Proposal Title: Biomechanical activation of pro-metastatic programs in circulating tumour cells by vasculature constriction forces.

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Proposal outline

Rationale: It is estimated that up to four million cancer cells per gram of tumour tissue enter the bloodstream per day in animal models(*1*), yet very few of these eventually establish metastases. We do not understand the factors that select the small minority of these cells to succeed. A number of studies have demonstrated that biomechanical forces exerted on nuclei cause significant nuclear deformation, rupture, DNA damage, and potentially mutagenic events that lead to metastasis(*2-5*). **Thus, disseminated tumour cells may gain metastatic competency as their nuclei are deformed during transit through narrow blood vessels.** In support of this idea, nuclear deformation caused by chromosome missegregation can drive epithelial-mesenchymal transition (EMT) and metastasis through upregulation of cGAS/STING-NFκB signalling axis(*6-9*).

Hypothesis: Forces exerted on non small cell lung cancer (NSCLC) circulating tumour cells (CTCs) during transit through the vasculature rupture nuclei; resulting in the activation of cGAS/STING-NFkB signalling axis which promotes EMT and metastasis (Fig. 1). This presents a therapeutic opportunity for suppressing metastasis by blocking the activity of this axis. To explore this hypothesis, a student will combine both cutting-edge bioengineering at ICL and quantitative phenotyping approaches at ICR:

Preliminary Evidence: We have generated preliminary data demonstrating that nuclei envelope rupture occurs during cancer cell transit through microfluidic capillary constrictions under physiological conditions. This phenomena has never been previously reported in cells undergoing capillary-like transit. and is important because rupture is a key event that leads to the release of DNA into the cytoplasm - a trigger of cGAS-STING activation.

Aim 1: Quantify the biomechanical activation of cGAS/STING – NFkB signalling axis, and engagement of EMT, in NSCLCs during physiological microvasculature transit.

Working in the Au laboratory, the student will develop a next generation multiscale microfluidic vasculature network platform capable of mimicking the unique hemodynamic environment experienced by CTCs transiting through the microcirculation. An advantage of microfluidic platforms is that cells which have experienced nuclear rupture can be selectively

isolated for downstream analyses (e.g. Aim 2). The proposed device will be the first device capable of the modelling the multi-pass transit of CTCs through vasculature of different scales. In parallel, the student will work in the Bakal laboratory to engineer a panel of reporter lines by which to monitor cGAS/STING, NFκB and EMT. These reporter lines will then be imaged at they are exposed to different hydrodynamic environments at the ICR's advanced imaging facility.

This aim will provide the first evidence that CTC nuclear envelope rupture may occur due to hemodynamic forces in the vasculature, and that these events can cause the upregulation of inflammation and induction of cGAS/STING.

Aim 2a: Characterize the genome, transcriptomes, and proteomes of lung cancer cells that have undergone nuclear rupture in different hemodynamic environments.
The student will identify, isolate and expand CTCs with promising responses from Aim 1 (i.e. cGAS/STING activation) from multiscale vasculature platforms. The student will then characterize how nuclear rupture affects the genomic sequence, and transcriptome of lung cancer cells at ICR's Tumour Profiling Unit. Working with the Laboratory of Jyoti Choudhary, the student will quantify the proteome and phosphoproteome of lung cancer cells that have undergone nuclear rupture.

Aim 2b: Quantify the metastatic potential of lung cancer cells that have undergone nuclear rupture in engineered devices.

The student will isolate and expand cells that have undergone nuclear rupture in the multiscale vasculature platforms to assess their metastatic potential in immunocompromised mice. Experiments will involve either tail-vein injection, xenografts, or orthotopic injection.

The outcome will be a mechanistic understanding of how nuclear rupture caused by hemodynamic forces can drive metastasis through cGAS/STING activation and will inform how genetic or chemical manipulation may prevent metastasis post-nuclear rupture.

Aim 3: Identify small-molecules which can prevent the engagement of inflammatory and pro-EMT programmes following nuclear rupture in hemodynamic environments.

The student will engineer a high throughput microfluidic vasculature drug screening platform where lung cancer cells exposed to physiological hemodynamic forces will be simultaneously exposed to panels of small-molecule inhibitors of relevant enzymes. The student will perform a screen of ~50-100 candidate small-molecules that may prevent cGAS/STING activation or downstream signalling cascades that result in pro-metastatic programmes.

This aim will provide initial candidates for the development of high-impact therapeutics that aim to prevent the engagement of pro-metastatic pathways.

