ONC201

Briefing Document for June 20, 2019
Oncologic Drugs Advisory Committee
Pediatric Subcommittee

Advisory Committee Briefing Materials:

Available for Public Release

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AUC	area under the concentration-time curve
BIOMEDE	Biological Medicine for Diffuse Intrinsic Pontine Glioma (DIPG) Eradication
CLIA	Clinical Laboratory Improvement Amendments
ClpP	caseinolytic protease P
CNS	central nervous system
COG	Children's Oncology Group
DIPG	diffuse intrinsic pontine glioma
DLT	dose-limiting toxicity
DRD2	dopamine receptor D2
DRD3	dopamine receptor D3
DRD5	dopamine receptor D5
ERK	extracellular signal-regulated kinase
HGG	high grade glioma
IHC	immunohistochemistry
IND	investigational new drug
KPS	Karnofsky Performance Status
LGG	low grade glioma
MAPK	mitogen-activated protein kinase
MRI	magnetic resonance imaging
NANO	Neurologic Assessment in Neuro-oncology
OS	overall survival
PD	progressive disease
PFS	progression free survival
PK	pharmacokinetics
PR	partial response
PRC2	polycomb repressive complex 2
RANO	Response Assessment in Neuro-oncology
RNA	ribonucleic acid
RNA-seq	RNA sequencing
SD	stable disease
WHO	World Health Organization

1.0 BACKGROUND

1.1 DRD2 Overview

Dopamine receptor D2 (DRD2) is overexpressed in many cancers, including high grade gliomas, and its expression carries a poor prognosis (Li et al., 2014; Prabhu et al., 2018). As a G protein-coupled receptor, DRD2 controls mitogenic and other signaling pathways that are associated with tumorigenesis, proliferation, and metastatic dissemination. Selective DRD2 antagonism causes inactivation of MAPK signaling and induces tumor cell death in preclinical models of high grade gliomas and other malignancies (Cheng et al., 2015; Li et al., 2014).

The dopamine receptor family consists of 5 members, categorized as D1-like and D2-like subfamilies based on coupling to different G-alpha proteins that cause opposing downstream signaling consequences (Prabhu et al., 2018). Therefore, oncogenic dysregulation and therapeutic targeting of dopamine receptors must be highly specific to achieve a profound net functional response.

1.2 ONC201 Overview

ONC201 is an orally administered, anti-cancer small molecule that selectively antagonizes DRD2 and DRD3 (Madhukar et al., 2017; Prabhu et al., 2018). DRD2 antagonism by ONC201 requires a combination of orthosteric and allosteric receptor residues that define it as the first bitopic DRD2 antagonist for clinical oncology (Figure 1A). This receptor pharmacology results in selective and atypical functional inhibition of the receptor involving both competitive and non-competitive antagonism. ONC201 exhibits a relatively slow association and rapid dissociation with DRD2, both of which have been reported as defining characteristics of DRD2 antagonists that are not generally associated with extrapyramidal side effects.

Downstream of target engagement, the compound induces p53-independent apoptosis involving integrated stress response activation and inactivation of Akt/ERK signaling in tumors cells (Figure 1B) (Allen et al., 2013; Ishida et al., 2018; Ishizawa et al., 2016; Kline et al., 2016). In addition to DRD2/3 antagonism, direct activation of caseinolytic protease P (ClpP) by ONC201 has recently been reported (Graves et al., 2019; Ishizawa et al., 2019). ClpP is a serine protease located in the mitochondrial matrix of human cells that plays a central role in mitochondrial protein quality control by degrading misfolded proteins. ClpP, in complex with other proteins, regulates oxidative phosphorylation by controlling the degradation of respiratory chain complex subunits and can trigger the mitochondrial unfolded protein response that converges with the integrated stress response. Therefore, ONC201 exhibits anti-cancer activity as a ClpP agonist to activate the integrated stress response, in addition to DRD2 antagonism that inactivates Akt/ERK signaling.

