ESOT Transplant Clinical Fellowship 2022 - Report

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GENERAL AIM

My principal goal for this 1-year fellowship has been to **enhance my knowledge in the clinical managing of kidney transplantation**, expanding my clinical skills especially **thanks to the integration of novel tools and technologies.** This goal has been attainable in a structure of excellence capable of providing a clinical experience deeply bound to the novel instruments delivered by clinical research.

HOSTING INSTITUTION

During this fellowship, I joined the team of the Kidney Transplant Unit at Saint Louis Hospital, Paris. With its transplant rate of 140 per-year, is one of the largest renal transplant centres in France. In particular, Saint Louis Kidney Transplant Unit, run by Prof. Lefaucheur, stands in France and in Europe as a leading and pioneering centre as it concerns its aims of expanding the kidney donor pool and of performing kidney transplantation against immunological barriers. Namely, SLS Kidney Transplant Unit is a globally famous centre for the handling of antibody-mediated rejection and, due to its strong connection with the histocompatibility laboratory Jean Dausset (one of the largest in Europe), has developed specific programs, exported all over the country, for the hyperimmunised patients. In addition, it has been one of the first centre to adopt and boost the usage of reperfusion machine and it is actually the first centre in France for the volume of DCD kidney transplants.

Moreover, Saint Louis Kidney Transplant Unit is a founding member of the Paris Transplantation Group, run by Prof. Loupy and Prof. Lefaucheur, and it lies so at the heart one of a dynamic European consortium as regards to the clinical research in the field organ transplantation, with several translational studies on-going focused latterly on the identification and validation of novel invasive and most importantly non-invasive biomarkers to build an integrated predictive tool of humoral rejection (STRONGER TOGETHER AWARDS 2017, 19, 21).

The combination of these two components, makes of Saint Louis Hospital the perfect environment which **allowed me to have access to the technological platforms (Axe 1) needed to operate on the patients I deal with in my daily clinical practice (Axe 2).**

PROJECT DESCRIPTION AND OUTCOMES ACHIEVED

Axe 1: Integration of new technologies

I had the opportunity to integrate into my clinical practice the breakthrough technologies coming from clinical research, available giving to the link of Saint Louis with Paris Transplant Group.

Considering the tracs I intend to carry on in my professional future and the expertise I wanted to bring back to Italy I was able to developed my knowledge around **three major points** around which I have planned, in agreement with the working team in my home institution, to strengthen my background.

A) *Molecular diagnostic by gene expression analysis in kidney allograft biopsy* and *novel peripherical biomarkers*

- Description

I had access to the first and so far, unique in Europe, **molecular diagnostic platform for allograft**: the "INSERM Paris microarray and PCR platform", available at Paris Transplant Group.

Every kidney transplanted biopsy at Saint Louis Hospital is frozen to be centrally analysed according to the classifiers derived from the Banff Human Organ Transplant (BHOT) platform; this is a 770 gene panel (validated and integrated in the latest Banff classification) which includes the genes related to rejection, tolerance, viral infections, and innate and adaptive immune responses.

Recent findings, demonstrated that the BHOT panel comprises the relevant genes and pathways associated with AMR and TCMR in kidney allograft tissue and that **BHOT-based classifiers** perform as well as those based on whole-transcriptome analysis.

In this sense, "INSERM Paris microarray and PCR platform" offered me the opportunity to exploit a "ready-to-use" and faster technology in order to maximize the diagnostical power as it concerns specifically the ABMR spectrum.

In addition, **I developed understanding and of peripherical transcriptomics (cellular-free DNA)** to assess serum RNA and mRNA related to genes that have been shown to be components of scores associated with operational tolerance or rejection.

- Outcomes achieved and progress in clinical research

During my period at Saint-Louis hospital, I was able to learn how to:

- **analyse and interpretate the "molecular" reports** for every kidney transplant biopsy performed in the "day hospital" section of the Kidney Transplantation Unit. The total number of biopsies performed were **roughly 250 with a same number for the reports analysed**;
- **solve blurred clinical conditions** in which the simple histological patters are not resolutive (for example patterns of microvascular inflammation without C4d positivity and no identifiable DSA/non-HLA antibodies or pattens of absent microvascular inflammation in presence of C4d positivity and DSA) in order to **deliver a satisfying reclassification of non-concluding diagnosis**;
- **to offer** a precise **therapeutical strategy derived from to the gene expression pattern** in a larger clinical-pathology and molecular interdisciplinary confrontation with the inclusion of several cases in specific protocols or international clinical trials;
- **identify patterns of AMBR related to non-HLA antibodies**, the diagnosis of which relies on the demonstration, at biopsy level, of the specific molecular signature of rejection;

