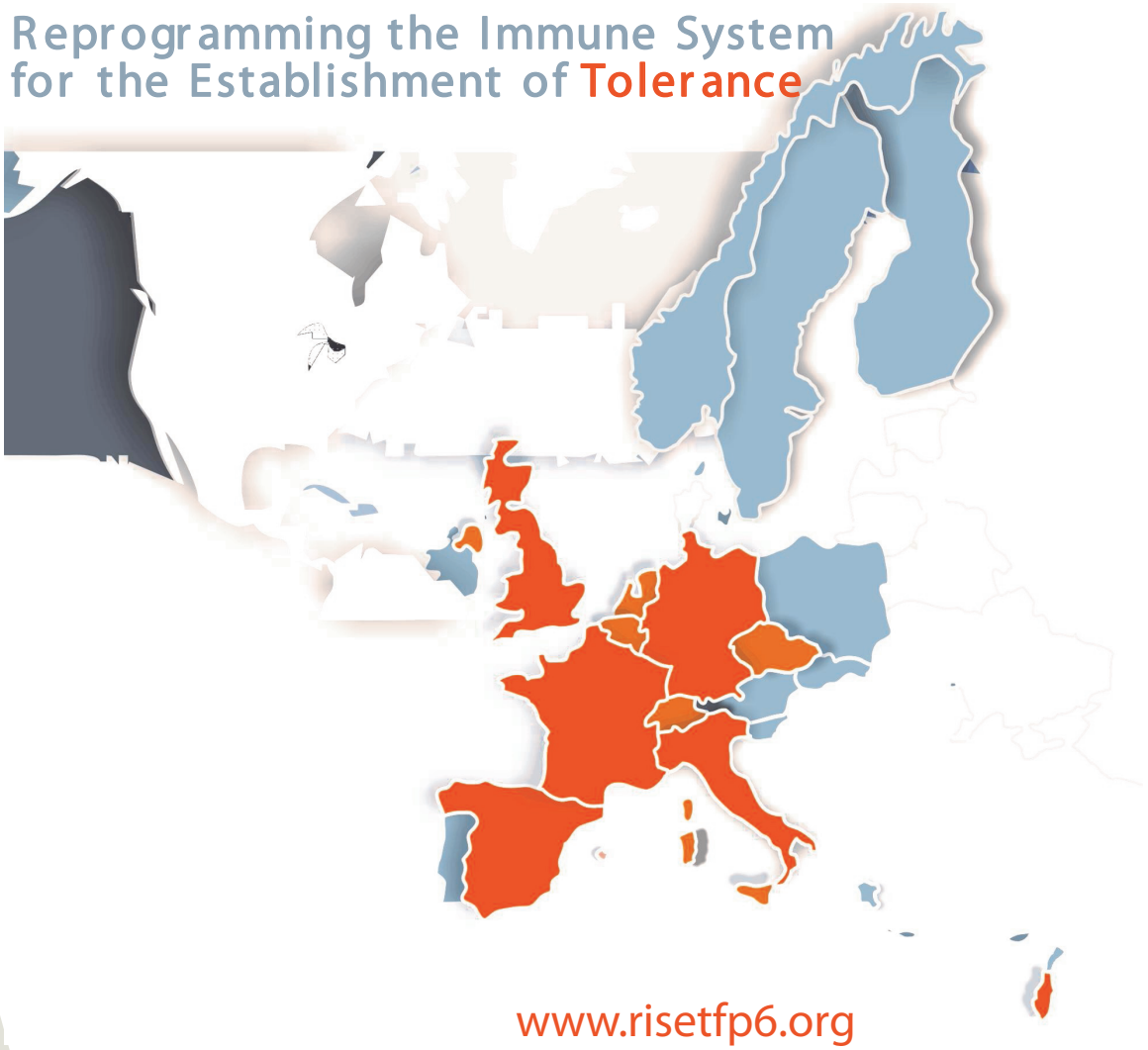
2009 Newsletter

Reprogramming the Immune System for the Establishment of Tolerance



www.risetfp6.org

1 What is RISET?

TRANSPLANTATION DRAMATICALLY IMPROVES THE SURVIVAL OF PATIENTS WITH ESTABLISHED ORGAN FAILURE. HOWEVER, AS A DIRECT CONSEQUENCE OF IMMUNOSUPPRESSIVE DRUGS, RECIPIENTS HAVE A SIGNIFICANTLY INCREASED RISK OF BOTH INFECTIONS AND MALIGNANCIES. RECENT SCIENTIFIC ADVANCES INDICATE THAT THE INDUCTION OF TOLERANCE, DEFINED AS PERMANENT ACCEPTANCE OF A TRANSPLANT IN THE ABSENCE OF CONTINUOUS IMMUNOSUPPRESSION, IS AN ACHIEVABLE GOAL.

RISET is a multinational European project financed by the European Commission within the Sixth Framework Programme that will focus on the translation of these advances in research into clinical practice and industrial development..

What are the aims of the RISET Project?

The RISET Project goals are the

- Development of reliable tests to predict tolerance, i.e., tests that allow identifying transplant recipients who are truly tolerant and/or require minimal immunosuppression
- Implementation of pilot clinical investigations to induce tolerance with strategies proved effective in the experimental setting, assessing the clinical and immunological outcome of enrolled patients
- Stimulation of debate about ethical aspects related to tolerance induction protocols to develop appropriate guidelines
- Establishment of educational programmes on transplantation tolerance for the patients and their families, for physicians, scientists and nurses
- Identification of new genes and molecules relevant for diagnosis and for the development of new therapies for tolerance induction in the future.

The RISET Project:The potential impact

The potential impact of the project includes:

- Improving the health and quality of life of transplant recipients
- Saving health-care costs related to transplantation
- Creating a competitive and innovative European task force to translate transplantation tolerance into clinical practice
- Building a new partnership between the academic and the private sector to promote industrial activities in the field of transplantation tolerance
- Establishing novel training programmes for physicians and scientists
- Involving stakeholders in the implementation of innovation therapies in transplantation

Potential Benefits

Transplant recipients will benefit from the outcome of research, and its translation into patient care, in the following ways:

- New and more effective ways to prevent organ rejection .
- Improved survival rates for organ recipients
- Improvements in patient care
- Extended life expectancy
- Better quality of life

* The generation of a first set of markers for tolerance (WP1) has been supported by the created in the Framework Programme 5 demonstration project (Contract QLRT-2001-02127-*Demonstrating the applicability of novel indices of immunological tolerance to clinical practice),* which is based on the study of samples from a small cohort of patients who retained a normal graft function despite discontinuation of conventional immunosuppression.

7 RISET work packages

Structure of the project

FP 5*: Demostration project: samples from spontaneously tolerant patients

Objective:
To define immunological and molecular phenotypes of transplantation tolerance success and/or failure in patients and clinically relevant experimental models

WP1

WP leader:
Prof. Hans Dieter Volk.
Charité-Université Medicine,
Berlin, Germany

WP 1A: BIOMARKERS DISCOVERY

WP 1B: VALIDATED ASSAYS AND ASSAYS

PROJECT MANAGEMENT

WP leader:
Michael Goldman
Université Libre de Bruxelles,
Gosselies, Belgium

WP2

Objective:
To gain insight into mechanisms of immune regulation and tolerance

WP leader:
Mª Christina Cuturi.
Center Hospitalier.
Université Nantes, France

WP3

Objective:
To develop innovative therapies for the induction of "operational transplant tolerance"

WP leader:
Lucienne Chatenoud.
Université René Descartes, Paris, France

WP5

Objective:
To identify key issues and potential problems or obstacles for the translation of the results and developments obtained in RISET in the terms of benefit to patients, the EU society and the EU economy
To identify solutions to the problems identified
To disseminate the results of the project where needed in order to accelerate the effective translation of findings and developments in terms of social benefits to the patients, the economy and the EU in general

WP4

WP leader:
Kathryn Wood.
Oxford University.
United Kingdom

Figure 1:
Structure of RISET.

