Protocol Title: A Phase II, Open-Label Study of ONC201 in Adults with Recurrent High-Grade Glioma

Protocol Number: ONC013 (NCT03295396)

Protocol Date: 19 November 2019 (Version 4.0)

PROTOCOL TITLE: A phase II, open-label study of ONC201 in adults with recurrent high-grade glioma

Protocol Version 04

Date: November 19, 2019

Oncoceutics Study Identifier: ONC013

Overall Principal Investigator:
Andrew B. Lassman, MD
Department of Neurology & Herbert Irving Comprehensive Cancer Center
Columbia University Irving Medical Center
710 West 168th Street
New York, NY 10032

Sponsor:
Oncoceutics, Inc.
3675 Market Suite
Suite 200
Philadelphia, PA 19104

Agent(s): ONC201

IND: 136090

Version 01: 10/19/17
Version 02 (Amendment 1): 04/16/18
Version 03 (Amendment 2): 04/23/18
Version 04 (Amendment 3): 11/19/2019

Study Summary

Title	A phase II, open-label study of ONC201 in adults with recurrent high-grade glioma
Short Title	ONC201 in recurrent high-grade glioma
Oncoceutics Protocol Number	ONC013
IND	136090
Overall Principal Investigator	Andrew B. Lassman, MD Department of Neurology & Herbert Irving Comprehensive Cancer Center Columbia University Irving Medical Center
Regulatory Sponsor	Oncoceutics, Inc.
Phase	Phase II
Methodology	Open-label, two arm
Study Duration	Approximately 24 months from start of screening to last subject processed and finishing the study
Objectives	**Primary Objective**: • To estimate the overall response rate (ORR) by RANO-HGG of ONC201 in adults with recurrent histone H3 K27M mutant glioma **Secondary Objectives:** • To estimate the overall response rate (ORR) by RANO-LGG of ONC201 in adults with recurrent histone H3 K27M mutant glioma • Assess the safety, toxicities and tolerability of ONC201 in adults with recurrent high-grade glioma (HGG); • Estimate the median progression-free survival (PFS), PFS at 2 and 6 months, overall survival (OS), and median duration of response;
Exploratory Objectives	• Assess the association of ORR with tumor markers including: location (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 ORR with circulating markers including: Induction of serum prolactin; early activation of NK cells; immune cytokines and effectors in the serial serum samples by enzyme-linked immunosorbent assay (ELISA);

Number of Subjects	Target enrollment is 95 evaluable subjects for the study. Thirty-nine (39) for Arm A and 56 for Arm B.

Major Inclusion/Exclusion Criteria	Key Inclusion criteria: Histologically confirmed diagnosis of HGG (any histology, including but not limited to glioblastoma, astrocytoma, and oligodendroglioma); presence of histone H3 K27M mutation; unequivocal evidence of progressive disease on contrast-enhanced brain CT or MRI as defined by RANO criteria; measurable disease by RANO-HGG criteria; previous therapy with at least radiotherapy; no more than two prior episodes of recurrence; interval of at least 90 days from the completion of radiotherapy to the first dose of ONC201; age ≥18 years. KPS ≥ 60. Key Exclusion Criteria: Presence of diffuse leptomeningeal disease or evidence of CSF dissemination; tumors with known *IDH1* (isocitrate dehydrogenase 1) or known *IDH2* mutations
Study Product, Dose, Route, Regimen	ONC201, 625 mg once weekly, oral
Duration of administration	ONC201 treatment will continue if tolerated until disease progression, unacceptable toxicity, or withdrawal of consent, whichever comes first.
Statistical Methodology	**Primary Efficacy Analysis**: Simon's two-stage design (Simon, 1989) will be used. The null hypothesis that the true response rate is 0.01 will be tested against a one-sided alternative. Arm A In the first stage, 17 patients will be accrued. If there are 0 responses in these 17 patients, the study will be stopped. Otherwise, 22 additional patients will be accrued for a total of 39. Arm B In the first stage of Arm B, 17 patients will be accrued. If there are 0 responses in these 17 patients, the study will be stopped. Otherwise, 39 additional patients will be accrued for a total of 56. At least 3 responses will be required in order for success to be claimed (i.e. for the null hypothesis ($p_0 \leq 0.01$) to be rejected in favor of the alternative ($p_1 \geq 0.10$) with 80% power, alpha=0.025). Patients will be analyzed separately in each arm.