- **I was the principal actor of periodical remote-meetings with my Home Institution in Italy to discuss the more difficult clinical cases**, the total of which were **included in the biopsies we received and analysed from my home Institution in Italy (60 biopsies)**;

Regarding the novel biomarkers I was able learn how to:

- **provide a risk classification** of kidney transplant patients stable (at low risk of rejection) or instable (at high risk of rejection). This classification can **prevent unnecessary invasive biopsies** and their costly procedure, while addressing to allograft biopsy only the instable cases;
- **manage a more performant follow-up** and non-invasive risk stratification for the patients in whom different clinical condition (such as BK virus nephropathy, bacterial/viral/fungi infections or cancer) have imposed **a minimisation of the immunosuppressive therapy**;

- **I participated actively to the inclusions and follow-up of the EU-TRAIN Clinical study.** This is a prospective observational cohort study actually running at Saint-Louis Hospital and in three other centres). The aim is to stratify kidney transplant recipients using non-invasive biomarkers for the risk of allograft rejection in the first-year post transplant (variance explanation modelling) and reclassify the rejection diagnoses made by standard of care

histopathology by the gene expression profiling in allograft biopsies (Low-risk" and "High-risk" clustering).

- Clinical examples of my activity

Case 1. Female patient, 24 years, end-stage-renal (ESRD) disease due to primitive FSGS. Second kidney transplantation in 2020 (living donor), induction Thymoglobulin (with five plasma-exchange) due to a pre-formed DSA (Cw17- MFI 12,000). Creatinine nadir at 58 µmol/L with normal 3-months post transplantation (M3) biopsy (Cw17-MFI 2,500). Maintenance: tacrolimus, mophetil-mycophenolate, steroids. Elevation of DSA (Cw17-MFI 5,000) at M12 with normal renal function. M12 biopsy: normal. Early diagnosis of "molecular rejection" on the same biopsy using Nanostring technology followed by a treatment with 3 PE + RTX (1g) and I.V. polyclonal immunoglobulins (IG). The control biopsy 3 months later showed a histological ABMR (g1, cpt1, cg0, c4d0) treated with 3 boluses of corticosteroids + 5 PE, continuing of I.V. IG. (Figure 1 shows the PCA of the patient's molecular score).

Case 2. Male patient, 39 years, with ESRD of unknown aetiology. First kidney transplantation in 2006 (living donor), induction Thymoglobulin, no preformed DSA. Maintenance: CyA, mophetil-mycophenolate, steroids. Evolution towards chronic allograft dysfunction: baseline creatinine 200 µmol/L in 2020 and "de novo" DSA DQ4 MFI 5400 (April 2018). Biopsy in December 2020 for elevation of creatinine up to 362 µmol/L with proteinuria 4.6 g/g, (DSA is MFI 5,650): g0 ptc0 i0 t0 c4d0, cg3 interpretated as CNI toxicity. The molecular score assessed by Nanostring showed a probability of ABMR: 99.6%. Reclassification as transplant glomerulopathy (cg3) with molecular profile of active ABMR. Treatment with 5 PP, RTX (1g), steroid pulses and IVIG 2g/kg during 6 months. Switch CyA to FK. (Figure 2 shows the biological pathways analysis of the patient).

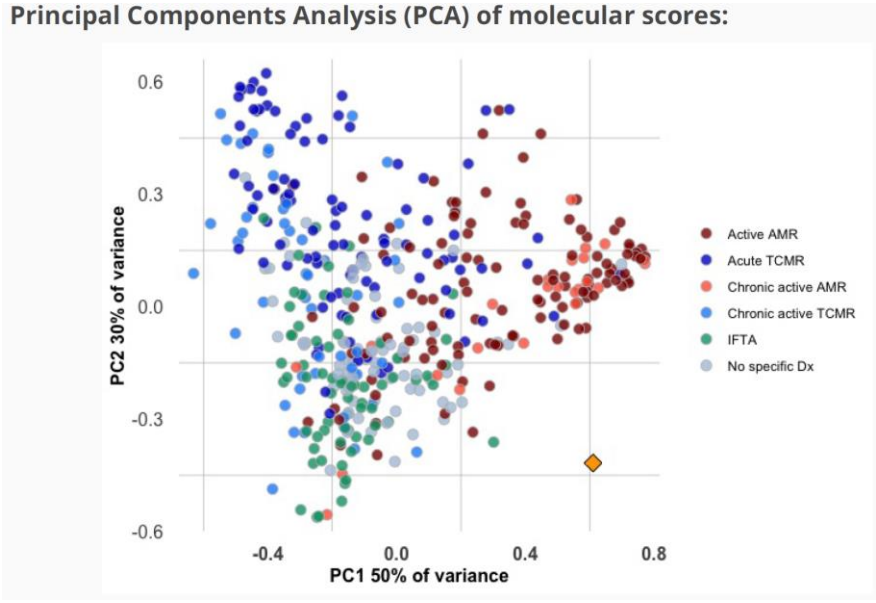

Figure 1.