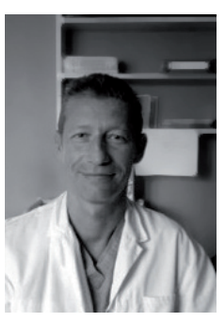
However, following the European Commission recommendation of the first RISET periodic reports, it has been decided to further specify the WP1 activities by splitting it into two groups. WP1a will be dedicated to the biomarkers discovery and then further interact with WP2 activities focused on basic science. WP1b will be dedicated to the validated assays and assays validations activities allowing testing samples of patients enrolled in the pilot clinical studies of WP3.

The ethical aspects of these investigations involving innovative therapies as well as collection and analysis of human samples will be considered in WP4. Research on the potential obstacles of social, ethical or regulatory nature to these activities will also be initiated early on in WP4 in order to facilitate the implementation of further clinical investigations at later stages.

3 potencial impact

of the achievement of immunotolerance in organ transplantation: The major challenge for improving the overall results in the future.

Prof. Vincent Donckier



The current situation of organ transplantation

In recent years organ transplantation has seen spectacular progress in peri-transplant care and development of very potent immunosuppressive drugs. At present, in most cases, transplantation represents the optimal treatment for patients suffering end-stage organ diseases. Either after pulmonary, cardiac, renal or hepatic transplantations, short-term results are now excellent, offering to the recipient both a potential cure of the pre-existing disease and a major improvement of quality of life. With the use of highly efficient immunosuppressive medications, acute rejection is virtually no longer a problem in clinical practice. However, middle- and long-term results of solid organ transplantations are less satisfactory. Immunosuppressive drugs generate major side effects due to their direct toxicity, such as diabetes, renal insufficiency and hypertension, as well as inducing global immunodeficiency, leading to the development of infections and cancers. Together, these side effects are the leading cause of death after the first year post-transplant. In addition, currently available immunosuppressive drugs are unable to prevent or control chronic rejection phenomenon which still represents a significant cause of long-term graft loss.

Apart from this, the main problem of organ transplantation worldwide remains the shortage of organs.Despite remarkable efforts to increase graft sources, such as the development of living donor programs, organ recovery from non heart beating donors or the extension of donor criteria, it is unlikely that organ supply will ever respond to the demand. Notably, the ineffectiveness of current immunosuppressive drugs on chronic rejection contributes to this situation because a significant proportion of patients on waiting lists, particularly in renal transplantation, are those requiring a re-transplantation for immunological chronic graft damages.

The potential of inducing immunotolerance in organ transplantation

The induction of transplantation tolerance, defined as the indefinite survival of an allograft without chronic immunosuppression and with preserved or restored overall immune capacity, would address the above questions (**Table 1**). First, elimination of the need for chronic immunosuppressive medications would solve the problem of their toxicity, as well as the issues of compliance and costs of prolonged treatments. Second, restoration of the recipient's immune capacity in the long-term would reduce the risks for infections and cancers and eventually contribute to the cure of the pre-existing disease (i.e. problem of hepatitis C recurrence after liver transplantation). Finally, true transplantation tolerance would allow long-term graft survival without chronic immune damage, therefore reducing the need for re-transplantation. The concept of transplantation tolerance is very well established in experimental models. However, despite extensive animal testing, clinical trials aiming at minimizing or withdrawing immunosuppression after solid organ transplantations remain relatively rare. At present, the critical questions to address are: Is clinical transplantation tolerance really an achievable goal?, Which would be the best therapeutic strategies for inducing tolerance? and How to conduct clinical studies that are acceptable from an ethical point of view?

Patients who tolerate their solid allograft in the long-term without immunosuppression exist. It is known that approximately 10 to 15% of the recipients of liver grafts may develop a spontaneous tolerance. Investigations performed in these patients and comparisons with those non-tolerant are highly informative, enabling the analyses of potential mechanisms of immunological tolerance and also the research of biological markers, able to differentiate tolerant from non-tolerant patients (biomarkers of tolerance). In this perspective, remarkable progress has recently been made by the Barcelona group, which established a discriminating tolerance-associated genetic profile in patients tolerating their liver grafts as compared with those requiring chronic immunosuppression. In another type of approach, transplantation tolerance has been successfully intentionally induced in pilot clinical trials in kidney and liver transplantations, using the infusion of donor hematopoietic cells after recipient's myeloconditioning. The mechanisms of tolerance in these conditions are not totally understood, but it appears to be dependent on engraftment of the donor cells (macrochimerism) for at least a transient period post-transplant.

Significant barriers still exist for a broad clinical application of this type of strategy, including the difficulty of collecting and selecting donor hematopoietic cells at the time of organ transplantation, toxicity of the conditioning regimen and risk of graft-*versus*-host disease after donor cells infusion, underlying the need of further research in this field.

The elucidation of the mechanisms leading to tolerance, either spontaneously or after induction protocols, and the identification of markers of tolerance will be instrumental in the design of clinical studies over the next several years. In particular, reliable biomarkers of tolerance would represent a crucial step in making procedures safer, especially if they are discriminative in patients under immunosuppression. In this context, European projects on tolerance in transplantation represent a unique opportunity, offering close collaborations between experimental and clinical researchers and the possibility to perform a large battery of specific tests in specialized laboratories.

Ultimately, independently of the strategy used, the only true test for the tolerance hypothesis after organ transplantation will remain the discontinuation of the immunosuppressive treatment. Therefore, the ethical question is central. Indeed, despite the limitations previously mentioned, current immunosuppressive drugs are effective in the prevention of acute rejection and still represent the standard of care in organ transplantation. In any case, immunosuppression weaning or withdrawal in a clinical tolerance trial will expose the patient to a risk of acute rejection and potentially to the dramatic consequence of a graft loss. Because of this, the selection of the patients is a key point. Liver transplantation is probably a favorable choice for such studies as liver grafts are globally well tolerated. Moreover, rapidly detected and quickly treated acute rejection episodes are fully reversible and do not affect long-term graft function and survival.

Globally, even if our conviction is that tolerance is achievable and will represent a major benefit for organ recipients, the individual patient's risk/benefit ratio of a given protocol should be carefully weighed.

Of course, the first responsibilities of the investigators are to give to the patient complete and precise information on the potential risks and to carry out an extensive follow-up. European collaboration such as in the RISET consortium also offers the optimal ethical environment, through open discussions in working groups and presentations of the results to independent external advisory boards.

Table 1:
Current limitations of immunosuppression in solid organ transplantation and potential impact of transplantation tolerance

Organ transplantation under chronic immunosuppression	Transplantation tolerance
Toxicity of immunosuppressive medications	No need for chronic administration of immunosuppressive medications
Long-term global immunodeficiency favoring infections and cancers	Specific tolerance to the graft but immunocompetence to other antigens
Absence of prevention of chronic rejection	Long-term tolerance to the graft
Problems of the compliance to and the cost of long-term immunosuppressive treatment	No need for chronic administration of immunosuppressive medications
Problem of organ shortage	Reduction of the demand for re-transplantation in patient suffering from chronic rejection

Hans-Dieter Volk

WP 1 Leader

Advances within RISET An update

Diagnostic tests for transplantation tolerance.

One significant part of the RISET project is the development of reliable tests or biomarkers as immune monitoring tools in clinical trials. Based on robust validation data and preliminary biological data we selected the most promising tests for the RISET immune monitoring portfolio. Our selection includes safety markers (EBV/CMV/Polyoma viral load, HLA-DR expression on monocytes, CMV/EBV spec. T-cell) as well as "tolerance/rejection" markers detected by gene expression profiling by Agilent Microarray and real-time RT-PCR of selected RISET genes, tests to characterize humoral and cellular sensitizations (screening of HLA alloantibodies, IFN-gamma ELISPOT, CTLp) or particular expansions and response states (TcLandscape; HMOX-1 polymorphism).

During the last months we have achieved significant progress in this workpackage.

