B-012: In vivo Mini Screen to assess the Antitumor Effect of Carboxyamidotriazole Orotate (CTO) and Temozolomide (TMZ) in Monotherapy in Three ITCC-P4's High-grade Glioma (HGG) Pediatric Patient -Derived Xenograft (PDX) Tumor Models

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Background

- Pediatric high-grade glioma represents approximately 9.3% of all primary malignant and nonmalignant brain and other CNS tumors diagnosed in children and adolescents ≤19 years. Treatment of pediatric high-grade glioma depends on the type of tumor, its location and size, how far it has spread and the age and overall health of the patient.
- The main treatment paradigm includes surgery followed by systemic therapy with or without radiotherapy. The use of RT with concurrent TMZ followed by adjuvant TMZ and lomustine for patients ≥3 years was recommended based on Phase II Children's Oncology Group ACNS 0423 trial.
- Targeted therapies have been used in the treatment of pediatric patients with high-grade gliomas with various molecular signatures: the BRAF V600E point mutation is detected in 10%-15% and PDGFR is altered in some pediatric high-grade glioma patients.
- Despite aggressive primary management, most patients experience recurrence or disease progression. Patients with recurrent or progressive disease have a median overall survival of ≤6months, and no effective therapies currently exist. We propose using CTO, a novel oral inhibitor of non-voltage-dependent store-operated Ca2+ release -activated Ca2+ channels, blocking Ca2+ influx and Ca2+ release from intracellular stores, resulting in disruption of multiple signal transduction pathways, including genomic and epigenetic factors PI3K/AKT/PTEN/mTOR, RAS/RAF/MAPK/MEK, WNT/B-catenin, HDAC and others. currently under development for treatment of adult glioblastoma (GBM) and glioma patients.
- There is an unmet need for improving the overall survival in pediatric high-grade glioma with an oral drug exhibiting a safe toxicity profile that crosses the blood-brain barrier, regulates multiple tyrosine kinases and has the potential to overcome drug resistance

Study Design

- **Study Objectives**
  - In vivo screen to assess the anti-tumoral effect of CTO and TMZ in monotherapy in three pediatric high-grade glioma tumor models
  - Studies contracted with Charles River Labs (currently IQVIA Labs) and ITCC-P4. All tumor modes were derived from a childhood cohort and characterized by the ITCC-P4 (Innovative Therapies for Children with Cancer Pediatric Preclinical Proof of Concept Platform)
  - Three HGG PDX Models:
    - Represent models with genes targetable with CTO, such as BRAF, EGFR, HDAC1, HDAC2, HSP90AB1, KRAS, NRAS, PIK3CA, PTEN, TP53.
    - The characteristics of the human tumor xenografts and the clinical information on PDX growth condition are described in **Table 1A**.
    - The mutational profile of the tumor models is given in **Table 1B**. Specifically, the HG0067_PP01 exhibited BRAF mut, the HG0230_PR06 exhibited PIK3CA mut, and the HG0356_pp01 exhibited BRAF mut and HDAC2 loss.
    - The detailed molecular information on gene expression data of target biomarkers is given in **Table 1C.**
    - The summary of assessments of transcriptional signature behavior in CTO treated anagen hair RNA in vitro is given in **Figure 1**.
- **Study Design**
  - All experiments were performed in NSG mice bearing subcutaneous tumor xenografts using 3 experimental cohorts (n=3 mice/group/model).
  - Group 1 served as the control and received a vehicle consisting of 40% PEG 400 in water for injection (WFI) on a schedule of 5 days followed by 2-day treatment hiatus.
  - Group 2 was administered CTO orally at dose of 513mg/kg/day using the same 5-days-on, 2-day-off weekly cycle.(28- day cycle)
  - Group 3 received temozolomide (TMZ) via oral gavage at a dose of 17mg/kg/day, following a cyclic dosing of two five-day treatment blocks (Days 1-5 and Days 15-19).
  - Mice were monitored for effect on body weight (to signal any potential toxicities) tumor regression, tumor growth inhibition (TGI) and survival.
- **Study Treatment**
  - CTO administered daily, 5-days on, 2 day-off, 28-day cycle
  - TMZ administered days 1-5 and days 15-19 of each cycle

