CHILDREN'S CANCER INSTITUTE

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DISCOVERING NEW EPIGENETIC PLAYERS IN DIPG ONCOGENESIS

Translational
DIPG, Childhood (Brain Cancer)
Affiliation: The Cure Starts Now
Requested Funding: $100,000

EXECUTIVE SUMMARY

1. **Scientific Merit**

Diffuse intrinsic pontine glioma (DIPG) is the most aggressive brain tumour found in children. The median survival time after diagnosis is approximately one year with no cure in sight. Recent studies have identified, in more than 60% of DIPG cases, a somatic mutation of the H3F3A gene, that leads to a lysine 27 to methionine mutation at the histone variant H3.3 (H3.3K27M). The presence of this mutation is associated with an intense epigenetic deregulation, including a decrease on the levels of the repressive histone mark H3K27me3 and an increase on the levels of the active histone mark H3K27ac, however *epigenomic studies beyond H3K27 modifications are needed*. Mechanistic studies on how this mutation further affects chromatin, gene expression and how it drives oncogenesis are currently under intense investigation, but it is still far from complete. Histone variant H2A.Z and H3.3 can form double variant nucleosomes which are associated with active chromatin and can drive gene transcription. Overexpression of H2A.Z and the acetylated form of H2A.Z (H2A.Zac) have been found in several cancer and is associated with poor prognosis[1,2]. Moreover, we have found that H2A.Zac is significantly re-distributed at enhancer and promoter-associated nucleosomes in cancer and is associated with aberrant gene activation[3,4].

Project Rationale: Despite the physiological known collaborative role between the histone variants H2A.Z and H3, the pro-oncogenic role of H2A.Z in DIPG has never been explored before.

Hypothesis: *H2A.Z acetylation contributes to DIPG by promoting the formation of H3.3K27M-H2A.Zac nucleosomes. These onconucleosomes are then mislocalised to form cancer-related neo-enhancers. The therapeutic modulation of H2A.Zac could improve DIPG outcomes.*

The **overall aim** of this project is to establish the genome-wide relationship between the histone variants H2A.Z(ac) and H3.3K27M in DIPG and to generate proof of principal data for the use of H2A.Zac-based inhibitors in combination

with other epigenetic drugs as a novel treatment strategy for these incurable paediatric tumours.

- **Aim 1. To study the genome-wide distribution of H2A.Z/H2A.Zac in H3K27M DIPG cell cultures.** We will build a complete epigenetic roadmap of DIPG cell lines and derived patient cultures mining public and newly generated epigenomic data**.** We will study the genomic distribution of H2A.Z(ac) and its association with H3K27M and aberrant gene expression. For this we will use DIPG patient-derived cell cultures with different mutation status: H3.3WT, H3.1K27M and H3.3K27M.

- **Aim 2. Combinatorial epigenetic therapy based on H2A.Zac-targeting.** We will test H2A.Zac inhibitors in combination with other epigenetic drugs using *in vitro* and *in vivo* models. We will then investigate the mechanisms of the epigenetic reprograming of the most cytotoxic drug combination.

Innovation and creativity: This project will investigate for the first time if H2A.Z is an active player in H3.3K27M-driving DIPG oncogenesis using a combination of state of the art epigenomic techniques and in vitro and in vivo DIPG models.

Significance and outcome: The use of H2A.Zac modulators to treat DIPG will open a new door for novel therapeutic interventions of this devastating paediatric cancer.

2. **Feasibility:**

All of the techniques and collaborations required for the project have been established and are demonstrated on the preliminary data of the proposal (see next section). This project will be performed at the Children Cancer Institute, located in the world class UNSW Lowy Cancer Research Centre and affiliated with Sydney's Children's Hospital Network and UNSW Sydney. The institute has all the infrastructure required for molecular biology, cell culture and animal work. As part of the UNSW campus we have access to the Ramaccioti Center for Genomics that is equipped with the latest next-generation sequencing technology (NovaSeq and NextSeq).

3. **Expertise:**

PI Valdes Mora expertise

Dr Fatima Valdes Mora is an accomplished mid-career researcher (two career disruption periods in the last 6 years) arising as an authority in Cancer Epigenetics and Genomics who holds a CINSW Career development Fellowship (2019-2021). She obtained her PhD in Molecular Biology, Biochemistry and Biomedicine from CSIC (Autonoma University of Madrid, Spain) in December 2008. She completed her postdoctoral research at Prof Susan Clark's lab (Garvan Institute in 2013). Valdes-Mora's leadership is evidenced by her Group Leader appointment at Garvan in January 2014 to lead the Histone Variant Group only five years after her PhD was awarded. She has recently obtained a highly competitive position as Team Leader at the Children's Cancer Institute (Sydney, Australia), where she has established the Cancer Epigenetic Biology and Therapeutics group.

Valdes Mora's research has made major impact on histone variant cancer research; she has played an integral role in discovering novel genome-wide roles of the acetylation of the histone variant H2A.Z and enable of international significance. She has a strong profile on molecular biology, epigenetics, NGS and bioinformatics interpretation of genome-wide data. Dr Valdes-Mora has published 26 peer-reviewed papers since 2004 (17 corresponding/first author), from which 13 has been published in the last 5 years. She has attracted more than A$3.5M of competitive funding including NHMRC project grants, CINSW, PCFA, NBCF and Cure Cancer.