1. METHODICAL VALIDATION OF SET-1/2 TESTS

Standardization and methodical validation has been a crucial task since the start of the project and is still one of our main objectives. All set-1 and set-2 tests have been methodically validated. For this purpose, 2 types of Standard Operating Procedures (SOPs) have been developed by the core labs in Berlin, Nantes, Cologne, Leiden, and Prague:

- SOP with a step-by-step description of how to collect the necessary amount of samples of a clearly defined source and how to handle, store and transport these samples
- SOP with an equally detailed description of how to perform the assay, a preceding statement of its purpose, duration time and indications concerning the pre-analytics.

All these SOPs have been evaluated and approved by the RISET validation body (Univ. Konstanz in collaboration with the CHARITE Berlin). They have also evaluated and approved the validation data (reproducibility, sensitivity, specificity) that has been generated and documented, once the operating procedure of each assay has been set up in its final version.
Theoretical standardization of the assays obtained by the development, evaluation and approbation of the above mentioned documents has been completed by on-site audits to ensure that standardization went into practice and is going to be maintained.

All in all it has been a complex procedure to obtain and control quality. It has been more time consuming than expected. One interesting assay is the ELISPOT analysis to detect donor-specific memory T cells that are associated with poor graft survival prognosis.

As an example, the progress with this interesting assay can be summarized as follows:

- Delivery of new ELISPOT readers to 5 centers to make the data better comparable (sponsored by industry)
- Release and validation of the new machine
- Development of consensus SOP
- Cross-Validation of SOP at Berlin and Barcelona

⇨ Outlook: Transfer to Leiden, London, Paris, and cross-validation between all 5 centers
⇨ Aim: Development of a standardized RISET ELISPOT assay that can be used for multicenter-trials on-site

2. CLINICAL VALIDATION OF SET-1/2 TESTS

As clinical validation requires a huge set of clinical samples, it has been necessary to find a way that guarantees the availability of enough material. Focusing on a reduced pool of assays seemed to be the best solution. Therefore, the established tests were subdivided into obligatory and optional assays: a pool of tests that need to be applied and a pool of tests that can be applied.

As only some of the assays are performed by the clinical partners themselves, sample transfer from the clinical (WP3) to the test-performing partners (WP1) has been organized in collective sample shipments with centralized monitoring (CHARITE), allowing sample tracking via the RISET Website http://www.risetfp6.org (Intranet: WPs´ sections > WP1 > Sample shipment).

Eight collective shipments as well as some single shipments made it possible to follow-up:

1. the Epstein-Barr virus/ Cytomegalovirus viral load of 63 patients,
2. the gene expression profiling by real-time Polymerase Chain Reaction on blood samples of 182 patients and custom designed microarray analyses of 156 patients,
3. the serum of 173 patients for HLA antibodies,
4. the CTLp analyses on the PBMC of 36 patients,
5. the ELISPOT analyses on the PBMC of 57 patients,
6. the TcLandscaping on the samples of 46 patients, and
7. the urinary IP-10 level of 61 patients.

In addition, several optional RISET tests and on-site tests have been performed.

The results of the analyses have all been transmitted to the clinical partners for clinical validation. In order to launch a more effective validation process, the CHARITE organized two meetings, where the principle investigators of the clinical trials and partners of the test-performing centers could directly discuss first results. The structure of the meetings can be summarized as such:

- More in depth explanation of the assays, interpretation from the methodical point of view, pitfalls and possible misinterpretations
- More in depth explanation of the underlying clinical protocol, clinical questions
- Discussion on a future RISET-trial based on biomarkers

Six months later, we organized a follow-up meeting with the additional aim of preparing the setup of a data management and data analysis structure. Whatever data analysis strategy is applied (exploratory and hypothesis-driven analysis, discrimination analysis, trial specific/combined analysis, etc.), they are all based on the exchange and use of information collected by the clinical investigators and produced by the laboratories.

Strengthening the collaboration between WP1 and WP3 to correlate biological and clinical data and to implement a structuring body has not been the only outcome of the meeting. The translation of basic research to clinical protocols and/or lab facilities has also been discussed in the presence of the WP2 leader.

The preliminary data look very promising. We hope that the first conclusive results will be available very soon.

Maria Christina Cuturi

WP2 Leader

Inducing allograft tolerance.

The overall objective of WP2 is to gain insight into mechanisms of immune regulation. We are studying different models of tolerance and regulation at the cellular and molecular level and we have identified biomarkers that could represent tools to diagnose or induce tolerance (with WP1 and WP3) (**Figure 2**).

According to gene expression analysis by DNA chip from some tolerant or stable patient samples, WP2 partners have selected a precise list of genes to be studied by quantitative RT/PCR in Dendritic Cells (DC), T cells or tolerance models. We found four interesting molecules which were upregulated in regulatory T cells, immature DC or tolerated allografts and we started to develop tools to analyze their function and their potential in tolerance for immuno-therapy (antibodies, KO mice…). Moreover, these molecules are analyzed by quantitative RT/PCR in the new clinical samples (with WP1 and 3) that are generated in the RISET consortium in order to validate these biomarkers.

In addition, we are developing cell-therapy strategies with tolerogeneic DCs, regulatory T cells or with anti-CD3 or CD52 antibodies to establish a good pre-clinical model of tolerance induction. So far, we haveobtained encouraging data in auto-immune and transplantation settings by combining different treatments. Indeed, it appears that tolerogenic DCs are more effective at inducing graft acceptance when they are administrated in combination with regulatory T cells or antibody treatment. A joint manuscript on "Tolerogeneic DC" is in preparation. This would lead to the proposal of protocols aiming at inducing allospecific long-term tolerance that could be applied in the clinic.

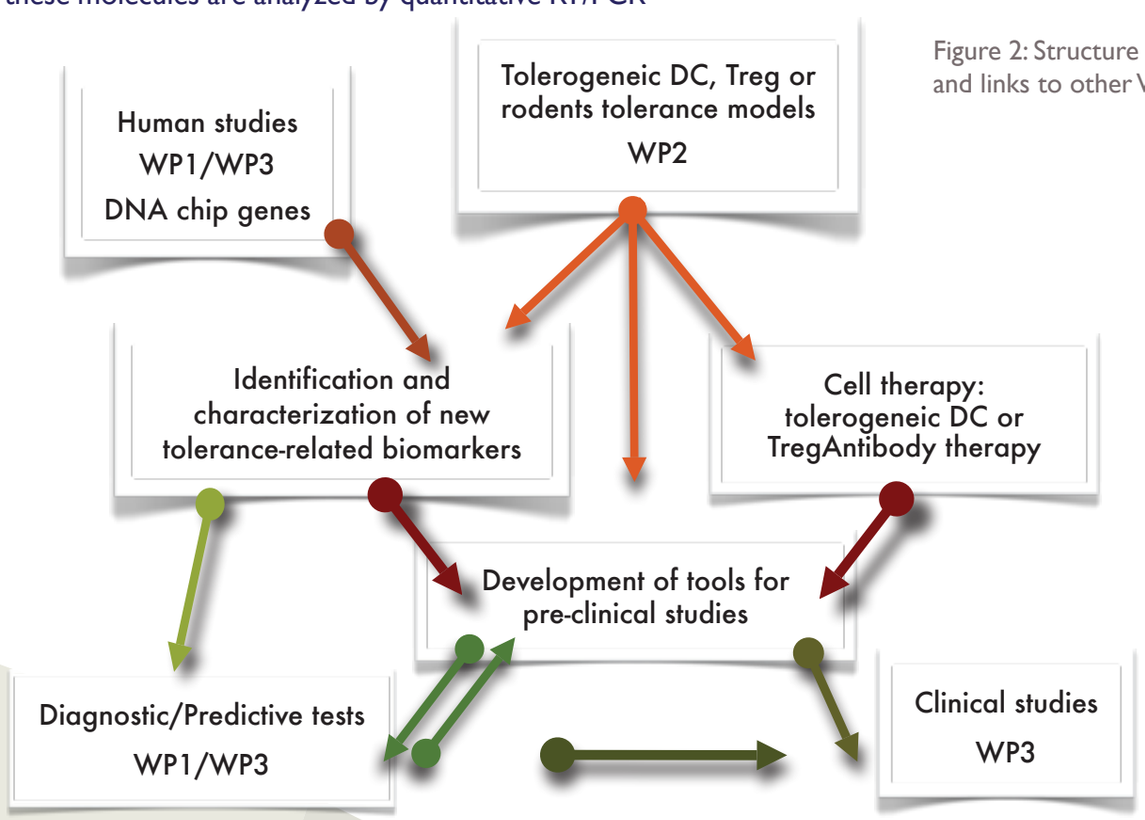


Figure 2: Structure of WP 2 and links to other WPs.