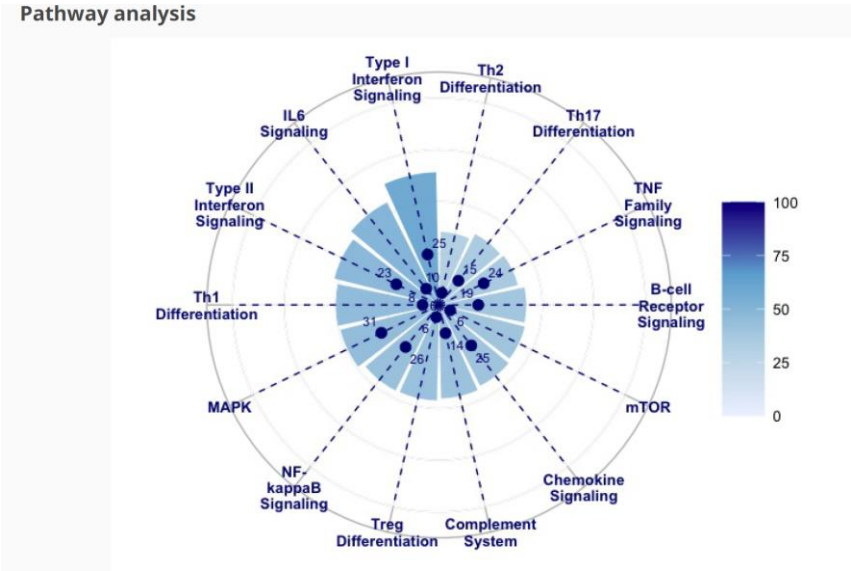

Figure 2.

B) *Usage of **artificial intelligence.***

- Description

I have developed an active usage of predictive tools apprehended by Paris Transplantation Group. In particular I have taken extensive **knowledge of the iBOX.** It is a predictive algorithm which represents a novel integration of demographic, functional, histological, and immunological factors that can be implemented in routine clinical practice. The algorithm predicts allograft survival at 3-, 5- and 7- years post risk evaluation. This system, unlike other tools aiming at predicting allograft rejection or loss, tested all relevant parameters of an extensively phenotyped database of transplanted patients. It was validated in a European cohort of more than 2,000 recipients and a US cohort of more than 1,400 recipients.

- Outcomes achieved and progress in clinical research

During my period at Saint-Louis hospital, I was able to:

- **effectuate a real-time evaluation** by the iBOX technology in order of predict the risk of graft failure and ABMR **pre and post treatment** to inform and individualise clinical strategy;

- **boost up the development of an active project of remote monitoring** of a large cohort of kidney transplant recipients (particularly important in this period in which we face the COVID-19 pandemic);
- **participated actively to the inclusions and the follow-up of the clinical study CIBIL** (Clinical Impact of the iBox as an early Intervention tooL: A prospective randomised controlled trial to assess the use of a software predicting allograft survival in the follow-up of kidney transplanted patients).

*** The CIBIL study**

Background:
In the standard of care pathway, once transplanted, patients have regular appointments with their nephrologist, with a high frequency in the first year and less frequently in the next years.

A significant number of clinical, biological, histological, and immunological parameters are looked into by the clinician during these appointments. The advent of the "big data" era has submerged the medical field with patients' information that cannot be processed in the most efficient way by the clinician without the help of the machine.

The Predigraft software:
In order to address this problem, Cibiltech (a start-up) has developed a software tool for clinicians in kidney transplantation, called Predigraft, which allows optimization of patients' follow-up, providing alerts on patients' status as well as easily accessible, integrated and essential information to the clinician for evidence-based medical decisions and which is based to the iBOX tool (described above).

The Predigraft "alert system":
Upon basing on a French retrospective cohort comprising 1,125 patients transplanted between January 1st, 2011, and January 1st, 2020. All the biological results performed from the first months post-transplantation up to the end of the follow-up and all the kidney allograft biopsies performed were included. For each biological result, an iBox score was performed and a probability of kidney allograft survival was given. When the association between the decrease in kidney allograft survival probability (i.e. decrease in the kidney allograft survival probability between two evaluations) and the biopsy results was assessed, we found a significant and higher proportion of abnormal biopsies when the decrease was higher than 5%.