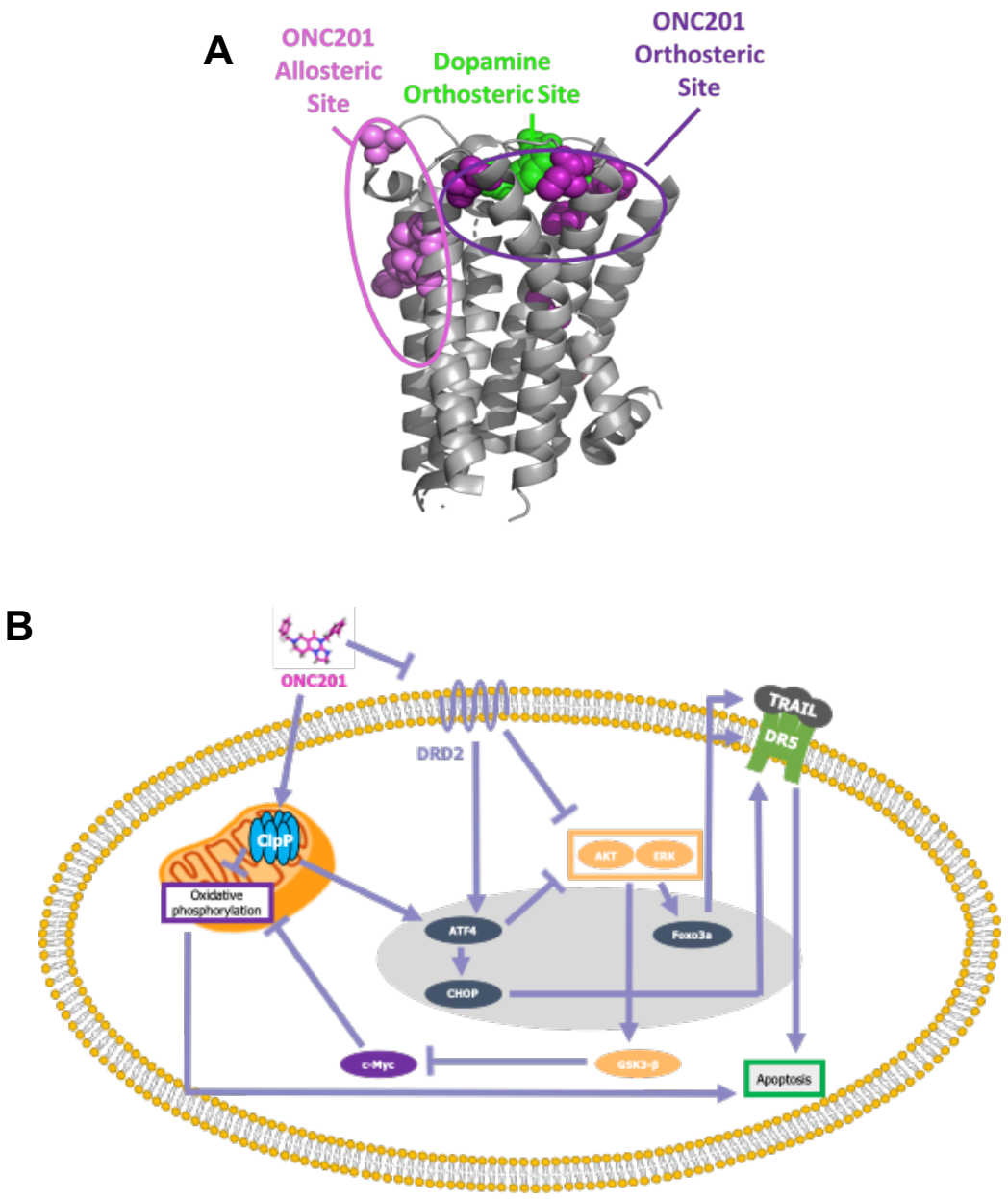

Figure 1. ONC201 mechanism of action. (A) DRD2 amino acids required for functional antagonism by ONC201 shown as purple spheres. Adapted from PBD 6C38. (B) Summary of mechanism of action of ONC201.

The small molecule has demonstrated efficacy in a range of preclinical models of advanced cancers, with the most pronounced in vivo efficacy shown in high grade gliomas (Allen et al, 2013) (Figure 2). These downstream effects are persistent after drug washout in vitro and in vivo, which rationalizes the weekly schedule of ONC201 that yields saturable efficacy in preclinical models (Allen et al., 2016).

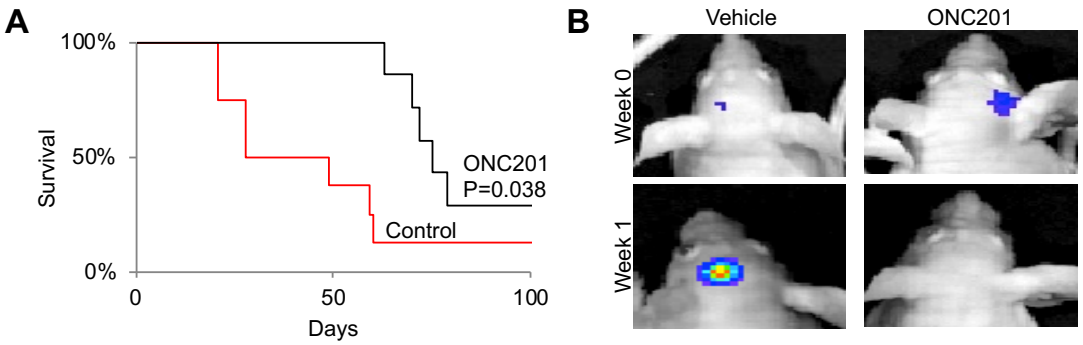

Figure 2. Efficacy of ONC201 in preclinical high grade glioma models. (A) Overall survival of mice harboring luciferase-labelled SF767 intracranial tumors treated with a single oral dose of vehicle (n = 8) or ONC201 (25 mg/kg, n = 7) at 2 weeks after implantation. (B) Bioluminescence imaging of an exemplary control mouse and a ONC201-treated mouse from the experiment described in (A).

In Phase I clinical trials, dose-limiting toxicities were not observed and 625mg ONC201 administered once every one week or once every three weeks was selected as a biologically optimal dose based on pharmacokinetic and pharmacodynamic profiles (Stein et al., 2017). This dose and schedule has exhibited a favorable safety profile in >350 patients enrolled in 13 clinical trials that have enrolled a range of advanced cancers. Dose-limiting toxicities (DLTs) have not been reported across the various clinical trials and administration schedules with dose levels ranging from 125 mg to 625 mg. No study drug discontinuation due to drug-related toxicity has been reported. One dose reduction (625mg to 500mg) occurred in 1 patient with a Grade 3 neutropenia assessed by the investigator as possibly related to ONC201 that, upon re-challenge, did not recur.

In accordance with the broad applicability of the mechanism of action of ONC201 for the treatment of many types of cancer, the preclinical activity of ONC201 has been demonstrated in a variety of cancers. Correspondingly, there are currently 13 ongoing clinical trials with ONC201 evaluating a wide spectrum of tumor types: 3 clinical trials enroll high grade glioma patients and the other clinical trials enroll patients with a range of solid tumors and hematological malignancies that include neuroendocrine tumors, endometrial cancer, breast cancer, multiple myeloma, acute myeloid leukemia, and non-Hodgkin's lymphoma. While the activity of ONC201 across this array of cancers continues to be evaluated as a single agent and/or in combination with other anti-cancer therapies, high grade gliomas has been prioritized as the lead indication due to the emergence of objective responses as a single agent in clinical trials in the recurrent setting that are outlined below. The potential utility of ONC201 in pediatric indications beyond high grade gliomas is being evaluated in preclinical studies in collaboration with the NCI Pediatric Preclinical Testing Consortium.

1.3 H3 K27M-mutant Glioma Overview

Gliomas that harbor the H3 K27M mutation have been identified as a molecular subset of brain tumors that are responsive to ONC201 (Chi et al., 2017). H3 K27M is a missense mutation that occurs in one of several genes encoding for the histone H3 protein. The mutant form of histone H3 acts as a dominant negative inhibitor of the PRC2 histone methyltransferase complex, which normally trimethylates lysine 27 of histone H3, among other substrates. Consequently, tumor cells harboring this mutation exhibit a histone hypomethylation profile that causes epigenetic dysregulation of the expression of many genes associated with cancer (Piunti et al., 2017; Schwartzentruber et al., 2012).

The H3 K27M mutation defines a distinct form of Grade IV glioma codified in the 2016 WHO classification of CNS tumors (Khuong-Quang et al., 2012; Louis et al., 2016). H3 K27M-mutant diffuse midline glioma is characterized by a poor prognosis and a high prevalence in midline gliomas that predominantly afflict children and young adults (Kleinschmidt-DeMasters and Mulcahy Levy, 2018): 75% of thalamic tumors, ~54% of brainstem tumors and 55% of spinal cord tumors; 24% of pediatric gliomas and 8% of adult gliomas. The H3 K27M mutation occurs in a unique spatiotemporal pattern, with midline gliomas involving the pons (i.e. DIPG) tending to occur in pediatric patients (<18 years of age) and midline gliomas involving the thalamus and spinal cord tending to occur in young adult patients.