Lucienne Chatenoud & Sylvaine You

RISET: The challenge of clinical trials aiming at transplant tolerance.

Inducing "operational tolerance" is the challenge that the RISET consortium wished to address. Twenty six European groups involved in experimental and clinical transplantation joined the Consortium. To understand the challenges and the way in which the clinically oriented part of the program (Work package 3) was redirected along the way based on the results obtained, it is important to retrace the first salient steps of the work the Consortium performed.

1 An Initial Ambitious Goal.

The programme was launched with three hypothesis-driven pilot clinical investigations aiming at inducing "operational transplant tolerance" defined as a state of lasting antigen-specific unresponsiveness in the absence of generalised immunosuppression. The rationale was based on the clinical transfer of pre-defined protocols which proved effective in inducing transplant tolerance in the experimental setting and for which compelling evidence was accumulated to demonstrate that peripheral tolerance mechanisms, mostly based on T lymphocyte-mediated immunoregulatory circuits, played a key role in the induction and maintenance of such tolerance states. These protocols were cell therapy-based involving the use of:

Anergised interleukin (IL-) 10 driven T lymphocytes (Tr1 cells) of donor origin in recipients of allogeneic bone marrow (R. Bachetta, MG. Roncarolo; San Raffaele-TIGET center, Milano, Italy). The patients received HLA-haploidentical hematopoietic stem cells on day 0 and an infusion of IL-10-anergised donor T cells one month later. No immunosuppression was given at the time of the transplant or the infusion of Tr1. A total of 7 patients were included in this trial. Overall the treatment proved safe: the low dose of $CD3^+$ anergised cells infused gave rise, in some patients, to moderate acute graft *versus* host disease (GvHD) which in all cases was responsive to therapy. In parallel, the low frequency of infectious episodes observed, the absence of Epstein Barr Virus and Cytomegalovirus replication, together with the complete remission of the disease, provided the proof of concept of the efficacy of the adoptive transfer of Tr1 cells.

Mobilised immature CD34+ bone marrow stem cells of donor origin in recipients of allogeneic liver transplants (V. Donckier, M. Goldman; Université Libre de Bruxelles, Brussels, Belgium). The rationale of the study was based on compelling experimental evidence to show that donor cells, when presented in adequate conditions, may render the host's immune system tolerant to donor antigens through different mechanisms such as deletion or regulation of anti-donor T cells and/or generation of suppressive cells. The trial performed included patients with intra-hepatic malignancies, not complying with the criteria for deceased liver transplantation due to high risk of cancer recurrence following conventional immunosuppression. Patients received a living-related liver graft (from a family member, including spouses) followed by the infusion of mobilized donor stem cells on day 7. These cells were harvested after G-CSF treatment and infused in recipients conditioned with cyclophosphamide and Thymoglobulin^R. The immunosuppressive regimen included steroids for 3 days and sirolimus starting on day 0 and followed until liver function tests returned to normal, a point in time when immunosuppression was discontinued. Four patients were included. In all patients reversible acute rejection occurred when immunosuppression was discontinued. Relapse of the original tumor occurred in 2/4 patients. Global hyporesponsiveness and donor-specific hyporeactivity was assessed in 2/4 patients after discontinuation of immunosuppression.

Monocyte-derived Transplant Acceptance Inducing cells (TAIC) of donor origin in recipients of allogeneic kidneys (F. Fändrich; University Schleswig-Holstein & Blasticon, Kiel, Germany). TAIC are derived from human monocytes following in vitro culture in presence of M-CSF and interferon (IFN) . Two major mechanisms have been proposed to explain the therapeutic effect of TAIC based on numerous experiments. Firstly, allogeneic in vivo injection of TAIC into non-immunosuppressed recipients leads to transient mixed chimerism lasting for a few weeks indicating that TAIC are not acutely rejected within the allogeneic environment. Secondly, cell-to-cell interaction between TAIC and host T-cells induces regulatory T-cells, as demonstrated by the induction of $CD4^+CD25^+$ T cells in these recipients. Adoptive transfer experiments with these $CD4^+$/$CD25^+$ T-cells demonstrated their capacity to down-regulate alloreactive graft-specific immune responses. These experiments led to the assumption that TAIC could induce donor-specific peripheral tolerance by inducing regulatory T-cells when used in clinical trials. The first trial proposed in the context of RISET was a single centre open-label study of the administration of TAIC and autologous regulatory cells in recipients of a living donor renal allograft. Five days before transplantation TAIC were administered. On the day of transplantation, patients received triple immunosuppression with tacrolimus, anti-thymocyte globulin and corticosteroids. Anti-thymocyte globulin was given postoperative only on Day 0, 1 and 2. Corticosteroids were tapered off during weeks 9 to 10. If the reduction in creatinine clearance at the last visit on week 12 was ≤ 25 % compared to Day 56, tacrolimus was reduced during week 13. Tacrolimus was tapered during weeks 25 to 28 if there were no histological, biological or clinical signs of rejection. A total of 6 patients were included. Acute rejection that was responsive to treatment was observed in one patient. In none of the patients GvHD was observed. In 4 of the patients a rejection episode appeared when all the immunosuppressive drugs were stopped. After adequate treatment, reversion of the rejection episodes was observed and the long term follow-up evidenced a satisfactory graft survival rate. One patient showed normal graft function for 8 consecutive months after withdrawal of all immunosuppressants.

In order to fulfil the requirements of FP6 projects, 18 months after starting the project the data were reviewed by both an external advisory board, as well as by a panel of experts gathered by the Commission. The comments that were related to the commitment and efforts provided by the clinical teams in recruiting patients were largely positive but also highlighted were two relevant issues which, if implemented, could represent a further leap for the project.

- The first was to pursue the cell therapy strategies undertaken but by redirecting all three protocols, attempting to improve effectiveness and therefore the benefit to the patients and the project.
- The second recommendation was an emphasis on the absolute necessity to meet, in the context of this clinical endeavour, the main objective of RISET which is to provide a set of validated monitoring tools to test for "operational tolerance" in transplanted patients. The practical problem which became evident was that in no case did the protocols mentioned above, in spite of their intrinsic "richness" of clinical experience, provide a sufficient number of samples to the RISET core laboratories running the standardised monitoring tests to achieve a reliable, statistically significant validation of the individual methods. The only way to tackle this problem was to increase the number of patients available for monitoring meaning an increase in the number of protocols within RISET. For obvious reasons, most of these new trials, that are now ongoing, belong to a second category of clinical investigations aiming at **minimization of immunosuppression** which would be based, if possible, in the not too distant future on a selected panel of **biomarkers**.

2 Redirecting The Clinical Objectives To Better Address The Complex Goals Of Riset

A total of 7 additional clinical trials are presently ongoing in the context of RISET; four of them are focussing on protocols aiming at drug minimisation, two are devoted to innovative strategies to promote transplant tolerance and one focuses on a more explorative aspect of kidney biopsies to predict and monitor chronic kidney allograft nephropathy.