Those results showed that a decrease in kidney allograft survival probabilities between two observations shows a patient instability and is associated with more diseases. **Based on those results, a warning has been created in Predigraft when the probability of kidney allograft survival was decreased by at least 5% between two evaluations to alert the physician on his patient's instability to better and earlier detect a treatable disease.**

Study design (figure 3):
450 Patients transplanted for at least 3 months prior to inclusion in the study are being included and randomized 1:1 in two groups.

● **Group Predigraft (Standard of care + Predigraft)**: Subjects have a clinical followup based on site standard of care and benefit from follow-up using Predigraft in addition of the standard of care: the investigator receives an alert every time there is a subject's instability, instability based on the following criteria: allograft survival assessed by iBox decreased by at least 5% in the last 12 months.
● **Group Standard of care**: Subjects have a clinical follow-up based on site standard of care.
Subjects from both groups will be followed up for a total period of 18 months.

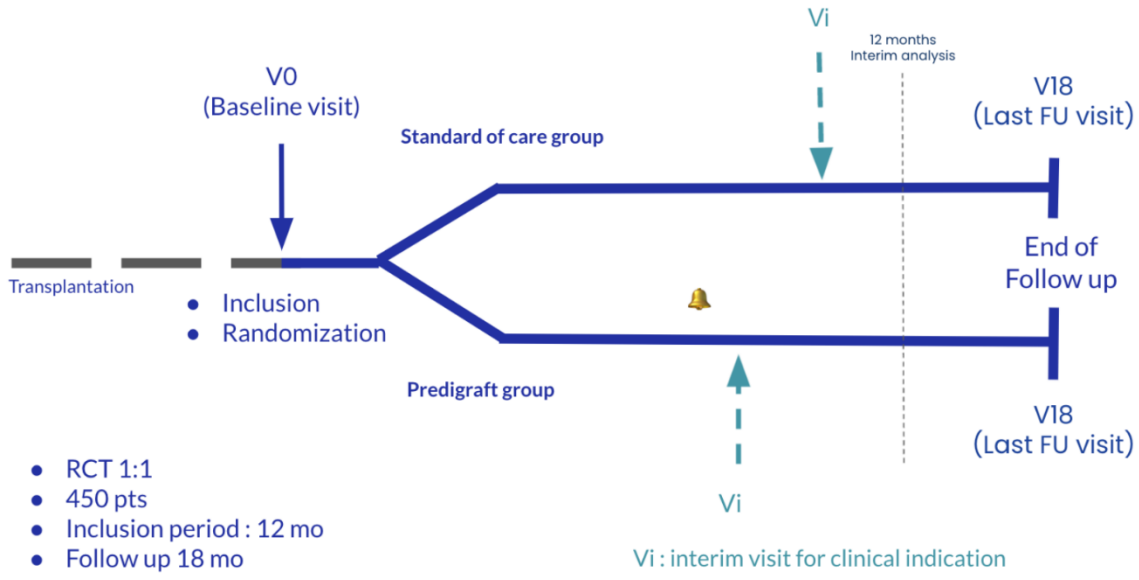

Figure 3.

Study objectives:
Main objective of the study is to **improve the prevalence of biopsies leading to therapeutic change in the Predigraft group compared to the Standard of care group in kidney transplanted patients.**

The secondary objectives of the study are:
- To decrease time to biopsy (investigation delay) in the Predigraft group compared to the Standard of care group;
- To improve renal function in the Predigraft group compared to the Standard of care group by the end of the follow-up.

- Clinical examples of my activity

Case 1. Twenty-year-old patient. Second kidney transplant (12/2021) for segmental and focal hyalinosis (without genetic mutation). 2nd transplant on 02/12/2021 with nadir serum creatinine at 158 µmol/L. Maintenance: Neoral 225mg x 2, Cellcept 1g x 2, Cortancyl 10mg. Precocious FSGS recurrence with proteinuria at 2 g/g. Treatment in January 2022 by immunoadsorption (30 sessions) + 4 injections of Daratumumab + 1 injection of Obinutuzumab. Proteinuria reduced to 0.4 g/g in 3/2022. March 2022: Inclusion in the CIBIL PREDIGRAFT branch study. No systematic kidney biopsy. Difficult follow-up, absence to several appointments.
In September 2022 a blood test is taken in town and uploaded to the Predigraft application. The system shows an alert for significant reduction in graft survival (Figure 4 and 5). Biopsy performed: active TCMR with a single FSGS lesion. Treatment: bolus of methylprednisolone 500 mg over 3 days. Decrease in creatinine to 205 µmol/L on discharge. Subsequent stabilization of graft survival in the following months (figure 6).

