**Background**

Diffuse low-grade astrocytomas (DA), a glioma subgroup, are slow-growing primary brain tumours (WHO Grade II) that arise predominantly in the cerebral hemisphere of young adults (30-40 years old) (1). Accounting for over 10% of all astrocytic tumours, DA is classified based on its cytologically atypical appearance (2) as they infiltrate the brain parenchyma and house reactive gliosis around their margins (3). Patients commonly present with symptoms such as changes in vision and sensation, as well as headaches and seizures (4). While treatment options vary depending on the patient, evidence suggests that early microsurgical resection of DA predicts a more favourable prognosis (5). Stratification of low-grade gliomas by The Cancer Genome Atlas (TCGA) has provided great insight into the molecular profile of DA and patterns of gene expression with prognostic or predictive significance. Many genes involved in the control of metabolism, proliferation and DNA regulation have been implicated in DA including IDH1/2 mutations, ATRX and TP53. (1,2,11,20). Although IDH1 mutations reveal a prognostically favourable subclassification of DA, they have been associated with resistance to apoptosis, increased proliferative and self-renewal capacity, immune suppression, gliomagenesis and a higher risk of MT, supporting the hypothesis that IDH1 mutations impact early glioma development and malignant progression (20-21). Inhibition of IDH1 has shown to be efficacious in impairing stem cell self-renewal and reduced tumorigenesis (22). Inhibitors of mutated IDH1 (IDH1-mt) thus pose as a possible therapeutic modality in DA for both treatment and as a preventative approach to MT. However, even more concerning is that current chemotherapeutics have been associated with inducing mutagenesis and subsequent MT (23-25). Current therapeutic landscapes have yet to assess markers of TMZ-induced mutagenesis on MT.

**Hypothesis**

We hypothesize that cells harboring IDH1-mt status have increased tumorigenic, proliferative, self-renewal and malignant transformation capacity. When used in combination with current treatment options, IDH1 inhibitors may provide a novel therapeutic modality and drive preventative treatment options for high-grade glioma transition for patients with diffuse astrocytoma. Furthermore, we hypothesize that induction of a high grade phenotype may elucidate novel cell signalling pathways and subsequent targeting of markers in malignant transformation.

**Specific Aims**

**Aim 1:** Functionally profile the effects of small molecule inhibitors against IDH1-mt through a multitude of in vitro stem cell assays.

**Aim 2:** Validate the inhibitor with the greatest in vitro efficacy in established mouse adapted patient-derived therapy models via inhibitory agent mono- and combinatorial therapy at various time points through tumour progression.

**Aim 3:** Perform genome wide CRISPR-barcoding screens to identify therapy-resistant genes of emerging clonal populations that drive malignant transformation for subsequent co-targeting with IDH1-mt.

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Methodologies

**Aim 1:** To study the functional effects of IDH1-mt reduction in diffuse astrocytoma, an initial dose response study will be conducted in multiple patient-derived lines. Once an effective therapeutic dose is established, a variety of in vitro assays will be performed including: (i) dose response curve with 2-fold dilution of inhibitor ranging from 20µM to 39nM to determine an IC50, or the concentration in which half the cells die; (ii) mixing experiment to validate that IDH1-mt inhibitors spare normal neural stem cells (NSCs), such that NSCs and luciferase-tagged patient-derived diffuse astrocytoma cells are treated with an inhibitor, at a concentration relative to tumour cells, mixed and evaluated based on luciferase levels; (iii) western immunoblotting to confirm a reduction in IDH1-mt; (iv) perform mass spectrometry to confirm reduction of onco-metabolic products of IDH1-mt; (v) secondary sphere formation and limiting dilution assays to identify the effects of small molecule inhibitors on self-renewal capacity/differentiation of treated cells; (vi) cell cycle analysis via flow cytometry to determine if inhibitor treated cells lose their uncontrolled proliferative potential and arrest in S phase.

**Aim 2:** We have uniquely modified the existing chemoradiotherapy protocol in DA patients for treatment of immune-deficient NOD-SCID mice engrafted with DA cells. With the identification of the most promising small molecule IDH1-mt inhibitor, we will further test its ability to reduce tumorigenic potential of DA cells in vivo. Intracranial injection of DA will be followed by an in vivo treatment regimen with a small molecule inhibitor targeting IDH1-mt. Vehicle alone (control arm), chemoradiotherapy (2 Gy cranial irradiation and 50mg/kg TMZ), IDH1-mt inhibitor alone, and chemoradiotherapy combined with IDH1-mt inhibitor. Tumour burden will be evaluated with magnetic resonance imaging (MRI) and immunohistochemistry (IHC) through collection of mice brains at various time points: (i) engraftment, (ii) post-radiation, (iii) post-chemotherapy, (iv) post-inhibitor treatment and (v) post-combinatorial therapy. Other organs will be collected for pharmacokinetic/pharmacodynamic (PK/PD) studies to investigate toxicity.

**Aim 3:** We will perform a genome-wide CRISPR-barcode screening to identify genes of clonal populations that drive malignant transformation for subsequent co-targeting with IDH1-mt. (i) Cells will be transduced with a single CRISPR-barcode lentiviral construct with a GFP-tag; (ii) GFP labelled DA cells will be treated with TMZ in vitro, to induce malignant transformation through conversion to Warburg phenotype, as indicated by the literature; (iii) perform CRISPR KO in order to identify dysregulated genes in clones driving this transformative process. The effects of these knockouts will be evaluated using established stem cell assays indicated in Aim 1, along with testing such samples through the in vivo protocol outlined in Aim 2 with greater emphasis on the combinatorial cohort. Genes from the screen will be co-targeted with IDH1-mt in subsequent in vivo trial.

Hypothesized Results

**Aim 1:** In vitro experiments will further elucidate IDH1-mt inhibitors to sufficiently protein levels of the target of preclinical models.

**Aim 2:** Combinatorial in vivo model with a IDH1-mt inhibitor holds greater potential for improved survival benefit and tumour burden reduction, as chemoradiotherapy will be able to work with a small molecule inhibitor.

**Aim 3:** Analysis of CRISPR-barcode screens on DA cells will lead to identification of emerging clonal populations in malignant transformation as well as dysregulated genes and pathways that govern this transformative process to develop a preventative treatment modality that can be used in combination with primary inhibitors.

Rationale for Proposed Research/ Therapeutic Relevance

The malignant transformation of low-grade diffuse astrocytomas into higher grade gliomas presents a major complications in patients’ mode of treatment and prognosis. However, by successfully inhibiting major therapeutic targets such as IDH1 mutants using small molecules, we can develop preventative measures against such invasive tumour progression. Our proposal aims to validate a novel therapeutic target, IDH1 mutants, whose inhibition would effectively reduce the tumorigenic properties of DA and halt their progression into higher grade gliomas. Even more, we aim to identify novel targets of treatment induced MT through the use of small molecule inhibitors along for optimal eradication of tumour. As there are no proven or effective preventative options for patients with malignant diffuse astrocytoma at risk of developing higher grade gliomas, our proposal is all the more relevant and helpful to the clinical population.
References


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