Trials On Drug Minimisation

Clinical study to evaluate the efficacy in kidney allograft recipients of an immunosuppression regimen based on induction therapy combining anti-CD52 (Campath-1H) and anti-TNF monoclonal antibodies followed by a maintenance regimen based on either tacrolimus or sirolimus (O. Viklicky, IKEM, Prague, Czechoslovakia in collaboration with P. Reinke, D. Volk, Charite hospital, Berlin Germany). The clinical center in Prague will enroll 20 patients receiving peri-operatively 2 injections of the anti-CD52 antibody alemtuzumab (Campath-1H) to induce massive depletion of both T and B lymphocytes in combination with a single injection of Infliximab (anti-TNF) and followed by either tacrolimus or sirolimus low-dose monotherapy. The aim of the trial is to assess whether sirolimus monotherapy in the absence of any treatment with calcineurin inhibitors (CNI) and/or steroids is safe and allows promoting long term survival of well functioning kidney allografts. Infliximab is added to the treatment regimen to decrease the risk of humoral rejection which has been one of the problems reported by various groups using Campath-1H as induction therapy.

A study on Minimization of immunosuppression in renal allograft recipients (I. ten Berge, Department of Internal Medicine, Renal Transplant Unit, Academic Medical Center, Amsterdam, The Netherlands). Renal transplant recipients are treated with an induction regimen including CD25 monoclonal antibody, corticosteroids, mycophenolic acid and cyclosporine. After 6 months, the patients are randomized in 3 treatment groups, receiving either prednisolone and mycophenolic acid; prednisolone and cyclosporin, or prednisolone and sirolimus. Protocol biopsies are performed before transplantation and at 6 and 24 months transplantation. Mononuclear cells, serum and urine samples are collected before transplantation and at frequent intervals thereafter will be provided to the RISET core laboratories. A total of approximately 30 patients will be enrolled. In addition, this center developed over the last years an "in house" mixed lymphocyte culture (MLC)-5-(and -6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) test they would apply in parallel and, if possible, bring to the stage of validation with the help of the consortium. In this patient cohort, the alloimmune response before transplantation and during conversion from triple to double drug treatment is measured by the multiparameter MLC-CFSE assay, which enables us to determine a combination of quantitative and qualitative properties of alloreactive T cells in one assay. In MLCs, CFSE labelled recipient cells are stimulated with donor specific or with third party cells. $CD4^+$ and $CD8^+$ precursor frequencies and number of divisions are deduced. The intracellular content of effector molecules is measured and responder cells are analysed for expression of cytokine- chemokine- and integrin receptors. Data will be related to the clinical course and evaluated on their usefulness as predictor of alloreactivity after minimisation of immunosuppression.

A study addressing the *Validation of an IFN-γ ELISPOT to better adapt immunosuppression in kidney allograft recipients* (J. Grinyo, Hospital Universitari de Bellvitge, University of Barcelona, Spain). As part of the potential techniques used to assess T-cell alloreactivity the interferon (IFN)-enzyme-linked immunospot (ELispot) assay has currently emerged as a highly sensitive method which may help optimising immunosuppression. The working hypothesis is that a prospective assessment of donor-specific cellular alloreactivity using the IFN-γ Elispot assay will be helpful to identify those patients that could benefit from a CNI-free immunosuppressive strategy. This is a non randomized, pilot, prospective,

open-label, multicenter trial including 60 patients to study whether allocation of a CNI based or CNI-free immunosuppressive regimen is feasible depending on the donor-specific cellular alloresponse as evaluated by IFN-γ ELISPOT assay in low risk renal transplant patients.
From the day of transplantation patients receive Thymoglobuline starting on that day up to day 5 post-transplantation, mofetil mycophenolate, corticosteroids in principle for 12 months. **In addition,** depending on pre-transplant IFN-γ Elispot, treatment will be:

- CNI-free (sirolimus) if the donor-specific IFN-γ Elispot is negative (**Group A**)
- Tacrolimus if the donor-specific IFN-γ Elispot is positive (**Group B**)

At 6 months post-transplant: a new donor-specific IFN-γ Elispot and a renal allograft biopsy will be performed in order to adapt the immunosuppressive treatment.

Group A Negative pre-transplant donor-specific IFN-γ Elispot

- if the donor-specific IFN-γ Elispot is positive and/or there are histological signs of rejection: treatment will be switched from sirolimus to tacrolimus.
- if the donor-specific IFN-γ Elispot is negative and there are no histological signs of rejection: treatment with mycophenolate and corticosteroids will be discontinued.

Group B Positive pre-transplant donor-specific IFN-γ Elispot

- if the donor-specific IFN-γ Elispot is positive and/or there are histological signs of rejection: dosage of tacrolimus is increased, mycophenolate and corticosteroids are maintained.
- if the donor-specific IFN-γ Elispot is negative and there are no histological signs of rejection: progressive discontinuation of corticosteroids will be applied for the following 3 months, tacrolimus and mycophenolate are maintained.

- **A study on the Search for the immunological signature of operational tolerance in liver transplant recipients** (A. Sanchez-Fueyo, Liver Transplant Unit, Hospital Clinic Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain). The objective is to prospectively validate the diagnostic accuracy of a series of previously identified biological markers of operational tolerance ("signature of tolerance") by exploring an independent cohort of 81 liver allograft recipients receiving conventional immunosuppressive therapy. Patients are recruited from several European liver transplant units and are selected based on a set of common clinical criteria. Participating centers are: Hospital Clínic Barcelona (Dr Sánchez-Fueyo), Catholic University Louvaine(Dr Pirenne) and University Tor Vergata Rome (Dr G. Tisone). Subsequently, the plan is to include 20-30. In addition, this study recently incorporated its objective with that described above performed by the Brussel's team (V. Donckier, M. Goldman).

Innovative Approaches To Promote Transplant Tolerance

There are two studies in this group:

- **Combined Pharmacologic and T Cell-Mediated Immunosuppression approach for the Induction of Donor-Specific Unresponsiveness in Liver Transplant Recipients** (Geissler, Resenburg, Germany). This is a pilot study the main objective of which is to propose a novel cell therapy strategy, based on *in vitro* expanded $CD4+CD25^{high}$ regulatory T cells to establish a safe immunosuppression minimization regimen in liver transplant recipients thus setting the basis for protocols aiming at inducing immunologic tolerance. A treatment regimen associating anti-thymocyte globulin and sirolimus will be administered in the early post-transplant period, followed by an infusion of autologous (recipients') $CD4+CD25^{high}$ regulatory T cells. This trial includes 3 successive phases:
  - **Phase I**: 18 consecutive liver transplant recipients (including recipients of organs from both living and deceased donors) treated with standard immunosuppression (cyclosporine, daclizumab, prednisolone) will be sequentially monitored to assess the proportions and the functional capacity of regulatory T cells. Using the standardized monitoring tools provided by the RISET core laboratories, the analysis of blood and tissue samples recovered from these patients allowed the collection of informative data which meant it was appropriate to embark on the second phase of the study.

- **Phase II**: Here the aim will be to test the feasibility and safety of a thymoglobulin/sirolimus induction protocol on 10 consecutive liver transplant recipients (from living or deceased donors). Read-out parameters will include clinical criteria (i.e. frequency of rejection episodes requiring pharmacologic intervention, ability to taper immunosuppression, and organ-directed toxicity). Immunological monitoring performed in the RISET core laboratories will be used to define differences in terms of immune reconstitution and specific alloreactive responses in these patients. Results will be compared to those received from patients included in phase I of the study.
- **Phase III**: Once the feasibility and safety of the thymoglobulin/sirolimus immunosuppressive induction protocol is assessed (within the frame of the Phase II study), this treatment will be implemented with the cell therapy approach based on the adoptive transfer of CD4+CD25^{high} regulatory T cells. Drs. Edinger and Hoffmann recently described protocols for the isolation of human CD4+CD25^{high} regulatory T cells in GMP-conditions and they have developed efficient *in vitro* expansion protocols for this T cell subset. Upon approval of the cell therapy trial by ethical and regulatory authorities they plan to enroll a total of 6-8 patients in this pilot trial.

