The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Photoacoustic imaging for the optimisation of CAR T-cell cancer therapy of soft-tissue tumours – reporter-gene studies**
Short Project Title:	**Reporters for photoacoustic imaging of CAR T-cell cancer therapy**
SUPERVISORY TEAM	
PhD registered at:	Institute of Cancer Research
Application webpage:	https://www.icr.ac.uk/phds
Primary Supervisor(s):	Prof. Jeff Bamber (Institute of Cancer Research) Dr. Lucia Florescu (University of Surrey)
Associate Supervisor(s):	Dr. Anant Shah (National Physical Laboratory) Dr. Astero Klampatsa (Institute of Cancer Research)
Secondary Supervisor:	Dr. Emma Harris (Institute of Cancer Research)
Lead contact person for the project:	Prof. Jeff Bamber
DIVISIONAL AFFILIATION	
Primary Division:	Radiotherapy and Imaging
Primary Team:	Ultrasound and Optical Imaging
PROJECT PROPOSAL	

BACKGROUND TO THE PROJECT

This is one of two projects offered to develop photoacoustic imaging for the optimisation of CAR (chimeric antigen receptor) T-cell cancer therapy. This student will be registered at the Institute of Cancer Research (ICR) in Sutton within the Division of Radiotherapy and Imaging (https://www.icr.ac.uk/our-research/research-divisions/radiotherapy-and-imaging) but carry out the work in collaboration with the Centre for Vision Speech and Signal Processing at the University of Surrey (https://www.surrey.ac.uk/centre-vision-speech-signal-processing), and will spend at least three months with the National Physical Laboratory (NPL) in Teddington (https://www.npl.co.uk/). Further technical advice will come from iThera Medical (https://www.ithera-medical.com/), the company that manufactures the (MSOT™) equipment that will be used for photoacoustic imaging in the project.

In 2018, NHS England and the FDA in the USA approved the availability of CAR T-cell therapy for cancer patients. The therapy involves removal of the patient's T cells from the blood, followed by their genetic modification to express CARs that specifically target tumour cells, and re-infusion of the cells back into the patient. The access to

this “living drug” therapy has set the scene for major development in the field of personalised medicine, with more than 800 CAR T cell therapies being investigated in clinical trials (ClinicalTrials.gov).

Preclinical assessment of the efficacy, pharmacokinetic profile and toxicity profile of CAR T cells is of critical importance in the development and optimisation of such therapies, prior to clinical trials. This is particularly so for application to the treatment solid tumours (Klampatsa et al 2017, Martinez et al 2019, Sacchetti et al 2019). Ideally, this requires an ability to noninvasively image and track the CAR-T cells in vivo. Current imaging approaches (optical, PET, SPECT, MRI) have limitations such as low tissue-penetration depth, poor sensitivity, poor quantification, inadequate resolution, lack of 3D information, or a sensitivity to CAR T cells that fades with time.

The two students will work on related projects which aim to develop and evaluate the use of photoacoustic imaging for determining the biodistribution of the CAR T cells *in vivo*. Photoacoustic imaging is an exciting relatively new biomedical imaging method which employs an ultrasound scanner in combination with a pulsed laser to create high-resolution 3D images of the optical absorption-properties of tissues and cells (Attia et al 2019, Wang and Yao 2016). The CAR T cells will be genetically modified to express proteins that are detectable using photoacoustic imaging. Both projects are multidisciplinary, but the emphasis of this project is in the biological aspects of the new technology development and evaluation, whereas the other project focuses more on the physics and engineering aspects.

PROJECT AIMS

- Screen reporter gene/cell-line combinations for best and most stable photoacoustic detectability, and minimum effect on cell viability and behaviour.
- Use selected reporter genes and tumour models to determine sensitivities and linearities for cell number detectability by photoacoustic imaging *in vivo*.
- Determine whether incorporation of photoacoustic reporter genes into CAR T cells alters treatment efficacy.
- Explore *in vivo* the application of photoacoustic reporter gene imaging to the dynamic assessment of CAR T-cell numbers as they penetrate tumours to be treated and to determining where else the CAR-T cells accumulate.
- Evaluate whether photoacoustic reporter gene imaging has potential for predicting treatment efficacy in an individual patient.

RESEARCH PROPOSAL

This is an exciting multidisciplinary convergence-science project involving development of a novel non-invasive imaging approach for cell tracking *in vivo* and will require the use of biological techniques applied in gene transduction as well as inventive applications of photoacoustic imaging. This high-level and multi-faceted skill set will be developed over the duration of the PhD and represents excellent training in research techniques and methodology. The need to develop creative solutions for addressing challenges in the project, in liaison with researchers at the ICR, the NPL and the University of Surrey, will provide outstanding doctoral training.

The project will begin by learning ultrasound, optical and photoacoustic physics and practical methods, cell culture, transduction, CAR-T cell fluorescence-activated cell sorting and other techniques.