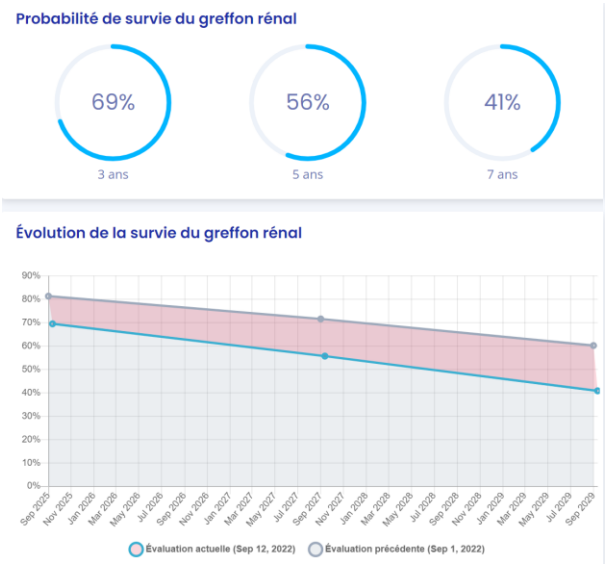

Figure 4.

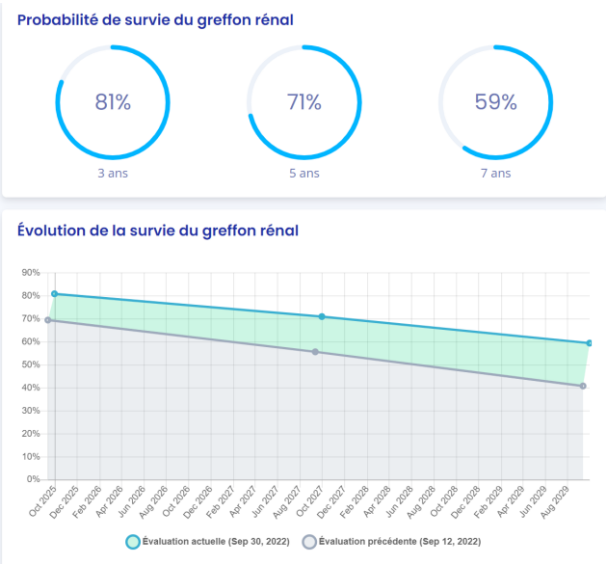

Figure 5.

Figure 6.

C) ***Integrate exploitation*** *of Nanostring technology, extensive phenotyping of anti-HLA antibodies and artificial intelligence.*

- Description

The combined usage of these two abovementioned tools has allowed me to learn an **active management of the kidney transplantation list.**

- Outcomes achieved and progresses in clinical research

During my activity as consultant nephrology (outpatients' pre-transplantation evaluation) I was able to:

- **promote a better allocation and a more precise risk evaluation at the time of allograft performance with a "virtual biopsy" developed on base of the integration of new technologies**; in particular, I was able to understand the principles underlying the **Antigène permis" ("Authorised antigen") program (HAP)** of which Saint Louis Hospital was one of the principal contributors. This specific program maximises the access to transplant for the high-sensitized patients minimising, on the other hand, their immunological risk;
- set the indication for the **inclusion in novel specific desensitization protocols** (Saint Louis Kidney Transplant Unit is internationally known for its ground-breaking experience in

desensitization of highly-immunized patients, and it is the actual regional reference centre for desensitization protocols) most notably the **Imlifidase protocol**.

- Specific protocols and daily clinical activity

*** The HAP ("Authorised antigen program")**

The aim of the program is to increase the number of HLA-compatible proposals without increasing the risk of immunological loss of the graft by allowing a less demanding level of HLA incompatibility but on condition that each HLA incompatibility corresponds to an antigen permitted according to the SAB technique.
Associated with the concept of virtual cross match, this priority makes it possible to increase the pool of compatible donors and to limit the impact of geographic graft transfer on the extension of the duration of cold ischemia.
I took an active role in including, during my activity in the "pre-transplant" outpatient's activity, the pool of hypersensitized patients into the program.

Concerning the results of the transplant via the HAP priority, the failure rate at 1 year by the Kaplan Meier method is ever comparable to the failure rate observed for hyperimmunized patients outside PAH and for non-hyperimmunized immunized patients.

