Progress Report

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Date: 31 May 2017

Title of Project: Novel combination therapy for brain tumours

Summary:

In Australia, brain cancer is the leading cause of cancer-associated death for adults under the age of 40 and children under the age of 10, with one person dying every eight hours. The most common malignant primary brain tumour is glioblastoma. The current standard of care for glioblastoma patients is a combination of surgery, radiation and chemotherapy. This is the only therapeutic option, and one that is never curative. For patients receiving this treatment the 5-year survival rate is less than 5%, with the vast majority succumbing to the disease within two years. Thus, the need for new therapeutic strategies is urgent.

My team is developing a promising strategy involving degradation of the epidermal growth factor receptor (EGFR), which is amplified and mutated in 60% of glioblastomas. Inhibitors that block the kinase activity of the EGFR are approved for other cancers, but have failed in glioblastoma trials thus far. Yet recent evidence suggests that to target EGFR effectively it is necessary not only to block its kinase activity, but also to degrade the protein itself. A novel and unique way to do this is by inhibiting the dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) that regulates EGFR degradation.

In ***Aim 1*** we will determine the anti-cancer activity of the DYRK1A and EGFR inhibitors combination in patient-derived glioblastoma cells. We will correlate activity of the combined treatment with the mutational profile of the patient. This will identify which subgroup of patients could benefit most from our approach.

In ***Aim 2*** we will examine the anti-cancer activity of the DYRK1A and EGFR inhibitors combination in an animal model of glioblastoma. This will demonstrate the translational potential of this novel combination therapy.

In ***Aim 3*** we will examine expression of DYRK1A in microarrays of Australian glioblastoma tissue bank. Through an established international collaboration, we also have access to an outstanding resource of brain cancer tissue microarrays constructed from a library of >3,500 specimens. These microarrays will be used to correlate DYRK1A expression with EGFR expression, histopathological and patient survival data.

The outcomes of this first ever evaluation of a combination of DYRK1A and EGFR inhibitors will have profound implications for the design of efficacious, personalised therapy for glioblastoma. With this work we will also contribute to the understanding of resistance to EGFR inhibitors that has plagued glioblastoma trials to date. This project will open up the possibility of improving glioblastoma therapy with DYRK1A inhibitors that have already been identified and are now in clinical development. As a result, translation of the preclinical evidence generated by this project could be achieved in the near future.

Hypothesis vs Findings

To address the experiments outlined in Aim 1, we developed novel and better DYRK1A inhibitors with excellent cellular efficacy. The novel DYRK1A inhibitors were tested for anti-cancer efficacy in established and patient-derived glioblastoma cells representing four subtypes of glioblastoma tumours: classical, mesenchymal, neural and pro-neural. We determined that glioblastoma tumours of classical and mesenchymal subtypes are most sensitive to DYRK1A inhibitors. We also used novel DYRK1A inhibitors to perform EGFR degradation assays. We demonstrate for the first time that EGFR degradation can be induced with drugs inhibiting DYRK1A activity.

The DYRK1A inhibitor development and EGFR degradation studies have been recently published in the prestigious Journal of Medicinal Chemistry (IF 5.6, ranked #3 of 59 medicinal chemistry journals). Ms Athena Phoa, PhD student under my supervision working on EGFR degradation through DYRK1A inhibition (research partially funded by the Brain Foundation grant) received an international travel award from *Ligue contre le cancer* (France) to give an oral presentation at the "DYRK1A related kinases & human disease" conference in Saint-Malo, France. In June 2017, I will be presenting this work at the AACR-EACR-SIG Special Conference: The Challenges of Optimizing Immuno- and Targeted Therapies: From Cancer Biology to the Clinic (Florence, Italy).

Publication reference: Zhou R, Phoa AF, Abbassi R, Hoque M, Stringer BW, Day BW, Font P, Ryan RM, **Johns TG**, **Munoz L,** Kassiou M. Structural optimization and pharmacological evaluation of inhibitors targeting dual-specificity tyrosine phosphorylation-regulated kinases (DYRK) and CDC-like kinases (CLK) in glioblastoma. **Journal of Medicinal Chemistry** 60 (2017) 2052 - 2070

Unanswered Questions

We are currently

- Completing combination studies of DYRK1A and EGFR inhibitor in a panel of patient-derived glioblastoma cell lines (Aim 1)
- Performing pharmacokinetic and toxicity studies necessary to initiate testing of novel DYRK1A inhibitors in glioblastoma xenografts (Aim 2).

What these research outcomes mean

The over-expressed and mutated EGFR underlies proliferation and self-renewal capacity of glioblastoma cells, reinforcing that EGFR is a prime driver of glioblastomas. However, EGFR kinase inhibitors (e.g. lapatinib, dacomitinib) or EGFR antibodies (e.g. cetuximab, panitumumab) that have been approved for lung and colorectal cancers have not yet succeeded for glioblastoma therapy as single agents. One emerging explanation for this failure is the fact that these drugs do not down-regulate EGFR expression. Importantly, EGFR expressed at the cell surface, even if catalytically inactive, promotes survival of cancer cells. These studies suggest that successful anti-EGFR approach requires both inhibition of kinase activity and degradation of EGFR. To stimulate EGFR degradation is not a trivial task, because the majority of proteins regulating EGFR degradation are not druggable proteins. Therefore, our work showing that EGFR degradation and inhibition of EGFR non-kinase functions can be induced with DYRK1A inhibitors has significant implication for glioblastoma therapy.

Please include any appropriate photos or diagrams.

Attached to the email is the 1st publication stemming from this grant. The financial support from Brain Foundation is acknowledged at the end of the paper.