- **Use of a combination of anti-CD3 and anti-CD7 immunotoxins to induce long-standing remission of severe high grade graft-versus-host disease** (I. VJM van Oosterhout, Henogen, Gosselies, Belgium). For logistic reasons this protocol is only about to start; the company had to fulfill the various requirements of regulatory authorities, namely EMEA and RIVM (Dutch body responsible for clinical evaluation of immunological products) since participating clinical centers are based in Holland. The batches of the GMP products have been produced and made available to the centers. This study will be performed in bone marrow transplant recipients presenting with severe GvHD non responsive to previous conventional therapies. The objective is to provide a novel alternative therapeutic strategy to allow successful long-term engraftment of bone marrow transplants in a particularly difficult subset of patients presenting a life-threatening condition. Results from a pilot study proved highly encouraging. The centres which will recruit the patients have now been selected in Maastricht, Nijmegen, Rotterdam, Utrecht. Clinical Trial Submission to relevant competent authorities and ethic committee has been completed for some of the centers and is ongoing for the others. A total of 12 patients will be enrolled.

Histological Markers Of Chronic Allograft Nephropathy

A multicenter study is being led by E. Rondeau, Hôpital Tenon, Paris, France and S. Florquin, Amsterdam, Holland on the **Development of validated analysis of renal transplant biopsies to identify local markers of tolerance and to predict the development of fibrosis.**
Cellular infiltrates in renal biopsies are the hallmark of rejection but are also frequently observed in biopsies of good functioning grafts. The significance of these infiltrates is still a matter of debate. Their characterization may give an insight into the function of these cells and provide additional surrogate markers for immune reactivity of a renal transplant recipient towards his graft, and for renal outcome. The team would like to develop predictive diagnostic tools to assess the fibrogenic properties of the infiltrates. More specifically, they propose to investigate for the presence of epithelial to mesenchymal transition (EMT) markers expressed by tubular epithelial cells in the vicinity of the infiltrating cells. The aim is thus to confirm that in a prospective study and in a population of kidney transplanted patients, the type of cellular infiltrates and the epithelial behaviour mirror the degree of graft acceptance, and that the biomarkers we have selected will be relevant tools to identify the tolerant or near-tolerant patients in whom immunosuppressive regimen could be minimized. Renal biopsies (200) will be obtained from patients included in I. Ten Berge's study as well as protocol renal biopsies (250 biopsies recovered at 3 and 12 months after transplantation) from patients treated at Hôpital Tenon.

3 After Riset: The Next Steps

The RISET project will end in March 2010. It is important to realise, independently of the informative data which will be recovered from this extensive collaborative endeavour (analysis of a total of 272 patients plus an independent analysis of 450 kidney biopsies) the important precedent that the RISET networks represent for the future. This type of research certainly provides the proof of concept that high quality interaction can be achieved between clinicians and biologists committed to transplant immunology. It also indicates that validating tests which may turn out to be the clue to how transplant patients should be managed in the future can only be achieved when a sufficient critical mass is gathered, not only in terms of various clinical centers co-ordinating their efforts but also with the active participation of laboratories combining their expertise in fundamental and applied immunology.

The European Union has proven, once more, that it is certainly equipped with tools that are unique to provide not only the financial support but also the visibility needed to this perform this type of work at its optimum. It is the responsibility of the European transplant community to join forces in order to allow the perpetuation of such initiatives.

WP4

Aurélie Mahalatchimy & Anne Cambon-Thomsen

RISET: Legal and ethical update

A number of new and updated texts relevant to RISET have appeared in 2008 and the European Commission (EC) as well as the European medicines agency (EMEA) are very active in this area. The RISET consortium is regularly informed of developments and has participated in a number of the public consultations proposed. They relate to Transplantation regulation, cell therapy regulation, immunosuppression regulation and to clinical assays in the domain of immunosuppression and cell therapy. (Those consultations in which RISET participated are marked with an asterisk **. For full details of the schedule of events for each consultation please see the RISET website . Two documents of particular importance to RISET are marked # #. Full summaries of these are available on the RISET website).
www.risetfp6.org

1. Public Consultation Papers

25/01/2007: PUBLIC CONSULTATION PAPER: DRAFT OF THE COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP): "GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS": **

10/04/2008: PUBLIC CONSULTATION PAPER: PROPOSALS TO AMEND ANNEX I TO DIRECTIVE 2001/83/CE REGARDING ADVANCED THERAPY MEDICINAL PRODUCTS (Implementation of the "advanced therapies" regulation, Regulation (EC) No 1394/2007[1])**

24/04/2008: PUBLIC CONSULTATION PAPER: DRAFT OF THE COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP): "BIOMARKERS QUALIFICATION: GUIDANCE TO APPLICANTS": # #.

07/05/2008: PUBLIC CONSULTATION PAPER ON THE CERTIFICATION OF QUALITY & NON-CLINICAL DATA FOR SMALL AND MEDIUM-SIZED ENTERPRISES:

04/07/2008: PUBLIC CONSULTATION PAPER ON GOOD CLINICAL PRACTICE SPECIFIC TO ADVANCED THERAPY MEDICINAL PRODUCTS: **

22/07/2008: PUBLIC CONSULTATION PAPER ON THE REVISED CLINICAL TRIAL APPLICATION FORM REGARDING ADVANCED THERAPY INVESTIGATIONAL MEDICINAL PRODUCTS: **

03/07/2008: PUBLIC CONSULTATION PAPER ON THE DATA FIELDS CONTAINED IN THE "EUDRA CT" CLINICAL TRIALS DATABASE TO BE INCLUDED IN THE "EUDRAPHARM" DATABASE ON MEDICINAL PRODUCTS AND MADE PUBLIC:

1._Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004, OJ L324, 10.12.2007, p.121.

2. European Union (Eu) Legislation

08/12/2008: EU LEGISLATION: PROPOSAL OF A DIRECTIVE ON STANDARDS OF QUALITY AND SAFETY OF HUMAN ORGANS INTENDED FOR TRANSPLANTATION: # #.

I- **EU GUIDELINES:**

- **30/05/2008: ADOPTION OF THE FINAL GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS[2]:**
- **05/11/2008: PUBLICATION OF ADDITIONAL GUIDELINES ON GOOD CLINICAL PRACTICES (GCP) INSPECTIONS:**
- **16/12/2008: PUBLICATION OF GUIDANCE DOCUMENTS APPLYING TO CLINICAL TRIALS:**

II- **CONFERENCE AND WORKSHOPS:**

- **29-30/09/2008: "INNOVATION FORUM" organised by the Drug Information Association (DIA) in London:**
- **03/02/2009: 1st EMEA WORKSHOP ON ADVANCED THERAPY MEDICINAL PRODUCTS IN LONDON[3]:**

2._http://www.emea.europa.eu/pdfs/human/cpwp/41086906enfin.pdf
3._Agenda: http://www.emea.europa.eu/pdfs/conferenceflyers/sme3/61666908en.pdf

WP4

Christiane Kapitz & Anne Cambon-Thomsen

A survey on the state of public debate on immunosuppression and tolerance induction in the context of human transplantation.

As part of the RISET project on transplantation tolerance, an analysis of the state of the public debate on immunosuppression and tolerance induction was performed, in order to explore questions emerging or circulating in the public arena. . The studied areas were media, institutions, associations and personal exchanges. A corpus of texts and other resources was assembled: articles (daily press, medical press), TV, internet sources (national transplant agencies, patient associations, forums/lists of discussion of patients). The social needs for information were addressed by analyzing the information delivered through various channels, the exchanges between patients or their families and the needs they expressed. The countries involved were France, Netherlands, Belgium, Germany and Italy. In the field of health, information potentially allows for debate to be possible within society. It is hoped that the analysis of the data collected will provide an indication of what it is that the public feel it is desirable and / or important for people to know and what might be considered taboo.

