ER-β Agonist as a Cure for High-Grade Gliomas

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Introduction

The most aggressive, treatment-resistant central nervous system (CNS) malignancy are high-grade gliomas (Grade III, IV); maintaining the worst prognosis of all intracranial tumors with a mean survival of 15 months and 5-year survival rate of <5% [1-5]. Even though over 50% of patients with high-grade gliomas receiving standard-of-care (SOC) treatment regimens experience virtually no improvement, the SOC has not been altered or enhanced in over a decade[6]. In animal models, estrogen (17β-estradiol; E2), a steroidal sex hormone, has been shown to suppress glioma ontogenesis by binding Estrogen Receptor alpha (ERα), beta (ERβ), and the G-protein Coupled Estrogen Receptor (GPER)[10,11]. Despite therapeutic efficacy in various malignancies, estrogen therapies increase the risk of the development of other diseases overtime and non-specifically bind and agonize ERα, causing additional adverse side effects[10,12–18]. ERβ is understood to have tumor-suppressive effects in glioblastoma, as increased expression correlates with better prognosis and less-aggressive tumor growth[10,19,20].

We recently published that a novel, potent and selective ERβ agonist, *ent*-28: a) reduces cell proliferation at a 50-fold lower concentration and b) induces apoptosis at a 7-fold lower concentration when compared to TMZ in GBM cell lines[26]. We have demonstrated that as a selective ERβ agonist, ent-28 suppresses tumor growth and induces apoptosis of tumor cells derived from glioma patients in vitro[26]. In addition, our preliminary data demonstrate that *ent*-28 attenuates growth of patient-derived xenografts (PDX) and has no adverse side effects in immune-competent mice at acute and chronic dosing schedules. Thus, the specificity of *ent*-28 for ERβ over ERα will allow us to minimize side-effects of non-selective ER-targeted therapy and maximize therapeutic benefit[32].

Methodology

Patient-derived cell lines. Freshly resected tumors from glioma patients enrolled in an ongoing prospective, multi-institutional Alliance Network clinical trial (Study Chair: Dr. Arti Gaur) were used to generate tumor-derived stem cells and adherent glioma cell lines.

Preparation of ent-28. ERβ agonist, ent-28, was designed by and made in the Micalizio laboratory. We used 90% propylene glycol (PG) to dissolve the powdered compound into solution. Compound formulations were calculated using the average body weight of the treatment mice to create a 50mg/kg (mpk) dose.

Antitumor activity of ent-28 on patient-derived xenografts. Flank tumors were established in immune compromised NOD-scid IL2rγnull (NSG) mice (n=13) by subcutaneous injection of 1 x 106 patient cells in 200uL of DMEM. When tumors were palpable, mice were either treated via oral gavage 3 times per week with 100uL of 50mg/kg (mpk) ent-28 diluted in 90%PG (n=10) or with 100uL of 90% PG alone (n=3). Length and width of tumors were measured twice a week with a caliper (Mitutoyo, Kanagawa, Japan) and tumor volumes were calculated using the formula: (Length) x (Width)2 / 2. We conducted a log-rank test to calculate the p-value and hazard ratio for the Kaplan-Meier analysis. To establish orthotopic patient derived xenografts 500,000 glioma cells were stereotactically injected into the dorsal striatum of male NSG mice (n=20). 2 days after stereotactic injections, tumors were evaluated through 3T gadolinium contrast-enhanced MR imaging and mice were randomized into treatment groups. Mice were oral gavaged with either 90% propylene glycol (PG) (n=10) or 50 mg/kg ent-28 (n=10) 3 times/week and MR imaged weekly. We conducted survival analysis using the log-rank test to calculate significance.

Single-dose pharmacokinetic study. Male CD-1 mice (n=24) were oral gavaged with 50mg/kg ent-28 and tissues and plasma were harvested at 6 different time points (n=3 per timepoint). The tissues collected: kidney, spleen, liver, lung, heart, small intestine, pons/medulla, midbrain, olfactory bulb, hypothalamus, cerebellum, and cortex. The timepoints included: 0 hour, 15-minutes, 30-minutes, 1-hour, 6-hour, 24-hour, and 48-hour. The samples were distributed to our collaborator, Dr. Thomas Burris, Washington University, St. Louis, and LC-MS-MS analysis was conducted onsite.

Immunohistochemistry. Organs from ent-28-treated (n=3) and untreated (n=3) mice were fixed in 10% formalin for 24 hours, rehydrated in 70% ethanol, and paraffin-embedded. The images were quantified using the average of three independent tumor core and edge regions using QuPath.

Clinical data collection and analysis. Patient data was collected manually from the electronic medical record system utilized at DHMC, EPIC. Analysis is ongoing and is anticipated to incorporate multivariate ANCOVA.