Feasibility (*up to 250 words*)

The projected project timeline is outlined in Figure 3. This project involves integrating cell biology and bioengineering technologies which have been developed by the Bakal and Au laboratories respectively. The expertise that the Co-Is have in these areas will provided a uniquely qualified training environment that will ensure the success of the PhD student For example, the student will benefit from the extensive expertise in the Bakal laboratory in engineering reporter lines, quantitative cell imaging, and the genomic and proteomic characterization of characterization of cancer cell lines. Moreover, the student will be

trained by Dr Au in the bioengineering of novel physiologically-relevant microfluidic and organ-on-a-chip devices. Furthermore, the the student will have extensive access to relevant facilities for carrying out the work such as microfabrication (to two state-of-the-art cleanrooms at Imperial College, advanced microscopy (numerous inverted fluorescent and confocal microscopes exist at ICL and ICR), cell culture and data processing through the Bakal and Au laboratories. We anticipate the main technical challenge will be to adapt novel microfluidic devices to image single living cellsand signalling events in real time using the the advanced imaging platforms at the ICR. However, given the history of both the Bakal and Au labs in succeeding at overcoming similar technical challenges, we do not anticipate this will be a problem.

On a day-to-day basis this work is made very feasible by the close proximity of the Au and Bakal labs, which are located a short 15 minute walk from each other in South Kensington.

Multidisciplinary approach

Roles and Contributions

The proposed studentship involves extensive cross-disciplinary training in cancer cell biology especially quantitative cell imaging, the biology of metastasis, and systems biology (ICR); as well as cellular biomechanics, biomedical device design and the development of organ-on-a-chip platforms (Imperial). Notably, by combining advanced microfluidics with cutting-edge quantitative cell imaging approaches to identify new means to prevent metastasis will, to our knowledge, be a first of its kind project.

Dr. Sam Au was appointed lecturer in the Department of Bioengineering in 2017 following a Tosteson Postdoctoral Fellowship at Harvard Medical School in the labs of Prof. Mehmet Toner and Prof. Daniel Haber. During his time at Harvard, Dr. Au developed numerous microfluidic platforms for isolating and studying the behaviour of metastatic cancer cells in the circulation. Dr. Au's laboratory at Imperial College focuses on developing biomimetic organ-on-chip platforms and translational lab-on-chip devices specifically for improving our understanding and treatment of cancer. Dr. Chris Bakal is a Reader at the Institute of Cancer Research whose laboratory is focused on describing the signalling networks regulating cancer cell shape determination. Dr Bakal is a pioneer in the use of Artificial Intelligence based methods to analyse image based screens, and over the last 10 years has developed numerous quantitative technologies and statistical tools for the high-throughput analysis of cell shape, and signalling dynamics at the single cell level. Recently, Dr Bakal has developed new approaches to integrate quantitative imaging data with genomic and proteomic datasets. By working in these two laboratories, we envision the student will truly be trained as a "scientist of the future" who is able to seamlessly integrate bioengineering and cancer biology methods in translational research.

Dr. Au will be responsible for training and guiding the PhD student along all stages of microfluidic platform development including: conceptualisation, CAD device design, SU-8 master mold microfabrication, PDMS replica soft lithography, microfluidic device operation and device validation. These skills are highly desired in many academic fields and industries including microfluidics, MEMS, medical device developments, semiconductors, aerospace & aeronautics, and will be immensely valuable for advancing the candidate's career.

Dr. Bakal will be responsible for providing the student training in a wide-range of cell cancer biology techniques including cell-line engineering (CRISPR-mediated tagging), quantitative live-cell imaging, genomic and proteomic analysis, and *in vivo* cancer models.

Multidisciplinary Training Strategy

To gain broader training applicable to a PhD studentship in bioengineering and cancer research, the student will also enroll in modules and workshops for both professional and technical development. **Dr. Au will teach a graduate-level cancer engineering module** beginning October 2019 that will be highly relevant for the student. Furthermore, the Graduate School of Imperial College offers such courses tailored to doctoral students that include topics such as, “Writing for Success: Publication”, “Perfecting Presentations: Conferences and Seminars”, “Careers: An Introduction to Career Planning for 1st year PhDs” and “Statistics: Regression Modelling”, which will be immensely beneficial for gaining relevant skills.

The ICR provides mandatory training in cancer biology via coursework, as well as additional training in programming, statistics, imaging, and mouse husbandry. Moreover, three weekly seminars presented by an international range of speakers, as well as ICR students and postdocs, will provide the student will outstanding exposure to the cutting edge of cancer research.

All this work is made possible by the close proximity of the Au and Bakal labs, which are located a short 15 minute walk from each other in South Kensington. The projected project timeline and sharing of student time across laboratories is outlined in Fig. 2.

Coordination of Student Time

Because all project aims involve coordination between the Bakal and Au labs, the student will spend time working with both teams throughout all three aims and he/she will be provided adequate laboratory space and resources throughout the duration of the PhD. During a typical week, we expect that the student will spend ~2 entire days in the Au lab working on devices, ~2 days in the Bakal laboratory on cell biology work (culturing, imaging, etc.) and ~.5 days in each laboratory meeting with Dr Bakal and Dr Au individually, as well as attending *both* laboratories’ weekly lab meetings and departmental seminars. Dr. Bakal, Dr. Au and the student will hold joints meeting once every 2 months to guide the overall direction of the project.The projected project timeline for the student time across both laboratories is outlined in Figure 3.

