PhD thesis project:

"Understanding viral escape to immunity and antivirals from whole genome sequencing of Human Cytomegalovirus genome "

Director: Professor Sophie Alain MD, PhD co-director of UMR INSERM 1092, RESINFIT Limoges University and of the French National Reference Center for Herpes Viruses, Limoges University Hospital
Partners: Medical Genomic platform and bioinformatics unit of the Limoges University Hospital

Beginning: September-October 2023

Cytomegalovirus (CMV), an herpesvirus, is a major pathogen in transplantation, both because of its indirect immunosuppressive and proinflammatory effects and because of its direct effects, particularly on the transplanted organ. It also remains the leading cause of congenital neurological deficit. The available antivirals are virustatic, and the control of viral replication by the immune response is an essential adjuvant in the therapeutic or vaccine effectiveness. To date, the correlates of protection or control of infection are poorly understood. For a virus of this size, which encodes more than 150 proteins, and interacts closely with cells by adapting to the cell type, identifying the genes and more precisely the polymorphisms involved in the immune response escape is extremely difficult. Despite increasing knowledge of many CMV genes in immune escape, or in the control of latency, the results remain partial. Only a "global" approach can map all the mechanisms simultaneously involved. The recent development of high-throughput technologies now makes it possible to study both the variations in the viral genome and thus the selection of pathogenic strains and the transcription of viral messengers and miRNAs in specific pathological situations, directly from human samples at the site of infection. The bioinformatics analysis of these large data sets is being developed at the National Reference Center for Herpesviruses in Limoges and will make it possible to identify activation patterns and genetic particularities of isolates during therapeutic escape, depending on the pathologies studied. This opens the way to new preventive and curative approaches. The HORUS project aims to "Casting light on **HO**st-cytomegalovi**RU**s interaction in **S**olid organ transplantation" and received European funding to begin in November 2022 with the aim of identifying kidney recipients at high risk of CMV infection and of refractory infection through the development of an algorithm. This algorithm being further validated by an experimental cohort.
The HORUS project from Bordeaux University aims to "Casting light on **HO**st-cytomegalovi**RU**s interaction in **S**olid organ transplantation" and received European funding to begin in November 2022 with the aim of identifying kidney recipients at high risk of CMV infection and of refractory infection through the development of an algorithm. This algorithm being further validated by an experimental cohort.
This project involved 19 clinical renal transplant and research teams, from France, Switzerland, Poland, Belgium, Italy and Germany. The overall goal of "HORUS" is to improve our understanding of the host-virus relationship between CMV and solid organ transplant recipients for the discovery of signatures integrating viral, clinical and immunological characteristics associated with CMV control, with the ultimate aim to decreasing the incidence of CMV, better managing difficult- to treat infection and avoiding the use of unnecessary antiviral therapy and for the discovery of new molecules able to targets specifically CMV-immune response without increase acute-rejection risk. This project will answer to three unaddressed questions:
1. How to identify the host-virus interactions that impact the incidence of CMV infection after solid-organ
transplantation?

2. How to identify the host-virus interaction associated with efficient control of CMV infection?
3. How to improve the prevention and management of CMV infection using immunomodulatory regimens that enhance immune response to CMV?

The "HORUS-exploratory cohort" will be a homogeneous cohort of kidney transplant recipients with homogeneous immunosuppression coming from the 450 patients of day 0 of graft cohort and will consist in:

- 60 D+R- patients: - 20 without CMV replication post transplantation
- 20 with CMV replication with fast clearance
- 20 with "difficult-to-treat" CMV replication (mutation, viral or clinical relapse)
- 40 D+R+ patients: - 20 without CMV replication post transplantation
- 20 with CMV replication.

The reference center will be specifically involved in the following task:

Explore viral characteristic (especially viral strains evolution and their ability to escape the immune response and the treatment during complicated infections) and viral-host interaction to understand why patients undergo CMV infection and difficult-to-treat infection.

The Reference Center will analyse whole genome of CMV circulating isolates during patient's follow-up and in case of emergence of resistance, to better understand the role of emerging CMV variants. In parallel, she/he will analyse the capacity of HCMV to reactivate from donor's renal tissue and from donor's lymphocytes collected from kidney conservation liquid before grating. This task involves both optimization of NGS in low viral load samples and analysis of HCMV RNAs from kidney tissues or lymphocyte to identify HCMV reactivation.