*** The Imlifidase desensitisation protocol**

In February 2022, the French High Commition for Healthcare (Haute Autorité de Santé) HAS authorized post-MA early access for Imlifidase (Idefirix) in the following indication: 'Deimmunization treatment of adult patients hyperimmunized patients awaiting kidney transplantation with a positive crossmatch towards an available graft from a deceased donor and not eligible for current deimmunization.
A group of experts has defined the criteria for selection of patients eligible for this treatment. These criteria have been approved by the Francophone Society of Transplantation (SFT) and the Francophone Society of Nephrology Dialysis and Transplantation (SFNDT) which decided on the continuity of this work to unite their efforts to propose treatment recommendations and monitoring in order to standardize practices in France. The objective of these recommendations is to propose a common framework to the teams that will use Imlifidase in order to be able to analyse the results and tolerance of this new treatment.

I have the selected and included three hyperimmunised patients at Saint-Louis Hospital, <u>including the first one at national level</u> in which Imflifidase was used.

- Clinical examples of my activity (<u>first deceased donor kidney transplantation in France with Imlifidase desensitisation</u>)

<u>Case 1.</u> Female patient, 22 years old. Group O -. In 2004: ESRD due to a systemic vasculitis associated with a cortico-resistant nephrotic syndrome. In 2009: 1st kidney transplantation. Induction: SIMULECT, TACROLIMUS, CELLCEPT, Steroids. In 2012: ABMR + TCMR treated with steroids boluses, immunoadsorption, 2 injection of RTX, Eculizumab. In 2016: late kidney transplant dysfunction in ABMR context leading to HD. De-transplantation in 2017. Major anti-HLA immunization: cPRA 100%, LCT PRA 100%. Simulations show a total maximum of 1 potential donors at national level in 5 years. Serum tested for 1:10 dilution showing a favorable profile for inclusion in the protocol (with a cPRA passing from 100% to 70%). In November 2022: deceased donor kidney transplantation proposal with 4 major anti-HLA DSA (MFI from 15,000 to 17,000) and a positive cross-match (LCT and cyto-flow) pre-Imflifidase. Four hours post-Imflifidase injection: all the for majors DSA pass to an MFI < 1,000 and the cross-match is negative (LCT and cyto-flow). Transplantation is performed with the protocol showed in figure 7.

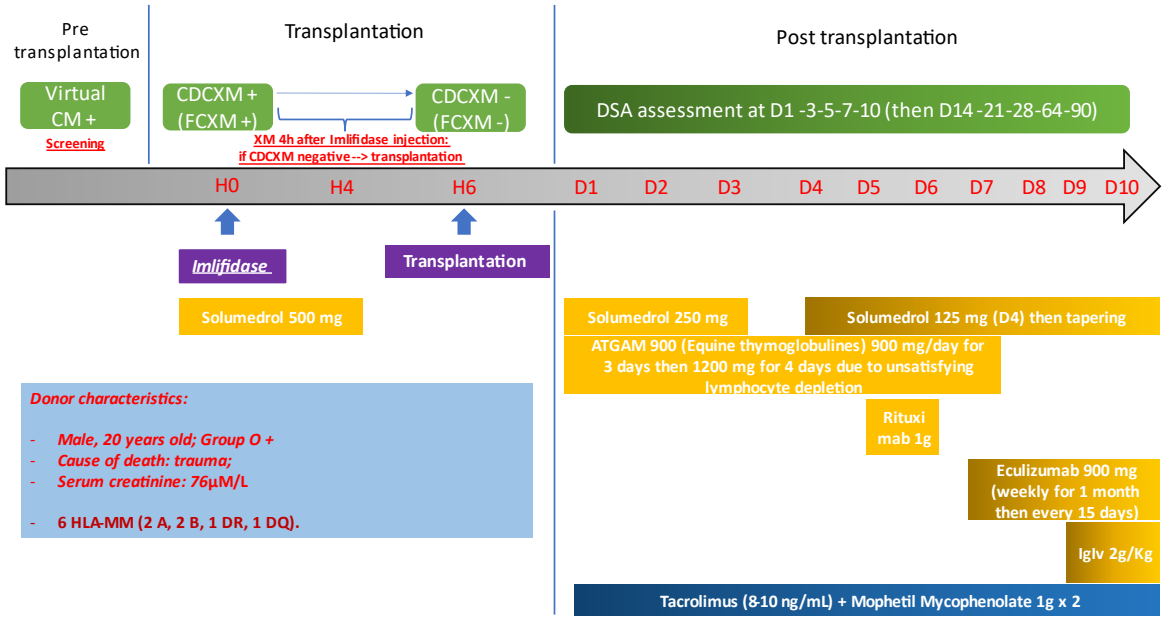

Figure 7.

