Progress Report

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Title of Project: *The role of immune cells (microglia) and a cell membrane protein (P2X7 receptor) in human brain tumours – a bed (hospital) to bench side (laboratory) research study.*

Summary:
Human brain tumours (gliomas) generally afflict young individuals in their 30s-40s with significant implications for the person as well as their family / carers. Gliomas are characterized in 4 grades and the most severe form (grade IV; Glioblastoma; GBM) show very aggressive growth potential with capacity to divide and infiltrate the surrounding normal brain tissue. There are no cures for grade III and grade IV human brain tumours and the current conventional therapies only improve survival by a few months. Individuals suffering from glioblastoma (grade IV tumour) have a life expectancy of 9-18 months. Hence therapies that combat this devastating disease are desperately needed.

Gliomas (grade III and IV) are made up of highly dividing **tumour cells** as well as **microglia**. Microglia are the immune cells of the brain. In normal healthy brain microglia survey the surrounding brain tissue for signs of infection or inflammation. Our prior work has revealed that microglia are an intricate part of human gliomas and they exhibit an **activated** phenotype (appearance). As oppose to inactivated state, activated microglia are capable of releasing various biological factors that can affect the function of other neighboring microglia as well as tumour cells. In the setting of brain tumours the roles of microglia are not entirely clear? ***Its not known if microglial function halts or promotes tumour growth?***

We hypothesise that activated microglia can release various soluble factors (proteins) that can further **enhance the growth, proliferation and invasion of brain tumours**.

How microglia can alter tumour growth is unknown? Our preliminary results indicate that this effect is achieved through a key membrane protein, **P2X7 receptor (P2X7R).** P2X7R is expressed on the cell surface of microglia. Our previous work in rats has shown that stimulation of P2X7R on the surface of microglia can induce the **activation of microglia**. Once activated microglia can produce various factors which we propose to induce tumour growth and proliferation. P2X7R is over-expressed in a number of cancers including human gliomas and for the current study we would be studying its **function** in gliomas and ***seeing if P2X7R function can induce glioma growth and proliferation?***

For this study with ethics approval from Royal Melbourne Hospital, we will be obtaining human brain tumour specimens from individuals who are undergoing surgery as part of treatment of their brain cancer. The piece of brain tumour obtained at the operating theatre (headed by associate investigator Prof Kate Drummond) will be taken by CI (Dr Mastura Monif) to the laboratory so it can be dissociated and cultured for further examination in the laboratory. We would be looking at the cellular composition of the tumour (i.e., presence of tumour cells and presence of activated microglia) as well as expression of P2X7R (on the cell surface). Thereafter we will use specialized microscopy techniques to see if P2X7R is functional? By **inhibiting P2X7R function** with specific ***molecules, we aim to see if we can reduce microglial activation? Also with P2X7R inhibition we will try to see if the number of tumour cells are reduced?***

This project is very collaborative in nature and it brings together various health and academic centers in Victoria. We have extensive knowledge and expertise in culturing of human brain tumours and we would like to extend our previous findings to decipher if P2X7R function is crucial in glioma growth and proliferation? Our findings are hoped to lead to development of **anti- P2X7R therapies to combat the devastating condition of human glioma.**

Hypothesis vs Findings

We hypothesised that P2X7R is expressed in glioma cells as well as glioma associated microgila and that the receptor is functional.

- Our results indicated that the receptor is indeed expressed in human glioma cells
- It is also expressed in human tumour associated microglia
- Also in a U251 glioma cell line we were able to show that receptor is expressed and functional

We hypothesised that P2X7R inhibition would have an effect in tumour growth and proliferation

- *Our findings revealed that inhibition of P2X7R in a glioma cell line as well as human glioma cultures results in a reduction in tumour cell proliferation*
- *Interesting (and with huge excitement) we were able to show that P2X7R inhibition was even better than the conventional chemotherapy in reducing tumour cells number.*

Research papers thus far:

- Liyen Katrina Kana, David Williams, Kate Drummond, Terence O'Brien, **Mastura Monif**, The role of microglia and P2X7 receptors in gliomas, Journal of Neuroimmunology. Volume 332, 15 July 2019, Pages 138-146
  This review highlights the role of P2X7R and microglia in human brain cancer, summarising previously published literature and focusing on future directions for therapeutic interventions.

- Liyen Katrina Kana, David Williams, Kate Drummond, Terence O'Brien, **Mastura Monif**, P2X7R antagonism can reduce glioblastoma proliferation, Purinergic signalling, In submission (Sept 2

