Despite progress in diagnostics and medicine, diffuse astrocytomas pose a significant clinical challenge due to their infiltrative character \(^1\). Therefore, resection of the tumour mass solves the problem only partially as the infiltrated tumour residue is considered the source of regrowth and patient’s relapse \(^2\). This is complicated by the fact that at a cellular level astrocytoma is very heterogeneous and can contain genomically and proteomically diverse populations of immature and aggressive Brain Tumour Initiating Cells (BTICs) that can contribute to drug resistance and therapy evasion \(^3\). To control recurrence and improve patients’ prognosis and survival, identification and targeting of BTIC populations is a high priority goal.

The World Health Organization has divided diffuse astrocytomas into two classes, IDH1/2 (Isocitrate dehydrogenase 1 and 2) wildtype and IDH1/2 R132H mutant tumours \(^4\). IDH1/2 status is an established prognostic marker for glial tumours, with the IDH1/2 mutations warranting better prognosis as well as more therapy options and better drug response \(^5,6\). In opposition, tumours identified as IDH1/2 wildtype tend to recur as high-grade gliomas and the therapy options are limited \(^5,6\). This proposal aims to compare the levels/presence and genomic/proteomic characteristics of BTICs in these subtypes toward the goal of revealing novel potential therapeutic strategies for the treatment of this aggressive disease.

**Hypothesis:** Targeting the unique differences in IDH1/2 wild-type BTICs will present novel therapeutic targets both for advanced glioma (including glioblastoma multiforme) and in preventing the progression of early-stage diffuse astrocytoma.

**Aims:**

1. To conduct a genomic and proteomic comparison between BTIC populations isolated from low grade primary IDH1/2 wildtype or IDH1/2 mutant astrocytoma patient vs. those collected from recurrent (typically high grade) astrocytoma patient samples.
2. To screen the response of the BTIC population on chemotherapy *in vitro* and compare to genomic/proteomic properties.
3. To determine tumourigenic potential of the BTIC population *in vivo* and compare to genomic/proteomic properties.

**Aim 1.** In collaboration with the Henry Ford Health System (HFHS), MI (USA), we will establish a panel of patient derived, primary and recurrent pair-matched BTIC populations from patients with low and high grade diffuse astrocytoma as we have conducted for glioblastoma \(^11\). BTICs will be identified and selected for, using well-established surface antigens/markers such as CD133, CD44 and CD15, linked to enhanced brain tumour aggressiveness and therapy resistance \(^7,8\). Such approach provides marker enriched cultures of live BTICs which can be further studied in functional, *in vitro*, and tumourigenic, *in vivo*, assays. Patient clinical data and follow up will be made available to us by the HFHS.

Whole exome and RNA-sequencing will be performed on the isolated BTIC population in collaboration with Eran Andrechek (MSU) and quantitative proteomics will be conducted in collaboration with Dr. O.Vactratsis (UWindsor). Genomic and proteomic changes between the BTIC population and whole tumour will be determined. This analysis will determine differences in stemness and differentiation marker expression as well as IDH1/2 status. This aim will characterize the differences between the BTIC population of
primary and recurrent astrocytoma as well as generating a bank of patient BTICs which will serve as a platform for this proposal.

**Aim 2.** The optimal management of patients with diffuse astrocytoma, after tumour resection, poses a significant challenge due to the high incidence of relapse and high-grade gliomas being the most frequently recurring tumours in those patients $^9,^{10}$. The more aggressive nature of the newly arising tumours, within a relatively short period of time and under the burden of chemotherapy, suggests apparent selection of highly immature populations of BTICs and their clonal expansion as a secondary tumour mass. To determine the potential of the BTIC populations of evading therapy, we will perform a resistant BTIC enrichment assay, utilizing selected drug treatments. The primary tumour lines will be subjected to long-term treatments with therapeutic doses of standard of care used by HFHS, upon tumour resection, along with vehicle controls. Surviving populations will be tested, over the time-course of the 4-week treatment for any occurring changes to the CD133+, CD44+ or CD15+ cell population content, using FACS, which will also allow for subsequent cell sorting. We will determine essential characteristics of surviving sorted populations at each time point using assays proposed in Aim1 and compare them back to recurrent BTIC population genomic/proteomic data (Aim1).

**Aim3.** We will test whether resistant populations emerging from Aim 2 are capable of driving relapse in vivo by performing orthotopic xenografts into immunodeficient NOD-SCID mice. We will compare these to xenografts of populations prior to treatment. We hypothesize that treatment will select for a more aggressive population of cells. We will then compare resulting tumours using pathology as well as genomics and proteomics and determine differences from original tumour and isolated BTIC population.

**Predictions:** We predict that the BTIC population isolated from low-grade IDH wild-type patients will be more similar to those found in high-grade/relapsed disease in both gene/protein characterization, response to treatment and the characteristics of tumors formed in vivo. We predict that the gene/protein changes will represent novel therapeutic and/or diagnostic targets.

**Importance/Impact:** Dissecting the identity and characteristics of the cell population driving relapse and drug resistance in diffuse astrocytoma is of utmost importance to continuing advances to treat this aggressive disease and to prevent progression to aggressive high-grade glioma.

**References:**