

ESOT Transplant Fellowship 2020 - Report

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Host institution: Pr Oriol Bestard group, Vall d'Hebron Research Institute, Barcelona, Spain

Project description and outcomes achieved:

The project during my fellowship in the team of Pr Bestard, in Barcelona (first in the IDIBELL laboratory, then at the Vall d'Hebron Research Institute), was to develop an expertise in immune monitoring strategies in the field of solid organ transplantation. In order to do so, two main technologies were targeted: lymphocyte function assessment through Enzyme Linked Immune absorbent Spot (ELISpot), and traditional flow-cytometry to detail immune phenotypes.

The initial scientific project was nicknamed IMAGINATION (Infectious Memory Along kidney transplantation), focusing on the reconstitution of anti-infectious immunity in kidney transplant recipients.

The foundations of this project were the immunomonitoring expertise of the team of Pr Bestard, and two patients' cohorts from Grenoble University Hospital.

I – Immunomonitoring expertise

During my stay in Pr Bestard's team, I could participate in several projects that focused on immunomonitoring, both as a biologist and as a statistician. Below are listed a selection of these projects I had the chance to participate in.

a) Panel Reactive T-cells project

The Panel Reactive T-cells project (PRT) is an ongoing project focusing on the detection and identification of HLA-specific T cells against the donor (Donor Specific T cells, DST). In the CELLIMIN trial, previously published by the team¹, these DST evaluated pre-transplantation were shown to be associated with a higher risk of rejection. In the PRT study, we focus on a panel of potential donors, similar to the Panel Reactive Antibodies. This PRT evaluation could provide a donor non-specific evaluation of the T alloreactivity, as

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a new rejection risk biomarker. To do this, the team used splenocytes from multiple donors, depleted them in CD3 cells to eliminate donor cells reactivity and used them as stimulants in traditional Interferon-gamma ELISpot assays. By performing a count of positive ELISpot assays against 10 different donors, we define the PRT value. For example, if a patient had a positive T cell ELISpot against 6 out of 10 different donors, using a predefined cutoff value for positivity, this patient was considered to have a 60% PRT value.

In parallel, we also performed more classical virus-specific ELISpot, against CMV and EBV, and Donor Specific T-cell ELISpots. This allows to investigate the correlations between PRT, DST reactivity (in terms of number of cells) and anti-viral reactivity. Also, we associate the evaluation of Donor Specific Antibodies by traditional Luminex assays. This allows to investigate the association between DST and DSA. Using these two assays, we can better understand the triggers of allospecific memory. The PRT study is still ongoing, recruiting patients with various immunization profiles.

For this PRT study, I was involved in samples manipulations, CD3 depletion, performing the ELISpot assays and analyzing the first results.

b) Flu-specific B-cells response to vaccination

Investigating further the determinants of immunological memory in kidney transplant recipients, the Belavac study focused on evaluating the flu-specific memory B cell populations. The core idea was to compare the evolution of a flu-specific memory B cell populations, after a flu vaccine injection, in kidney transplant recipients treated either with tacrolimus or with belatacept. This work showed that belatacept-treated patients are less likely to develop a novel vaccine-induced immunity against influenza, as defined by memory B cells. To detect these memory B cells, a specific fluorescent-labeled influenza antigen was developed. This antigen was used for characterization of CD20+CD27+IgD- cells, considered to represent the memory B cells compartment. However, recall responses, for patients already vaccinated against the same influenza strain, do have an increase in flu-specific memory B cells. This work was recently published².