Since the midline region of the brain is involved in critical physiological functions, many of these tumors are not operable (especially in the brainstem where the pons is located). This means that until recently, many midline gliomas such as diffuse intrinsic pontine glioma (DIPG) were diagnosed solely on a radiographic basis. Recent advances in neurosurgical techniques and increased patient consent to post-mortem tumor tissue retrieval have led to systematic molecular evaluations of DIPG and other midline gliomas. Standard therapy for midline gliomas involves neurosurgery, if feasible, followed by fractionated external beam radiotherapy. Radiotherapy remains the sole standard-of-care alone that is considered palliative, as it transiently improves symptoms and tumor burden in DIPG.

1.4 Identification of H3 K27M-mutant Glioma as a Candidate Indication for ONC201

The identification of H3 K27M-mutant glioma as an ONC201-responsive subset of brain tumors began with a positive outlier radiographic tumor response that occurred in the first Phase II clinical trial of the compound (Arrillaga-Romany et al., 2017). This adult recurrent glioblastoma trial demonstrated that ONC201 exceeded therapeutic thresholds and achieved pharmacodynamic activity in resected glioblastoma samples following the second weekly dose of ONC201 at the 625mg recommended Phase II dose (Figure 3).

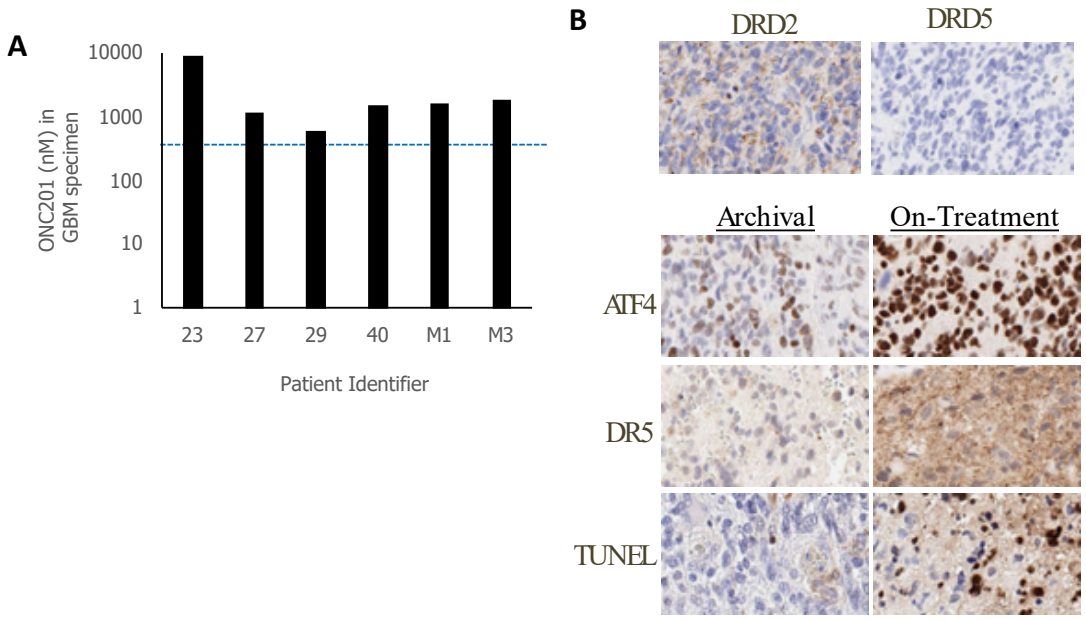

Figure 3. Intratumoral ONC201 concentrations and pharmacodynamics in resected recurrent glioblastoma specimens. (A) ONC201 concentrations in tumor tissue homogenates of adult recurrent glioblastoma tissue resected ~24 hours after the second weekly dose of 625mg ONC201. (B) Immunohistochemical (IHC) analysis of archival DRD2 and DRD5 expression (top) and expression of pharmacodynamic biomarkers (bottom) in archival and on-treatment glioblastoma tissue resected, as described in (A).

Among the first cohort of adult recurrent glioblastoma patients to receive ONC201, one patient underwent a deep and durable tumor regression who remains on therapy for >3 years (Figure 4). Subsequent investigations revealed that her tumor exclusively harbored the H3 K27M mutation among the cohort. RNA-seq in a panel of patient samples showed that glioma cells with the H3 K27M mutation exhibit DRD2 overexpression and suppressed DRD5 expression (Figure 5) (Chi et al., 2017), a biomarker signature associated with enhanced tumor cell sensitivity to ONC201 (Prabhu et al., 2018). Furthermore, the prevalence of these gliomas in midline brain structures overlaps with some of the highest expression of dopamine pathway members and dopaminergic neuron projections.

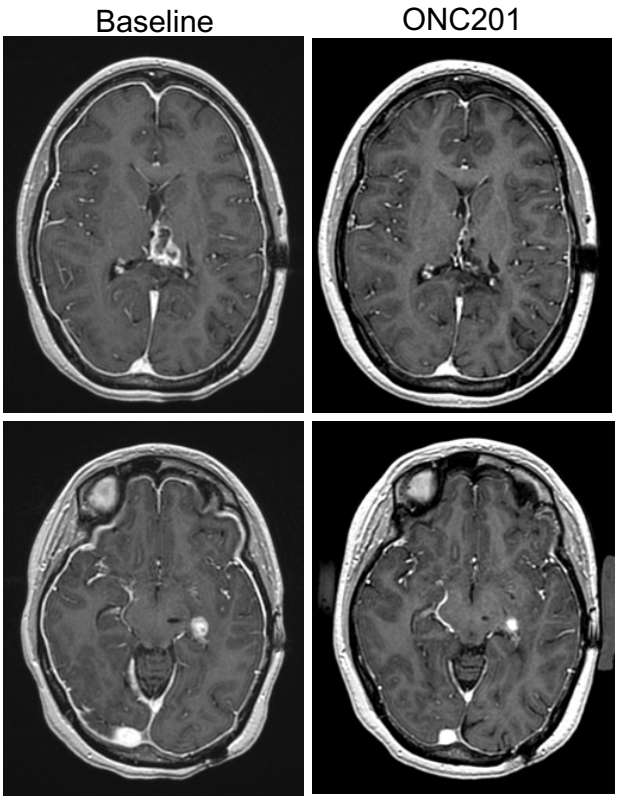

Figure 4. Objective response in first H3 K27M-mutant glioma patient treated with ONC201. Gadolinium-enhanced MRIs shown at baseline and 72 weeks post-initiation of ONC201.