This represents a great opportunity for a PHD student to develop his skills in whole genome sequencing transcriptomics and bioinformatics in our team, in the context of a global multicentric project.

Year 1-2: Best method or best combination of methods for reliable and sensitive CMV whole genome sequencing

Whole genome analysis optimization:

Several methods from our team and from others yet gave some light on hypervariable regions of the genome and natural geographic variability. Although they are limited to date in terms of sensitivity for complex samples with high human genome load, and in long genome reconstruction.

Awaiting Horus recruitment of patients and sample collection, the PhD student will first work on CMV strains and isolates, and on various samples from the Reference Center biocollection to develop the CMV whole genome analysis by complementary methods (illumina capture, Ampliseq Ion torrent and long range Pac Bio analysis). She/He will work within an international collaborative network to first explore the sensitivity and reliability of each method, with the challenge of samples of varying complexity (saliva, urine, plasma and whole blood) and varying viral loads. Illumina capture methods, used by several laboratories are easier to implement but their sensitivity can be low and must be tested on complex matrices. Ampliseq method is very sensitive but PCR can increase the error rate and PCR efficiency can vary between primers. It could be used as a control for difficult regions. PacBio could help solve alignment difficulties with a long-range analysis. The student will learn bioinformatics of whole genome analysis using the Reference center's AspiCMV pipeline, background removal, genome variability analysis using the Graçy pipeline for identification of variants of interest and reconstruction of genomes using Harold pipeline recently published by J Breuer team (Royal London Univ. UK). The PhD student will then test the method on samples from well-documented patients from the Reference Center biocollection. And finally applied to Horus patient's samples.

HCMV reactivation assays from tissues and leukocytes will also have to be optimized during these two years. For CMV reactivation potential transcripts from IE genes will be quantified, and also genome copies by digital PCR, after stimulation of leukocytes or tissues). And if possible, transcriptomic single cell analysis will be performed. This model will be developed at the Inserm U1092.

Years 2-3 will be devoted to the Horus exploratory cohort samples analysis, using a combination of selected methods for whole genome analysis of CMV genome variability in kidney transplant samples from patients with refractory infection or multiple recurrences of infection, compared with sustained responders to antiviral therapy by statistics and artificial intelligence methods in conjunction with clinical and immunological data from Consortium members.
Lastly, samples from the exploratory cohort will be explored for HCMV reactivation.

Collaborations:
With the medical genomics platform (Emilie Guerin) and the Reference Center team (Mélissa Mayeras et Sébastien Hantz) at Limoges Univ Hospital including biostatisticians Valentin Tilloy and Daniel Diaz.
In the context of whole genome sequencing CMV whole genome capture and bioinformatics analysis, notably with HAROLD, is developed with Professor Judy Breuer's Laboratory at the Royal London Hospital. The Ampliseq method is currently in development at the reference center, in collaboration with Timothy Kowalik group, UMASS, Boston, and Pac Bio analysis will be developed locally by the student with the genomics engineers.
Finally, the PhD student will interact regularly with the Horus Consortium members.

SKILLS REQUIRED:
Master 2 level
English level: Fluent
Technical skills
Experience in capture sequencing or ampliseq sequencing would be appreciated, same as previous experience in bioinformatics analysis.
Experience in cell-culture.
Personal qualities: highly motivated, rigorous, organized, ability to work independently and in team, ability to synthetize and communicate results to the team and to the Consortium.

BENEFITS
3-year contract - monthly gross salary 2 046,71 €

APPLICATION
Application should include:
1. Letter of motivation and why the applicant considers her/himself a good match for the position.
2. Curriculum vitae, including a description of relevant skills and experiences.
3. Copy of Master diploma as well as grades and ranking of Master (1 and 2 when available).
4. Names, e-mail addresses and telephone numbers to 1-2 references.
Master of Microbiology, virology (or Biology or Biochemistry or Molecular biology) including NGS
It should be sent to Sophie Alain (sophie.alain@unilim.fr) and Valentin Tilloy (valentin.tilloy@chu-limoges.fr) and Cindy Demay (cindy.demay@unilim.fr)
Applicants will be selected after an interview with the team.

FUNDING: The PhD student salary will be covered by European funding for Horus project and by the University of Limoges. And the student will depend on the Inserm unit 1092 with an Inserm convention.

BIBLIOGRAPHY:

RESINFIT Website: https://www.unilim.fr/resinfit/
CNR Website: cnr-herpesvirus.fr

HORUS project, Horizon EU 2021

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