TABLE OF CONTENTS

1. OBJECTIVES

The primary objective of this trial is to determine the efficacy and safety of ONC201, an oral small molecule imipridone DRD2 antagonist, in adult subjects with recurrent HGG.

1.1 General Study Design

This is an open-label, two arm study. The trial will enroll a total of up to 95 subjects. Arm A will enroll up to a total of 39 evaluable subjects and Arm B will enroll up to a total of 56 evaluable subjects. A Bayesian monitoring approach will be applied to assess safety throughout the trial.

After screening procedures and registration, all subjects will be treated with oral ONC201 (625 mg) once every week. All subjects will remain on study until confirmed progressive disease, 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 28 days (4 weeks), corresponding to 4 doses of ONC201.

Subjects will be evaluated for efficacy approximately every 8 weeks with neuroimaging and clinical evaluation. Neuroimaging studies contrast-enhanced brain MRI or CT for patients unable to undergo 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, both criteria for HGG and low-grade glioma (LGG).

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.

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, fax, or mail.

1.2 Primary Objective

- To estimate the overall response rate of ONC201, assessed by RANO-HGG criteria, in adult patients with recurrent histone H3 K27M glioma.

1.3 Secondary Objectives

- Assess ORR. according to RANO-LGG, criteria for non-contrast enhancing lesions.
- Assess the safety, toxicities and tolerability of ONC201 in adults with recurrent high- grade glioma;

- Estimate the median PFS, PFS at 2 and 6 months,median OS, and median duration of response;

1.4 Exploratory Objectives

- Assess the association of ORR with tumor markers including: location (thalamus, brainstem, spinal cord); Histone H3 mutation (*H3F3A* / H3.3 vs *HIST1H3B* / H3.1); DRD2 and DRD5 expression;

- Assess the association of ORR with circulating markers including: Induction of serum prolactin; early activation of NK cells; immune cytokines and effectors in the serial serum samples by ELISA;

1.5 Endpoints

-Efficacy
- Response by RANO-HGG
- Response 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

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

-Other
- Quality of life Assessment by MDASI Patient Reported Outcomes Questionnaire
- NANO (Neurologic Assessment in Neuro-Oncology)

2. BACKGROUND

2.1 Study Disease

According to the 2007 World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS), astrocytoma (a major type of glioma) is classified into grades I, II, III and IV based on histology. Grade I and II astrocytoma are considered low grade glioma (LGG), while grade III (anaplastic astrocytoma) and IV astrocytoma/gliomas as well as Grade III oligodendroglioma are considered HGG. Grade IV glioma, or Glioblastoma Multiforme (GBM), is the most common and lethal primary CNS malignancy and accounts for over 15% of all brain cancers (Patel et al., 2014). The 5-year survival rate for GBM remains at <10% (Stupp et al., 2009). In 2014, there were over 23,300 new cases of cancer involving the brain and nervous system and over 14,000 deaths (Siegel et al., 2014). Although initial treatment typically includes surgery, radiation and chemotherapy, HGG usually recurs less than 6-8 months from initial diagnosis.(Gorlia, et. al. 2012). There are limited choices for treating recurrent disease. Bevacizumab was approved for treating recurrent GBM, but studies repeatedly demonstrated outcomes lacking in overall survival benefit from its use.(Wick, et. al. 2017, Gilbert, et. al. 2014) Thus, recurrent HGG has an unmet clinical need with poor survival and limited treatment options that offer meaningful therapeutic benefit.

In the 2016 edition of WHO classification of CNS tumors, Diffuse Midline Glioma (DMG) with Histone H3 K27M mutant is recognized as a distinct disease entity. Any DMG with the H3 K27M mutation is classified as grade IV (Louis et al, 2016). Histone H3 is one of the proteins that comprise the octameric nucleosome and there are 3 variants: H3.1, H3.2, and H3.3. 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 (DIPG) and HGG, primarily restricted to midline locations (spinal cord, thalamus, pons, brainstem) in children and younger adults (Schwartzentruber et al, 2012; Sturm et al, 2012; Fontebasso et al, 2014; 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.

The H3K27M mutation may present in patients with leptomeningeal dissemination as

well as tumors not originating from the midline CNS structures. No prospective trials exclusive to histone H3 K27M mutant gliomas have been conducted and therefore prospectively collected outcome data are lacking. However, survival data are well published for 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, 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 samples that are refractory to chemotherapy and targeted therapies. This is independent of mutations 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 use in *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 complete details of 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.