Collaborators expertise:

- **Prof Nada Jabado** is a Professor of Pediatrics and Human Genetics at McGill University and a staff physician in the Division of Hematology and Oncology at the Montreal Children's Hospital. She began her career as an independent investigator in 2003 at the RI-MUHC, pioneering a research program in pediatric brain tumors, which is now unparalleled. Her research group was among the first to identify a histone mutation in human disease which has revolutionized this field. The epigenome was a previously unsuspected hallmark of oncogenesis and this discovery linked development and what we now know are epigenetic-driven cancers. Dr. Jabado has over 150 peer-reviewed publications to her credit in such prominent journals as Nature Genetics, Nature, Science and Cancer Cell. In 2015, Dr. Jabado was named as a Fellow of the Royal Society of Canada in the Life Sciences Division.

- **Dr Maria Tsoli** graduated with First Class Honours in Microbiology and Immunology from the University of New South Wales in Sydney in 1999 and received a PhD with High Distinction in Molecular Modelling and Tumour Biology from the University of Duisburg-Essen, in Germany in 2004. After her PhD, she worked as a postdoctoral scientist in the field of Cancer Cachexia Syndrome at Anzac Research Institute in Sydney. In 2009 she accepted a postdoc position at the Sanford-Burnham Institute for Medical research in San Diego, USA where her research efforts focused on the fields of Colorectal Cancer and Paediatric Bone Disorders. In 2011 she joined the Children's Cancer Institute where she has been has worked as a senior scientist and managing the Brain Tumour Group under the mentorship of the Paediatric Oncologist A/Prof David Ziegler (Sydney Children's Hospital). She is the lead scientist responsible for establishing state of the art techniques for research in Diffuse Intrinsic Pontine Gliomas (DIPG) and the development of aggressive glioma PDX models. Key achievements include the (a) establishment of primary glioma neurospheres (DIPG, glioblastoma, medulloblastoma) and orthotopic animal models from paediatric overseas and Australian patient's biopsies/autopsies; and (b) establishment of single-agent and combination high-throughput screening performed on primary DIPG neurosphere-forming cells. She has been a chief investigator in grants from CBCF, TDC and DIPG collaborative worth over 500K worth and CIC/CID in Cancer Australia grants. She has published overall 24 manuscripts in journals including Cancer Research, Cell Metabolism, Cancer Discovery and Neuro-Oncology.

Research Team

The Research Team will comprise PI lab members: one Honours student and one postdoctoral researcher, Dr Yolanda Colino Sanguino, an early career researcher and an arising start in cancer epigenetics and bioinformatics; and PI's collaborators: Dr Maria Tsoli (CCI, member of A/Prof David Ziegler's group): world-expert in DIPG models and therapy and Prof Nada Jabado, MD (RI-MUHC, Canada), a world-renowned expert on DIPG, who made the ground-breaking discovery of oncohistones in high-grade gliomas and she is the international reference in DIPG molecular mechanisms; and associated investigators: A/Prof David Ziegler (CCI): a paediatric oncologist with expertise in neuro-oncology, early phase clinical trials and preclinical development of novel therapies for high risk paediatric cancers; and Dr Brendon Mohanan (Cancer Therapeutics CRC): expert in cancer therapeutics, CEO of Cancer Therapeutics CRC, a successful cancer research organisation with a primary focus on small molecule drug discovery.

Our collaborators and associate investigators will provide reagents: DIPG cell cultures (Tsoli and Ziegler), epigenetic drugs (Monahan) and epigenetic biochemical reagents (Jabado); and they will also provide input and expertise during the progress of the project.

PI-Valdes Mora will oversee the research team interaction by organising weekly meetings to discuss results, troubleshooting and project direction with her lab members. Dr Colino-Sanguino will perform the experiments and bioinformatic analyses at Valdes-Mora's group. Valdes-Mora and Colino-Sanguino will supervise an Honours student for the grow, maintenance and cell fixation of the DIPG cell cultures. Colino-Sanguino will perform ChIP-seq studies, including the bioinformatic analysis and integration. We will have a close relationship with the Brain Tumours group (A/Prof Ziegler and Dr Tsoli) as this lab is located in the same Research Institute than PI's group, we will have fortnightly meetings for the coordination of the project and result discussion. We will follow already established protocols by Dr Tsoli from A/Prof Ziegler's group for the drug treatments and cell assays of DIPG cell cultures.

PI will also coordinate monthly meetings with our other collaborators, Prof Jabado and Dr Monahan for advice and update on research progression during the duration of the project.

References:

1 Buschbeck, M. & Hake, S. B. Variants of core histones and their roles in cell fate decisions, development and cancer. *Nat Rev Mol Cell Biol* **18**, 299-314, doi:10.1038/nrm.2016.166 (2017).

2 Valdes-Mora, F. *et al.* Clinical relevance of the transcriptional signature regulated by CDC42 in colorectal cancer. *Oncotarget* **8**, 26755-26770, doi:10.18632/oncotarget.15815 (2017).

3 Valdes-Mora, F. *et al.* Acetylated histone variant H2A.Z is involved in the activation of neo-enhancers in prostate cancer. *Nat Commun* **8**, 1346, doi:10.1038/s41467-017-01393-8 (2017).

4 Valdes-Mora, F. *et al.* Acetylation of H2A.Z is a key epigenetic modification associated with gene deregulation and epigenetic remodeling in cancer. *Genome Res* **22**, 307-321, doi:10.1101/gr.118919.110 (2012).