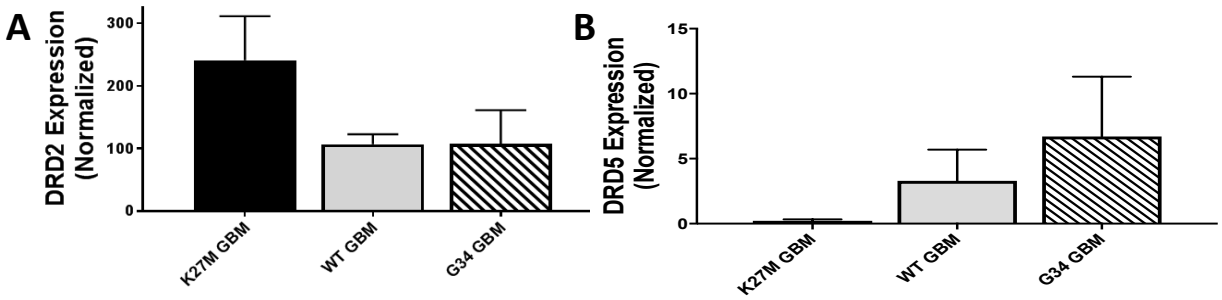

Figure 5. Dopamine receptor expression in gliomas by histone H3 mutation status. (A) DRD2 and (B) DRD5 expression by RNA-seq on patient biopsies. The panel included samples with H3 K27M (n=8), H3 wild-type (n=28), and H3 G34R (n=3).

2.0 CLINICAL DEVELOPMENT OF ONC201 FOR ADULT RECURRENT H3 K27M-MUTANT GLIOMA

Based on the positive outlier clinical response and corroborating preclinical results, H3 K27M-mutant glioma patients were enriched in the ongoing adult glioma trial. An additional clinical trial dedicated to evaluating this adult patient population was initiated. Compassionate use was permitted while the clinical program was expanded. As of December 15, 2018, 15 H3 K27M-mutant glioma patients had received ONC201 who meet the following criteria that are designed to isolate the observed effects to single agent ONC201: age >18 years old, recurrent and measurable disease by RANO-HGG criteria, contrast-enhancing disease not involving the pons or spine, H3 K27M mutation detected in a glioma sample by IHC or sequencing method performed in a CLIA setting, >90 days from prior radiation, and administration of single agent ONC201 without concurrent anti-cancer therapies (including bevacizumab) until progression.

The characteristics of the 15 patients who met the criteria outlined above are summarized in Table 1. Nine were enrolled to NCT03295396, 5 to NCT02525692, and 1 to an expanded access protocol conducted under the Sponsor's IND. Median age was 28-years old (range: 17-58), median number of recurrences was 1 (range: 1-3). Eight (53%) patients' primary lesion was in the thalamus, four (27%) brainstem, one (7%) cerebellum, one (7%) basal ganglia, and one (7%) frontal lobe. Seven (47%) of these patients had multi-focal disease at baseline. Histologies were reported as diffuse gliomas for several (47%) of these patients, three (20%) glioblastoma, three (20%) astrocytoma, one (7%) pilocytic astrocytoma, and one (7%) gliosarcoma. All patients received at least prior radiation with a median time from prior radiation of 51.7 weeks (range 12-101.4).

Table 1. Demographics of adult recurrent H3 K27M-mutant glioma patients treated with ONC201 (N=15).

	All Patients	ONC006	ONC013	Expanded Access
	N=15	N=5	N=9	N=1
Gender (N%)				
Female	7 (47%)	2 (40%)	5 (56%)	-
Male	8 (53%)	3 (60%)	4 (44%)	1 (100%)
Age, years, median (range)	28 (17-58)	28 (17-58)	28 (19-55)	37
Weight, kilograms, median (range)	71.5 (56.2-106.1)	71.5 (67.3-89.9)	71 (56.2-106.1)	95.5
KPS, median (range)	90 (70-90)	90 (70-90)	90 (80-90)	80
Primary tumor location (N%)				
Thalamus	8 (53%)	2 (40%)	5 (55%)	1 (100%)
Brainstem	4 (27%)	2 (40%)	2 (22%)	-
Cerebellum	1 (7%)	-	1 (11%)	-
Frontal lobe	1 (7%)	1(20%)	-	-
Basal ganglia	1 (7%)	-	1 (11%)	-
Histology (N%)				
Diffuse glioma	7 (46%)	1(20%)	6 (66%)	-
Glioblastoma	3 (20%)	2 (40%)	1(12%)	-
Astrocytoma	3 (20%)	-	2(22%)	1(100%)
Pilocytic astrocytoma	1 (7%)	1(20%)	-	-
Gliosarcoma	1 (7%)	1(20%)	-	-
Multifocal disease (N%)				
Yes	7 (47%)	2 (40%)	4 (44%)	1 (100%)
No	8 (53%)	3(60%)	5 (56%)	-
Number of lesions, median, (range)	1 (1-3)	1 (1-3)	2 (1-3)	2 (2)
Number of recurrences, median (range)	1 (1-3)	2 (1-3)	1 (1-2)	3
Time from prior radiation, weeks, median, (range)	51.7 (12-101.4)	44 (19.0-52)	58 (12-101.4)	38.4
Levetiracetam (N%)				
Yes	3 (20%)	2 (40%)	1 (11%)	-
No	12 (80%)	3 (60%)	8 (89%)	1 (100%)
Dexamethasone, mg per day, median (range)	4 (0-16)	3(2.5-4)	4 (0-16)	0 (0)

ONC201 was orally administered at 625 mg once per week, except for one patient dosed on the initial once per 3 weeks schedule. No dose-limiting toxicities or treatment discontinuations due to toxicity occurred.

RANO-HGG criteria are routinely used for high grade gliomas that are contrast-enhancing and RANO-LGG criteria are used for low grade gliomas that often do not contrast enhance. Midline glioma sometimes exhibit minimal contrast enhancement and frequently exhibit a mixture of contrast-enhancing and non-contrast enhancing regions in the tumor. In light of these considerations, blinded independent central review of tumor response was assessed by RANO-HGG and RANO-LGG for each patient to assess contrast-enhancing and non-contrast-enhancing disease (Figure 6-7).

Best response by RANO-HGG criteria is at least 27% (95% confidence interval [CI], 8% to 55%) (Figure 6): 1 complete response (CR), 3 partial responses (PR), 7 stable disease (SD), and 4 progressive disease (PD). Two SD patients remain on study and therefore ORR has not been determined.

