Final Report

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Title of Project: Oncogenic transformation of human neural stem cells into medulloblastoma-forming cells

Summary

Medulloblastoma is the most common malignant brain tumour in children and the most significant cause of childhood cancer-related death. If we are to improve the outcomes of children with these cancers it is imperative that we have a good understanding of what drives tumour growth, which will allow us to design better treatments.

We have known for a long time that some patients with medulloblastoma respond well to treatment, but others do not and are likely to die from their disease. In the past we haven't had enough information to understand why this is, but recent research has revealed that although medulloblastomas may look the same down a microscope, when examined more closely at the genetic level, they are not all the same and in fact there are at least four distinct subgroups of these tumours (Sonic Hedgehog/Shh, Wingless/Wnt, Group 3 and Group 4), each arising when certain subsets of genes malfunction in specific cells within the brain.

Previous laboratory studies have shown that mimicking the genetic changes of Group 3 medulloblastoma in mouse neural stem cells can cause these cells to behave like that specific medulloblastoma subtype, however this has never been shown in human cells. While the mouse model provides proof of principle of this concept, it is important that we create models using human neural stem cells as targeted therapies that will be moved forward to the clinic are raised against the human genetic targets. Whilst some homology exists between human and mouse genetics, we need to ensure that we have the best, most appropriate models when these models are used to test targeted agents in order to best predict outcomes in the clinic.

In this study, we aimed to transform normal human neural stem cells into cancer-causing cells by altering specific genes that are implicated in medulloblastoma. These genes were selected from a list of known mutated genes or genes found to be overexpressed in medulloblastoma, including ß-catenin, the catalytic subunit of PI3-

kinase (PIK3CA), C-MYC, N-MYC and GLI2. This was to be achieved by first making the cells "tumour-prone" via the use of a short-hairpin RNA to downregulate the tumour suppressor gene TP53.

During the process of preparing these constructs, we discovered that a research group led by Dr Charles Eberhart at The Johns Hopkins Hospital in Baltimore (Maryland, USA) was working on a similar approach to modelling medulloblastoma in the laboratory and had successfully created a Group 3 (MYC-driven) model of medulloblastoma. This model was achieved using human neural stem cells transformed using lentivirus coding for a dominant negative R248W p53 (similar in concept to our short-hairpin RNA approach), hTERT to immortalise the cells and c-MYC which is associated with Group 3 medulloblatoma. Additionally, they also transformed the cells with a constitutively active AKT which was found to decrease latency and increase penetrance of tumours. When implanted into mouse brains, these cells recapitulated Group 3 medulloblastoma histologically, pathologically and genetically.

We were excited to see that this as yet unpublished research demonstrated that our approach was sound, and saw this as a great opportunity to collaborate with like-minded international researchers. We proposed a collaboration using the Johns Hopkins hTERT and dominant negative p53 constructs in combination with the ß-catenin, PIK3CA, N-MYC and GLI2 constructs prepared in our laboratory, which Dr Eberhart enthusiastically agreed to.

Unfortunately due to strict regulatory issues within the Johns Hopkins Hospital, we experienced significant delays in obtaining the constructs and the cells, and have just received the transformed cells in May of this year. However, during this time in our laboratory, the coding region for each target gene (with the exception of GLI2 where we experiences technical difficulties) has been amplified and cloned into separate lentiviral constructs that co-express the gene luciferase to enable non-invasive monitoring of tumour growth using a Xenogen IVIS Spectrum. Each gene has also been cloned into lentiviral constructs that co-express the green fluorescent protein gene to allow both *in vitro* and *ex vivo* identification of tumour cells. We are currently in the process of optimising lentiviral production in order to ensure the best possible transduction rate of the human neural stem cells.

Once established, these cell lines will be implanted into the cerebellum of immunodeficient (nude) mice (10^6 cells per mouse) (Figure 1). Ten mice will be implanted per cell line. This number is based on our laboratory's previous experience with implantation of cells, and takes into account the risk that some cell lines do not have a 100% establishment rate (as low as 50% is expected in some cell lines based on published mouse modelling data).

Tumour growth in implanted mice will be monitored via measurement of luciferase activity using an IVIS Spectrum imager and traditional phenotypic monitoring. The endpoint of the study is tumour development, thus time to morbidity and Kaplan-Meier analyses will be used to assess the outcome. Morbidity will be assessed based on weight loss, ataxia and/or doming of the head. At morbidity mice will be sacrificed and brains harvested for histological analysis. Comparisons of survival will be made between each oncogene and its corresponding control cell line using a log-rank test.