The student will then focus on screening via literature review and selected focused experiments, reporter gene/cell-line combinations that should provide stable expression of proteins expected to produce a strong photoacoustic

signal with a unique and identifiable spectrum and/or temporal switching signature. This will include CAR T cells but not be limited to them because there will be considerable added value in having preclinical tumour models that are intrinsically visible with high resolution on 3D photoacoustic imaging, for noninvasive monitoring of tumour status prior to and following treatment (Whilding et al, 2019). Early publishable data will come from experiments to compare these combinations in terms of: the relative expression levels, stability and persistence of expression, cell viability and behaviour in 3D culture after transduction (as assessed with both a Celigo™ 3D fluorescence imaging cytometer and using the photoacoustic signal on three-dimensional photoacoustic microscopy at NPL), strength and spectral/switching properties of the photoacoustic signal, and minimum numbers of cells that can be detected above noise and tissue background photoacoustic signals in tissue mimicking phantoms.

These skills and data will contribute to the first-year report and transfer viva assessment of the student. After completion of any necessary unfinished components to the work, papers will be submitted to peer-reviewed journals, and the findings will be presented at appropriate international/national conferences.

The student will then undertake training and receive certification for skills including those for growing subcutaneous and orthotopic tumours and *in-vivo* imaging. The project will continue with *in-vivo* extensions of the year-1 studies, using a selected subset of tumour models and reporter genes based on the year-1 findings. Minimum tumour size (i.e. number of cells in a localised collection expressing the reporter gene) for photoacoustic visibility for several models *in vivo* will be established and tumour growth monitored, with and without corrections using a method developed by the other student, evaluated in comparison with callipers (subcutaneous only), ultrasound imaging and fluorescence imaging using an IVIS™ scanner in the frequently encountered situation that the photoacoustic signal-generating proteins are also fluorescent. Further publications and presentations are expected at this stage.

In collaboration with Dr. Klampatsa (ICR) CAR T-cell techniques will be learnt. *In-vitro* studies will be conducted to confirm that incorporation of reporter genes for imaging is not to the detriment of treatment efficacy. Persistence of the reporter-gene signal *in vitro* and *in vivo* will be studied. The performance of photoacoustic imaging for studying the whole-body biodistribution of CAR T-cells, including determining the extent to which they penetrate the target tumours, will be studied as a function of time after infusion, using *in-vivo* optical (IVIS™) imaging for dynamic comparison. After termination of *in-vivo* studies at an appropriate time (to be determined), tumour and various organs and tissues will be sampled, histological sections prepared, and fluorescence microscopy and NPL's photoacoustic microscope used to validate the cell numbers predicted by ICR's in-vivo photoacoustic imaging. The effect on quantification of cell numbers of theoretical modelling-based correction for system-dependent factors developed by the other student, will also be studied. Opportunities will exist for extending the studies to determine the likely usefulness of photoacoustic visualisation of CAR T-cell distribution early during treatment as a predictor of treatment efficacy. Additional publications are expected at this stage.

LITERATURE REFERENCES

Attia ABE, Balasundaram G, Moothanchery M, Dinish US, Bi R, Ntziachristos V, Olivo M. A review of clinical photoacoustic imaging: Current and future trends. Photoacoustics.16:100144, 2019.

ClinicalTrials.gov. https://clinicaltrials.gov/ (2021, accessed 5 February 2021)

Klampatsa A, Haas AR, Moon EK, Albelda. Chimeric antigent receptor (CAR) T cell therapy for malignant pleural mesothelioma (MPM). Cancers;0:115, 2017.

Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, inifiltrating and surviving in the tumor microenvironment. Front Immunol;10:128, 2019.

Sacchetti B, Botticelli A, Pierelli L, Nuti M, Alimandi M. CAR-T with license to kill solid tumors in search of a winning strategy. Int J Mol Sci;20:1903, 2019.

Wang LV, Yao J. A Practical Guide to Photoacoustic Tomography in the Life Sciences. Nat Methods. 28;13:627–638, 2016.

Whilding LM, Halim L, Draper B, Parente-Pereira AC, Zabinski T, Davies DM, Maher J. CAR T-cells targeting the integrin αvβ6 and co-expressing the chmokine receptor CXCR2 demonstrate enhanced homing and efficacy against several solid malignancies. Cancers;11,674, 2019.

CANDIDATE PROFILE	
Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)	
Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc in Biological Sciences/Physics/Engineering (1st/2i), optionally Masters with a biomedical application.
Intended learning outcomes:	• Knowledge of advanced and novel biomedical imaging techniques, including ultrasound and photoacoustic imaging, and photoacoustic microscopy. • In-depth developmental experience and skills in medical image data and signal processing. • Knowledge of experimental methods in reporter gene transduction, assays and imaging, and practical experience of applying them in a preclinical context. • Cell culture and quantification methods. • Knowledge and skills in the planning and execution of preclinical in-vivo hypothesis-testing experiments, and corresponding statistical analysis methods, to evaluate various imaging strategies in the context of CAR-T cell therapy. • The skills necessary to become an independent self-directed research scientist, including the design of hypotheses and the planning and execution of studies to test them.
ADVERTISING DETAILS	
Project suitable for a student with a background in:	☑ Biological Sciences ☑ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or Epidemiology ☐ Computer Science ☐ Other (provide details)
Keywords:	**1.** quantitative cancer imaging biomarkers
	2. adoptive cell immunotherapy
	3. photoacoustic/optoacoustic/ultrasound imaging
	4. reporter gene imaging

	5. cell tracking
	6. physics/engineering/biology/convergence science PhD London