For this Belavac study, I was involved in the flow cytometry analysis and the statistical analysis, as well as in the writing of the manuscript.

c) Phenotypic investigation of the CELLIMIN trial

The CELLIMIN trial¹, already mentioned above, was a trial including new kidney transplant recipients, with a follow-up over the first year post-transplantation. This follow-up included mainly ELISpot assays at the time of transplantation, then regularly post-transplantation. Samples for a flow cytometry analysis were also harvested and then analyzed using several immunological panels for Peripheral Blood Mononucleated Cells (PBMC), focusing on

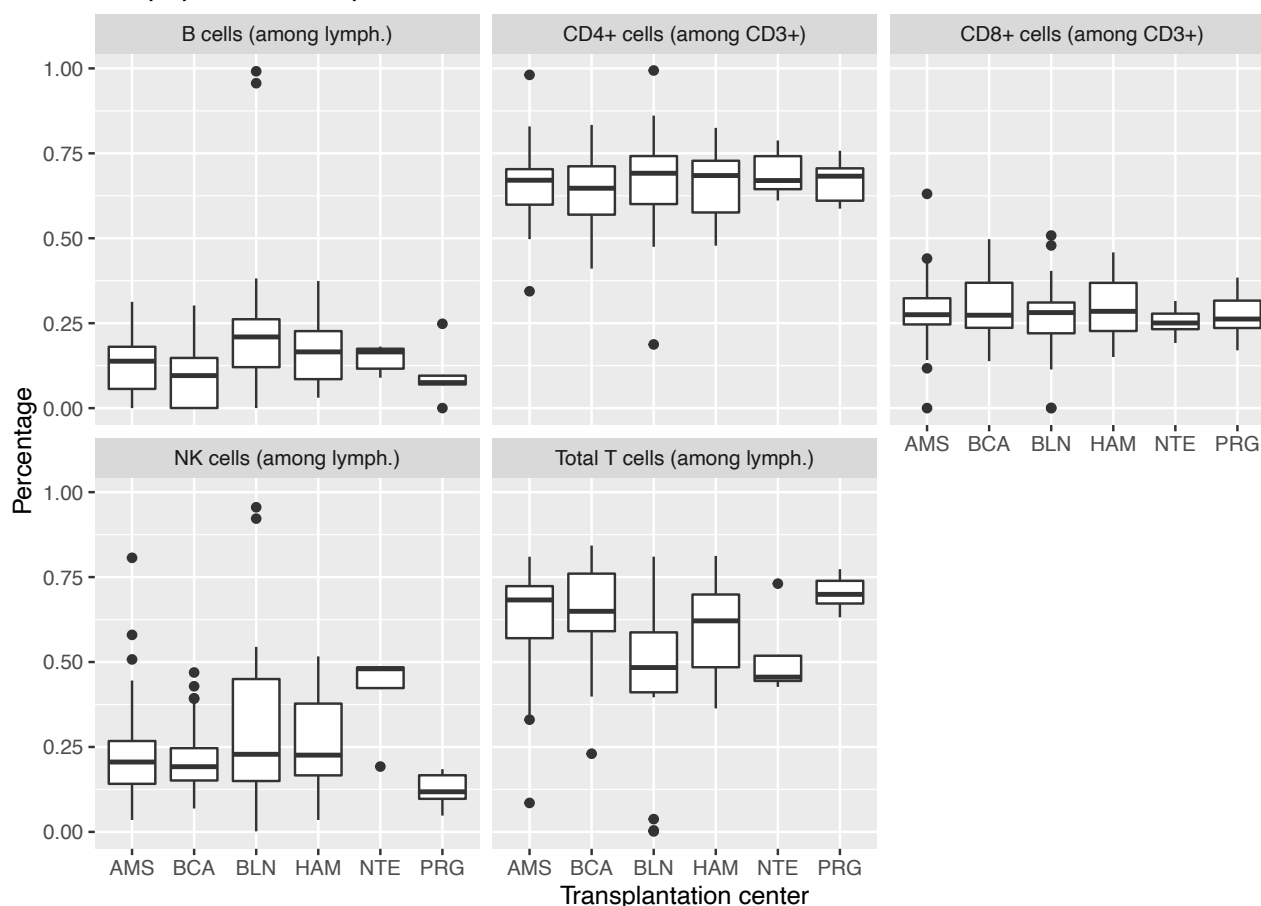
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various immunological compartments: basic immunological cells, T cells, B cells, dendritic cells, NK cells and Tregs.

