer Name: Michael Chiarella
Signing Reason: I am the author of this document
Signing Time: 5/22/2020 | 7:40:00 AM PDT
B9D5E73F5C20462CB84AE2A81D3B4DA2

Signed: _____ Date: 5/22/2020

Michael Chiarella
VP, Clinical Operations
Oncoceutics, Inc.

This clinical trial protocol was reviewed and approved by:

DocuSigned by:
Josh Allen
Signer Name: Josh Allen
Signing Reason: I approve this document
Signing Time: 5/22/2020 | 1:41:57 PM PDT
9645D4DF7BAD4D76A5CDBF85B3CC3D82

Signed: _____ Date: 5/22/2020

Josh Allen, PhD
Chief Scientific Officer
Oncoceutics, Inc.

15. PROTOCOL INVESTIGATOR SIGNATURE PAGE

PROTOCOL INVESTIGATOR SIGNATURE PAGE

Study Title:	ONC201 in Newly Diagnosed Diffuse Intrinsic Pontine Glioma and Recurrent/Refractory Pediatric H3 K27M Gliomas
Study Number:	ONC014
Version:	5
Date:	14 May 2020

I have read this protocol, and I agree that it contains all necessary details for me and my staff to conduct this study as described.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with the Declaration of Helsinki, International Conference of Harmonisation guidelines on Good Clinical Practice (ICH GCP), and applicable regional regulatory requirements.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

_____

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APPENDIX A: KARNOFSKY AND LANSKY PERFORMANCE STATUS CRITERIA

	Karnofsky Scale	Lansky Scale
100	Normal, no complaints, no evidence of disease	Fully active
90	Able to carry on normal activity	Minor restriction in physically strenuous play
80	Normal activity with effort	Restricted in strenuous play, tires more easily, otherwise active
70	Cares for self, unable to carry on normal activity or to do active work	Both greater restrictions of, and less time spent in active play
60	Requires occasional assistance but is able to care for most needs	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	Considerable assistance required for any active play, fully able to engage in quiet play
40	Disabled, requires special care and assistance	Able to initiate quite activities
30	**30** Severely disabled, hospitalization indicated, although death not imminent	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	Limited to very passive activity initiated by others (e.g., TV)
10	Moribund, fatal process progressing rapidly	Completely disabled, not even passive play

APPENDIX B: TABLE OF EIAEDS AND NON-EIAEDS*

CYP3A4 enzyme-inducing antiepileptic drug (EIAEDs)	Non-CYP3A4 enzyme-inducing antiepileptic drugs (Non-EIAEDs)
Carbamazipine Oxcarbazepine Phenytoin Fosphenytoin Phenobarbital Primidone	Levetiracetam Valproic acid Lacosamide Gabapentin Topriamate Lamotrigine Tiagabine Zonisamide Clonazepam Clonozam Pregabalin

* This is a partial list. A comprehensive list of EIAEDs can be found at The University of Indiana (http://medicine.iupui.edu/clinpharm/ddis/) has a comprehensive list of EIAEDs which should be
consulted.

APPENDIX C: CRANIAL NERVE PALSY SCORING

Cranial Nerve	Exam Finding	Score (0 - normal, 1 - mild/partial, 2 - severe/complete)
III (oculomotor)	Eye movement - all directions except lateral (CN III) Eye position: down and out Can't move eye up or in	
	Ptosis -upper eyelid droop	
IV (trochlear)	Eye movement - inferior & medial (CN IV) Eye may not move down & in, toward nose (though ocular motility usually looks normal)	
V (trigeminal)	Facial Sensation (CN V) Facial numbness/ decreased sensation to light touch	
	Jaw (masseter) movement (CN V) Decreased mouth opening against resistance	
VI (abducens)	Lateral eye movement (CN VI) Eye deviated medially (toward nose) Eye can't move to the side (laterally)	
VII (facial)	Facial movement (CN VII) Facial asymmetry: Flattening of nasolabial fold Larger palpebral fissure (eye open wider)/weak eyelid closure Drooling from one side of mouth	
VIII (vestibulocochlear)	Cochlear N - Hearing (VIII) Decreased hearing (assessed by finger rub or rustling paper close to one ear	
	Vestibular N - Balance (VIII) Vertigo Horizontal nystagmus Truncal unsteadiness/ ataxia	
IX (glossopharyngeal) and X (vagus)	Swallowing/palate movement (CN IX & X) Decreased palate elevation on phonation Decreased/absent gag reflex. Drooling /pooling of saliva suggests dysfunction Hoarse voice	
XI (spinal accessory)	Shoulder and Neck movement/strength (CN XI) Decreased shoulder shrug strength (Trapezius) Decreased neck turning against resistance.(Sternocleidomastoid)	
XII (hypoglossal)	Tongue movement (CN XII) Tongue protrusion: deviates to one side	
Total Score		