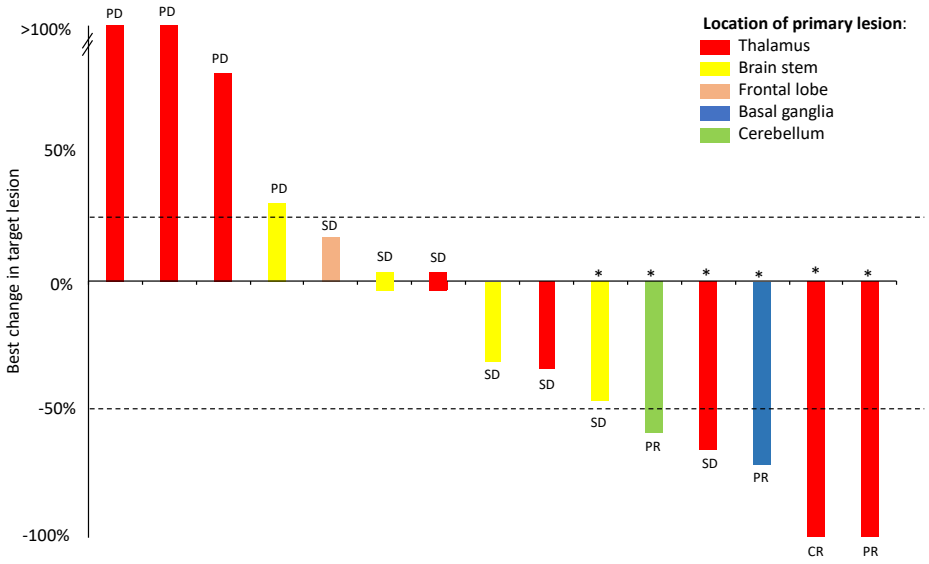

Figure 6. Waterfall plot for contrast-enhancing assessment of adult recurrent H3 K27M-mutant glioma patients treated with ONC201. Best change in target lesion(s) size relative to baseline by RANO-HGG. Enrollment cutoff is December 15, 2018. CR – complete response, PR – partial response, SD – stable disease, PD – progression disease. *Patient remains on study.

Best response by RANO-LGG is at least 36% (95% CI, 13% to 65%) (Figure 7): 1 CR, 1 PR, 3 minor response (MR), 4 SD, 5 PD, 1 unevaluable (UE). Two SD patients remain on study and therefore ORR has not been determined.

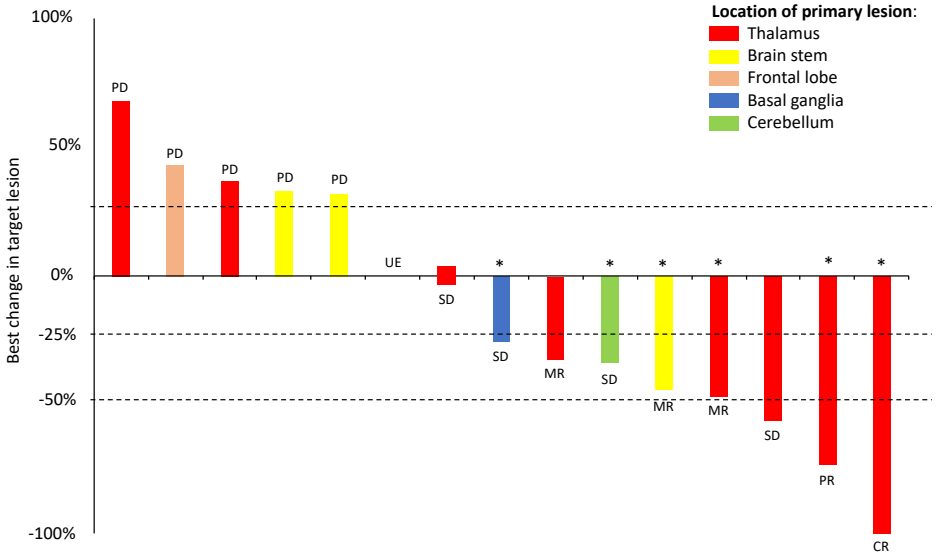

Figure 7. Waterfall plot for non-contrast-enhancing assessment of adult recurrent H3 K27M-mutant glioma patients treated with ONC201. Best change in target lesion(s) size relative to baseline by RANO-LGG. CR – complete response, PR – partial response, MR – minor response, SD – stable disease, PD – progression disease, UE - unevaluable. *Patient remains on study. Enrollment cutoff is December 15, 2018.

Best response by either RANO-HGG or RANO-LGG is 47% (95% CI, 21% to 73%): 2 CR, 2 PR, 3 MR, 5 SD, 3 PD. Median onset of response by RANO-HGG is 2.6 months (range 1.3-3.4) (Figure 8-9). Median duration of response by RANO-HGG has not been reached with a median follow-up of 7.7 months (range 1.8-29.8).

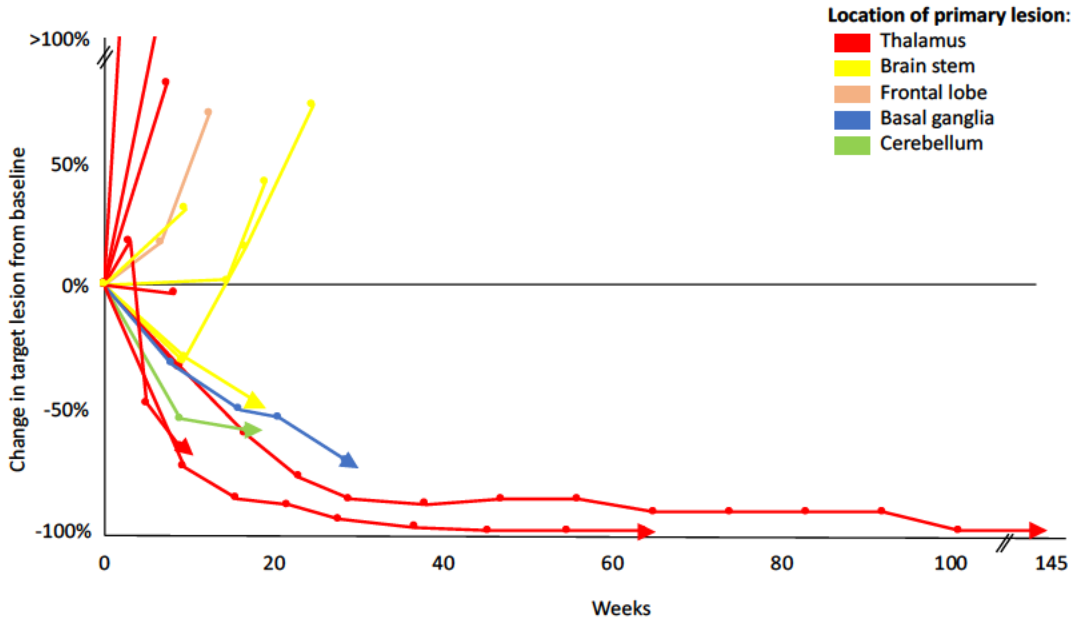

Figure 8. Spider plot for contrast-enhancing assessment of adult recurrent H3 K27M-mutant glioma patients treated with ONC201. Change in target lesion(s) size relative to baseline by RANO-HGG since initiating ONC201. Enrollment cutoff is December 15, 2018.

