PhD PROJECT PROPOSAL

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Monitoring the transendothelial migration of T-ALL leukemic cells using innovative biophysical imaging approaches

Lead supervisor: **Arnauld SERGÉ** (MCU AMU, HDR) - *Laboratory Adhesion and Inflammation* (LAI) *Molecular interactions* group

Biophysicist, specialized in single molecule tracking, membrane protein dynamics, image processing

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Co-supervisor: **Magali IRLA** (DR2 Inserm, HDR) - *Center of Immunology Marseille-Luminy* (CIML) *Immune tolerance and T cell differentiation* team leader

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Abstract (10 lines)*

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive cancer, with cancer dissemination within organs being a major driver of poor prognosis. The mechanisms facilitating T-ALL dissemination remain poorly understood, although this process represents a therapeutic target of choice. Preliminary data indicate that T-ALL cells overexpress the cytokine RANK ligand (RANKL), which interacts with its receptor RANK on endothelial cells, likely promoting transendothelial migration within organs. This project aims to elucidate the role of the RANK/RANKL axis in T-ALL dissemination and assess the therapeutic potential of repurposing Denosumab, a neutralizing anti-RANKL antibody, to limit disease dissemination and progression. Using advanced biophysical methods as well as leukemic cell line and primary cells from T-ALL patients, the project will investigate the molecular mechanisms driving T-ALL transendothelial migration. This interdisciplinary project focuses on leukemia, transendothelial migration, and cutting-edge imaging techniques, aiming to establish RANKL neutralization as a novel strategy to prevent T-ALL progression.

Keywords*

T-cell acute lymphoblastic leukemia, endothelial cells, blocking antibody, RANK/RANKL interactions, nanoscopy, single molecule tracking, super-resolution, 2-color analyses, molecular interactions, membrane dynamics, flow chamber assays

Scientific question and Objectives (10 lines)*

The dissemination of T-ALL cells into organs is a key determinant of disease severity and relapse. These cells infiltrate multiple organs, including the bone marrow, spleen, and, most critically, the central nervous system. However, the mechanisms underlying their transendothelial migration remain unclear. Our data reveal that T-ALL cells overexpress RANKL, which interacts with its receptor RANK on endothelial cells, stimulating their functional properties. This suggests that the RANK/RANKL axis between endothelial and T-ALL cells promotes leukemia dissemination. The objectives of this project are to address the role of the RANK/RANKL axis in (1) T-ALL transendothelial migration using Transwell assays, and in (2) T-ALL adhesion to endothelial cells using laminar flow chamber, as well as (3) the dynamics of RANK/RANKL at T-ALL/ endothelial cell contacts using single molecule tracking. The therapeutic potential of neutralizing the RANK/RANKL axis will be evaluated using an anti-RANKL antibody to hamper T-ALL transendothelial migration. Altogether, this project by exploring these mechanisms and validating Denosumab as a potential strategy to limit T-ALL progression is expected to pave the way for a novel therapeutic approach to improve T-ALL management and patient outcomes.

Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

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This interdisciplinary project seeks to unravel the mechanisms underlying T-ALL dissemination, with a particular focus on how leukemic cells extravasate into tissues. Specifically, it investigates the role of the RANK/RANKL axis in promoting T-ALL adhesion to and transmigration across endothelial barriers. Using Transwell and flow chamber assays, this project aims to investigate how T-ALL cells migrate through endothelial monolayers and evaluate the potential of the anti-RANKL antibody to inhibit this process. RANKL expression levels will also be monitored by flow cytometry in primary cells from T-ALL patients provided by clinicians to determine whether it can serve as a severity marker for the disease.

Single-molecule tracking and super-resolution microscopy will provide high-resolution insights into the dynamic interactions between RANK and RANKL at cell-cell interfaces. The experimental model includes T-ALL cell lines (Jurkat and HSB-2), patient-derived primary T-ALL cells, and human umbilical vein endothelial cells (HUVEC), while peripheral blood mononuclear cells (PBMCs) and naïve T cells from healthy donors will be used as controls. Flow chamber assays will simulate blood shear flow conditions to assess leukemic cell rolling, adhesion, and extravasation, while testing the efficacy of anti-RANKL antibody in disrupting these processes.