Ent-28 is a Highly Selective ER-β Agonist and crosses the blood-brain barrier

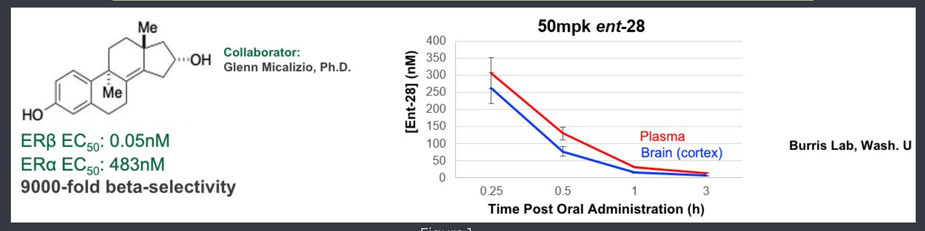

Figure 1

High dose, chronic treatment of immune competent mice with *ent*-28 is not toxic

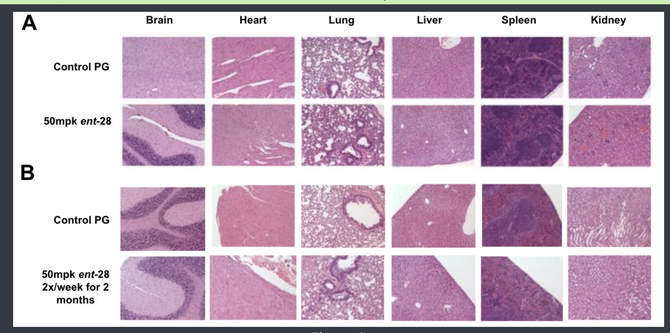

Figure 2

Ent-28 promotes survival and attenuates tumor growth

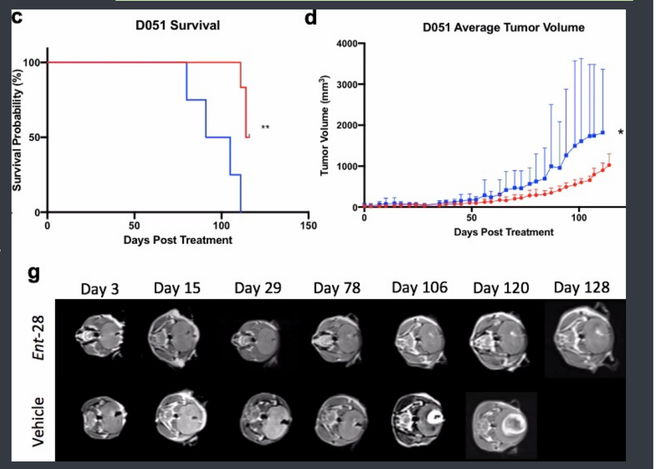

Figure 3

Analysis of variables in the clinical(patient) data

Patient ID	Sex	Diagnosis	WHO Grade	Tumor location	Cellularity	Pleiomorphism	Endothelial proliferation	Necrosis	GFAP	Ki67	ATRX	BRAF	IDH	P-53	MIB-1	1p/19q Codeletion	MGMT Methylation	Blood Type	RH Status
I	M	GBM	4	R Temporal					+		WT		WT	30% of cells	80%				
II	F	AA	3	L Frontal	Mod-High	+	-	-	+	20% +	M		M	Strong, diffuse			-	A	-
III	M	DA	2	L Occipital	Moderate	+	-	-	+	5% +	M	-	M	30%	5%	-	-	O	-
IV	M	GBM	4	R Frontal	Moderate	moderate	+	-	+	5% +	M	-	M		3-4%		-		
V	M	GBM	4	L Parietal	Mod-High	moderate	+	+	+	15% +	WT	+	WT	-	10%	-	-	O	+
VI	F	GBM	4	R Parietal	Increased			+	+	30% +	WT		WT	>70% of cells		-	-	A	+
VII	M	GBM	4	R Sup. Temporal	High	+	+	+	+	30% +	WT	+	WT		30%	-	-	A	+
VIII	M	GBM	4	R Insular Mass	High	High	+	+	+	30% +	WT		WT	+				A	-
IX	M	AA	3	L Temporal	Moderate	moderate	-	-	+	7% +	M		M	+					
X	M	AO	3	R Frontal	High	+	+	-		25% +	WT		M			+	+		

GBM: Glioblastoma; AA: Anaplastic Astrocytoma; DA: Diffuse Astrocytoma; AO: Anaplastic Oligodendroglioma; WT: Wildtype; M: Mutant

Table 1

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Results

- **ENT-28 INHIBITS** HETEROGENOUS PATIENT-DERIVED **GLIOMA CELL LINES** WITH **NO EFFECT ON HEALTHY CELLS**[FIGURE 1]
- HIGH DOSE, CHRONIC TREATMENT OF IMMUNE COMPETENT MICE WITH **ENT-28 IS NON-TOXIC** [FIGURE 2]
- **ENT-28 CROSSES THE BBB, PROMOTES SURVIVAL, AND ATTENUATES TUMOR GROWTH** IN PATIENT CELL LINE-DERIVED XENOGRAFTS [FIGURE 3] **REGARDLESS OF TUMOR GRADE, MUTATIONAL STATUS, SEX, CELLULAR AND/OR MOLECULAR HETEROGENEITY** [TABLE 1]

Conclusion

We have demonstrated that *ent-28 has anti-tumor activity in patient-derived glioma cells in vitro and in vivo regardless of tumor grade and mutation status. Additionally, ent-28 crosses the blood brain barrier with 85% penetrance in vivo.Ent-28 promotes survival and attenuates tumor growth five patient cell line-derived orthotopic xenografts. Furthermore, irrespective of tumor grade, mutational status, sex, cellular and/or molecular heterogeneity, Ent-28 attenuates glioma patient-derived tumor cells.*

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