See more at RISET website…
http://www.risetfp6.org/cgi-bin/WebObjects/Awo3.woa

www.risetfp6.org

4 The Patient forum

THE RISET PROJECT AIMS TO ADDRESS THE ETHICAL ASPECTS RELATED TO THE STEPS NEEDED TO ACHIEVE IMMUNOTOLERANCE AND TO ENSURE WIDE DISSEMINATION OF ALL ITS RESULTS. PATIENTS' PARTICIPATION IS ESSENTIAL IN THIS PROCESS. EVALUATING HOW INFORMATION ON IMMUNOTOLERANCE REACHES THE PATIENTS, AS WELL AS PROMOTING DIALOGUE BETWEEN THEM AND THE RISET EUROPEAN TASK FORCE IS EXTREMELY IMPORTANT. THE RISET NEWSLETTER PROVIDES A UNIQUE MEDIUM FOR THE PATIENTS TO EXPRESS THEIR CONCERNS AND VIEWS ON THE TOPICS ADDRESSED BY THE RISET CONSORTIUM.

Terry Mangan, Chairman, European Heart and Lung Transplant Federation.

As a heart transplant recipient and Chairman of the European Heart and Lung Transplant Federation (EHLTF) I have read with great interest of the EU funded RISET research project.

The EHLTF is an organisation of seventeen National Heart and Lung transplant patient support groups representing almost 10,000 recipients, any of whom could benefit from the successful outcome of this project.

The leaders of the individual National Associations join with the managing committee in welcoming and supporting the work of this exciting and important research project.

As transplant recipients we are of course aware of the many side effects and problems that present post transplantation. It is fair to say that many of these are fully explained by the Transplant hospital or clinic when our drug and medication regime begins. However knowing the consequences of the sometimes serious and dangerous side effects of long term immunosuppressive medication does not lessen the effects or related worry.

The side effects of immunosuppressive therapies are well known and can include,

- Impaired and deteriorating Renal function.
- Skin and other malignancies.
- High Blood Pressure.
- Weight Gain and Moon Face.
- Hirsutism
- Osteoporosis.
- Diabetes.

From a patients perspective the prospect of greatly reduced immunosuppressive medication through the induction of tolerance is to be warmly welcomed and supported.
It is hard not to see great potential in a successful RISET project not only for recipients but also for health care providers. Some of these could be,
Improved survival rates.

- Improved quality of life.
- Less Long Term Side effects.
- Improved social activities.
- Substantial health care economies.
- Significant economies on post transplant maintenance.
- Less usage of hospital beds, angiography, blood tests, biopsies.

As transplant recipients we are of course grateful to have received a second chance of life and despite the side effects we would sooner endure those than suffer the alternative.!!

It is also fair to say that the majority of transplant recipients go on to lead successful, active and fulfilling lives.

The EHLTF wish great success to those involved in this worthy RISET project.

EHLTF

EUROPEAN HEART AND LUNG TRANSPLANT FEDERATION

In conclusion may I, with respect and humility, take this opportunity to acknowledge Organ Donors and their families without whose goodness and compassion there would be no transplant programs.

"Talk to your family today about Organ Donation"

Patient Views on Improvements in Immune Tolerance for Organ Transplantation by Suzanne R. Pattee, J.D. Vice President, Regulatory and Patient Affairs Cystic Fibrosis Foundation

Living with CF, I have often contemplated the future decision about obtaining a lung transplant when my health significantly declines. The option of lung transplantation has not always been open to me. When I was diagnosed with CF 45 years ago, my parents were told that I had a 50 percent chance to live to be 5 years old. Fortunately, I successfully battled the odds as a child, and continue to do so today. In the interim, many of my friends with CF have received lung transplants. Instead of viewing this surgery as a release from the burdens of living with CF, it is more properly viewed as switching from one intensive chronic disease to another. I have some friends who are happy and healthy at least 10 years after receiving lung transplants. However, I also have lost some friends on the waiting list for a transplant, or within a few years after the procedure. Despite the tremendous potential benefit of transplantation, it is never an easy decision.

To further complicate the transplantation decision, lung transplant recipients often have much lower survival rates than those receiving other solid organs, such as kidneys. For years, transplanted lungs were very difficult to harvest from cadavers and more prone to complications and rejection than other organs. Currently, 60 percent of individuals who have received transplanted lungs survive 5 years.

RISET's goal to improve the ability of the body's immune system to accept transplanted organs would be a tremendous benefit to people with CF, like me and my friends. If progress could be made to improve the acceptance of new lungs in particular, people with CF would have a much greater chance for longer, healthier lives.

Thanks to the hard work of the CF Foundation and the international CF science community, the median predicted age of survival for people with CF in the U.S. has improved from early childhood in the 1950s to age 37 today. However, the median age of the population remains at 16 years in the US, and many folks with CF die in their mid-twenties. As a result, many young people with CF consider the option of lung transplantation when faced with serious health decline. Last year, 178 people with CF were transplanted in the U.S., according to the U.S. CF Foundation National Patient Registry. But, many also die after transplant. In 2007, 12 percent of the deaths for people with CF were from transplant complications.

Following transplantation, people with CF often have a better chance of survival than people who receive transplants due to other lung diseases. This can be attributed in part to their experience following a complex, life-sustaining medical regimen for CF. Thus, they are used to taking pills daily, frequently visiting their physician and supplementing their diet to combat CF prior to a transplant. Post transplant, this becomes even more urgent with immunosuppressive drugs, such as cyclosporine, to fight organ rejection.

One way to lower the risk of infections and chronic rejection is to find ways to safely and successfully reduce the levels of immunosuppressive drugs for people with lung transplants. Some progress is being made in this area with an inhaled form of cyclosporine. RISET's efforts to improve immune tolerance also would be a tremendous benefit to people with CF who obtain lung transplants. In addition to improved health and longevity, improved immune tolerance would reduce the side effects of the transplants and the treatment burden for patients. Overall, it would be an amazing gift to patients with CF to have a better chance of keeping their donated organs healthy and enjoying longer lives.

Identifying biomarkers of reduced immune tolerance would be another benefit to patients with CF who receive lung transplants. Just as testing blood glucose for people with diabetes can help individuals identify health trends and target treatment needs, biomarkers of changes in individual immune tolerance would help lung transplant recipients to closely monitor their health with their health care team and seek treatment options as necessary.

For people with end-stage CF, lung transplantation is an important option to consider. However, the specter of chronic organ rejection overshadows the choice. **RISET's contributions to solving the puzzle of immune tolerance for organ rejection would make this difficult choice easier.**

| Adding *tomorrows* every day.

Cystic Fibrosis Foundation

Nadine Stohler. President of the European Kidney Patients´Federation (CEAPIR)

European Kidney Patients' Federation
CEAPIR

In this stage of research, we have not enough information about the mechanism of immunotolerance in organ transplantation. As a kidney transplanted patient, transplant researcher and as President of CEAPIR, I think it is very dangerous to inform patients about immunotolerance. The risk that even a single patient is losing his graft after reducing or stopping the treatment by her/him self is too big. This is a decision which can only be made by a specialist for an individual patient and it must be done under very strict regulations and of course regular check up's in the clinic.

5 International Networks in Transplantation Tolerance: RISET and ITN

María Hernández, Robert Lechler, Alberto Sánchez Fueyo and Vicki Seyfert-Margolis

Recent therapeutical advances in transplantation aim to induce donor-specific tolerance, defined as permanent acceptance of allogeneic transplanted cells or tissues in the absence of continuous immunosuppression. The magnitude of this task presented to researchers and clinicians was such that it would only be achievable through the establishment of international networks of excellence. With this in mind different funding agencies were generated to channel and overview these endeavours. RISET (*Reprogramming the Immune System for Establishment of Tolerance*) is a multinational European project financed by the European Commission within the Sixth Framework Programme, that focuses on the translation of advances in research in tolerance into clinical practice and industrial development (www.risetfp6.org). ITN (*Immune Tolerance Network*) is an international collaboration of researchers aiming to accelerate the clinical development of immune tolerance therapies. In kidney transplantation clinical tolerance is considered when patients experience stable allograft function despite having ceased all immunosuppression for an extended period of time. This state, defined as operational tolerance, is very rarely observed, the common experience being that patients that stop their immunosuppression would lose their graft. It was therefore very important to determine if tolerance was identifiable. To this end two complementary studies, funded by these agencies, have been recently submitted for publication in which biological signatures or biomarkers indicative of tolerance have been described. These biomarkers need to be tested in the context of immunosuppression withdrawal studies to determine if they will be useful to predict good outcome before or during weaning of immunosuppression.