APPENDIX D: PATIENT STUDY DRUG DIARY

OTHER MEDICATIONS TAKEN

Drug Name	Dose	Dates Taken	Reason Taken

Study Participant Initials __________ Date __________

FOR OFFICE USE	
Staff Initials:	
Date of First Dose:	Date of Last Dose:
Date Dispensed:	Date Returned:
# capsules/ora sweet dispensed:	# capsules/ora sweet returned:
# capsules/ora sweet that should have been taken:	
Discrepancy Notes:	

Study Participant Study Drug Diary

Participant Identifier: ______________________________
Protocol #______________________________
Assigned Arm____________________ ☐ Not Applicable
Assigned Dose______________________________
Your MD____________________ Phone__________________
Your RN____________________ Phone__________________

Study Treatment Instructions

ONC201

- Your dose of ONC201 is made up of 125-mg capsules.
- You will take the study medication orally once every week.
- ONC201 capsules should be taken at approximately the same day each week at the same time on that day
- ONC201 should be taken on an empty stomach: No food for 2 hours prior to dosing or 2 hours following dosing. ONC201 should be taken with a glass of water and consumed over as short a time as possible.
- ONC201 capsules should be swallowed as a whole and not chewed. Do not crush or empty the capsule.
- Do NOT drink or eat any grapefruit juice and/or grapefruit-related citrus fruits (e.g., Seville orange, pomelos) while you are on this trial
- If treatment with ONC201 is interrupted (including vomiting), missed doses should NOT be replaced.
- Store at room temperature of 15 to 30°C (59°F and 86°F).
- Protect from moisture.
- Doses should be taken as described here for as long as you are taking study medication.
- This study treatment is yours and should not be taken by anyone else.

ONC201 in Ora Sweet Instructions

- One (1) amber plastic oval prescription bottle contains 1 dose of ONC201 at the assigned dose provided by your treating physician.
- You will take the study medication orally once every week on the same day and approximately the same time each week.
- ONC201 should be taken on an empty stomach: No food for 2 hours prior to dosing or 2 hours following dosing. ONC201 should be taken with a glass of water and consumed over as short a time as possible.
- Follow all other instructions listed above.
- Store at room temperature of 15 to 30°C (59°F and 86°F).

Study Drug Diary Version 2.0 10NOV2019

DOSING LOG

ONC201 For each dose, take ________________________

Please indicate the date, time, amount taken and any comments.

Date	Capsule or Ora Sweet Time Taken	Comments
06/01/09	*8:05pm*	*Vomited capsule*
06/08/09	*8:15pm*	*None*

SYMPTOMS/SIDE EFFECTS

Please record any side effects experienced during this cycle. Include the date the particular symptom started and when it ended. Please evaluate the severity of the symptom according to the following scale:

Mild: Awareness of sign or symptom; easily tolerated and did not affect ability to perform normal daily activities. Symptom did not require medication or therapeutic intervention.

Moderate: Significant discomfort which interfered with ability to perform normal daily activities. Symptom was easily resolved with at home medication or simple therapeutic intervention.

Severe: Marked discomfort with an inability to carry out normal daily activities. Symptom required new medication and/or therapeutic intervention in order to resolve.

Please Note: The severity should reflect the most severe level experienced during the time period.

Study Drug Diary Version 2.0 10NOV2019