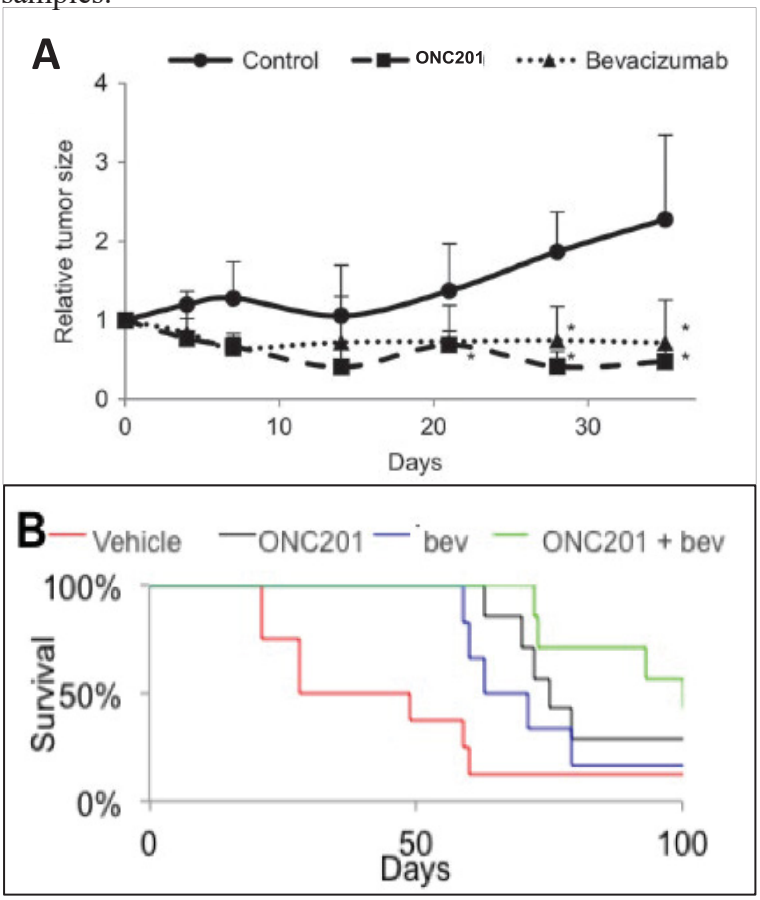


Figure 1 (A) Relative tumor size (compared to day 0) in a subcutaneous xenograft of T98G in mice treated with a single dose of vehicle, ONC201 (30 mg/kg, orally), or bevacizumab (10 mg/kg, iv) on day 0 (n = 8). **(B)** Overall survival of mice harboring SF767 intracranial tumors treated with a single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

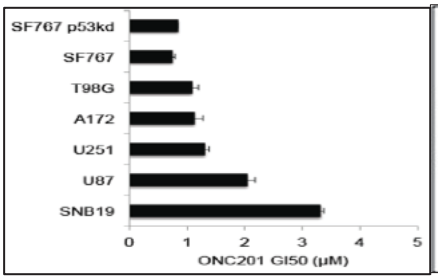


Figure 2 GI50 GBM cell lines at 72 hours after treatment with ONC201 or DMSO (n = 3).

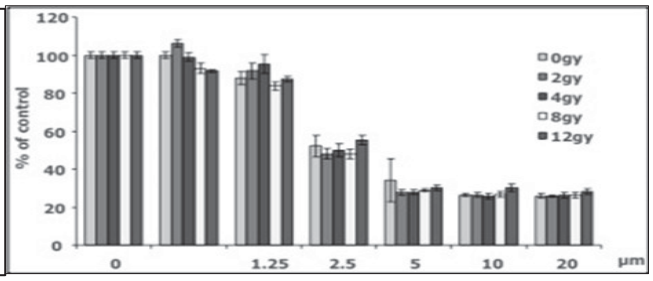


Figure 3 T98G TMZ-resistant and radio-resistant GBM cells following treatment with ONC201 at indicated concentrations (72 hr).

ONC201 demonstrates p53-independent activity in human GBM cell lines in the low micromolar range (Figure 2). ONC201 exerts 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).

