Joint PhD Project Proposal Lyon 1 University / Tohoku University

InnovInOnco Graduate Program

Title : Role of hypoxia in human tumor infiltrating dendritic cells migration

Co-supervision:

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Scientific context and objectives

The history of cancer immunotherapy began over a century ago with early, modest successes using host immune modulation. In the past decade, the discovery of immune checkpoints and drugs targeting them has revolutionized treatment for many cancers. Immune checkpoint inhibitors (ICIs) restore antitumor immunity suppressed by the tumor microenvironment. While ICIs are effective against “inflamed” tumors containing preactivated T cells, “cold” tumors lacking immune infiltration require alternative strategies.

Cancer vaccines can generate new tumor-reactive T cells, enhancing ICI responses or inducing immunity in non-inflamed tumors. Cellular immune amplification and epitope spreading are essential for vaccine efficacy. Clinical and preclinical studies show that therapeutic responses are associated with cytotoxic T cell (CTL) reactivation targeting both vaccine and additional tumor antigens. This spreading results **from dendritic cells (DCs) capturing and presenting tumor antigens released by CTL-mediated lysis**. Many tumors associated DCs were described such as cDC1 and cDC2, DC3, DC derived from monocytes or langerhans cell-like DC [1]. Among DC subsets, type 1 conventional DCs (cDC1) are the most efficient at antigen cross-presentation, a process that primes CD8+ T cells[2]

We have demonstrated that this particular cDC1 subset infiltrates breast and ovarian tumors[3] . Hypoxia significantly shapes dendritic cell (DC) behavior, enhancing their maturation, migratory capacity, and altering immune function. The hypoxic microenvironment—common in inflamed, tumor, or lymphoid tissues—activates pathways in DCs, most notably through hypoxia-inducible transcription factor HIF-1α, impacting survival, activation, and T cell stimulation[4]. Nevertheless, whether oxygen gradients guide dendritic cells directionally (aerotaxis) or modulate their motility (aerokinesis) is unknown in human cDC1 and in other described tumor associated DC subsets. Understanding how oxygen landscapes shape different DC subset trafficking between tumor core and lymphoid structures is essential to improve cancer vaccines and combinatorial immunotherapies.

The central objective of this PhD is to decipher how controlled oxygen microenvironments regulate the migration of human cDC1 (versus others DC subsets) and to identify physical strategies to steer their trafficking in tumors. This interdisciplinary project bridges tumor immunology, biophysics of cell migration, and microfluidic engineering.

Original approach and methodology

1) Immunological characterization

At CRCL, human cDC1,cDC2 , monocytes derived DC and Langerhans cell like DC will be generated from hematopoietic progenitors[5,6]and characterized by:

- Flow cytometry
- Maturation and activation markers

- Functional assays such as transwell migration in response to chemokine gradient (in place) and antigen presentation to CD8 T cells (in place)

2) Reconstitution of controlled oxygen landscapes using microfluidics and biophysical characterization of cDC1 migration.

We propose to investigate how the oxygenated landscape could modulate the migration of human cDCs using migration and aerotaxis tests developed jointly over the past year at the iLM and IFS (see references [8–11]). We will use advanced two-layer microfluidic platforms enabling an independent control of O_2 concentration in two gas channels (from about 0.5% to 21% pO_2). We will either impose homogeneous O_2 level, either stable or dynamic (oscillatory, flipping…) O_2 gradients. This platform is compatible with 2D (on fibronection or collagen coated surfaces) and 3D (in collagen gels) migration studies. It can reproduce in vivo-like tumor oxygen heterogeneity and allow quantitative live-cell imaging of migration.

Using high-resolution videomicroscopy and automated cell tracking, we will quantify at iLM: (1) cell speed, persistence, (2) directional bias and aerotactic index under gradients, (3) cytoskeletal dynamics, and eventual transitions between amoeboid and constrained modes in gels *Vs.* coated surfaces. Models (biased random walk, reaction-diffusion) will identify whether hypoxia induces aerotaxis or modulates effective cellular random space exploration (aerokinesis) or both.
Microfluidic migration capacity will be correlated with immune function to determine whether oxygen-driven motility states impact T cell priming capacity.

3) Characterization of underlying signaling mechanisms driving the migratory changes of various DC subsets under hypoxia.

We will evaluate the activation of oxygen-sensing pathways, cytoskeletal regulation, and cell-ECM adhesion using fluorescence reporter directing introduced in cDC by lentiviral infection (done on progenitor during the differenciation phase; in place in the CRCL). For example, real-time analysis include HIFα/HIFβ dimerization biosensors or peptide-based fluorescent probes. Visualization of cytoskeletal elements (actin, microtubules) via immunofluorescence with phalloidin (F-actin), anti-tubulin, or anti-myosin antibodies, followed by microscopy to quantify organization and dynamics will be done. Image analysis and Fourier transform, structure tensors can quantify fiber orientation to monitor cytoskeleton modifications.

The impact of the tumor microenvironment will be also modelized using supernatant of fresh dilacerated human tumors (from breast and ovarian cancers, Collaboration CLB hospital; see *Sakref et al.*[7]).

Expected outcomes and innovation:

1. Establish the first quantitative characterization of oxygen-guided migration in human cDC1 (in comparison to others DCs such as cDC2 and monocyte-derived DCs).
2. Discriminate aerotaxis versus aerokinesis in immune cells.
3. Provide a predictive physical framework linking tumor oxygen maps to immune cell trafficking.
4. Open translational perspectives to enhance dendritic cell–based immunotherapies by engineering oxygen niches or combining with vascular normalization strategies.

The originality lies in coupling clinical tumor immunology (CRCL), quantitative biophysics and cell tracking (iLM) and state-of-the-art oxygen-controllable organ-on-chip technology (IFS, Tohoku)

Candidate profile, feasibility and collaboration structure:

We seek an excellent international candidate (new to a French university) with a background in biophysics, biomedical engineering, immunology, or related fields, motivated to work at the interface of physics and cancer immunotherapy. Sabreena Khan, a cancer bioengineering master 2 student

that arrived in September 2025 in France from Pakistan,has already started a~6-month internship both at CRCL and iLM on that project. She will perfectly fit with the proposed PhD internship.

The collaboration between IFS and iLM started in 2019 when K. Funamoto together with a master student spend two months in Lyon. Since then, more than 5 collaborative papers were published on the control of O_2 for cell migration studies (see references [8–11]). A master 2 student of Funamoto Lab, Taishi Nakamura is visiting iLM for 2 months in Feb.-March 2026. He brought various molds of two-layer microfluidic platforms and started to make devices in iLM clean room. He will perform preliminary experiments with cDC1 cells jointly with S. Khan. He will start a PhD at IFS from April 2026 insuring a smooth transfer of knowledge between France and Japan. **Therefore, this tripartite consortium between IFS, CRCL and iLM is already launched!**

The Lyon–Tohoku partnership is structurally supported by the IFS LyC Collaborative Research Project (600,000 JPY/year), ensuring mobility and experimental continuity for the PhD student. We plan an annual visit in Sendai of about 3 months /year for the PhD student in order to implement and optimize devices for human immune cells. The consortium will also respond to national and international funding calls and the PhD student may also apply to JSPS programs. The student will be co-supervised with structured annual mobility (France ↔ Japan), joint lab meetings, participation to the InnovInOnco session of the annual ICFD international conference in Sendai (November), and co-authored publications.

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