Cancer Research Centre of Excellence: **PhD Studentship Project**

Project Title **Ultrasound drug delivery across the blood-brain barrier for the treatment of paediatric brain cancer**	
Lead Supervisors	Dr. James J Choi (Main contact) Imperial College London / Department of Bioengineering Room 4.06 Royal School of Mines Building E-mail: j.choi@imperial.ac.uk Phone: +44 (0)2075 941 777 Prof. Gail ter Haar ICR / Radiotherapy and Imaging / Therapeutic Ultrasound Gail.terhaar@icr.ac.uk 0208 722 4020
Other supervisory team members:	**Backup Supervisors:** Dr. Mengxing Tang, Imperial / Department of Bioengineering Dr. Simon Robinson, ICR/ Radiotherapy Imaging/ Magnetic Resonance **Associate supervisors:** Prof. Chris Jones, ICR/ Molecular Pathology/ Glioma Team Dr. Gerard Hernandez Mir, Imperial / Bioengineering Dr. Ian Rivens, ICR/ Radiotherapy and Imaging/ Therapeutic Ultrasound Dr. Jessica Boult, ICR/ Radiotherapy and Imaging/ Magnetic Resonance
Pre-requisites of applicants	A good degree in Physics or Engineering (with particular emphasis on biological or medical applications) or Biology (with strength in physics)

Project Summary

Children diagnosed with diffuse intrinsic pontine gliomas (DIPGs) have an 18-month survival rate of only 10%. Over time, nearly every one of these children will die from their cancer. This devastating prognosis comes from the diffuse spread through healthy tissue, reducing the impact of localised surgery and radiotherapy. In such a diffuse cancer, chemotherapy would normally be the better treatment option; but DIPG *spreads behind an intact blood-brain barrier (BBB)*, thus limiting drug efficacy.

We have shown that short pulses of ultrasound can produce controlled, temporary changes to BBB permeability. This project will develop and test the first short-pulse drug delivery method for DIPG. By allowing drugs to cross the BBB, it has the potential to revive previously abandoned therapies that showed great promise for the treatment of DIPG.

Current Ultrasound Methods

Ultrasound applied after the systemic administration of microbubbles, can alter BBB permeability. This non-invasive technique can deliver peptides, antibodies, and nanoparticles, and has been tested on animal models of neurological diseases. Yet, despite these demonstrations, state-of-the-art ultrasound enhanced drug

delivery – using long pulses emitted at a slow rate – delivers drugs in a heterogeneous 'spot-like' pattern; and produces collateral damage (erythrocyte extravasation, microvascular rupture, arterial damage). Such side effects should be avoided in diffuse diseases where the surrounding tissue must be preserved, such as in paediatric DIPG.

Our Short-Pulse Ultrasound Technology

We are developing a new ultrasound emission sequence – rapid short pulses (RaSP) emitted at a fast rate – that delivers drugs across the BBB. RaSP give exceptional control over the microbubbles flowing through the vasculature, gently stimulating them into oscillations, and transporting co-injected drugs across the BBB. RaSP sequences have unique safety & performance features:

- **Diffuse drug delivery.** RaSP deliver drugs diffusely throughout the parenchyma (Figure 1), ensuring that every cancer cell is exposed to the drug.
- **Size-selective BBB opening.** RaSP delivers drugs but not erythrocytes and blood proteins (immunoglobulins), thus minimising the brain's exposure to unwanted cells and proteins.
- **Temporary BBB opening.** The BBB returns to normal permeability within 4 h (Figure 1). This is the shortest reported BBB permeability change.

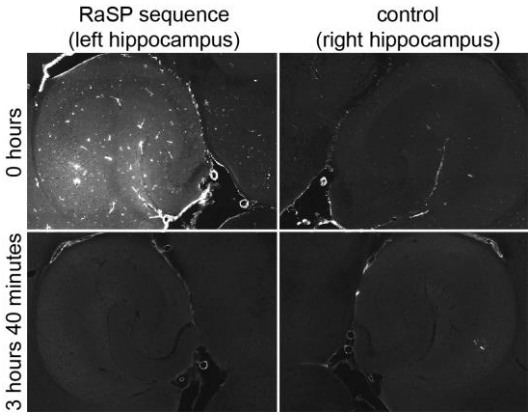

Figure 1. Our RaSP sequence produced a temporary (< 4h) alteration of BBB permeability. A fluorescently-tagged model drug (3-kDa dextran) was delivered across the BBB when systemically administered during ultrasound sonication, but not when injected 3h 40min after. **[preliminary results]**

Programme of Work

This project will develop and test RaSP ultrasound delivery of a drug for the effective treatment of DIPG modelled in a mouse.

Aim 1: Optimise RaSP sequences for safe drug delivery (ICL)

We will maximise the payload delivered to the brain without damage by optimising RaSP parameters *in vitro* and *in vivo*.