Table 1 Characteristics of the Human Tumor Xenografts

Table 1A Clinical Information and PDX Growth Condition

Tumor Type	Tumor Designations	Histology/ Differentiation	Patient Histology	Origin	Age (Sex) of Pt	TR [%]	IT [Days]	Td [Days]
Glioblastoma	CNXF 4134 // hg0067_pp01	Glioblastoma not known	high grade glioma, anaplastic astrocytoma, grade III	Primary	12 (F)	83	21	2.4
Glioblastoma	CNXF HG0230 // hg0230_pp06	high grade glioma not known	high grade glioma, GBM, grade IV	Primary	15 (M)	100	35	-
Glioblastoma	CNXF 4286 // hg0356_pp01	Glioblastoma undifferentiated	HGG - Pleomorphic Xanthoastrocytoma with anaplastic features	Primary	- (M)	67	26	2.3

TR = tumor take rate (proportion of implanted tumors suitable for randomization); IT = induction time (time interval between tumor implantation and randomization/start of dosing); Td = tumor volume doubling time; Stage according to the TNM Staging Guide or according to the International Classification of Diseases for Oncology (ICD-O, issued by the World Health Organization) by the World Health Organization)

Table 1B Molecular Information: Mutation Profile of Selected Tumor Models (Oncoprint)

Based on whole exome, whole genome and RNAseq analysis, the molecular landscape of the models was assessed and the driver genes of interest are highlighted per model.

ITCC-P4 PDX Models	Mutations
HG0067_PP01	*BRAF mut*
HG0230_PR06	*PIK3CA mut*
HG0356_PP01	*BRAF mut, HDAC2 loss*

Figure 1. The transcriptional behavior of signatures on CTO treatment (expressed relative to 0 mM baseline) Of Anagen Hair in vitro. Mol Cancer Ther 2013,12:A233.



	μM CTO					
	2	5	10	2	5	10
EGFR	-0.6	-0.6	-0.6	-0.8	-0.8	-0.8
MEK	-0.5	-0.5	-0.5	-0.5	-0.7	-0.7
HSP90	-0.2	-0.2	-0.2	-0.4	-0.4	-0.4
P53 stabilisation	0.15	0.15	0.15	0.4	0.4	0.4
WNT / β-catenin	0	0	0	-0.14	-0.14	-0.14
HDACi	-0.1	-0.12	-0.12	-0.08	-0.16	-0.16
GF	-0.1	-0.1	-0.16	-0.07	-0.01	-0.07
RAS	-0.12	-0.14	-0.14	0	0	0
PI3K	0	0	0	0	0	0
PI3K/mTOR	0	0	0	0	0	0
NOTCH / GSI	0	0	0	0	0	0

Table 1C Molecular Information: Gene Expression data of target biomarkers

Log 2 gene expression of biomarkers per tumor model are shown on a heat map (top panel) and in a plot (bottom panel)

ITCC-P4 PDX-Models	BRAF (ENSG00000157764.8)	EGFR (ENSG00000146648.11)	HDAC1 (ENSG00000116478.7)	HDAC2 (ENSG00000196591.7)	HSP90AB1 (ENSG00000096384.15)
HG0067_PP01	3.40690396	7.16539445	7.01615308	4.88547675	11.5107475
HG0230_PR06	4.61399715	9.24681445	6.31088897	4.50968318	9.83550158
HG0356_PP01	4.91047844	3.06101577	6.31980035	4.76160974	11.1875607

ITCC-P4 PDX-Models	KRAS (ENSG00000133703.7)	NRAS (ENSG00000213281.4)	PIK3CA (ENSG00000121879.3)	PTEN (ENSG00000171862.5)	TP53 (ENSG00000141510.11)
HG0067_PP01	3.21938539	5.56061714	3.64713027	3.95738533	6.20105549
HG0230_PR06	6.27081671	6.28995616	4.42123003	5.52755207	6.62249408
HG0356_PP01	5.15403226	7.48570375	4.99724272	6.04874624	6.33041498

Table 2 Overview of Experiments

Tumor Designation/ Passage[1] // ITCC-P4 ID	Exp. No.	Mouse Strain	Date of Tumor Implantation	Number (Sex) of Animals	Tumors Implanted per Animal	Date of Randomization (Day 0)	Number of Randomized Animals	Group Median Tumor Volume[2]	Group Mean Tumor Volume[2]
CNXF 4134 / 9N2 // HG0067_PP01	V1906	NSG	02-Jul-25	16 / female	1	16-Jul-25	9	81.5–109.4	94.2–118.6
CNXF HG0230/ 11N2// HG0230_PR06	V1907	NSG	18-Jul-25	15 / female	1	19-Aug-25	9	113.8–120.2	116.2–116.7
CNXF 4286 / 7N2 // HG0356_PP01	V1908	NSG	02-Jul-25	15 / female	1	22-Jul-25	9	103.4–117.6	139.9–148.7

