Delivery of OT-101 - TGF-β2 Antisense for the Treatment of Glioblastoma

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ABSTRACT

OT-101 - a TGF-β antisense- is active against recurrent glioblastoma in G004- a phase 2 clinical trial. OT-101 was delivered intratumorally by Convection Enhanced Delivery (CED). To further expand the application of OT-101, we explored the intrathecal delivery of tritiated OT-101 into Sprague-Dawley CD (albino) rats. Throughout the studies, there were no sex differences. After 1 hr intracerebral infusion in rats, OT-101 was limited to the infusion site. Whereas for the 1 hr intraventricular infusion, OT-101 was more widespread with 35X, 19X, 12X higher concentration found in cerebellum, remaining cerebrum, cerebrospinal fluid (CSF), respectively. OT-101 concentration was stable for the first 4 hours post infusion and decayed biexponentially with a slow terminal half life for tissue but not for CSF, suggestive of rapid penetration of the underlying tissue away from the CSF compartment. Minimum amount of OT-101 was detected in the plasma compartment. Intrathecal bolus administration of 0.1mL of OT-101 at 14, 30, 200, 300, and 500 µM into cynomolgus monkeys did not result in any single dose toxicity. Histopathology examination revealed no substance-related histomorphological lesions in the cavum subarachnoideale of the lumbar region. No changes were noted in the grey and white matter of the spinal cord, the nerve trunk, and nerve cells did not show any abnormalities. These data suggest that intrathecal administration of OT-101 is a potentially effective delivery route for antisense therapeutics such as OT-101 to the midline ie. for Diffuse Midline Glioma (DMG). A phase 1b/2 clinical trial evaluating OT-101 against DMG is proposed and the trial design will be presented

BACKGROUND

OT-101: Antisense oligodeoxynucleotides are short strings of DNA that are designed to downregulate gene expression by interfering with the translation of a specific encoded protein at the mRNA level. Several RNA therapeutics, including anti-sense oligonucleotides have been evaluated in clinical trials and some approved. OT-101 is a synthetic 18-mer phosphorothioate oligodeoxynucleotide (S-ODN) in which a non-bridging oxygen of each phosphate moiety is substituted by a sulfur atom. OT-101 was designed to be complementary to a specific sequence of human TGF-β2 mRNA following expression of the gene. It is a first-in-class RNA therapeutic designed to abrogate the immunosuppressive actions of TGF-β2 and reduce the level of TGF-β2 in malignant gliomas, and thereby delay the progression of disease.

Diffuse midline glioma (DMG) is a highly morbid pediatric central nervous system (CNS) tumor for which there is currently no effective treatment. DMG is responsible for 50% of all childhood HGG. Due to their anatomic location and infiltrative nature, DMGs are not amenable to surgical resection and are most often diagnosed radiographically and treated with radiation therapy, with no effect on survival. The median age at diagnosis is 5 to 11 years with tumors that arise in the pons occurring at a younger age (~7 years) than those that arise in the thalamus (~11 years). DMG patients face a very poor median overall survival (OS) of just 9–11-months, with <10% of patients with pontine tumors surviving two years post-diagnosis. Radiation remains the mainstay of therapy, though it is only palliative, and is expected to increase survival by an average of 3 months.

RATIONALE FOR USE OF OT-101 IN DMG

- It is safe and effective during extended (7-day) high flow perfusion of the brain in adult gliomas. As single agent it is as effective as the most active drug in adult gliomas – TMZ for chemo naïve patients BCNU/CCNU in chemo failure patients.
- TGF-β2, is expressed at high levels in both pediatric GBM (WHO Grade IV) and pediatric DIPG (WHO Grade IV) patients.
- Expression analyses of pediatric brainstem cases of the TCGA database yielded highly significant survival benefit across all four quartiles of TGF-β2 expression. This was not observed for either TGF-β1 and less so for TGF-β3.
- Expression analyses of all gliomas cases treated with radiation yielded highly significant survival benefit across all quartiles of TGF-β2 expression. This was not observed for either TGF-β1 nor TGF-β3.

ADME After Intracerebral/Intraventricular Infusion in Rats

Dosing by intracerebral infusion or intraventricular infusion resulted in distribution of OT-101 throughout the CNS. Dosing was done at 12 ug/rat. The distribution profiles between cerebral and ventricular administration were similar except for a higher drug concentration in cerebellum and CSF with direct administration into the CSF compartment with intraventricular administration. The similarity of intraventricular administration versus intracerebral administration allows for the reliance on preclinical and clinical safety data on intracerebral administration of OT-101 for the intraventricular clinical program for OT-101 in DMG.

