EXECUTIVE SUMMARY

Targeting hypoxia and mitochondrial metabolism with repurposing drugs as an approach of radiosensitization for diffuse intrinsic pontine glioma

Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating, aggressive childhood brain tumor arising in the ventral pons, comprising approximately 10-15% of pediatric brain tumors [6]. Half of all malignant pediatric gliomas occur in the brainstem, with DIPG being the most common tumor subtype in this anatomical region, constituting 80% of brainstem gliomas overall [7]. With an estimated 200-300 children affected by DIPG annually in the United States and 20-30 children in Australia, it is the second most common malignant brain tumor of childhood. In the absence of effective therapies, the prognosis of DIPG is bleak. The median age at diagnosis is 6-9 years, with median survival of 9 months; 90% of children will die from the disease within 2 years of initial diagnosis, with less than 1% surviving after 5 years [8]. To date, RT is the only form of treatment that offers a transient benet in DIPG. Unfortunately, almost all DIPGs recur locally within 12 months secondary to radioresistance. Therefore, improving the effect of RT remains the most promising avenue to better outcomes in children with DIPGs.

Hypoxia is a common microenvironmental feature of solid tumors [9] that exists because the supply of oxygen is insufcient to meet the metabolic demand of the tumor [10]. Recent reports showed that DIPGs are hypoperfused compared to surrounding brain tissue, suggesting that the tumor cells are exposed to a hypoxic microenvironment [2]. This stimulus may induce widespread transcriptional changes through activation of hypoxia inducible factor (HIF), which have been associated with invasion, metastasis, angiogenesis, and resistance to therapeutics. Hypoxia has been recognized as a barrier to effective RT long time ago [11], because molecular oxygen (O) is an electrophile that enhances the efcacy of RT by xing radiation-induced DNA damage. It has also been reported that drugs that target metabolic diseases are clinically important in the treatment of cancer. Biguanide (metformin/phenformin) is a class of hypoglycemic agent currently used in clinic that can also modify tumor mitochondrial metabolism to reduce oxygen consumption rate. By targeting mitochondrial complex I, biguanide reduces oxygen consumption rate of tumor cells, which in turn alleviates the hypoxic condition. Our preliminary data have shown that metformin signicantly improve the radiosensitivity of DIPGs to extend the median survival of an orthotopic model bearing patient-derived DIPG cells. Strikingly, phenformin, another biguanide derivative, demonstrated a much more potent anti-DIPG activity which reduced the half maximal inhibitory concentration (IC) up to 30 times compared to metformin. To minimize lactic acidosis induced by phenformin, a drug combination was developed by combining phenformin with DCA. Not only did this unique combination signicantly alleviate phenformin's glycolytic effect, it also augmented cell-killing effect via a mechanism of inducing energy crisis, apoptosis and DNA double-strand breaks. Moreover, molecular signaling analysis revealed that this combination effectively inhibited HIF-1 and c-Myc, two master regulators that collaboratively enhance the cancer cell growth/survival and metabolic needs through increased uptake of sugar and contribute signicantly to radioresistance. Additionally, a signicant upregulation of TKT, a gene encoding the key enzyme transketolase that controls pentose phosphate pathway (PPP), was also observed in treatment resistant clones, suggesting the inhibition of PPP may also be needed when acquired resistance is developed. These ndings indicate that targeting hypoxia and mitochondrial metabolism with repurposing drugs represents a novel radiosensitising approach for incurable DIPG.

Overall, we have strong preliminary data suggesting that targeting hypoxia and mitochondrial metabolism potently improve the radiosensitivity of DIPG cells, thus providing an innovative therapeutic opportunity for the treatment of DIPG. Children currently diagnosed with DIPG have no hope of cure and are offered palliative treatment only. Both phenformin and DCA are generic drugs that are clinically available and have been well characterised as treatments for Type II diabetes and congenital mitochondrial deciency, respectively. Compared to other novel targeted anti-neoplastic agents, these repurposed drugs will signicantly reduce the cost of treatment and shorten the time of translating positive results generated from our proposed project to clinical testing. Importantly, as a potential treatment for brain cancer, both phenformin and DCA have been shown to readily cross the BBB via oral administration [12, 13]. We will perform the rst comprehensive analysis to assess efcacy of this novel treatment, identify potential metabolic signatures, optimize

the timing of sequential treatment, and explore resistant mechanism for developing salvage therapy using in vitro and in vivo DIPG models. In doing so, we aim to develop the quantum of preclinical data required for personalized treatment and rapidly translate this novel therapy to the clinic.

Our team has all the necessary expertise that will ensure the success of the proposed project and ultimately the implementation of the discoveries into the clinic. In collaboration with Prof Michelle Monje (Stanford University, USA) and Dr Ángel Montero Carcaboso (Hospital Sant Joan de Déuin, Spain), we have 10 patient-derived DIPG cells cultured in our lab, consisting of tumors that were established from biopsies at diagnosis and those from early post-mortem autopsies. Using this panel of DIPG isolates allows us to identify the capability of our proposed treatment to overcome the intrinsic (biopsies at diagnosis) and acquired (early post-mortem autopsies) radioresistance of DIPGs both in vitro and in vivo. Dr Han Shen (Westmead Institute for Medical Research) is an early career post-doctoral researcher with particular expertise in DIPG cell culture and xenograft models, cancer cell biology, drug discovery and radiation oncology. Dr Shen will perform most of the experiments proposed in this application. Dr Raluca Maltesen (Aalborg University Hospital, Denmark) is a metabolomics and data mining scientist specialized with 7 years' experience in systematic biology and multivariate data analysis. She will provide the guidance and analyze the data from metabolomics studies. Dr Eric Hau (Westmead Institute for Medical Research, Crown Princess Mary Cancer Centre Westmead Hospital) is a clinician-scientist and leader in Radiation Oncology with 10 years' experience in treating patients with high-grade brain tumors. Dr Hau will supervise the progression of whole project and facilitate the translation of our laboratory ndings from this project into clinical practice with the already established collaborations at Westmead Hospital and the adjacent Westmead Children's Hospital. We will also be collaborating with Sydney Informatics Hub based at University of Sydney for analysis of single cell transcriptomics data. The support from Dr Raluca Maltesen, Dr Eric Hau, Sydney Informatics Hub, Sydney West Radiation Oncology Network, and Westmead Institute for Medical Research provides an invaluable environment to ensure the success of this novel research project.