[1] The number preceding the N represents the total number of passages and the number following the N represents the passage number after the last freeze-thaw cycle.
[2] Range at randomization [mm³]

Table 3 Antitumor Efficacy

Gr. ID	Treatment[1]	Dose Level [mg/kg/day]	Schedule [Day]	Route	Min.T/C [%(Day)][2]	Efficacy Rating[3]	RECIST R [%] (Day)[4]	Response[4]	TGI_{last}	TGI_{Max}	Td [Days]	Tg [Days]
Tumor Model CNXF 4134 – Exp. V1906 - HG0067_PP01												
1	Vehicle	10 ml/kg	1-5,8-12, 15-19,22,23	po	n/a	n/a	2120.2 (23)	PD	-	-	3.4	7.4
2	Carboxyamidotriazole Orotate	513	1-5,8-12, 15-19,22-26	po	49.5 (23)	+	2050.7 (27)	PD	17.2 (23)	59.0 (6)	6.5	10.1
3	Temozolomide	17	1-5,15-19	po	78.8 (6)	–	1658.4 (20)	PD	-60.1 (20)	24.0 (6)	3.2	8.5
Tumor Model CNXF HG0230 – Exp. V1907 - HG0230_PR06												
1	Vehicle	10 ml/kg	1-5,8-12, 15-19,22,23	po	n/a	n/a	1431.5 (21)	PD	-	-	3.0	11.0
2	Carboxyamidotriazole Orotate	513	1-5,8-12,1519,22-26,29-33	po	40.9 (21)	+	1777.7 (35)	PD	62.7 (21)	74.8 (3)	9.9	16.9
3	Temozolomide	17	1-5,15-19	po	79.5 (3)	–	1563.4 (21)	PD	-9.7 (21)	39.7 (3)	4.6	9.9
Tumor Model CNXF 4286 – Exp. V1908 - HG0356_PP01												
1	Vehicle	10 ml/kg	1-5,8-12,15-19	po	n/a	n/a	1034.1 (14)	PD	-	-	2.5	6.0
2	Carboxyamidotriazole Orotate	513	1-5,8-12, 15-19,22-24	po	31.5 (14)	+	1372.8 (24)	PD	45.1 (14)	55.4 (3)	3.8	7.1
3	Temozolomide	17	1-5,15-19	po	67.7 (14)	–	1300.3 (21)	PD	38.7 (14)	38.7 (14)	2.3	6.9

n/a = not applicable; n.r. = not reached (i.e. group median RTVs always < 200%/400%)
[1] Vehicle in Group 1 and for carboxyamidotriazole orotate: 40% PEG 400 in WFI; vehicle for temozolomide: 10% DMSO, 90% of 0.9% saline in deionized water
[2] Minimum T/C values were calculated from mean RTV values.
[3] Efficacy rating: +++= T/C <5%; ++= ≤ 5% ≤ T/C< 10%; +=: 10% ≤ T/C < 25%; +: 25% ≤ T/C <50%; +/–: 50% ≤ T/C ≤ 65%; –: T/C >65%
[4] Response R evaluation for **whole groups** are graded according to RECIST criteria (see also Section 5.4.4): complete response (CR; R = -100%); partial response (PR; -100% < R < -50%); stable disease (SD; -50% ≤ R < +35%); progressive disease (PD; R > 35%).

Figure 2. Efficacy of Test Article Treatment (Overview)

Figure 2A Group Antitumor Efficacy

The minimum T/C (A1), TGI_{max} (A2) and TGI_{Last} (A3) values for all experiments are shown.

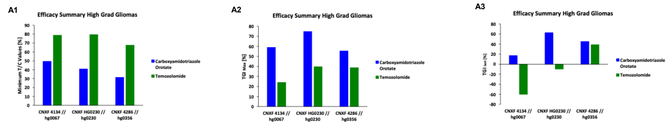


Figure 3 Antitumor Efficacy of Test Article Treatments (Exp. V1906 Tumor Model CNXF 4134 // HG0067)

Overview figure of absolute (A) and relative (B) mean tumor volumes of all treatment groups over time +/- SEM

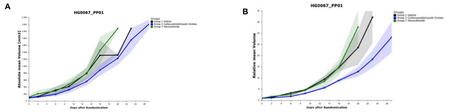


Figure 4 Antitumor Efficacy of Test Article Treatments (Exp. V1907 Tumor Model CNXF HG0230 // HG0230)

Overview figure of absolute (A) and relative (B) mean tumor volumes of all treatment groups over time +/- SEM. Please note, that graphs in (A) and (B) were truncated where less than 50% of animals were left in a group.