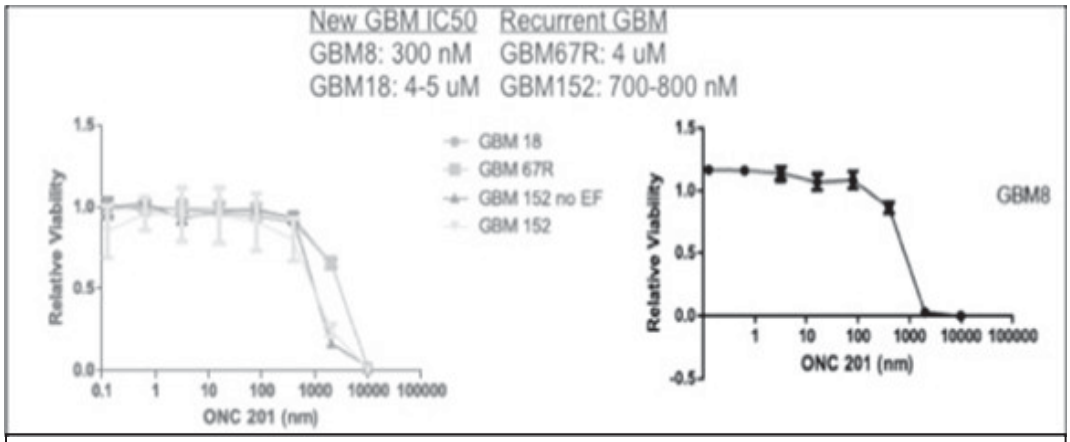


Figure 4 ONC201 kills newly diagnosed and recurrent GBM neurospheres. Cell viability of neurosphere GBM cultures following ONC201 treatment (72 hr)

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.

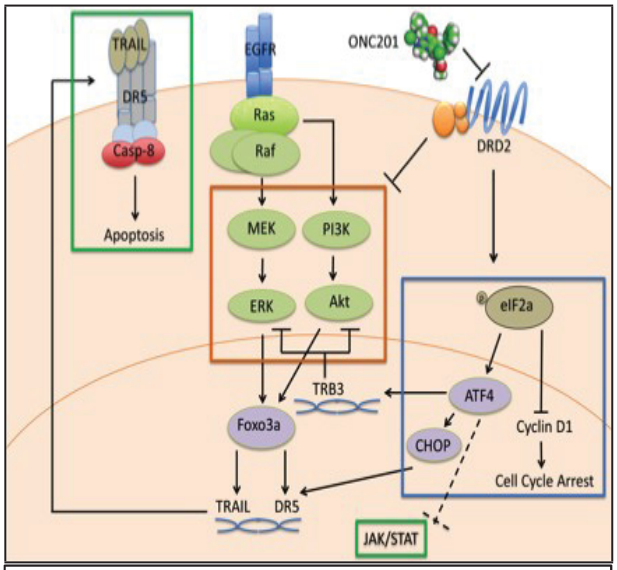


Figure 5 Proposed model of ONC201 mechanism of action in

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 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 a slight increase 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.

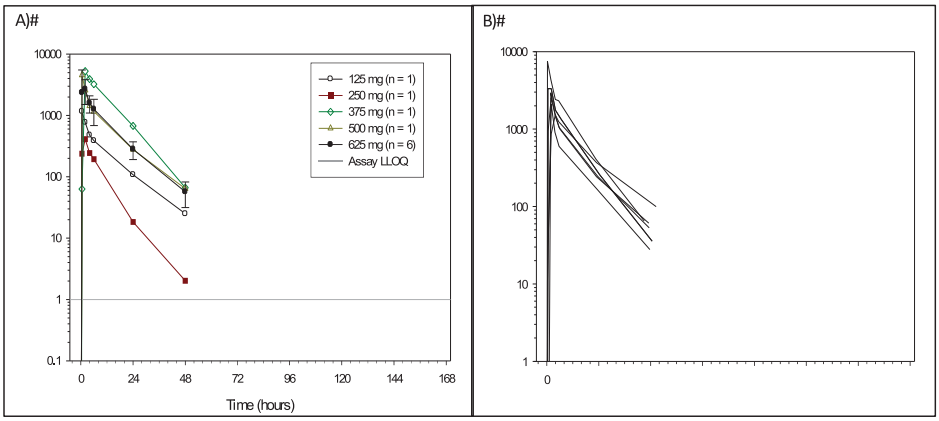
In dogs, exposure to ONC201 following oral gavage dosing at 4.2, 42, and 120 mg/kg ONC201 was dose-dependent and increased with greater ONC201 dose levels. Exposure to ONC201 was similar in male and female dogs with the observation that all mean male Cmax and AUC values were slightly greater than those 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 $T_{1/2,e}$ 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 advanced solid tumors, 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 (Fig 6; Table 2.1). 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 at1.8 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.

Figure 6 ONC201 pharmacokinetic parameters



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