Literature references:

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6. S. F. Bakhoum *et al.*, Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* **553**, 467-472 (2018).
7. G. Dunphy *et al.*, Non-canonical Activation of the DNA Sensing Adaptor STING by ATM and IFI16 Mediates NF-κB Signaling after Nuclear DNA Damage. *Mol Cell* **71**, 745-760 e745 (2018).
8. L. Galluzzi, C. Vanpouille-Box, S. F. Bakhoum, S. Demaria, SnapShot: CGAS-STING Signaling. *Cell* **173**, 276-276.e271 (2018).
9. J. F. Almine *et al.*, IFI16 and cGAS cooperate in the activation of STING during DNA sensing in human keratinocytes. *Nature Communications* **8**, 14392 (2017).

Figures:

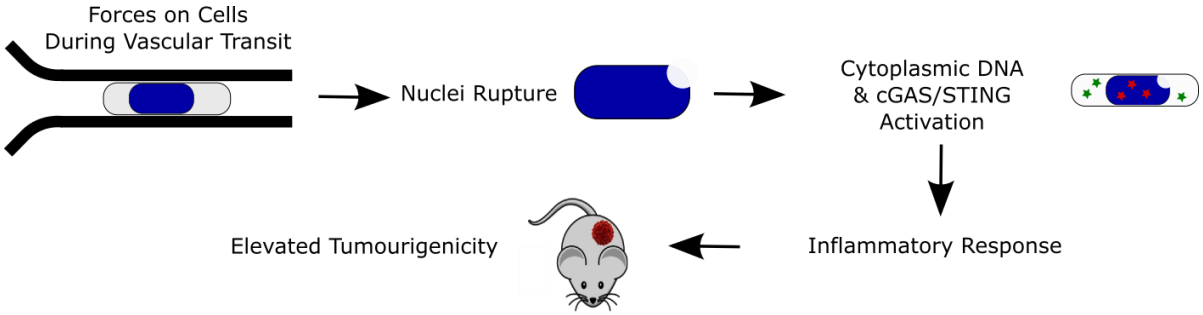

Figure 1: Hypothesised mode of cGAS/STING pathway activation in cancer cells circulating through the vasculature

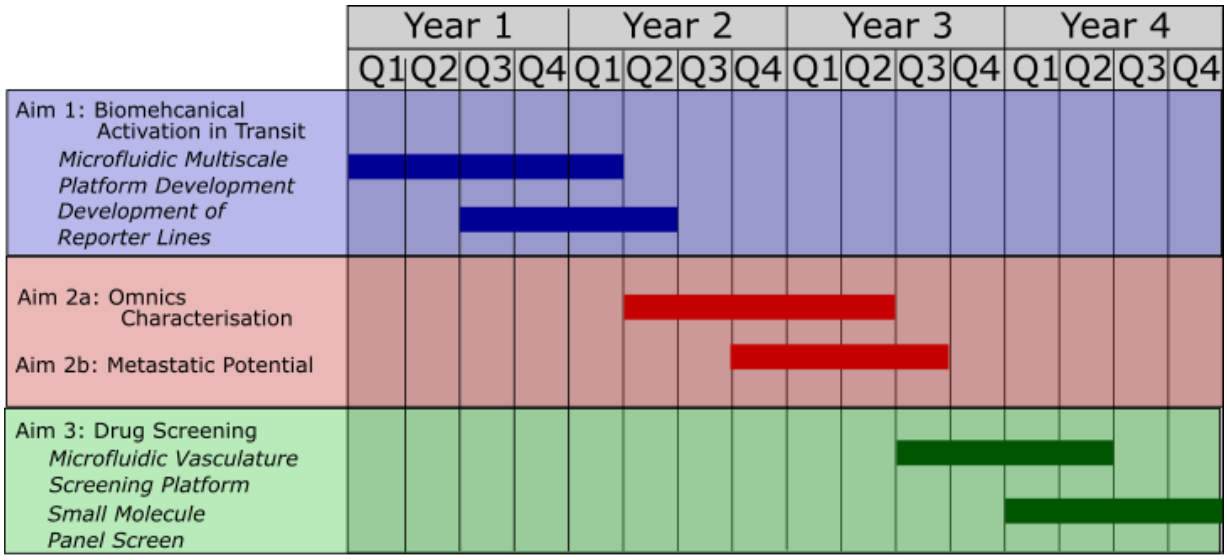

Figure 2: Gantt chart of proposed timeline and joint training plan for student.