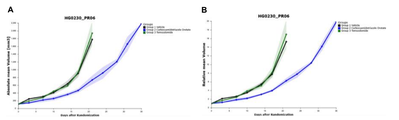


Figure 5 Antitumor Efficacy of Test Article Treatments (Exp. V1908, Tumor Model Tumor Model CNXF 4286 // HG0356)

Overview figure of absolute (A) and relative (B) mean tumor volumes of all treatment groups over time +/- SEM. Please note, that graphs in (A) and (B) were truncated where less than 50% of animals were left in a group.

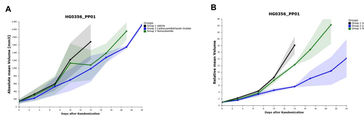


Figure 6 Bioinformatical Analysis

Figure 6A

Mutational Landscape of high-grade glioma PDX models investigated in this study

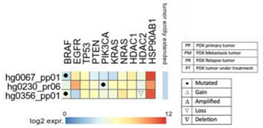
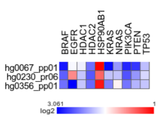


Figure 6B

Gene expression analysis showing Log2 expression values of biomarkers of tumor models



Results

Antitumor Efficacy: Tumor growth delay was observed for 3 PDX models with CTO administration, while TMZ failed to elicit an inhibitory effect on tumor progression in any of the models evaluated.

The minimum T/C ratios (Test Agent/Control; derived from mean relative tumor volumes) for CTO were 49.5%, 40.9%, and 31.5% across the three models, demonstrating moderate efficacy of CTO. The results are presented in:

- Table 2 gives the Overview of Experiments.
- Table 3 described the detailed data for anti-tumor activity.
- Figure 1 describes the transcriptional signature behavior of CTO.

- Figure 2 describes the anti-tumor efficacy for all three models expressed as minimum T/C (Test Agent/Control) in Figure A1, expressed as (TGI-Tumor Growth Inhibition) TGI_{max} in Figure A2 and TGI_{last} in Figure A3.

- Figure 3 describes the antitumor efficacy of CTO in the V1906Tumor Model CNXF4134//HG0067 model as (A) Absolute mean volume (mm^3) and (B) relative mean volume. The minimum T/C ratio was 49.5%. Based on the whole exome, whole genome, and RNAseq analysis, the driver gene of interest for this model is BRAF mut.

- Figure 4 describes the antitumor efficacy of CTO in the V1907Tumor Model CNXF//HG0230 model as (A) Absolute mean volume (mm^3) and (B) relative mean volume. The minimum T/C ratio was 40.9%. Based on the whole exome, whole genome and RNAseq analysis, the driver gene of interest for this model is PIK3CA mut.

- Figure 5 describes the antitumor efficacy of CTO in the V1908 Tumor Model CNXF4286//HG0356 model as (A) Absolute mean volume (mm^3) and (B) relative mean volume. The minimum T/C ratio was 31.5%. Based on the whole exome, whole genome and RNAseq analysis, the driver gene of interest for this model is BRAF mut, HDAC2 loss.

- Figure 6 presents bioinformatical analysis in: Figure 6A - Mutational Landscape of High-Grade Glioma PDX Models investigated in this study, and Figure 6B - Gene Expression Analysis Showing Log 2 Expression Values of Biomarkers of Tumor Models investigated in this study.

CTO demonstrated moderate efficacy, characterized by significant tumor growth delay across all 3 subcutaneous high-grade glioma (HGG) models. Conversely, TMZ failed to exhibit any detectable anti-tumor activity. Due to the small sample size (n=3) subtle molecular changes could not be analyzed statistically.

Conclusions

In conclusion, CTO yielded moderate antitumor activity with tumor growth delay observed consistently across the 3 pediatric high-grade glioma models, having BRAF mut, PIK3CA mut or BRRAFmut-HDAC2loss (targets of CTO), while no antitumor response was elicited by temozolomide in any of these models tested. Studies using larger sample size (n=10) are planned

First-in human Phase I trials are planned using biomarker-based trial design in pediatric high-grade glioma patients.