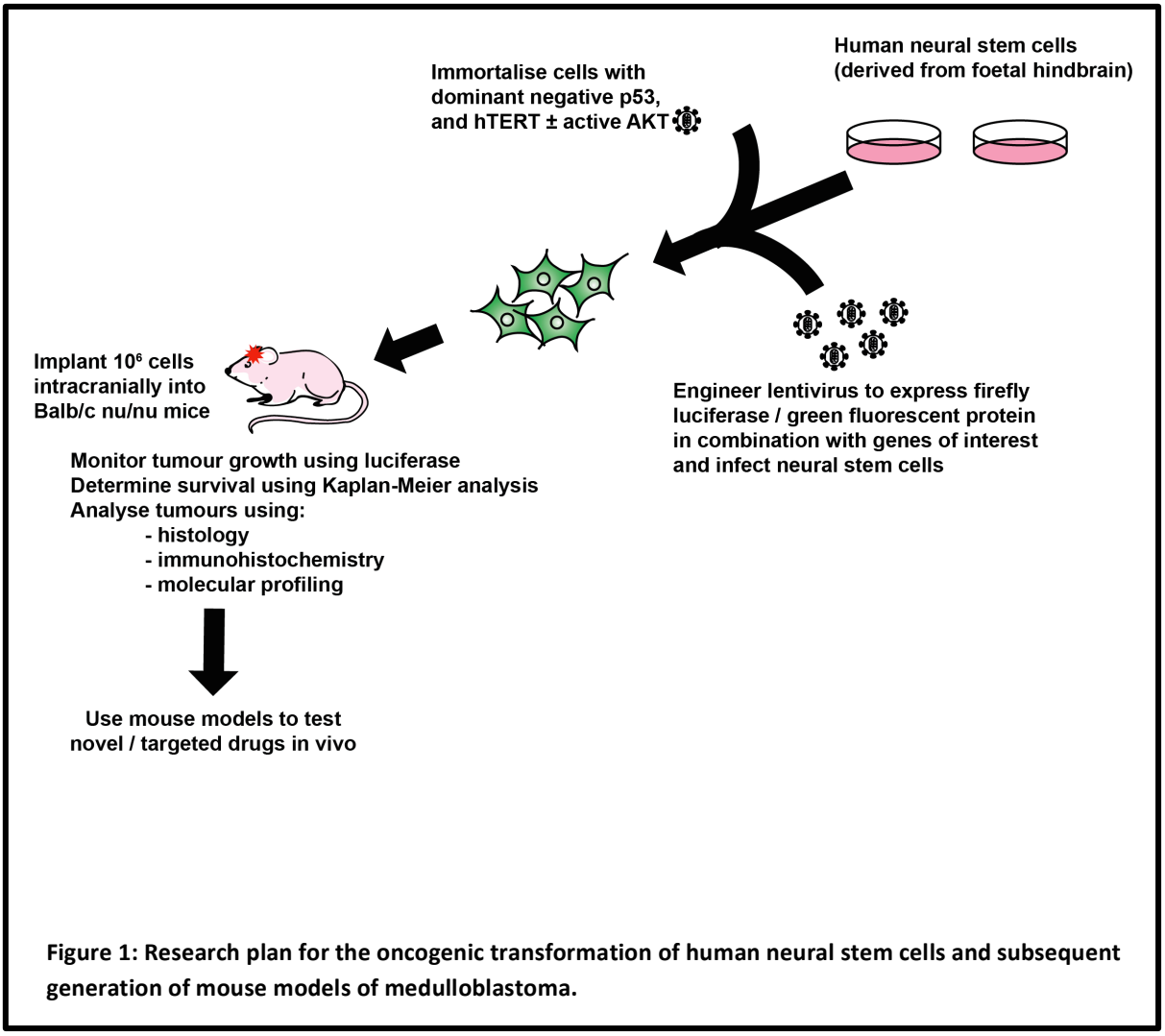

Figure 1: Research plan for the oncogenic transformation of human neural stem cells and subsequent generation of mouse models of medulloblastoma.

Tumour tissue will be subjected to routine histopathological analyses to examine the types of tumours formed and confirm the expression of each oncogene. Furthermore, DNA and RNA will be harvested from resulting tumours to enable molecular profiling to determine if the tumours match the molecular characteristics of the human disease.

Hypothesis vs Findings

We hypothesised that genes frequently altered in human medulloblastoma contribute to the initiation and progression of this disease. Moreover, based on evidence from mouse models, we hypothesised that normal human neural stem cells are the cell of origin for this disease. Thus, we proposed that the introduction of genes/mutations recurrently found in human medulloblastoma to normal human neural stem cells will recapitulate tumorigenesis upon intracranial implantation into mouse brains.

Based on the findings of our collaborators at Johns Hopkins Hospital, our hypothesis was correct in the case of C-MYC overexpression in a Group 3 model of

medulloblastoma using human neural stem cells. We are currently in the process of determining if this is also the case using our genes of interest in the remaining three subtypes of medulloblastoma in our laboratory.

Unanswered Questions

Whilst a Group 3 mouse model of medulloblastoma has been established and characterised, we are yet to determine if our selected genes of interest will faithfully replicate Shh, Wnt or Group 4 medulloblastoma.

What these research outcomes mean

Medulloblastoma comprises 20% of childhood cancer, yet is the major cause of childhood cancer-related morbidity.