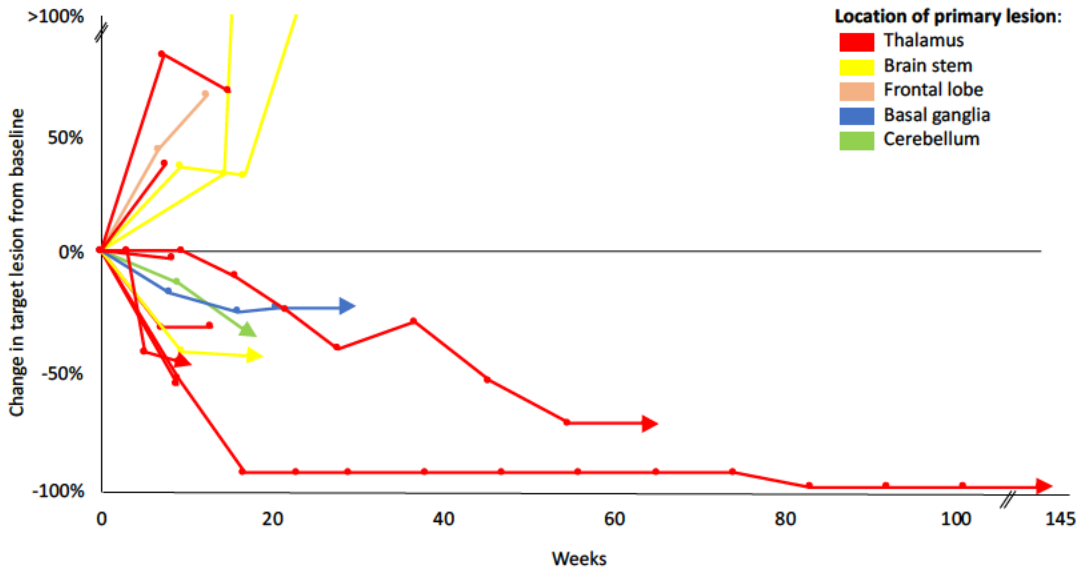

Figure 9. Spider plot for non-contrast-enhancing assessment of adult recurrent H3 K27M-mutant glioma patients treated with ONC201. Change in target lesion(s) size relative to baseline by RANO-LGG since initiating ONC201. Enrollment cutoff is December 15, 2018.

Two patients with objective responses by RANO-HGG have reported improvement in disease-associated symptoms that was evident with NANO criteria: one patient had improved gait, facial strength, and language; the other patient had improved vision and behavior. One patient who was not eligible for this analysis due to sub-cm lesions is not shown in the plots above, however she experienced a complete regression of the three target lesions that has remained durable for >18 months (Figure 10).

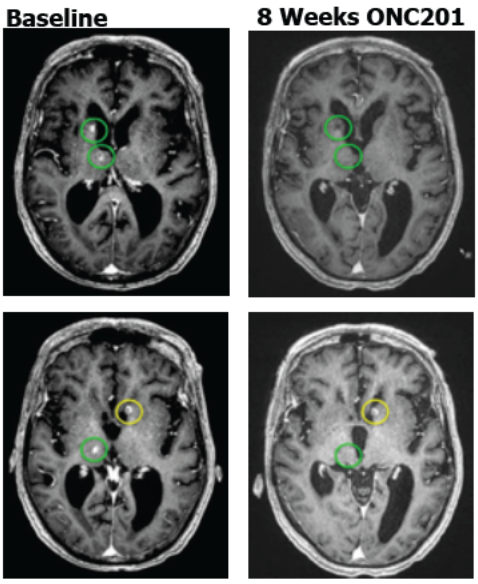

Figure 10. Gadolinium-enhanced MRIs of 74-year-old H3 K27M-mutant glioma at baseline and 8 weeks after initiating ONC201. Three target lesions are shown at baseline in green circles. The lesion in the yellow circle is related to a prior stroke.

Progression-free survival at 6 months by RANO-HGG is 33% (Figure 11A). Median overall survival has not been reached with a median follow-up of 9.1 months (Figure 11B).

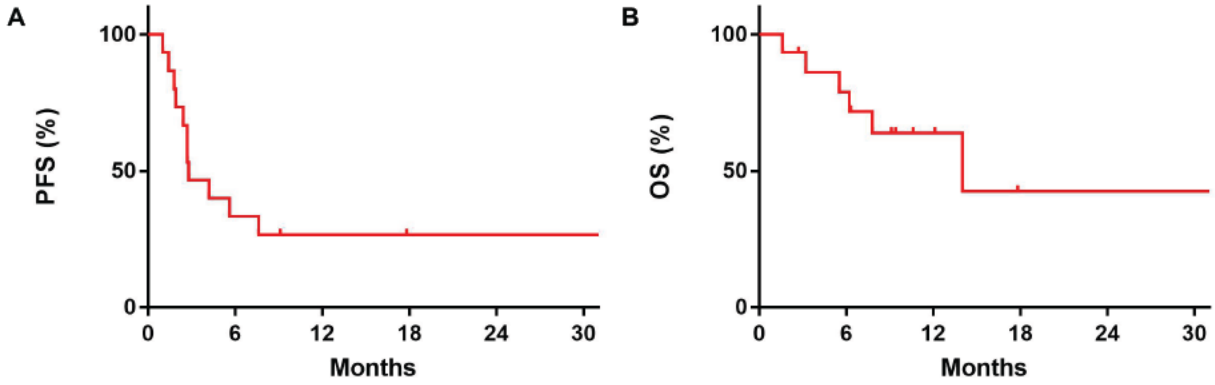

Figure 11. (A) Progression-free survival by RANO-HGG and (B) overall survival of adult recurrent H3 K27M-mutant glioma patients treated with ONC201.

The two single agent ONC201 clinical trials for adult recurrent H3 K27M-mutant glioma continue accrual (NCT03295396, NCT02525692). The clinical development of ONC201 for adult H3 K27M-mutant glioma will be discussed with FDA separately from the pediatric development program outlined below.

3.0 CLINICAL DEVELOPMENT OF ONC201 FOR PEDIATRIC H3 K27M-MUTANT GLIOMA AND DIPG

3.1 DIPG Background

The most common midline glioma in children occurs in the pons, called DIPG based on its radiographic appearance (Piunti et al., 2017). DIPG is generally not resectable and a 6 week course of palliative radiation remains the sole standard-of-care. The overall survival has not improved from a median of 8-9 months and the 2-year survival rate is <10%, despite decades of clinical trials. As a rare disease with an incidence and prevalence of ~300 patients annually in the United States, large clinical studies have not been feasible. Furthermore, randomized studies against radiation alone as the standard of care are not precedented, feasible, or ethical given the relatively homogeneous dismal outcome that has been documented for decades.