Favourable evolution with immediate recovering of renal function (stabilized at M1 at 70-80 µM/L of serum creatinine. Rebound of DSAs from D3 (at M3 the only significant being DQ6 with an MFI of roughly at 5,000 and stable and the other ones being < 1,000). At M1 sCr 91 µM/L; Histology: ABMR and TCMR

Banff classification: g0, pct 2, cg0, c4d3, i2, t2. Treatment with thymoglobulines (rabbit) 75 mg/days for 5 days + 3 steroids boluses of 500 mg + SOLIRIS. At M3 sCr 74 µM/L; Histology: absence of ABMR and borderline lesions of TCMR. Banff classification: g0, pct 0, cg0, c4d0, t1, i1; Nanostring molecular analysis showing high activity of ABMR but low activity of TCMR (figure 8). Excellent survival rate according to the iBOX score with improvement after the treatment (figure 9).

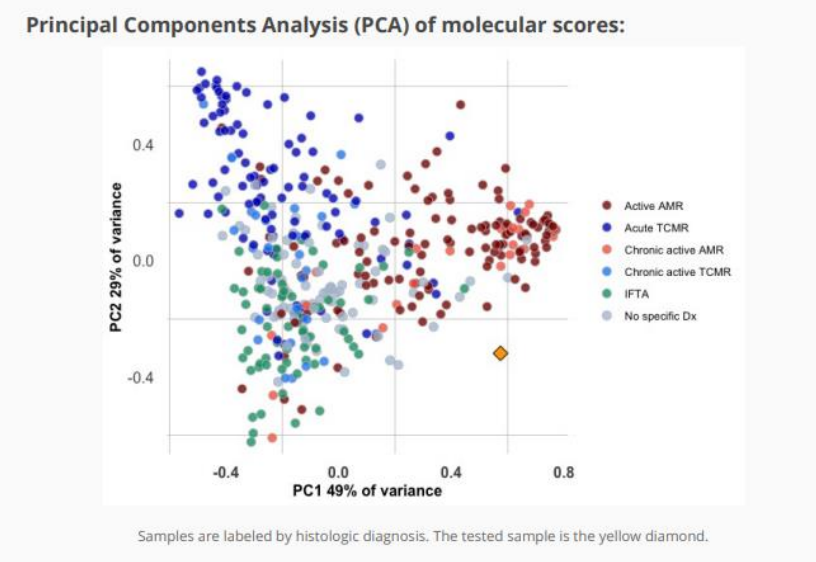

Figure 8.

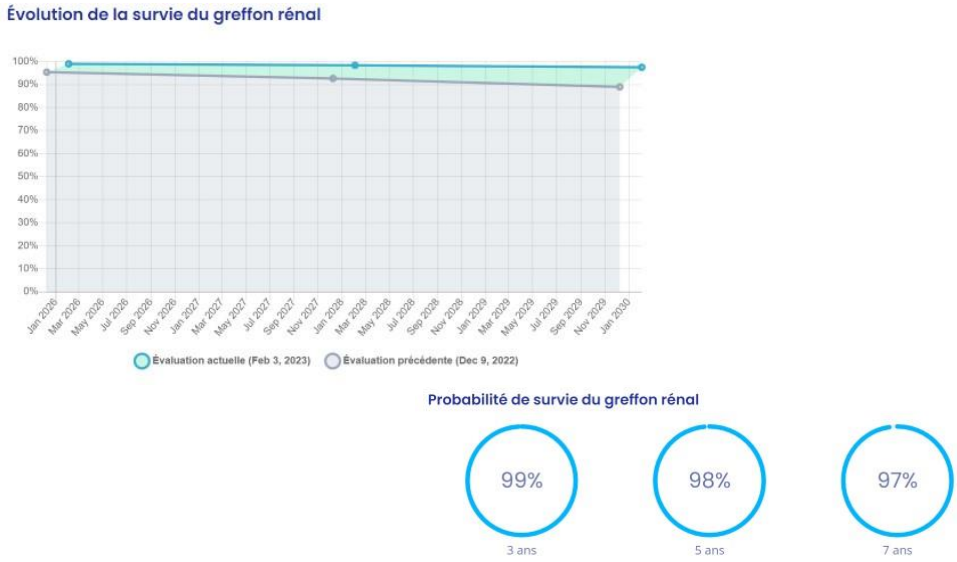

Figure 9.