Patients were included in 6 centers across Europe (Amsterdam, Barcelona, Berlin, Hamburg, Prague, Nantes), with a pre-transplantation assessment and 5 visits over the first-year post-transplantation. These data have not yet been explored and I started working on a R-based pipeline for an automated analysis of these numerous flow-cytometry file. Using this analysis pipeline, I could provide some first comparisons of centers and times. The next figure is a comparison of the 6 different transplant centers, depending on the lymphocyte subpopulation (B cells, T cells, CD4 and CD8 T-cells and NK cells), with PBMC sampled at the time of transplantation, before any immunosuppressive treatment.

Subpopulation comparisons AT BASELINE



d) ELISpot-based T-alloreactivity assessment and CYP genotype polymorphisms

The team performed an analysis of ELISpot-based allo-specific T cells (Donor Specific T cells, DST), like in the CELLIMIN trial, as a biomarker of the kidney transplant rejection risk. In this work, the CYP3A4 and CYP3A5 cytochrome genotypes were also assessed. These

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cytochromes are known to be involved in tacrolimus metabolism. As such, cytochromes polymorphisms impact tacrolimus exposure, as a pharmacokinetic parameter. The idea of this study linking ELISpot and CYP cytochrome was to evaluate the potential interaction between T-cell memory (evaluated by ELISpot) and tacrolimus underexposure (evaluated through genotyping). This allowed to confirm that DST assessment before transplantation was indeed a predictive biomarker of biopsy-proven allograft rejection within the 1st year post-transplantation, independently of CYP polymorphisms and more traditional risk factors such as preformed DSAs³.

For this study, I had the opportunity to perform a thorough statistical analysis, including multivariate models with interaction effects and I helped rewrite the initial paper to focus on the added value of CYP polymorphisms on top of DST analysis.

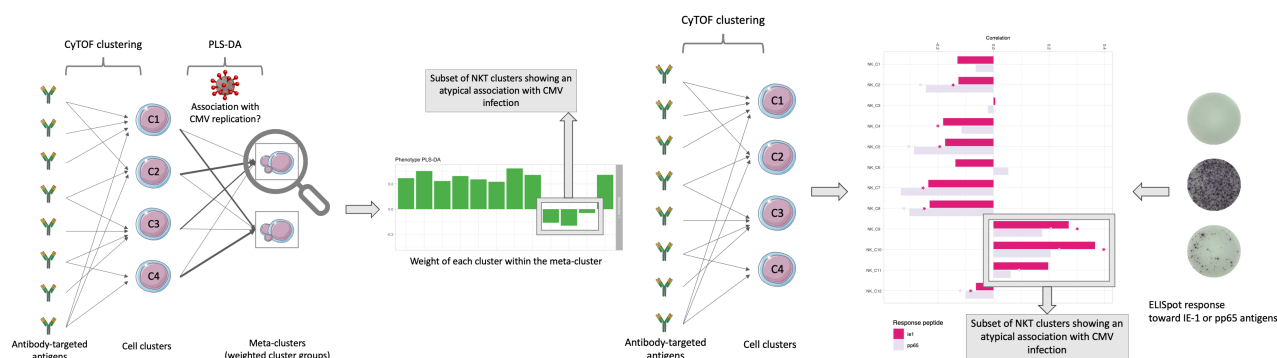
e) CICERONE study

Together with the team of Paolo Cravedi at Mount Sinai Hospital (New York, USA), Pr Bestard's team performed a deep phenotyping study of CMV-positive kidney transplant recipients. The Cicerone study offers a follow-up of 28 kidney transplant-recipients, over the first-year post-transplantation, with a focus on the CMV response. PBMCs were collected at the time of transplantation, then at 1-, 3- and 12-months post-transplantation. These PBMC were phenotyped using a high-dimensional Cytometry by Time Of Flight (CyTOF) analysis. This CyTOF analysis allows to investigate more than 40 surface markers and provide an unprecedented, detailed phenotype of the immune response depending on possible CMV infections occurring post-transplantation. In parallel, Pr Bestard's team provided a CMV-specific ELISpot analysis, which we could correlate with the immune phenotype to provide a better understanding of the immune response to CMV while under immunosuppression.