In the *in vitro* study, RaSP sequences will be designed to stimulate microbubble dynamics that produce safe BBB opening; and suppress harmful dynamics. Dynamics will be assessed with passive acoustic imaging and optical microscopy methods.

A select range of RaSP sequence parameters found in the *in vitro* study will be tested in normal mice. The left hemisphere of the mouse brain will be exposed to ultrasound after systemic administration of a microbubble

ultrasound imaging contrast agent (SonoVue®). We will evaluate the delivery payload and distribution by co-injection of fluorescently-labelled dextran (3 kDa); and evaluate safety using histology.

Aim 2: Optimise MRI sequences to monitor BBB permeability changes and cancer progression (ICL & ICR)

We will optimise existing and develop new MRI methods (including multiparametric sequences where appropriate) (Boult JKR et al., NMR Biomed 2016; Neoplasia 2017) that detect BBB permeability changes and track cancer progression.

We will optimise MRI sequences for the sensitive detection of a normally BBB-impermeable MRI contrast agent (Omniscan) that has crossed the BBB using RaSP (from Aim 1).

Progression of orthotopically-propagated DIPG in mice will be monitored using either MRI alone, MRI with a contrast agent, and MRI with ultrasound-delivered contrast agent. We will evaluate cancer progression in DIPG. If DIPG progression is not observable using MRI, we will use a hemispheric glioblastoma (Vinci M et al., *Neuro-Oncol* 2016; Fofana M et al., *Neuro-Oncol* 2016).

Aim 3: DIPG treatment in a mouse model (ICR)

We aim to reduce cancer progression in a DIPG mouse model using ultrasound enhanced drug delivery.

Using a DIPG mouse model, we will deliver a chemotherapeutic agent across the BBB with optimised RaSP and characterise treatment efficacy in terms of the (i) drug distribution in the tumour, (ii) upregulated or suppressed pathways by which the drug is effective, (iii) MRI-monitored tumour growth rate, and (iv) survival.

Feasibility

The ultrasound technology and equipment to be used are at an advanced stage of development at Imperial College for the treatment of Alzheimer's disease (funding from Alzheimer's Research UK). The Imperial team invented RaSP sequences and has an operational bench-top *in vivo* brain drug delivery system (previously funded by the Michael Uren foundation). Evidence that RaSP sequences improve drug delivery performance has been published by the applicants in a leading general journal (Choi et al., *PNAS* 2011) while more recent work explaining the mechanism for this improvement have been published in leading biomedical acoustics journals (Pouliopoulos et al., *Phys Med Biol* 2014, Pouliopoulos et al, *JASA* 2016). Better RaSP sequences continue to be developed in our laboratory; and evidence of improved performance and safety features were captured in on-going studies that will be reported in a journal article that will be submitted soon (Figure 1).

The proposed PhD project will translate the use of our ultrasound technology to cancer; and, more specifically, to novel paediatric high grade glioma models which exist at the ICR.

These goals are achievable within the period of a PhD project since the underpinning equipment and infrastructure already exists. The supervisory team are exceptionally well placed to supervise this timely project.

Our Team's Vision

The proposed PhD project transports a world-unique technology – RaSP ultrasound drug delivery – developed at Imperial College London (ICL) to world-leading experts in clinical therapeutic ultrasound, MR imaging, and paediatric glioma at the Institute of Cancer Research (ICR). The strategic objective of this studentship is to form a technological pipeline for novel noninvasive devices developed at the Noninvasive Surgery & Biopsy Laboratory (PI: Dr. Choi) at ICL to treat cancers with experts at ICR. Our long-term vision is to make

noninvasive microsurgery one of the top 4 cancer treatment options (amongst drugs, surgery, and radiotherapy).

Roles & Contributions

Dr. James Choi (PI; lead supervisor (biomedical-engineer); ICL) has been a leading contributor to the understanding of blood-brain barrier (BBB) opening mechanisms (12 publications) and the development of novel ultrasound drug delivery technologies for cancer and other diseases (+14 publications). His BBB opening research has been highly cited (Choi et al., *Ultrasound Med Biol* 2007: **193 citations;** Choi et al., *IEEE Biomed Eng* 2010: **115 citations**). He continues to publish in leading journals, such as PNAS (2011), the JNCI (2014), and Applied Physics Letters (2015).

Dr. Choi is the primary supervisor of this project and is responsible for the overall progression and training of the PhD student. Dr. Choi will train the student to develop ultrasound delivery technologies, to assess its physical and biological performance, and to use MRI and other methods for sensing BBB permeability changes.

Prof. Gail ter Haar (Co-I; lead supervisor; ICR) is a world-leading therapeutic ultrasound physicist recognised for bringing high-intensity focussed ultrasound (HIFU) into clinical use.

Prof. ter Haar and Dr. Rivens will co-supervise the student throughout the PhD; training the student in the calibration of devices, assessment of ultrasonic bioeffects, and use of ultrasound technologies.

Dr. Mengxing Tang (Co-I; backup ICL supervisor) is an expert in microbubble-based ultrasound imaging and develops ultrafast plane wave and super-resolution imaging technologies.