This project provides key mechanistic insight into the pathobiology of this disease and in the case of the Group 3 model has demonstrated that the candidate oncogenes investigated are directly involved in the transformation of normal human neural stem cells into cancerous cells. Furthermore, in combination with the results of our work with the remaining subgroups, this research will determine the role of the human neural stem cell as a cell of origin for medulloblastoma. This improved understanding of the underlying causes of tumour development will facilitate a more rational design of effective clinical treatments aimed at personalising cancer therapy by targeting specific cell types and gene mutations (Figure 2).

The new murine models of medulloblastoma generated by this project that exclusively overexpress the identified oncogenes will be an invaluable resource for the preclinical testing of novel targeted treatments. This essential information is required prior to translating this research to human patients and initiating clinical trials.

The data obtained from this small grant in collaboration with Dr Eberhart has been used in an application for further funding through the NHMRC to investigate the efficacy of drugs targeted to these oncogenes and/or their products. The production of new medulloblastoma models that complement our existing animal models will provide unique resources with which to implement a larger and more translational approach to our medulloblastoma research program. In combination with our ongoing collaborations with leaders in the field, these models will place us in an internationally unique and highly competitive position that will significantly strengthen future funding applications.

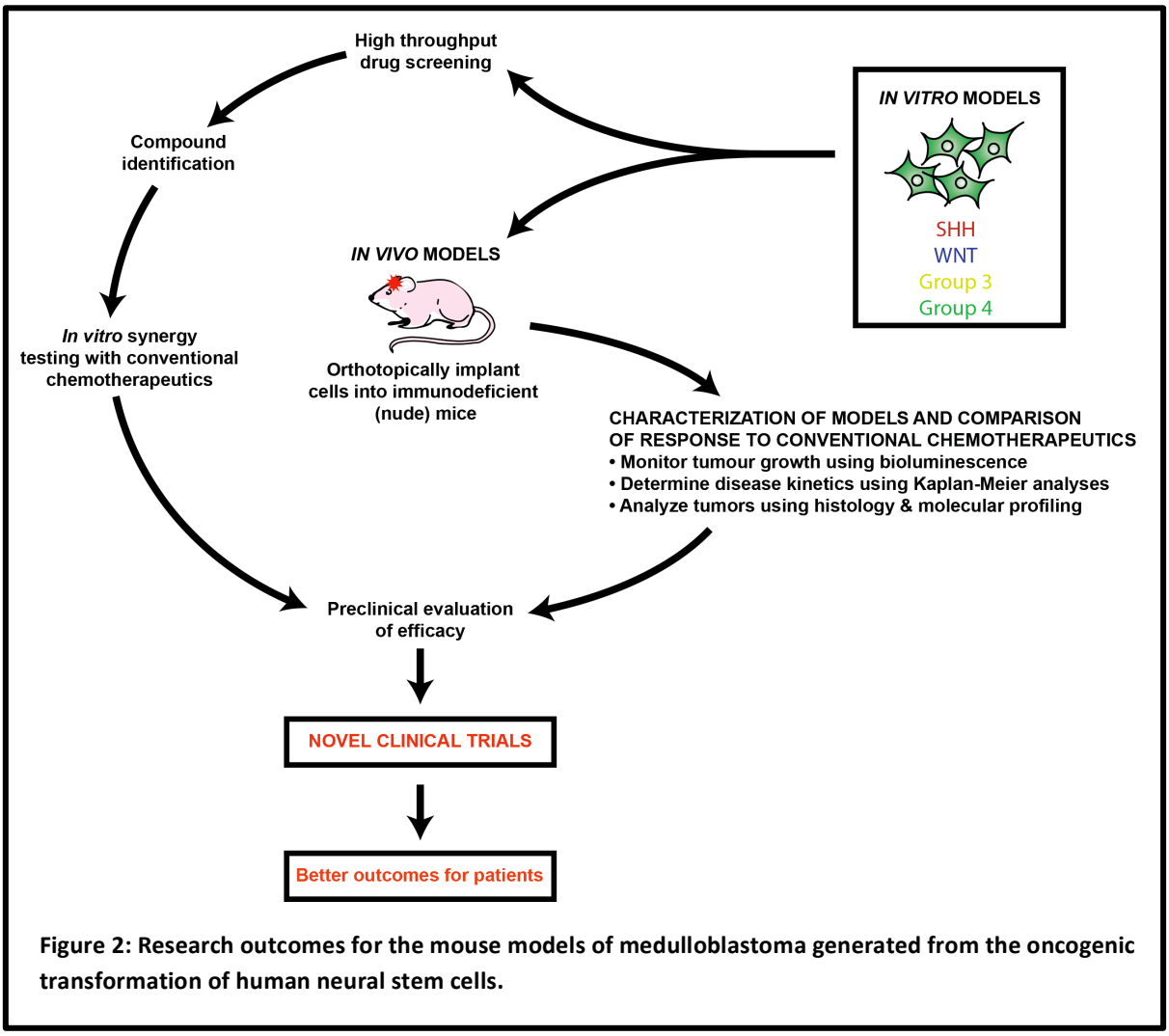

Figure 2: Research outcomes for the mouse models of medulloblastoma generated from the oncogenic transformation of human neural stem cells.