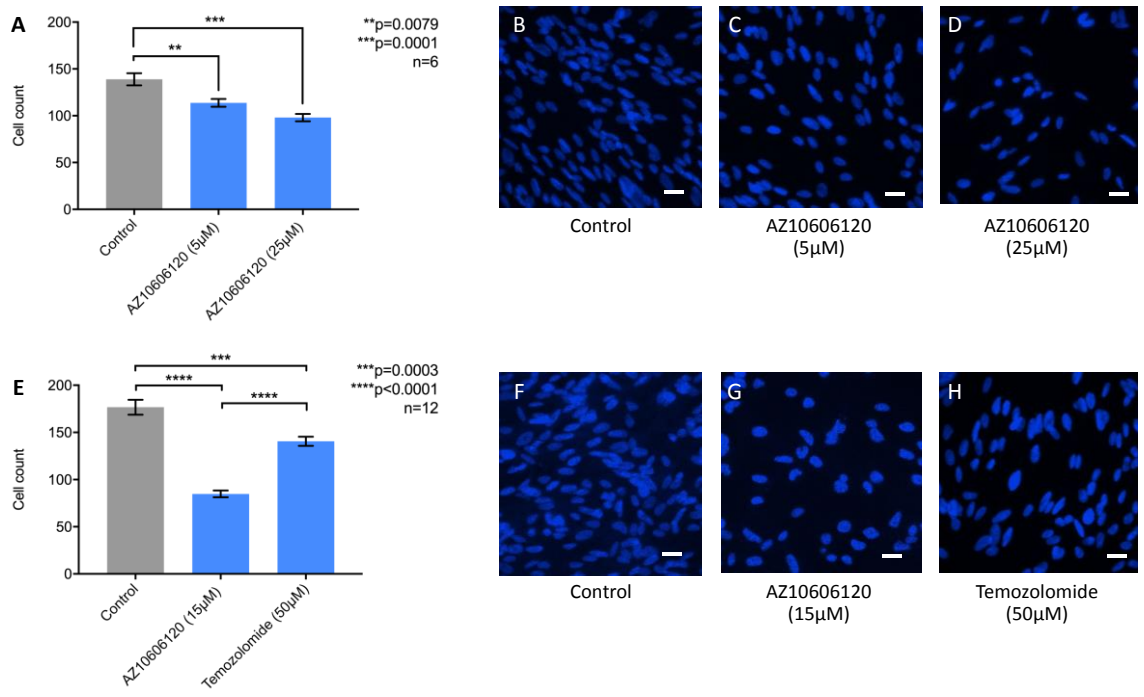

Figure 1 The P2X7 receptor (P2X7R) antagonist, AZ10606120, significantly inhibits tumour growth in U251 glioma cells and was more effective than the conventional chemotherapeutic agent, temozolomide.

(A) Manual cell quantification of U251 cells treated with AZ106 (5μM and 25μM) compared to control. A total of 16 fields were counted per treatment for n=6. Both concentrations of AZ106 significantly inhibited tumour growth, compared to the control; one-way ANOVA with Tukey's HSD, **P=0.0079 compared to control, ***P=0.0001 compared to control. Data are represented as mean ± SEM. Example U251 cell count images are provided for (B) control, (C) AZ10606120 5μM and (D) AZ10606120 25μM. (E) Manual cell quantification of U251 cells treated with AZ10605120 (15μM) and temozolomide (50μM) compared to control. A total of 16 fields were counted per treatment for n=12. Both AZ10606120 and temozolomide significantly inhibited tumour growth, compared to the control. Cell counts were also significantly lower in cells treated with 15μM AZ10606120 compared to temozolomide. One-way ANOVA with Tukey's HSD, ***P=0.0003, ****P<0.0001. Data are represented as mean ± SEM. Example U251 cell count images are provided for (F) control, (G) AZ10606120 15μM and (H) temozolomide 50μM. Scale bar: 5μm

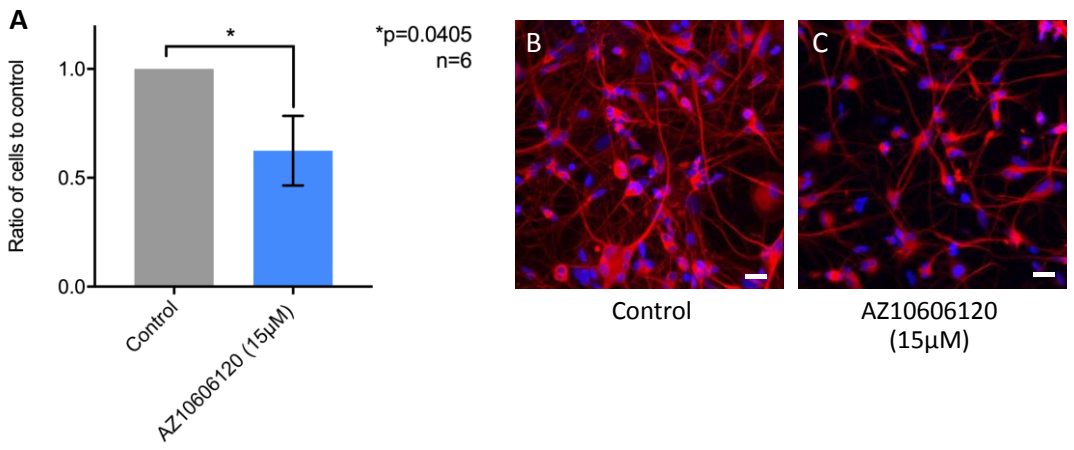

Figure 2 The P2X7 receptor (P2X7R) antagonist, AZ10606120 (AZ106), significantly inhibits tumour growth in human high-grade glioma samples.
(A) Manual cell quantification of human glioma samples treated with AZ10606120 (15μM), compared to control. Cells were stained with DAPI nuclear stain and fixed 72 hours post-treatment. Human tumour samples were pre-stained with glial fibrillary acidic protein (GFAP) for identification of tumour cells. A total of 16 fields were counted per treatment for 6 tumours. Cell counts were expressed as the ratio of treated cells to the control to account for any variability in initial culture cell numbers between tumours. AZ10606120 at 15μM significantly inhibited tumour proliferation, compared to the control; unpaired t test, *P=0.0405. Data are represented as mean ± SEM. Example cell count images are provided for (B) control and (C) AZ10606120 15μM. Scale bar:8μm

Unanswered Questions

- *Characterisation of the biomarkers in the setting of tumours treated and untreated with P2X7R inhibition*
- *Further characterization the interaction between microglia and tumour cells*

What these research outcomes mean

- *P2X7R is critical in glioblastoma proliferation*
- *Its inhibition could function as a therapeutic candidate in glioma eradication.*
- *Future studies (phase I and II) clinical trials as well as animla studies are warranted to assess this further*