

Final Report

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Date: 02/02/2026

Title of Project: Sphingosine kinase 2 as a new therapeutic target in glioblastoma

Summary

Glioblastoma (GBM) is the most commonly diagnosed primary brain tumour in adults. It is a highly aggressive tumour with poor patient prognosis; median survival from diagnosis is less than 15 months and less than 5% survival after 5 years. Surgical resection is the first line treatment for most newly diagnosed glioblastoma patients, but complete removal of the tumour is usually impossible because of its highly invasive and proliferative nature. Surgery is followed by radiation with concurrent and adjuvant chemotherapy with temozolomide. Despite aggressive therapy, the disease invariably progresses or recurs as resistance to temozolomide develops. Thus, more effective therapies are desperately needed for GBM.

Sphingolipids have emerged in the last few decades as key regulators of many fundamental cell functions that contribute to cancer. Indeed, one of the key enzymes that controls sphingolipid metabolism, sphingosine kinase 2 (SK2), has been implicated as an attractive therapeutic target in several solid and blood cancers. Because sphingolipids are highly enriched in the brain, we have examined the potential for SK2 to be a therapeutic target in GBM. Genetic removal of SK2 in GBM cells dramatically reduces tumour growth in the brains of mice. Furthermore, GBM cells implanted into the brains of mice lacking SK2 also appear to generate less aggressive tumours. Collectively, this suggests that SK2 in both the tumour and surrounding brain supports GBM tumour growth.

Hypothesis vs Findings

Our overarching hypothesis is that sphingosine kinase 2 (SK2) is dysregulated in GBM which contributes to enhanced survival of GBM cells, thus targeting SK2 in GBM could be therapeutically beneficial. To this end, we have tested our hypothesis as follows:

- To confirm our previous findings using mouse-derived SK2 knockout GBM cell lines, we have now generated CRISPR/Cas9 knockout of SK2 in five low passage patient-derived glioma neural stem (GNS) cell lines, which encompass the three molecular GBM subtypes. Notably amongst this cohort we have two matched pairs of primary and recurrent GNS cell lines, enabling us to assess the effect of SK2 knockout in a recurrent GBM setting. We have shown that knockout of SK2 in GNS cells dramatically reduces their proliferation and neoplastic growth; consistent with the mouse GBM cell data. Further to this, our preliminary findings suggest that a knockout of SK2 in recurrent GBM leads to a reduced proliferation of these cells.
- We have gained access to two structurally diverse SK2 inhibitors of clinical relevance and have been testing these inhibitors in our matched primary and recurrent low-passage GNS lines. We were able to show that both inhibitors can successfully kill the primary GNS lines. Further to this we have examined whether SK2 inhibition can synergise with

the current standard of care GBM chemotherapy, temozolomide (TMZ). Two primary GNS cell lines, were treated with different combinations of the SK2 inhibitor and chemotherapeutic drug TMZ and our findings indicate that SK2 inhibitors synergise moderately with TMZ to kill both primary GBM cell lines. Excitingly we were also able to show that the two tested SK2 inhibitors were also effective at killing the recurrent GBM cells. Recurrent GBM is notoriously hard to treat, with no standard of care available, so this is a significant finding.

- We have generated orthotopic patient-derived xenografts (oPDX) mouse models of our matched primary and recurrent GNS cell lines. The next steps of our project are to engraft the CRISPR/Cas9 SK2 KO primary and recurrent GNS cells into the brain of immunodeficient mice and assess the effects of SK2 on the growth of the brain tumours in an *in vivo* setting.

Unanswered Questions

The *in vitro* and *in vivo* findings we have made so far strongly suggest that SK2 is an attractive therapeutic target for GBM. However, the mechanistic effects of targeting SK2 on GBM biology still require further investigation. To determine the mechanistic effects of targeting SK2 we would like to carry out single-cell RNA sequencing of our CRISPR/Cas9 SK2KO patient-derived GNS lines, to help determine the effects of SK2KO on the overall transcriptome.

What these research outcomes mean

Our data strongly demonstrates that SK2 drives GBM tumour growth. We have established that clinically relevant SK2 inhibitors can block the growth of patient-derived tumour cells alone but notably can also enhance the potency of TMZ therapy for GBM. Furthermore, we have shown that SK2 inhibition may be a viable therapeutic avenue for recurrent GBM, of which currently no standard of care therapy exists.

Finally, I would like to thank all the donors and the Brian Foundation for generously supporting this research.