Additionally, advanced biophysical techniques like stimulated emission depletion microscopy (STED) and HaloTag fluorophore labeling will visualize molecular interactions at nanometer resolution. Complementary approaches, including fluorescence recovery after photobleaching, will further elucidate membrane diffusion dynamics, supported by rigorous statistical analyses to ensure data robustness. The therapeutic potential of blocking RANK/RANKL interactions, through anti-RANKL antibody, will be evaluated as a strategy to hinder T-ALL dissemination. This project integrates cutting-edge microscopy, molecular biology, and translational research to shed light on T-ALL disease and explore novel therapeutic interventions. Through its innovative methodologies and interdisciplinarity, this project is expected to advance our understanding of leukemia progression.

Interdisciplinarity and Implication of the two labs (15 lines)*

(In this section the collaboration of the two laboratories will be explained in detail to explain why the project cannot be conducted by one team alone)

Specialized in single-molecule tracking and image analysis at the *Laboratory Adhesion and Inflammation* (LAI), with a background in physics and in biology, Arnauld Sergé (Associate Professor at Aix-Marseille University) develops innovative imaging approaches targeting interactions within cell-cell contacts. The project will be conducted in collaboration with Magali Irla (Research director at Inserm) who leads a team entitled “Immune Tolerance and T Cell Differentiation” at the *Center of Immunology Marseille-Luminy* (CIML). M. Irla has an internationally recognized expertise in T-cell crosstalk in physiopathology. Arnauld Sergé and Magali Irla have already conducted several successful collaborative projects.

This biophysical project is fundamentally interdisciplinary. On one side, the project addresses the physics of cellular motion and interaction, together with molecular dynamics within the cell membrane, with a need to develop innovative imaging techniques (Team Arnauld Sergé, LAI). On the other side, it concerns the transendothelial migration of T-ALL cells that will be studied using Transwell assays with both cell lines and primary T-ALL cells, available in the partner team (Team Magali Irla, CIML). It will define whether RANKL expression levels monitored by flow cytometry in primary cells from T-ALL patients can serve as a severity marker for the disease.

To conduct this project, dedicated innovative tools will be developed for T-ALL cell motion, arrest on endothelial cells and extravasation, as well as single molecule labeling, automated single molecule tracking software, characterization and quantification of molecular trajectories. Such experimental and analytical developments are expected to ultimately beneficiate to the whole academic community. This PhD project is part of an interdisciplinary project at the interface between pathological T cells, physics and image analysis.

Specify with whom the person recruited will collaborate and on what aspects *

The PhD student recruited will collaborate in:

- Team of Dr. Arnauld Sergé, LAI with researchers, engineers, and students, experts in biophysics, including flow laminar chamber (fluidic and microscopy), single molecule and STED super-resolution experiments. The lab develops in-house analysis software to quantify rolling, adhesion and transmigration steps and single molecule tracking.

- Team of Dr. Magali Irla, CIML with researchers, engineers, and students, experts in immunology, including Transwell

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assays, with Jurkat leukemic cells transmigrating through HUVEC monolayers as well as flow cytometry analysis.

PhD student's expected profile*

We are seeking an interdisciplinary candidate in biophysics with a strong background in cell physics, imaging, and microfluidics, along with good experience in programming for image analysis. Additional knowledge in optics, statistics, and cell and molecular biology would be a bonus.

This project is entirely new

Two to five references related to the project*

Ahern, E., Smyth, M.J., Dougall, W.C. et al. Roles of the RANKL–RANK axis in antitumour immunity — implications for therapy. **Nat Rev Clin Oncol** 2018

Lyu A, Nam SH, Ryan S, Humphrey, Horton TM, Ehrlich LIR. *Cells and signals of the leukemic microenvironment that support progression of T-cell acute lymphoblastic leukemia (T-ALL).* **Exp Mol Med**. 2024 Nov;56(11):2337-2347.

Irla M. *RANK Signaling in the Differentiation and Regeneration of Thymic Epithelial Cells.* **Front Immunol.** 2021 Jan 22;11:623265.