3.2 Ongoing Pediatric Phase I Dose Escalation and Expansion Trial

Based on the initial clinical activity of ONC201 in adult recurrent H3 K27M-mutant glioma, the clinical evaluation of ONC201 in DIPG and other pediatric patients with H3 K27M-mutant glioma was warranted. A Phase I dose escalation and expansion clinical trial of ONC201 for previously-irradiated H3 K27M-mutant glioma and newly diagnosed DIPG patients ≤18 years of age was initiated. This study is designed to evaluate the safety of ONC201 as a single agent or in combination with radiation with scaling of the adult fixed, oral dose by body weight as a capsule or OraSweet formulation.

Arm A of this clinical trial has completed accrual, which confirmed the single agent RP2D of the 625mg once per week dose in adults scaled by body weight in pediatric H3 K27M-mutant glioma patients who received at least prior radiation. The characteristics of these patients are summarized below in Table 2.

Table 2. Demographics of Arm A patients of the pediatric H3 K27M-mutant glioma trial treated with ONC201 (N=21).

Gender (N%)	
Female	11 (52%)
Male	10 (48%)
Age, years, median (range)	8 (3-18)
Weight, kilograms, median (range)	39.4 (11.8-135.9)
KPS, median (range)	80 (50-90)
Primary tumor location (N%)	
Pons	14 (67%)
Thalamus	5 (23%)
Brainstem	1 (5%)
Posterior fossa	1 (5%)
Multifocal disease (N%)	
Yes	3 (14%)
No	18 (86%)
Number of lesions, median, (range)	1 (1-2)
Disease Status	
Recurrent	3 (14%)
Post radiation, not recurrent	18 (86%)
Time from prior radiation, weeks, median, (range)	10.1 (0.1-79.6)
Dexamethasone, mg per day, median (range)	2 (0.25-12)

One Grade 3/4 adverse event was reported as possibly-related to ONC201, which was one instance of elevated aspartate aminotransferase (Table 3). The majority of the low grade adverse events attributed by investigators as possibly related to study drug were nausea and headache that are commonly associated with the disease itself. The preliminary PK profile of ONC201 was similar to that observed in adults when the oral dose is scaled by body weight with a terminal half-life of ~ 8 hours, T_{max}~2.1 hours, C_{max}~2.1ug/mL (6uM), and AUC~2.3h*ug/mL.

Table 3. Adverse events reported in Arm A patients of the pediatric H3 K27M-mutant glioma trial treated with ONC201 (N=21). Adverse events reported in ≥10% of subjects are tabulated CTCAE version 5.0.

	All Attributions		Possibly or Probably Related	
	All Grades	Grade 3-4	All Grades	Grade 3-4
Nervous system disorders				
Headache	52%	0%	14%	0%
Nausea	43%	0%	24%	0%
6th nerve palsy	24%	0%	0%	0%
Ataxia	24%	0%	5%	0%
Dizziness	24%	0%	5%	0%
Vomiting	14%	0%	10%	0%
Dysarthria	10%	0%	0%	0%
Dysphagia	10%	0%	0%	0%
General disorders and administration site conditions				
Fatigue	19%	0%	10%	0%
Gait disturbance	10%	0%	5%	0%
Musculoskeletal and connective tissue disorders				
Muscle weakness right-sided	14%	5%	0%	0%
Right hemiparesis	10%	0%	0%	0%
Investigations				
Alanine aminotransferase elevated	10%	0%	10%	0%
Aspartate aminotransferase elevated	10%	5%	5%	5%
Eye disorders				
Diplopia	10%	0%	0%	0%
Respiratory, thoracic and mediastinal disorders				
Cough	10%	0%	0%	0%
Infections and infestations				
Pharyngitis	10%	0%	0%	0%

The largest group of patients across the pediatric clinical trial and expanded access program who are sufficiently interpretable for preliminary efficacy evaluation are 13 DIPG patients who received single agent ONC201 after completion of radiation, but prior to recurrence. Eleven patients were treated on the pediatric H3 K27M-mutant glioma trial and 2 patients were treated on an expanded access protocol conducted under the Sponsor's IND. Of these 13 patients, 6 patients had RNA-seq available: 5 H3.3 and 1 H3.1. Six other patients were identified as H3 K27M mutant by IHC and one patient's tumor has not been profiled. Median PFS and OS have not been reached with a median follow up of 13.2 months and OS12 is 69% (Figure 12). Historical outcomes for this disease include median PFS of 5-7 months, median OS of 8-12 months, and OS12 of 40-50% for DIPG patients.

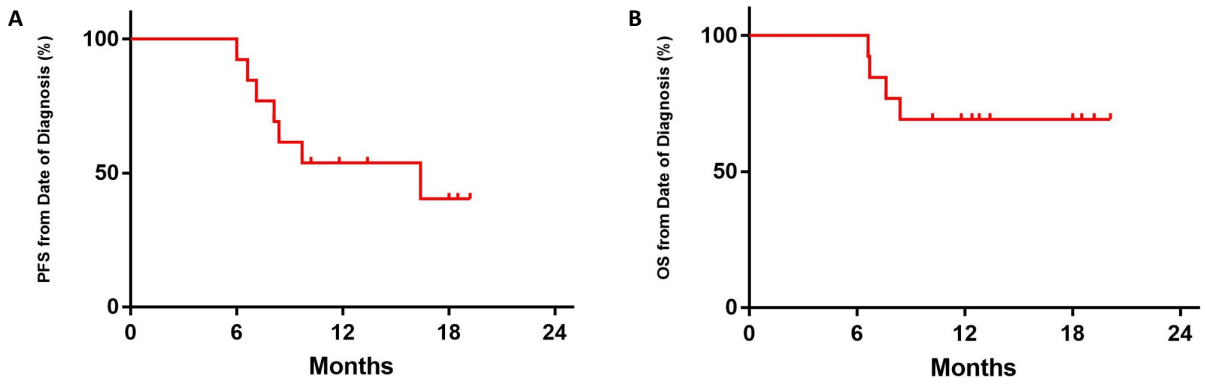

Figure 12. (A) Progression-free survival and (B) overall survival from diagnosis for DIPG patients treated with ONC201 following radiation prior to recurrence (n=13).

Interpreting time-to-event outcomes for non-DIPG H3 K27M-mutant glioma patients is difficult due to the lack of well-established historical controls and the fact that many of these patients initiate ONC201 prior to recurrence. Nevertheless, tumor regressions have been observed in patients with thalamic tumors while on treatment with kinetics that are atypical for the disease course (Figure 13).

