EXECUTIVE SUMMARY

Diffuse Intrinsic Pontine Gliomas (DIPGs) are the most aggressive of all childhood cancers. They are a type of brain tumour that peak in incidence at 5-7 years of age and are the most common form of malignant glioma to affect children. There are absolutely no effective treatments and current therapeutic strategies are palliative only. Due to their location within the brainstem the tumours cannot be removed surgically, they do not respond to chemotherapy, and radiotherapy only slows their growth temporarily. Novel and innovative treatment approaches are therefore urgently needed to counter these tumours.

Polyamines are small intracellular polycations, indispensable for cells. They are especially important for the survival of cancer cells, due to their increased metabolic needs, since they affect a range of biological processes, including DNA synthesis, transcription and translation, ribosome bio-genesis, protein phosphorylation and cellular proliferation. Polyamines are synthesized endogenously from ornithine. Ornithine decarboxylase ODC is the rate-limiting enzyme in polyamine synthesis, it is expressed abundantly in mammalian cells and is upregulated in cancer cells. However polyamines can also be taken up from intestinal microorganisms and dietary sources.

Difiuoromethylornithine (DFMO) is the best known and most well studied of all the polyamine synthesis inhibitors, and is an enzyme-activated, irreversible inhibitor of ODC. DFMO effectively crosses the blood-brain barrier with minimal toxicity, and is in a Phase I/II international clinical trial for neuroblastoma led by CI Ziegler. Our preliminary experiments have shown that DFMO significantly inhibits proliferation of DIPG neurospheres and formation of colonies. Furthermore we have found that a novel polyamine transport inhibitor AMXT-1501, which is currently in clinical development in adult phase 1 studies, synergistically increased the cytotoxicity of DFMO, reduced DIPG cell proliferation, colony formation and enhanced apoptosis. Treatment with AMXT-1501 led to reduced uptake of radiolabeled polyamine confirming the presence of an active polyamine transport system in DIPG cells. Most excitingly, and consistent with the *in vitro* results, combination of DFMO with AMXT-1501 significantly reduced tumour growth and led to unprecedented prolongation in the survival of mice bearing the SU-DIPG-VI and HSJD-DIPG007 orthografts. This combination represents the most effective combination treatment we have tested to date in our aggressive DIPG animal models with more than half of the mice surviving up to maximum holding time of 23 weeks post DIPG injection. These findings indicate that DIPG cells are sensitive to pharmacological inhibition of the polyamine pathway and that the combination of DFMO with AMXT-1501 represents an exciting new therapeutic strategy for DIPG.

We seek here to build upon these initial findings, and to develop the preclinical evidence required to urgently translate these novel discoveries to clinical trial to directly bene□t children with DIPG and other aggressive brain tumours. We aim to determine whether administration of DFMO/AMX-1501 directly inhibits polyamine synthesis in DIPG tumours. We also intend to evaluate whether the therapeutic efficacy of DFMO/AMXT-1501 combination can be further enhanced with radiotherapy and other anticancer drugs such histone deacetylase (HDAC) inhibitors, which are under clinical evaluation in DIPG patients. Our aims are to assess the efficacy of DFMO/AMXT-1501 combination together with

irradiation and subsequently in combination with panobinostat. Furthermore, in planning for a clinical trial, we intend to evaluate the therapeutic ef□cacy of DFMO with AMXT-1501 combination in three additional aggressive paediatric brain tumour orthtotopic animal models (GBM/HGG/Medulloblastoma). In doing so, we

aim to develop the quantum of preclinical data required to rapidly translate this therapy from the bench to the bedside for paediatric patients diagnosed with the most aggressive brain tumours.

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. Dr Maria Tsoli is a senior post-doctoral researcher with expertise in brain tumour and DIPG cell cultures and xenograft models, cancer cell biology and drug discovery and has been involved together with A/Prof Ziegler in the co-supervision of a PhD student who has provided the preliminary data described in this project. A/Prof Ziegler has preclinical expertise in paediatric malignant brain tumours and his clinical focus on early phase clinical trials will facilitate translation of positive results to the bedside. The support of A/Prof Ziegler and Prof Haber at the Children's Cancer Institute provides an invaluable environment to ensure the success of this novel research program, and with the ultimate goal of improving outcomes for children diagnosed with DIPG.