Imperial College London | ICR The Institute of Cancer Research

Project Title: Understanding systems and molecular mechanisms underlying cancer immunotherapy for the development of precision immunotherapy with informed strategies

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Proposal outline

Recent breakthroughs in immunotherapy development have established that anti-tumour immunity is a major exploitable mechanism to fight cancer [1]. Notably, immune checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 antibodies abrogate negative regulatory mechanisms in the T cell system and enhance anti-tumour immune response, and have been clinically approved for various cancer patients including melanoma, renal cell carcinoma, ovarian cancer, and Hodgkin's disease [2]. Our recent investigation using a single cell technology identified PD-1 and regulatory T cells (Treg) as two major suppressive mechanisms in tumour-infiltrating T cells from melanoma patients (malignant skin cancer) [3]. PD-1 is a surface protein that has a role in suppressing T cell receptor (TCR) signalling and thereby inhibiting T cell activation. PD-1 is highly expressed in over-activated T cells (often called 'exhausted T cells'), and inhibits their reactions to antigen. Thus the blocking of PD-1 and its ligand PD-L1/L2 can release the activity of tumour-specific T cells [4]. Treg specifically express the transcription factor Foxp3 and suppress anti-cancer immunity, and are a promising target for cancer immunotherapy [5]. Importantly, the immune check point inhibitor anti-CTLA4 antibody not only blocks costimulatory signalling (precisely, CD28 signalling), but also depletes regulatory T cells (Treg). However, anti-CTLA-4 increases the T cell responsiveness to not only cancer antigens but also self-antigens, inducing autoimmune reactions [2, 6].

In order to understand these dynamic processes during anti-tumour immune response, Masahiro Ono and his group used Fluorescent Timer protein, which changes its emission spectrum spontaneously and irreversibly, and thereby developed a new tool for analysing time-dependent changes in antigen-reactive effector T cells and Treg, designated as *Timer-of-Cell-Kinetics-and-Activity*,*Tocky* [7, 8]).

The proposed project aims to increase the precision of cancer immunotherapy by improving the understanding of T cell regulation in animal models and clinical samples using a multidisciplinary approach. Under this major aim, the project has the following objectives.

Objective 1: To understand differential effects of immunotherapy on T cells from tumour tissues using the Tocky technology. In order to understand dynamic changes in these cells upon immunotherapy, the project will use computational codes that have been developed in the Ono lab, and the student will be trained for both experimental methods (multicolour flow cytometry) and computational analysis. Immunotherapy models include immune checkpoint inhibitors and viral immunotherapies, which have been developed in the Melcher lab [9].

Objective 2: To investigate new T cell subpopulations in tumour tissues that have unique

immunological functions. Multidimensional methods [3] will be used to identify effector, regulatory, and activated T cell subpopulations in tumour tissues.

Objective 3: To identify clinically meaningful T cell subpopulations using clinical samples. The student will analyse tumour-infiltrating T cells using multicolour flow cytometry, gene expression analysis, and the multidimensional methods and other statistical methods.

Feasibility

Firstly, the studentship holder will be trained for molecular and systems immunology in the Ono lab, analysing immune checkpoint inhibitors. and will apply the newly acquired techniques to virus-based immunotherapy
Year 1 – Investigation of in vivo dynamics of tumour-infiltrating T cells using Tocky and a tumour inoculation model (Ono lab)
Year 2 - Investigation of the differential effects of virus-based immunothearpy on tumour-reactive T cells (Melcher lab)
Year 3 - Multidimensional single cell analysis of tumour-infiltrating T cells (Ono lab)
Year 4 – Investigation of clinical samples from cancer patients (Melcher lab)

Multidisciplinary approach

We will aim to train the studentship holder for multidisciplinary research methods, specifically focusing on the translational phase of immunotherapy development. During the PhD project, it is expected that the student will be trained for the following skills.

- Immunological analysis of tumour samples
- Multicolour flow cytometry
- Flow cytometric data analysis and Tocky (including the use of R codes)
- Immunotherapy and animal handling (including intraperitoneal injection)
- Tumour inoculation models
- Cancer cell culture
- Primary T cell culture
- Multidimensional analysis
- Gene expression analysis (qPCR and sequencing)
- Human sample analysis (flow cytometric analysis, qPCR)
- Statistical analysis of clinical data (including Kaplan-Meier survival analysis)

To be trained in these multidisciplinary methods, the student will receive trainings with the following structure:

In the first year, the studentship holder will be trained for basic experimental immunology and computational methods in the Ono lab. The skills will include the isolation of lymphocytes, tumour inoculation models, checkpoint inhibitors, multicolour flow cytometry, flow cytometric data analysis using the R language, which is required to analyse Tocky data [7]. The studentship holder will be supported for her/his daily experiments by post-doc, other PhD students in the Ono group. Technical training courses will include (1) flow cytometry training (provided by the core facility), (2) training courses for animal experiment (for personal license), and (3) introduction to the R language (Imperial).

In the second year, the studentship holder will be further trained for tumour immunotherapy in the Melcher lab. S/he will apply the Tocky tool to the tumour/ immunotherapy models in the Melcher lab, further developing the acquired skills. The student will be supported for her/his daily experiments by post-doc, other PhD students in the Melcher group, and also will keep an access to the Ono group for technical support.

In the third year the studentship holder will be trained for multidimensional analysis methods (e.g. t-SNE) and gene expression analysis (qPCR, and a small scale sequencing analysis).

In the fourth year, the studentship holder will be trained for analysing human samples from cancer patients. S/he will apply her/his skills in experimental and computational methods to address the problems in clinical immunology.

Literature references

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