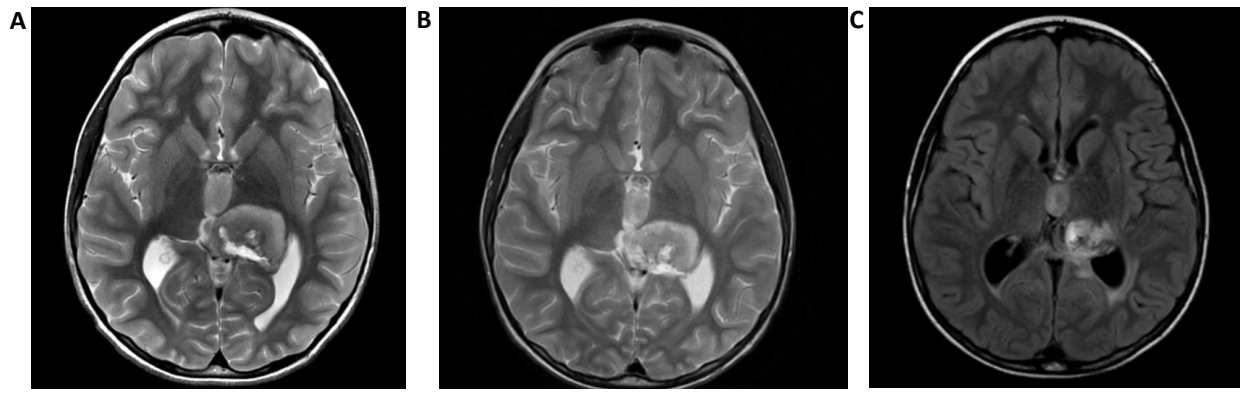

Figure 13. Gadolinium-enhanced MRIs of 6-year-old H3 K27M-mutant treated with ONC201. Imaging is shown at (A) diagnosis, (B) post-radiation before ONC201, and (C) 6 months after initiating ONC201. Panel C represents >50% regression relative to panel B.

The enrollment of this clinical trial continues (NCT03416530) to further establish the safety, PK, and efficacy profile of ONC201 in this patient population.

3.3 Planned Pediatric Trials

A series of additional pediatric clinical trials are in development that include Phase II clinical trials to evaluate the efficacy of ONC201 in newly diagnosed DIPG and H3 K27M-mutant glioma patients. A Phase I investigation of biomarker endpoints exploring alternative dose schedules in midline gliomas is also in development.

The most advanced study design in development is a NRG/COG cooperative group Phase II clinical trial investigating ONC201 in newly diagnosed DIPG, as well as newly diagnosed non-DIPG H3 K27M glioma in separate arms. Both arms will administer ONC201 concurrently with radiation, followed by maintenance ONC201. The newly diagnosed DIPG arm (age 1-21 years old) has the potential to confirm the hypothesis that ONC201 extends overall survival based on preclinical and early phase clinical data. The primary endpoint of this single arm is overall survival.

In Europe, ONC201 is being incorporated into the next version of the BIOMEDE clinical trial that is currently being designed. This version of the study is currently designed to randomize newly diagnosed H3 K27M-mutant and/or H3K27me3-negative diffuse midline glioma patients to receive ONC201 or another agent. The other agent will be selected from the first version of BIOMEDE involving everolimus, erlotinib, or dasatinib that have not been proven effective for this disease. Either drug will be administered concurrently and following radiation. The primary endpoint of this randomized study is overall survival.

3.4 Development Challenges

The development of a novel anti-cancer agent for pediatric H3 K27M-mutant glioma and/or DIPG faces a number of challenges involving the heterogeneity of the disease, lack of suitable radiographic assessment criteria, rare prevalence, and lack of feasibility to randomize against the current standard therapy that is palliative.

Regarding disease heterogeneity, the majority of the literature indicates that patients with midline gliomas who harbor the H3 K27M mutation have an inferior prognosis and different underlying tumor biology than those without this mutation (Feng et al., 2015; Karremann et al., 2018; Kleinschmidt-DeMasters and Mulcahy Levy, 2018). This recognition is embodied by the 2016 WHO criteria for central nervous system tumors where this mutation diagnoses a distinct form of Grade IV glioma (Louis et al., 2016). However, small published studies report disparate prognostic associations of H3 K27M outside of DIPG, particularly in adults. There are a number of factors that can contribute to heterogenous outcomes and symptoms among H3 K27M-mutant glioma, such as age and primary tumor location.

Radiographic assessments to measure objective response rate can sometimes be used to document the therapeutic effects of a targeted agent across a disease(s) with underlying heterogeneity. Unfortunately, RANO criteria that is widely used for brain tumors was developed for supratentorial glioblastoma and there are a number of features that limit its application to some midline gliomas. A major limitation is the reliance of RANO on contrast-enhanced imaging, as some midline gliomas such as DIPG exhibit minimal or no contrast-enhancement. Furthermore, standard criteria used in glioma clinical trials to isolate the treatment effect on radiographic imaging typically require a prolonged washout period from prior therapies after disease recurrence that is often not feasible for midline gliomas such as DIPG due to its associated dismal survival. The diffuse nature of DIPG and some other midline gliomas also makes quantifying tumor

dimensions subjective. Therefore, objective assessment of radiographic responses in this disease is challenging and often not feasible for some forms of H3 K27M-mutant glioma, such as DIPG.

In view of the outlined radiographic challenges and dismal survival outcomes, assessment of overall survival is positioned as the superior efficacy endpoint for documenting the potential efficacy of an investigational agent for DIPG. Historically, regulatory approval with overall survival as the primary endpoint has generally required randomized, controlled clinical trials. Conducting a randomized clinical trial that is sufficiently powered for regulatory purposes is extremely challenging for H3 K27M-mutant glioma while stratifying for various reported prognostic factors, as subsets of the disease, such as DIPG, have an annual incidence of ~300 patients in the United States and many of these patients will not be enrolled due to financial or geographic limitations, eligibility criteria, or competing trials. The lack of feasibility for a randomized, controlled trial is further pronounced in DIPG. Decades of clinical trials in this specific form of midline glioma have produced nearly superimposable survival curves with a median of 8-12 months and 2-year survival rate of <10%. Disease experts assert that a randomized trial against standard radiation is not necessary, due to the established consistent survival benchmarks documented in many trials, and that randomization of patients to palliative radiation is not ethical or feasible. BIOMEDE is one of the only randomized clinical trials to be conducted for DIPG. However, this study randomizes patients to drugs that do not have proven efficacy for the disease, rather than the standard of care that is the more appropriate comparison for regulatory purposes.

As a consequence of the challenges above, for regulatory purposes we propose to evaluate ONC201 for two subpopulations of H3 K27M-mutant glioma with different approaches based on feasibility: adult recurrent H3 K27M-mutant glioma using objective response rate and newly diagnosed DIPG using overall survival.

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