Axe 2: Daily clinical activity

I had the opportunity to develop the abovementioned points of my project, during this last year, thanks to my fully participation to the daily clinical practice of the Kidney Transplantation Unit at Saint-Louis hospital.

I was broadly involved in all the sections of the Unit, most notably:

- **Inpatient clinical ward** (during my on-call nights and week-ends):

  allocation of kidney, selection of the appropriate immunosuppressive protocol and management of infectious, surgical and other medical complications and technical usage of the reperfusion machines for both deceased and living donors, expanded-criteria donors, non-heart beating donors (Maastricht type III), ABO-incompatible, combined pancreas, liver and heart-kidney transplant patients with a total transplant rate of 150 per-year;

- **Inpatients clinical management** of the **cohort afferent to the day-hospital** section of the clinical ward (during 3 full days/week) for: **protocols allograft biopsies** performed at 3 and 12 months after transplantation and "**for cause" allograft biopsies**, **treatment of TCMR and ABMR** (including steroids, immunoglobulins, plasmapheresis, rituximab, complement inhibitors), **follow-up of a cohort of 85 patients treated with belatacept** for CNI toxicity and **inclusions in international clinical trials (most notably Imlifidase, CIBIL-study, EU-TRAIN study, but also anti-IL-6, C1 inhibitor);**

- **Outpatient pre-transplant evaluation** (1 day a week) on an active list of over 900 recipients, including the greatest cohort of HIV-positive recipients in France and including about 50% of highly-immunised patients; **I personally evaluated roughly 150 patients for pre-transplant evaluation, including the indication for inclusion in the abovementioned specific protocols;**

- **Outpatient post-transplant evaluation** (1 day/week) of a cohort of over 1500 kidney transplant patients**, including 300 patients on remote monitoring** (iBOX technology) and a large number of **immunologically "minimised" patients**;

- **Weekly meetings with HLA laboratory biologists and pathologist** which have been the fundamental moment **to transfer the implementation coming from the translational tools into the clinical frame** as regards to pre-transplant immunological risk evaluation, post-transplant immunological evaluation, confrontation between the histologic patterns with the biopsy gene expression profile.

Extra activities

Thanks to the fellowship grant I was able to further develop my knowledge in statistics and transplantation. Namely, I successfully attended the CESAM Diploma in statistics and epidemiology at Sorbonne University and the Paris-Saclay University Diploma in Transplantation course.
I also had the opportunity to participate, at the last SFT (Société Francophone de Transplantation) congress in Lyon (December 2022) as well as at The Second Joint meeting Brescia-Columbia University (September 2022) in my home institution, where I presented the first results of the molecular analysis of the biopsies performed.

OVERALL EXPERIENCE

It is nowadays clear how the scientific international organisations in solid organ transplantation (including ESOT and the Banff society) call for the filling of the growing gap between the new tools and technology of fundamental research and the clinical practice.

The urgency and the effort of the European community on the converging aim of integration between these two different but complementarian axes in the field of solid organ transplantation is fully expressed in the significative funding of the EU-TRAIN project (in the frame of the H2020 program) for the development of a new translational risk stratification system of allograft, in which Kidney Transplant Unit at Saint Louis Hospital and Paris Transplantation Group play a pivotal role.

The project for this clinical fellowship, that I had the privilege to win, originates from the same vision and is embedded in the same intention to form a class of clinicians capable of exploit the new tools available to enhance their clinical skills and deliver a more scientific and evidence-based clinical practice.

In this sense, this fellowship, beyond the 1-year time, has been and will be for me the opportunity to perpetuate, the usage of the new tools and technologies gained so far. In particular:

- I was able to export the iBOX technology for the evaluation of the risk of graft failure and ABMR pre and post treatment into my home institution cohort;
- I am the actual referent for the usage, in partnerships, of the of the "INSERM Paris microarray and PCR platform", available at Paris Transplant Group; in this sense, and thanks to the ESOT financial support, I was able to transfer biopsies samples from our Italian cohort, to be analysed by the Nanostring platform in Paris without any extra cost for my hosting institution. This helped my home institution in solving difficult and blurred clinical cases, and most importantly, has set the bases for a durable and profitable collaboration, in which data

from Italy could be integrated in a more broadly multicentric evaluation of the precision and performance of the molecular analysis over “classical” histology.

It was a great honour for me to receive this prestigious grant. I thank ESOT very much for giving me this immense opportunity. My experience at Saint-Louis hospital was one of the best of my life: I have learned from and enjoyed every moment of it. This fellowship will be of outmost interest and formative for my future projects and will help me to build a network of professional acquaintances, paving the way for future collaboration. A great thank you to ESOT for this wonderful opportunity.

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