For a better understanding of the mechanisms of alloreactivity responsible for complications following hematopoietic stem cell transplantation.

Initiated in 2017 by Doctor Alice Aarnink and Professor Marie-Thérèse Rubio, respectively Head of the HLA laboratory and Head of the Hematology Department's Transplant Unit within the Nancy University Hospital Center[1], this project benefited from financial support from CRYOSTEM[2] for access to biological resources and the consents of 50 geno-identical donor/recipientpairs. It is currently being carried out by IMoPA[3]'s "Cellular Engineering, Cellular Immunotherapy and Translational Approaches (CImIND[4])" team, with the contribution of Doctor Adèle Dhuyser, university hospital assistant and doctoral student, and benefits from the support of Professor Laurent Mesnard, Head of the Nephrology and Acute Kidney Intensive Care Service (SINRA[5]) and Hugues Richard, bioinformatician at the Robert Koch Institute in Berlin[6].

The Major Histocompatibility Complex (MHC) system, specifically known as the Human Leukocyte Antigen (HLA) system in humans, is a tissue compatibility system. Tissue transplants - whether of solid organs or blood tissue - require a certain level of compatibility between donor and recipient to be tolerated by the latter, whatever the level of immunosuppression prescribed.

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[1] Link on the Nancy University Hospital Center
[2] Link on CRYOSTEM website
[3] Link on the IMoPA unit
[4] Link on the CImIND team
[5] Link on the SINRA unit
[6] Link on RKI

In allogeneic hematopoietic stem cell transplantation, the compatibility of a potential donor/recipient pair is assessed by means of HLA compatibility. In clinical practice, the classic HLA class I molecules - HLA-A, -B and -C - and classic class II molecules - HLA-DR, -DQ - are taken into account, i.e. a total of five molecules, each contributed by the father and mother. Compatibility is thus based on 10 HLA molecules, or 12, if the classic class II molecule HLA-DP is also taken into account.

However, even in the case of a perfect match on all these molecules, as it is the case with a genotypic donor, the probability of graft-versus-host disease (GvHD) remains significant. There must therefore be polymorphisms responsible for this immunological mechanism, in addition to the already well-known HLA molecule polymorphism.

"From where can alloreactivity emerge in a geno-identical donor/recipient pair where all HLA molecules are identical?"

HLA molecules are present on the surface of all nucleated cells and platelets. They constantly present peptides[7] to T lymphocytes. T lymphocytes are the effectors of cellular immunity, helping to maintain the body's homeostasis by systematically eliminating abnormal cells considered to be different from "self".

In allogeneic stem cell transplantation, the recipient's blood tissue is replaced by that of the donor. The donor's T lymphocytes will be able to recognize the recipient's HLA molecules. If they are different, then they will exert their cytotoxic activity - hence the need for identical HLA molecules in hematopoietic stem cell transplantation! If the HLA molecules are identical, there is a second level of "self" recognition, via peptides presented by the HLA molecules to the T lymphocytes. If these peptides are not recognized as "self" by the T lymphocytes, they again exert their cytotoxic activity.

The peptides presented by HLA molecules reflect inter-individual genetic diversity, since they derive from proteins synthesized by our cells. Given inter-individual genetic diversity, even within the same family where

[7] A peptide is a small fraction of a protein, resulting from its degradation.

chromosomes are randomly distributed by parents to their offspring, allografting is always an immunological conflict, regardless of HLA compatibility.

Using exome studies to discover new polymorphisms

The exome refers to the set of exons, i.e. the parts of genes that are actually expressed in the form of proteins. It represents only 2% of the genome, but the sequencing effort required to study it remains extremely important. Exome sequencing generates a vast amount of information that needs to be sorted, organized and processed in order to be understood.

For this large-scale study, we benefited from the expertise of Professor Laurent Mesnard, a nephrologist and kidney transplant specialist, and Doctor Hugues Richard, a bioinformatician affiliated with the Sorbonne University and the Robert Koch Institute. Together, they developed a bioinformatics tool for comparing two exomes, in this case donor/recipient pairs. The tool was first applied to kidney transplantation, and revealed a correlation between the quantity of genetic variants in the pair and chronic graft rejection[8].

Our work involves translating this tool to allogeneic hematopoietic stem cell transplantation and optimizing it by introducing new filters, notably enabling analysis of the immunopeptidome, i.e. the set of peptides presented by HLA molecules to T lymphocytes.
Preliminary results obtained from the patient cohort made available by CRYOSTEM have enabled us to benefit from new funding to apply our research strategy to haploidentical donor/recipient pairs. In this type of transplant, which has recently emerged, the donor/recipient pair has only half the HLA molecules in common; this is made possible by innovative conditioning techniques. The use of haploidentical donors changes the paradigm we have been confronted with for many years, as there are often several possible haploidentical donors for a recipient, whereas the probability of finding an HLA-identical donor is around 60%, depending on the size of the siblings and the recipient's ethnic origins.

[8] Link on publication

The aim is therefore to define alloreactivity indicators that can be used to estimate the expected alloreactivity for each haploidentical donor, in order to determine which would have the "best alloreactivity", i.e. one that provides maximum Graft-versus-Leukemia (GVL) and a minimum GvHD effects.

Exploring other immunological systems outside the HLA system

We are also interested in other immunological systems, such as the Killer-cell immunoglobulin-like receptors (KIRs) gene family. Their polymorphism is more complex than that of the HLA system, due to the large number of genes involved. KIRs are expressed by natural killer (NK) cells, whose cytotoxicity is also involved in alloreactivity. This alloreactivity relies in particular on the presence of a mismatch between a donor's KIR receptors and the recipient's HLA class I molecules.

In summary, our work aims to gain a better understanding of the genetic polymorphisms responsible for alloreactivity after transplantation. Our research on KIRs and exome comparisons opens up new prospects for better anticipation of immunological risk, particularly in terms of GvHD.