[^{3}H]OT-101 Concentration (ng equivalent/g) median (min-max)

Hrs	Dose Site	Cerebellum	Remaining cerebrum	Remaining brain	CSF
Intracerebral infusion (N=8)					
0	30400 (20648-38800)	1320 (430-1913)	1360 (997-2618)	3135 (2358-3910)	13350 (3308-24325)
1	12850 (9950-14825)	851 (345-1453)	1610 (948-4384)	4310 (2815-5393)	2285 (1420-4325)
4	18400 (5800-30175)	692 (545-1045)	2565 (990-3341)	1883 (934-3610)	1056 (610-1185)
24	7820 (4504-11850)	432 (273-464)	962 (696-1703)	1141 (909-1775)	124 (89-140)
72	3145 (2562-4150)	165 (39-227)	375 (190-569)	287 (107-498)	24 (15-29)
Intraventricular infusion (N=1)					
1	30875	7938	2144	2944	5844
4	22250	1531	1069	2138	2094
24	2450	1413	284	534	71
72	850	401	250	317	26

At the end of the infusion period of 1hr, the rapidly perfused CNS components readily accessible by CSF (dose site, cerebellum, CSF, pituitary gland) achieved Cmax. Though readily accessible to CSF, the pineal organ actively taken up OT-101 and did not achieve Cmax until 4hr. The less perfused CNS components achieved Cmax at 1 (remaining cerebrum, remaining brain) and 4 hr (spinal cord).

A small fraction of administered OT-101 reached the plasma compartment with Cmax at 1hr. The organs readily perfused by blood achieve Cmax at 4 hr timepoint (thyroid and liver) and those actively taken up OT-101 did not achieved Cmax until 24 hr (kidney, spleen, bone marrow) or 72 hr (thymus).

Concentration of radioactivity in tissues following intracerebral administration of [^{3}H]OT-101 at dose level of 12 ug/rat.
Concentration = ng equivalent/g (mean, SD, N)
Infusion time = 1 hr.

Hours (post infusion)		0	1	4	24	72
Dose Site	mean	**28165**	12929.69	23018.36	8449.063	3025.68
	SD	**12950.03**	5534.462	21306.78	5105.702	1426.363
	N	**6**	8	8	8	8
Cerebellum	mean	**1239.167**	1238.359	847.5945	457.5092	141.8422
	SD	**790.1148**	1419.591	679.9536	363.2347	93.60506
	N	**6**	8	8	8	8
Remaining cerebrum	mean	1683	**2297.609**	2215.538	1062.155	381.9844
	SD	816.6407	**2121.81**	1380.145	654.7472	247.8907
	N	6	**8**	8	8	8
Remaining brain	mean	3056.833	**5108.281**	2020.409	1291.969	303.3984
	SD	1219.287	**3511.84**	1436.072	468.2549	214.2532
	N	6	**8**	8	8	8
CSF	mean	**14481.67**	3688.516	916.2813	118.6641	20.72031
	SD	**11839.01**	4316.185	472.7928	51.46708	10.8167
	N	**6**	8	8	8	8
Pituitary Gland	mean	**129.15**	109.2833	58.7	61.96667	9.793333
	SD	**97.75291**	120.1135	43.55865	46.16759	7.387562
	N	**6**	6	6	6	6
Spinal Cord	mean	255.5117	401.6667	**403**	175.25	70.41667
	SD	212.2003	327.0056	**135.6112**	83.68975	56.14148
	N	6	6	**6**	6	6
Thyroid	mean	48.19167	29.35167	**42.455**	16.15	9.025
	SD	65.89372	28.18978	**57.86549**	5.318928	3.068288
	N	6	6	**6**	6	6
Kidneys	mean	16.41	102.9667	221.4333	**368.6667**	155.5833
	SD	7.602187	93.99691	295.9934	**168.7539**	67.75132
	N	6	6	6	**6**	6
Bone Marrow	mean	3.243333	9.605	19.405	**49.71667**	31.63333
	SD	1.282336	8.159686	20.65162	**11.29751**	14.77656
	N	6	6	6	**6**	6
Liver	mean	5.396667	17.44667	**28.82167**	26.05	9.445
	SD	2.392201	14.68127	**41.37177**	17.74528	2.691875
	N	6	6	**6**	6	6
Plasma	mean	8.85	**16.015**	14.60167	12.86667	6.886667
	SD	4.799429	**10.76895**	6.639308	0.877876	1.750448
	N	6	**6**	6	6	6
Pineal body	mean	609.6667	1255	**1317.333**	1045.833	403.3833
	SD	549.5157	822.2618	**1440.409**	1804.737	542.7811
	N	6	6	**6**	6	6
Spleen	mean	3.325	15.045	8.606667	**22.25**	15.73833
	SD	1.386243	17.80838	2.688372	**7.181017**	4.627355
	N	6	6	6	**6**	6
Thymus	mean	2.531667	4.065	5.318333	13.38333	**13.92333**
	SD	0.836144	1.33614	1.38293	2.050772	**5.425649**
	N	6	6	6	6	**6**