Dr. Tang will supervise the sensing of microbubbles; and be available for scientific guidance on imaging-related topics. Dr. Tang will read the first year (transfer) report and provide pastoral care should it be needed.

Dr. Simon Robinson (Co-I; backup ICR supervisor).

Working in Dr. Robinson's pre-clinical MRI team, Dr. Boult has considerable experience in the propagation and subsequent MRI investigations of brain tumour models, and together will supervise the student's MRI work in the detection of BBB permeability changes and tumour progression.

Prof. Chris Jones (Co-I; associate ICR supervisor).

Prof. Jones will supervise the student in all aspects of DIPG biology

Tailored Training in Multidisciplinary Research

The PhD student will receive convergent training to become a Biomedical Acoustician & pre-clinical cancer scientist, and will develop the following expertise:

- **Mastery of biomedical acoustics:** a deep understanding of physical acoustics, microbubbles, pulse sequences, and bioeffects; skills in programming, signal processing, calibration, instrumentation, tissue phantoms, and microbubbles.

  Primary training supervisors: Dr. Choi, Prof. ter Haar
- **Expertise in general biology:** a deep understanding of the BBB and neuroscience; skills in sectioning, staining, western blotting, ELISA, microscopy, safety and toxicity assessment.

  Primary training supervisor: Dr. Mir, Dr. Boult
- **Expertise in animal handling and care:** A good understanding of animal health and behaviour; skills in animal handling, injections, isoflurane, and stereotactic frames.

  Primary training supervisors: Dr. Mir, Dr. Boult, Dr. Rivens

- **Expertise in MR imaging:** A good understanding of MRI sequences and contrast agents; skills in pre-clinical MRI, contrast agents, and image processing.

  Primary training supervisors: Dr. Boult, Dr. Robinson
- **Expertise in paediatric brain cancer:** a deep understanding of DIPG and chemotherapeutic agents; skills in cancer implantation, tumour growth monitoring, and drug efficacy assessment.

  Primary training supervisors: Prof. Jones, Dr. Boult

Tutorials. Dr. Choi will provide weekly 1h meetings with the PhD student. In the first 6 months, tutorials will be held weekly at ICL and monthly at ICR. The rate of tutorials will change as needed throughout the PhD study. The student can attend courses, seminars, and workshops held at ICL and ICR. The student will be required to obtain an animal license; and attend radiation and chemical safety training courses at both institutes.

Project Timeline

The student will split his or her time approximately 50:50 between ICL & ICR. The student will begin at ICL for Aim 1, developing the delivery technology, and then progressively increase work at ICR for Aims 2 and 3, characterising the delivery pattern using MRI and testing whether ultrasound delivery of a chemotherapeutic agent will produce a response in DIPG in an animal model.

Literature references

1. **Choi JJ**, Selert K, Vlachos F, Wong A, Konofagou EE. Noninvasive and localized neuronal delivery using short ultrasonic pulses and microbubbles. PNAS. 108(40):16539-44. 2011.
2. **Choi JJ**, Selert K, Gao Z, Samiotaki G, Baseri B, Konofagou EE. Noninvasive and localized blood-brain barrier disruption using focused ultrasound can be achieved at short pulse lengths and low pulse repetition frequencies. J Cereb Blood Flow Metab. 31(2):725-37. 2011.
3. **Choi JJ**, Coussios CC. Spatiotemporal evolution of cavitation dynamics exhibited by flowing microbubbles during ultrasound exposure. J Acoust Soc Am. 132(5):3538-49. 2012.
4. Pouliopoulos A, Bonaccorsi S, **Choi JJ**. Exploiting flow to control the in vitro spatiotemporal distribution of microbubble-seeded acoustic cavitation activity in ultrasound therapy. Phys Med Biol. 59(22):6941-57. 2014.
5. Pouliopoulos AN, Li C, Tinguely M, Garbin V, Tang MX, **Choi JJ**. Rapid short-pulse sequences enhance the spatiotemporal uniformity of acoustically driven microbubble activity during flow conditions. J. Acoust. Soc. Am. 140;2469. 2016.
6. **Boult JKR**, *et al.* Investigating intracranial tumour growth patterns with multiparametric MRI incorporating Gd-DTPA and USPIO-enhanced imaging. NMR Biomed 29:1608-1617. 2016.
7. **Boult JKR**, *et al*. Evaluation of the response of intracranial xenografts to VEGF signaling inhibition using multiparametric MRI. Neoplasia. 19:684-694. 2017.
8. Vinci M, *et al.* A patient-derived paediatric high grade glioma and DIPG cell culture panel recapitulating the genotypic and phenotypic diversity of the disease. Neuro-Oncol. 18:iii71. 2016.
9. Fofana M, *et al. In vivo* magnetic resonance imaging identifies clinical phenotypes of paediatric glioblastoma in an orthotopic mouse xenograft model. Neuro-Oncol. 18:iii140. 2016.