ESOT Transplant Fellowship 2022 - Report

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Project description and outcomes achieved: Malignancy is a frequent adverse event in renal transplant patients. The overall risk of developing cancer has been reported to be 2 - and 4 – fold higher compared to the general population. Immune checkpoint inhibitors (ICI) have revolutionized the treatment of cancer, with remarkable survival benefit. SOTR have been excluded from clinical trials owing to concerns about concomitant immunosuppressive therapy, alloimmunity and risk of transplant rejection. The risk of rejection in kidney transplant patients under ICI has been estimated to be around 42%. **Whether the mechanisms of ICI-induced allograft rejection are similar compared to other settings remains an unanswered question and so far, no predictive biomarkers are available.** The search for antitumoral responses without graft rejection is of paramount importance. Since the indications of ICI are expected to expand, it is important to determine the risk-benefit ratio of the use of ICI in patients with SOTR. With a combined approach of immune cell phenotyping, transcriptomic analysis and high-dimension immunostaining techniques of tissue, we hope to unravel pathways leading to allograft rejection in this setting and to create a risk-score to predict allograft rejection in this particular setting.

The project is divided into two parts.

In **the first part of** the study, we aim to analyze biopsy samples of patients with rejection under ICI with next-generation histopathology. **Exploring the renal transplant microenvironment can provide insights to unravel the mechanisms involved in transplant rejection under ICI therapy.**

We aimed to achieve the first part this year, but several aspects made it difficult to realize the analysis of the samples: slow inclusion because of the scarcity of the patients, slow responses from the different local ethical committees involved in the study and an unexpected and complicated pregnancy with forced bedrest.

To overcome some of the hurdles, we choose to collaborate with the Katholieke Universiteit Leuven Department of Imaging and Pathology for the analysis of the kidney biopsy samples. The MILAN method (acronym for Multiple iterative labeling by antibody neodeposition) is an established method for multiplexed immunohistochemistry characterized by (i) the use of conventional antibodies for immunofluorescence with secondary antibody amplification of the signal; (ii) the possibility to stain up to 80 markers at single cell level on the same slide; (iii) the possibility to stain at the same time batches of 10-15 slides; (iv) staining of the whole slide without the need to limit the analysis to regions of interest; (v) a rapid implementation of any project-specific antibody of interest, given that a suitable working antibody is commercially available. This technique is available within the KU Leuven Department of Imaging and Pathology and Leuven Institute for Single-cell Omics (LISCO).
We **created a kidney panel with 40 different markers** for this purpose and identified a total of 15 biopsy samples to be analyzed. This was possible because of the international collaborations between France, Belgium and the inclusion of patients from the Brigham and Women's Hospital in Boston.
The project will start in March 2023 and will end in December 2023. We hope to have an intermediate analysis in September 2023 for the ESOT congress

The **second part of the study** is the creation of a registry in parallel with a biobank composed of blood –, urine samples of ICI treated patients. This part is ongoing with the inclusion of 6 patients so far. A recent collaboration with Pr Moglie Le Quintrec in Montpellier will augment our patient number.

For the future of this project, it was of paramount importance to learn the cutting-edge techniques in the CR2TI laboratorium in Nantes. I integrated the CRTI team 4 for 4 months under supervision of Pr Nicolas Degauque and Pr Sophie Brouard in 2022. We worked on an ongoing project with the aim to define the contribution of the B cell compartment to the expansion of TEMRA CD8 associated pathogenic response in kidney transplant recipients. This opportunity taught me to work with flow cytometry, cell cultures, cell separation techniques as well as blood transcriptomics which is of utmost important for my project.
I will continue my work in the CR2TI laboratorium in Nantes for at least 2 years. When at least 10 patients included in the biocollection, the samples will be analyzed with similar techniques I learned so far.

I cannot thank ESOT enough for this opportunity and I will continue my clinical and fundamental research in the field of transplantation in the years to come.