Maillot L, Irla M, Sergé A. *Single Molecule Tracking Nanoscopy Extended to Two Colors with MTT2col for the Analysis of Cell-Cell Interactions in Leukemia.* **Bio Protoc.** 2022 Apr 20;12(8):e4390. doi: 10.21769/BioProtoc.4390.

Sergé, A., Bertaux, N., Rigneault, H., Marguet, D. *Dynamic multiple-target tracing to probe spatiotemporal cartography of cell membranes*. **Nature Methods** 2008

Two main publications from each PI over the last 5 years*

Arnauld Sergé (LAI):

1. Santamaria JC, Chevallier J, Dutour L, Picart A, Kergaravat C, Cieslak A, Amrane M, Vincentelli R, Puthier D, Clave E, Sergé A, Cohen-Solal M, Toubert A, Irla M. *RANKL treatment restores thymic function and improves T cell-mediated immune responses in aged mice*. **Science Translational Medicine 2024** Dec 4;16(776):eadp3171.
2. Gorshkova O, Cappaï J, Maillot L, Sergé A*. *Analyzing normal and disrupted leukemic stem cell adhesion to bone marrow stromal cells by single-molecule tracking nanoscopy*. **J Cell Sci. 2021** Sep 15;134(18):jcs258736. * corresponding

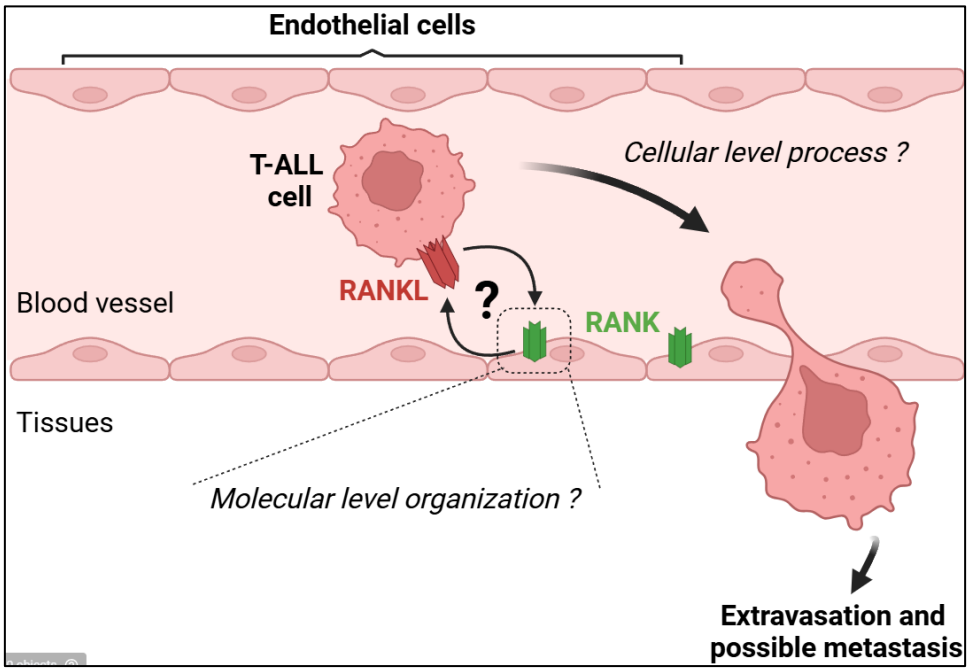
Magali Irla (CIML):

1. Santamaria JC, Chevallier J, Dutour L, Picart A, Kergaravat C, Cieslak A, Amrane M, Vincentelli R, Puthier D, Clave E, Sergé A, Cohen-Solal M, Toubert A, Irla M*. *RANKL treatment restores thymic function and improves T cell-mediated immune responses in aged mice*. **Science Translational Medicine 2024** Dec 4;16(776):eadp3171. * corresponding
2. Borelli A, Santamaria JC, Zamit C, Apert C, Chevallier J, Pierre P, Argüello RJ, Spinelli L, Irla M*. Lymphotoxin limits Foxp3+ regulatory T cell development from Foxp3lo precursors via IL-4 signaling. **Nature Communications 2024** Aug 14;15(1):6976. * corresponding

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Project's illustrating image



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