EXECUTIVE SUMMARY

Scientific merit:

Diffuse intrinsic pontine glioma (DIPG) is the most common brainstem tumour in children and a leading cause of paediatric brain tumour related deaths. Due to its location, surgical resection is not possible. It is invariably fatal, with a mean overall survival of 9 to 12 months from the time of diagnosis. Chemotherapy would normally be the preferred treatment option for such infiltrative disease, but such treatment of DIPG is complicated by its *diffuse in filtration and co-habitation* with healthy brain tissue, and *the presence of an intact blood-brain barrier* (BBB) throughout the majority of the tumour. Drugs including panobinostat, a histone deacetylase inhibitor currently in clinical trial for this disease [3], and EPZ6438, an inhibitor of the histone methyl transferase subunit of the PRC2 complex, EZH2 [4] have demonstrated potent *in vitro* efficacy, but local delivery, required to cause a survival benefit *in vivo,* has not be achieved, most likely due to poor CNS penetration of the compounds. Non-invasive focused ultrasound combined with systemic delivery of microbubbles (clinically approved for diagnostic ultrasound imaging) has the potential to deliver such drugs to the brain [2]. To achieve drug delivery, a focused ultrasound beam, generated using an externally placed source, travels through the intact skin and skull and into brain tissue. Microbubbles, administered systemically into the vasculature, are mechanically stimulated by the ultrasound beam only at the target site. One of the biological outcomes of this ultrasound-microbubble interaction is an increase in the permeability of blood vessels to therapeutic molecules. Where the target site is the brain, the changes in BBB permeability results in increased presence of drugs within the brain parenchyma [2, 3]. Current ultrasound methodology (see Fig. 1 in the attached Figure file) uses standard long-pulse sequences (10,000 cycles delivered at a slow rate (1 Hz)), which can result in adverse biological responses unrelated to the desired blood-tobrain- parenchymal drug delivery. These include: long BBB disruption times (up to 24 hours), release of neurotoxic species (e.g. albumin) into the brain, drug delivery to untargeted vessels and, occasionally, haemorrhage. These are particularly undesirable in the young, developing brain that is characteristic of the DIPG affected population. The delivery efficiency also varies, hence dose distribution heterogeneity is also an issue with long-pulse sequences. We have developed a new rapid short-pulse (RaSP) ultrasound method (5 cycles) with significant safety and performance advantages. In our proposed research, we will optimize this new technology to deliver drugs to reduce DIPG progression in an orthotopic, patient-derived xenograft model of DIPG. This new sequence enables a homogeneous dose delivery throughout the parenchyma. The safety concerns are also reduced, with a short duration BBB permeability change (<10 minutes) and reduced albumin and immunoglobulin release into the brain. In this project, we aim to assess the efficacy and safety profile of RaSP drug delivery in a DIPG animal model, using panobinostat. This is a necessary step before Phase I clinical implementation. This approach has the potential to provide the urgently required improvements in the safetreatment of this disease in the developing brain.

Feasibility: The breakthroughs in ultrasound delivery described above have been made possible with core technological advances made at ICL. The regime used in all current clinical trials of ultrasound enhanced drug delivery to the brain involves long-pulse sequences (10,000 cycles) [2, 3, 7-18] (Fig. 1, attached). . Choi et al have shown that surprisingly short pulses can also deliver drugs [6] and when incorporated into a rapid emission sequence can deliver a higher dose and more homogeneous distribution of drugs, as reported in *PNAS* [19]. We now have a better understanding of how ultrasound

stimulates microbubbles in vascular □ow conditions. Long pulses have been shown to produce a diverse and chaotic array of microbubble stimuli that could last as long as the full pulse duration (10,000 cycles) [20, 21] while short pulses produce less microbubble stimulus for a very short duration (5 cycles) [22, 23]. Furthermore, short pulses have been demonstrated to distribute the microbubble-mediated stimuli more uniformly throughout the acoustic field [22, 23]. We are now in a position to capitalize on these technological advances by combining the ultrasound engineering expertise available at ICL, with the preclinical facilities for DIPG studies available at ICR. Apart from the expertise listed below, we have in place all the necessary facilities for growing the DIPG model in mice, pre-clinical imaging and immunohistochemical analysis at ICR.

Expertise:

The proposed study is a joint effort between Imperial College London (ICL) and the Institute of Cancer Research (ICR). The project will begin at ICL where the technology will be optimised, and as the study progresses, the project will be increasingly conducted at ICR where the new technology will be tested on DIPG. This project will strengthen the link between ICL and ICR, which have recently jointly been awarded CRUK Major Cancer Centre status. Ultrasound pulse sequencing techniques under development at ICL (Dr. James Choi, co-I) will be made available for pre-clinical testing to world-leading cancer research groups in therapeutic ultrasound (Prof. Gail ter Haar, PI), cancer imaging using MRI (Dr. Simon Robinson, co-I), DIPG tumour models & histopathology (Dr Jessica Boult, co- I) and DIPG biology (Prof. Chris Jones, co-I).