CEP5003, a novel compound targeting Glioblastoma Cancer stem cells

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Introduction

Glioblastoma multiforme (GBM) is a genetically complex and aggressive primary adult brain tumor, with a median survival of 12 - 14 months. The heterogeneous nature of this disease has made the clinical treatment difficult. Using Patient Derived Xenograft (PDX) models of untreated GBM patients we have generated a model (and evaluated the efficacy of various compounds for GBM therapeutic treatments. We also generated a 3D GBM culture system for screening potential drug compounds for personalized clinical applications. The 3D GBM culture system of patient's survival model was constructed and validated on independent data sets that demonstrated an excellent prediction of GBM patients survival (log-rank test: p = 0.0001). Using a multivariate Cox proportional hazards model we identified that our 3D GBM model is distinct from other known GBM criteria (age at diagnosis, extent of surgical resection, post-operative Karnofsky Performance (KPS) score, treatment with temozolomide (TMZ) chemoradiation, and methylation of the MGMT gene). A five -fold cross-validation of our PDX model generation procedure confirmed validation of our 3D GBM model system. The model was further validated on an independent set of compounds such as CEP5003 that is capable of crossing the blood brain barrier and selectively targeting GBM Cancer Stem Cell (CSCs). Our data demonstrate that CEP5003 selectively targets GBM CSCs and we hypothesize that the clinical use of this class of compounds will improve quality of life and overall survival of GBM patients.

Methods:

The tumor samples obtained after surgery or biopsy, were placed immediately in Celprogen Tumor Transportation Media M36104-41S and shipped at 4-8˚C for processing. Tissues were washed with 1XPBS solution and aseptically cut into 0.5mm sections and cultured in 6 well tissue culture plates with an insert pre-coated with ECM E36104-41-3D. All cancer cell types remain viable and maintain their native architecture for at least 14 days and incorporated DNA measured by adding EdU (5-ethynyl-2'-deoxyuridine) to the culture. The efficacy of various therapeutic agents targeting major pathways (wnt, Notch, PI3K, MAPK, STAT) and chemotherapy agents were tested using DNA uptake and TUNNEL assay anti-cancer agents was calculated according to the inhibition index. The same compounds were tested for utilizing the patient's GBM Cancer Stem Cell Cultures established with Celprogen's Media M36104-41S and ECM E36104-41-T25. Expression of CD133, SHH, CD44, ALDH, CD90, OCT4, Ki67, P53, GFAP, O4, SSEA 3 / 4, and BRAF were quantified by qPCR or immunohistochemistry per cell culture.

In-vitro study: GBM CSCs and GBM tumor cells (parental) were isolated from 10 terminal donor patients that had under gone chemotherapy and gamma radiation treatments. The ages ranged from 35 years old to 65 years old, including both genders. The tissues were consented and obtain under IRB and HIPPA regulations and guidelines. The tissues were transported from the surgical suites to Celprogen in Human GBM CSC complete growth media (Cat# M36104-41S) within 24 hours after it had been surgically removed from the patients. Upon receipt of the tissue the tissue was sectioned into three equal sections and one section was processed into monolayer cell cultures selected for GBM CSC by Flow cytometer. The other section was maintained as the heterogeneous tumor population and cultured as parental cell culture. The GBM CSC section was processed further and isolated with CSC biomarkers, in Celprogen Media (M36115-42S) and ECM (E36115-42-T25) combination and further confirmed by Flow Cytometer. Once the monolayer cell cultures were established within 7-14 days; cells were characterized by Flow, IHC, Western Blot and Real Time PCR. Both the parental and the Human Pancreatic CSCs were check for tumorigenicity by injecting 1000 cells subcutaneously in SCID mice. Once the cells were characterized they were seeded at 10,000 cells per well in a 96 well format, pre-coated with Celprogen ECM (E36104-41-96Well) and cultured in complete growth media. The drugs were tested by incubating at various concentrations for 72 hours at 5% carbon dioxide and oxygen concentration, humidified 37˚C incubator. The cells were monitored by IncuCyte Zoom ESSEN Bioscience Real time imager, with an hour scan intervals continuously for the entire 72 hours. We utilized YoYo Red and YoYo Green viability dyes from Promega. CEP5003 was developed at Celprogen Inc. and are highly potent inhibitors for human GBM CSC. CEP5003 is currently in phase I clinical development for human GBM cancer patients. The IC50 curves were generated for the test compounds CEP5003.

In-vivo study: One thousand viable human GBM CSCs and parental cells were subcutaneously injected at the hind limb of SCID mice. After 10 days post injection, animals bearing established tumors (200-300 mm3) were divided and injected with test compound CEP5003 and carrier vehicle (control). The treatment was provided for the experimental group that received IP injections three times per week for a period of four weeks and followed for 4 weeks observation period . Each week the tumor growth and body weight measurements were performed and tabulated at regular intervals. At the end of the 8 weeks the mice were sacrificed and the tumor tissues were sectioned into three compartment; 1. One section was fixed and H&E stained, 2. One section was cultured into monolayer and IHC studies and flow studies with various Stem cell markers was performed, 3. One section was stored liquid nitrogen for genomic DNA and total RNA analysis for Real-time PCR.