For this study, I proposed a dimension reduction analysis using Projection to Latent Structures – Discriminant Analysis (PLS-DA), to select the immune cell clusters that were most associated with CMV infections. I also proposed to couple this approach with a correlation analysis between selected immune cell clusters and CMV-specific functional responses, showing that specific NKT cell subsets could be distinguished. Contrary to previous studies on the whole set of NKT cells, these specific NKT cell subsets were shown to be associated with a protective, anti-CMV response. A specific surface marker seems to discriminate NKT cells between a protective status and a risk status. This work is still in progress, but early results were presented at the 2021 American Transplant Congress (oral presentation) and at the ITS congress of the ESOT (Berlin, 2021, as a poster).

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II – Investigations on a patients' cohort from Grenoble

a) Belatacept cohort

The nephrology team at Grenoble University Hospital follows one of the biggest French Belatacept-treated kidney transplant recipients' cohort. Starting in 2017, this cohort now includes almost 300 patients. Early in the use of belatacept, we included patients in a research cohort, to investigate the immunological impact of a switch from tacrolimus to belatacept in these kidney transplant recipients. We collected PMBC at M0, M1 and M3 post-switch, in 30 patients.

The idea of this project is to offer a description of the immune system's evolution while undergoing a switch from tacrolimus to belatacept. Two perspectives are investigated: a functional perspective, using ELISpot assays, and a phenotypic perspective, using flow cytometry. The functional perspective uses CMV, EBV and Hepatitis B Virus (HBV)-specific ELISpot assays. The phenotypic description requires the definition of an antibody panel.

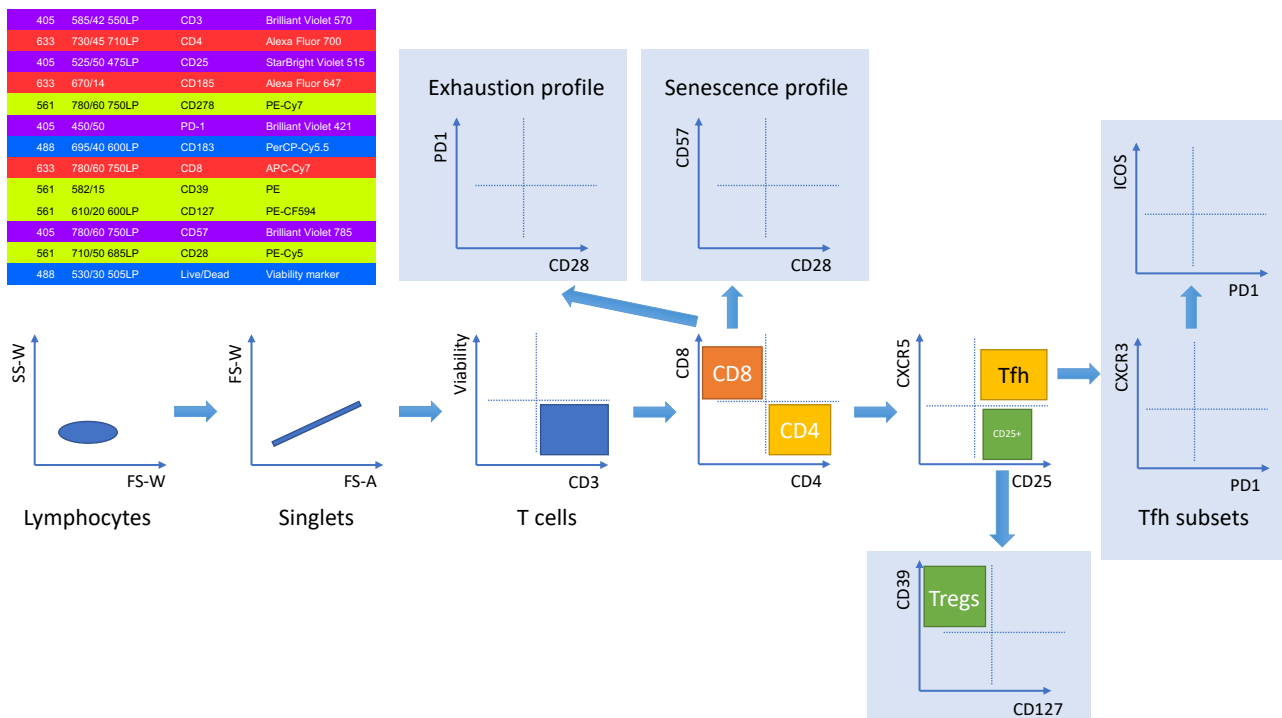
The first samples could be analyzed in March 2022 and the total cohort of 30 patients will be investigated early 2023.

