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 <15%.1,2 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 in in animal models, estrogen (TGF-α, E2), a steroid sex hormone, has been shown to suppress glioma growth by binding Estrogen Receptor alpha (ERα), beta (ERβ), and the G-protein Coupled Estrogen Receptor (GPER)1,2. Despite therapeutic efficacy in various malignancies, estrogen therapies increase the risk of the development of other diseases over time and non-specifically bind and agonize ERs, causing additional adverse side effects.1,2 ERβ is understood to have tumor suppressive effects in glioblastoma, as increased expression correlates with better prognosis and less-aggressive tumor growth.1,2

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. We have demonstrated that as a select ERagonist, ent-28 suppresses tumor growth and induces apoptosis of tumor cells derived from glioma patients in vitro.3 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 benefits.3

Methodology

Patient-derived cell lines

Freshly resorted 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

Ent-28 agonist, ent-28, was designed by and made in the Micalizzi laboratory. We used 90% propylene glycol (PG) to dissolve the powdered compound into solution. Compound formulation calculations 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 IL2rnull (NSC) mice (n=18) 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 10%PG and 90% PG alone (n=5). 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 Width2 / 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 stratum of male NSC mice (n=28). 2 days after stereotactic injections, tumors were evaluated through 3T gadolinium contrast-enhanced MR imaging and mice were randomized into treatment groups. Mice were orally gavaged with either 90% propylene glycol (PG) (n=10) or 50mg/kg ent-28 (n=18) 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 orally 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/mesulata, 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 Burns, Washington University, St. Louis, and LC-MS-MS analysis was conducted onsite.

Immunohistochemistry

Organs from ent-28-treated mice (n=3) and untreated mice (n=3) 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.

References