Results:

The results are indicated in the figures and graphs below:

Figure 1: Model for Human GBM CSC. Human GBM Cancer stem cells were inoculated subcutaneously (1000 cells/mouse).10 days post injection blood samples were obtained from animals 200 -300 mm^3 sized tumors for PK/PD and ex-vivo Biochemical/IHC analysis.

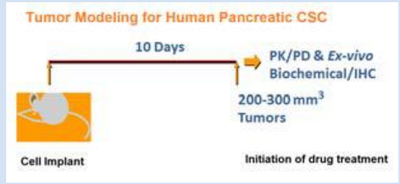


Figure 2. A. SCID mice injected with 1000 CD44+CD133+OCT4+ cells and 1000 CD44-CD133-OCT4- cells. Tumor formation was observed with positive markers within 20 days after subcutaneous injections. No visible tumor was observed with negative cells within the 20 day time frame. **B.** Human GBM CSC stained positive for CD133 marker from the tumor section.

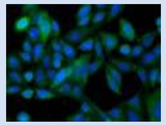


Table 1. Cancer Stem Cell general characterization Markers

Cancer Stem Cell Markers	Commonly Expressed Markers in Cell Culture
CD133	☑
Ability to for tumors <1000 cells in mice	☑
Telomerase	☑
SSEA ¾	☑
OCT-4	☑
SOX2	☑
CD44	☑

Table 2. Positive Cells Markers for Human GBM Parental Cancer cells and Cancer Stem Cells.

Parental Cancer	Cancer Stem Cells
CD133 (3-5%)	CD133, CD44, SSEA ¾ OCT4
Ability to for tumors <6000 cells in mice	Ability to for tumors <1000 cells in mice
Alkaline Phosphatase	Alkaline Phosphatase
Aldehyde Dehydrogenase	Aldehyde Dehydrogenase
Keratin	Telomerase
BRAF	Nestin, BRAF
Ki67	Ki67
GFAP	GFAP, O4
CD90	CD90, PDFR

Figure 3: Comparison of cell death of treated with untreated GBM Cancer by TUNEL assay

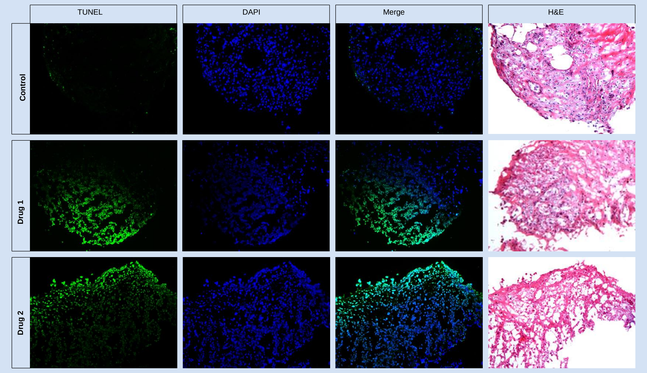


Figure 4: Survival curve of GBM PDX mice treated with CEP5003 and relative percent change in body weight

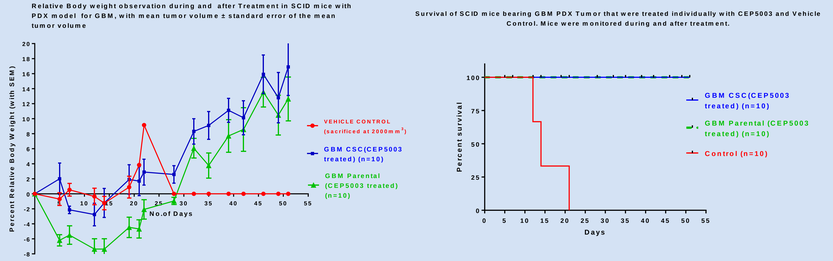
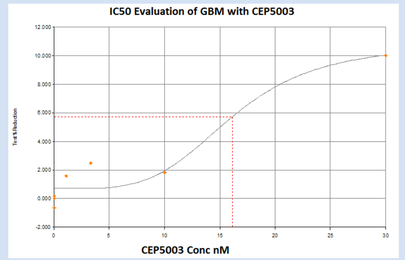
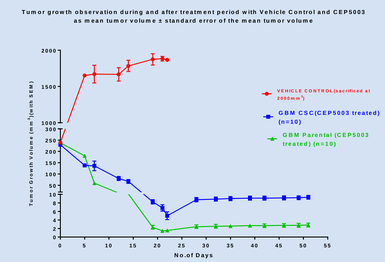


Figure 5: IC50 evaluation of GBM Cancer Stem Cells with CEP5003 and Tumor growth curves GBM PDX model





Conclusions:

The CEP5003 is a novel compound that is selective in targeting GBM CSCs. This molecule is able to transverse the blood brain barrier (BBB) and reach the target site. The ability of this molecule to cross the BBB and being highly selective to GBM CSCs makes it a potential drug candidate for treatment approaches for GBM advance stage cancer patients. We also have an orthotropic GBM PDX model where we have observed that CEP5003 was able to reduce the tumor size 80-90% from the original tumor mass. At present we are investigating through collaborations how to move this potential molecule faster to clinic.

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