NONCLINICAL SAFETY & PHARMACOLOGY

OT-101 has been subjected to a series of nonclinical safety and pharmacology studies. The salient features of the conclusions from these studies were as follows:

- Prolonged local administration of OT-101/AP 12009 may result in local tissue inflammation There was mild to moderate local toxicity observed in animals after infusion of a concentration of 500 µM without any macroscopic changes.
- When administered to 3 kg male or female rabbits as a bolus IT injection at a 0.12 mg/kg dose level (500 µM solution; 0.1 mL) with an estimated CSF concentration of 4.16 µM, OT-101 did not cause any clinical toxicity or drug-related macroscopic/microscopic changes consistent with sub-clinical toxicity.
- When administered to 6-7 kg cynomolgus monkeys as a bolus IT injection at a 0.05 mg/kg dose level (500 µM solution; 0.1 mL) with an estimated CSF concentration of 0.46 µM, OT-101 did not cause any clinical toxicity or drug-related macroscopic/microscopic changes consistent with sub-clinical toxicity.
- When given to rats via intraventricular administration, radiolabeled OT-101 (0.18 mg/kg) was detected within 1 hour after administration not only in the CSF but also in the cerebrum, cerebellum and pineal body. The CSF and brain tissue half-lives were <24 hours with <15% residual OT-101 remaining at 72 hours.

RATIONALE FOR INTRAVENTRICULAR INJECTION VIA THE OMMAYA RESERVOIR

- The intratumoral placement of the catheter posed potential risks avoidable with the use of Ommaya reservoir to access the ventricular space
- The delivery of OT-101 through intraventricular delivery is similar to that of intracerebral delivery allowing reliance on previous preclinical and clinical safety information
- Currently, Ommaya reservoirs have been successfully used in the treatment of leptinomenigeal cancers (LM) from multiple malignant tumors.
- OS was significantly higher in patients with Ommaya reservoir in spite of Karnofsky Performance Status (KPS) previous to chemotherapy. Therefore, intraventricular chemotherapy should be preferred over lumbar puncture chemotherapy administration if there are resources available.
- Ommaya is approved for administration of Brineura in pediatric patients. Brineura® (cerliponase alfa) is the only enzyme replacement therapy that helps treat CLN2 disease, a common form of Batten disease. Brineura is approved to slow loss of ability to walk or crawl (ambulation) in symptomatic pediatric patients 3 years of age and older with CLN2 disease.

DMG PHASE 1 STUDY DESIGN

Study Title: An Open-label Dose Escalation Study to Evaluate the Safety and Tolerability of Repeated Cycles of OT-101 in Pediatric Diffuse Midline Glioma (DMG) Patients, Administered Intraventricularly over A 7 Day Period at Weekly Intervals

Study Population:
Pediatric (≥ 2 to < 18 years) patients with Diffuse Midline Glioma (DMG)

Sample Size:

In general, a cohort of 3 evaluable patients has to be enrolled per treatment group. In the case of 2 patients experiencing dose-limiting toxicity, the number of evaluable patients may be increased to six to further evaluate toxicity.

Group	Conc.	Treatment Period (OT-101-infusion)		Flow Rate		Dose			Rest Period (no infusion)	
No.	µM	days	h/day	µl/min	ml/day	nmol/day	nmol/cycle	mg/cycle*	weeks	µl/min
1	10	7	24	4	5.76	57.6	403.2	2.476	1	0
2	80	7	24	4	5.76	460.8	3225.6	19.811	1	0

Study Objectives

Primary Objective: To determine the maximum tolerated dose (MTD) by assessing the dose-limiting toxicity (DLT) of two cycles of OT-101, administered intraventricularly at weekly intervals

Secondary Objectives:

- To determine the safety and tolerability of at least two cycles of OT-101, administered intraventricularly at weekly intervals
- To determine the change in tumor size measured in patients treated with at least two cycles of OT-101, administered intraventricularly at weekly intervals
- To determine the time to progression of patients treated with at least two cycles of OT-101, administered intraventricularly at weekly intervals
- To determine plasma concentration levels of OT-101

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