b) T-lymphocyte cytometry panel

The main technical challenge of this part of the IMAGINATION study was the definition of an adapted T lymphocyte panel. This panel and the appropriate antibodies were defined over my fellowship time and was first used in March 2022 in Barcelona. This panel is presented in the following figure.

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c) Fluorospot assessment

In order to evaluate the functional response of T cells in kidney transplantation, the team of Pr Bestard developed an expertise in ELISpot assays but also in Fluorospot assays. These Fluorospot assays improve on the classical colorimetric ELISpot by allowing the evaluation of up to 3 different cytokines at a single cell level. Beyond the classical Interferon-gamma, it is therefore possible to evaluate the Interleukin-2 and the TNF-alpha expressions. Using this Fluorospot assay, we will investigate both the quantitative and qualitative changes in EBV, CMV and HBV responses, over time, before and after the switch to belatacept.

Overall experience:

My overall experience for this year in Barcelona was excellent. Despite the coronavirus crisis, I could enjoy Barcelona and access to the laboratory. I had the privilege to work in a welcoming and wonderful team, to whom I am grateful for their help and support. The ESOT fellowship allowed me to take a full year off my hospital duties and fully enjoy a time dedicated to research.

This year in Barcelona was a tremendous experience to discover a new country, a new region, two languages (Castilian and Catalan), as well as two different laboratories. Indeed, I started my stay at Bellvitge Hospital (IDIBELL lab), before moving to Vall d'Hebron Hospital (Vall d'Hebron Research Institute) together with the nephrology team of Pr Bestard. I also had the chance to get a glimpse at a different health system, discover its strengths and weaknesses and get a perspective that will hopefully help me improve the health system in France.

My initial scientific project was not finished upon my departure, but this fellowship year is nevertheless a full success. I could earn an expertise in T-cell ELISpot performance and improve my knowledge of and ability in performing memory B-cell ELISpot. A virologist from my home institution (Dr Aurélie Truffot) could also come visit the team in Barcelona and benefit from their experience in performing ELISpot tests. Furthermore, together with the team in Vall d'Hebron Research Institute, I am completing a panel of experiences that will be the basis of an upcoming research publication.

As of 2022, the CMV T-cell ELISpot was validated in my original institution and is now fully operational for further research that are already under development. With the support of the team in Barcelona, Aurélie Truffot and I are working toward implementing further T-cell ELISpot and implementing the memory B-cell ELISpot.

These transfers were made possible by the ESOT fellowship, to whom I am also very grateful. I am a proud contributor to European Research, happy to build further the excellence research network between Europeans countries.

References

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