RISET is currently studying further tests to identify tolerance in different transplant settings. Samples from different trials are being collected and it will be possible to assess which biomarkers or tests are more useful to predict tolerance induction in kidney or bone marrow transplantation.

The ITN is currently conducting a clinical trial of mixed chimerism, a technique designed to replace the need for long-term immunosuppression in kidney transplantation. A repository of samples from this and other studies will form the basis to test biomarkers of tolerance that have proven to be useful to identify tolerance.

In liver transplantation approximately 20% of stable liver recipients can completely discontinue all immunosuppressive drugs without undergoing graft rejection. These patients appear to spontaneously develop transplantation tolerance while receiving conventional maintenance immunosuppressive drugs. The well-documented intrinsic tolerogenic properties of liver grafts are probably involved in the development of this phenomenon. Several clinical trials have been conducted to assess the feasibility of gradually tapering off immunosuppressive drugs in liver transplant recipients. These studies have shown that this strategy is reasonably safe, given that the occurrence of rejection can be easily reversed by the reinstitution of immunosuppressive therapy. However, none of these studies have identified markers that can be used to identify tolerant recipients before immunosuppressive drugs are weaned.

RISET is currently conducting a multi-center European clinical trial of complete immunosuppression withdrawal in adult liver transplant recipients to assess the safety of this strategy in a large cohort of recipients and to identify biomarkers associated with the tolerant state. According to the experimental design of the study, selected stable liver recipients (at least 3 years after transplantation) are gradually weaned from all immunosuppressiver therapy over a 6-month period, and then followed-up for 12 additional months. Recipients not undergoing rejection over this 18-month period are considered as tolerant. In order to conduct mechanistic studies through RISET core facilities, serial peripheral blood and liver tissue samples are collected before and after immunosuppression weaning is attempted.

The ITN is currently sponsoring in the US two clinical trials that target pediatric and adult liver transplant recipients, respectively, and address similar goals to the RISET liver study. In order to foster the discovery of novel biomarkers associated with tolerance in liver transplantation, RISET and ITN have signed a collaboration agreement that encompasses the exchange of scientific results and biological samples between the two research consortiums.

The collaboration here described will make available to RISET and ITN investigators data from a large number of tolerant and non-tolerant recipients, which will greatly enhance their capacity to identify and cross-validate potentially useful biomarkers.

During the last year, 3 new teams have become partners within the RISET consortium. This section briefly describes the profile of the teams and the specific projects.

New partners in

Barcelona

Renal Transplant Unit. Nephrology Department. Bellvitge Hospital.

Prof. Josep M. Grinyó, Dr. Oriol Bestard and Dr. Josep M. Cruzado

Our group has different areas of research in the field of renal transplantation. In 1992, our experimental nephrology laboratory was created and since that time, basic-experimental and clinical investigation projects have been carried out; the study and prevention of the ischemia-reperfusion injury, organ allocation-preservation, the development of new gene therapies not only in allo-models but also in a diabetic nephropathy model, the study of histological lesions in protocol allograft biopsies and the development of new immunosuppressive drugs/regimens have been the main topics focused by our group.

It should be noted that the study of transplant immunology is also a relevant area of research in our laboratory. In fact, the study of donor-specific alloreactivity was first evaluated using lymphocyte proliferative assays during the ninety's and is now being studied using new immune-monitoring tools, such as the ELISPOT assay and Facs analyses

The assessment of the donor-specific cellular alloimmune response of renal transplanted patients, using the ELISPOT IFN-gamma assay in order to be able to discriminate highly alloreactive patients at risk for immune-mediated graft injury, has been one of the main topics in this specific field and is the main role of our group in this European consortium. Over the last few years we have shown the relevant correlation of highly cellular alloreactive patients and worse graft function, suggesting an on-going immune-mediated graft injury among these patients.

We have been collaborating with one of the leading groups in this field in Charité, Berlin, led by Prof. Dr. Reinke and Prof. Dr. H-D Volk. Therefore, our group is involved in RISET in two main studies; the first is validating and standardizing the IFN-gamma ELISPOT assay for renal transplantation and the second is studying new biological markers as well as a potential different gene pattern profile in tolerant and highly alloreactive patients in an on-going pilot study performed in our Institution.

RISET

Amsterdam

Renal Transplant Unit in the Academic Medical Centre, the Netherlands

Prof. Ten Berge.

The Academic Medical Center is a tertiary reference center for nephrology and renal transplantation. We are affiliated with 15 dialysis centers and hospitals in the North and the Middle of the Netherlands. These centers refer their patients for transplantation screening and donor screening. In addition we have a significant number of dialysis patients in our own centre. We perform around 60 post-mortal transplantations and 40 living donor transplantations yearly. Due to the shortage of post-mortal donors, we expect that the number of live donor will grow by 20% each year. Patients can,if necessary, participate in the national paired-exchange transplantation program and we are currently working on a protocol to transplant across an ABO barrier.

Our center is headed by Prof. Dr. Ineke J.M. ten Berge, with staff members dr Frederike J. Bemelman, dr Karlijn A.M.I. van Donselaar-van der Pant and dr Anne van Tellingen. Our surgical team is headed by Prof. Dr. Dink Legemate and Dr. Mirza Idu. Our tissue typing laboratory is located in Sanquin and headed by Dr. N. Junior Lardy.

Our research involves four topics: (1) the cellular allo-immune response; (2) development of the antiviral immune response after renal transplantation; (3) diagnosis and significance of subclinical rejection; (4) optimization of the immunosuppressive drug therapy in renal transplantation.

AD 1

Currently, the multiparameter MLC-CFSE test is evaluated for its potential to establish pre-transplant differences between potential non-rejectors and rejectors. Results are expected in due course. We have alsodiscovered that CD103, used by allo-responsive $CD8^+$ T-cells to adhere to E-cadherin on transplant tubular cells, is also a marker of regulatory allo-reactive $CD8^+$ T-cells. This new subset of regulatory T-cells is increased by the immunosuppressive drug rapamycin. Recently we studied gene-expression profiles of $CD103^+$ as compared to $CD103^-$ T-cells and now we are analyzing the significance of molecules that have shown significantly different expression.

AD 2

With respect to immune responses to persisting herpes viruses in renal transplant recipients, we have studied both CD8+ and CD4+ T-cell responses to cytomegalovirus (CMV). The entrance of CD8+ CMV-specific T cells expanded the antigen-primed CD8+ T-cell compartment rather than competing for space with pre-existing memory T cells, specific for persistent or cleared viruses. To obtain insight in human CD4+ T-cell differentiation and selection *in vivo*, we longitudinally studied CMV-specific CD4+ T cells after primary infection. Dominant CMV-specific CD4+ clones during latency were only poorly represented in the acute phase, suggesting that strong selection and/or recruitment of novel clones after the initial clearance of the virus takes place in persistent infections in man.

AD 3

Regarding subclinical rejection, we questioned why the cytotoxic infiltrates, present in renal transplant biopsies of these patients don't lead to decreased renal function and found increased expression of the protease inhibitor serpin B-9 (SPB-9) in tubular epithelial cells of these biopsies. We hypothesized that SPB-9 protects renal tubular cells from apoptotic cell death. Currently we are studying the regulation of this molecule in primary tubular epithelial cells in vitro.

As participants of RISET we will enter clinical data in the RISET database. We will provide biological samples for RISET "obligatory tests"and we plan to implement data from our own immunological research.

AD 4

Clinical trials are being performed to determine the effectiveness and side-effects of different immunosuppressive drug regimens. As part of these studies, immunological, vascular and pharmacological studies are performed. In the past years, we have performed a multicentre randomized trial to study the effects of withdrawal of ciclosporin (CsA) at 6 months after transplantation, from a triple immunosuppressive regimen. Patients continued on prednisolon (P) and CsA, P and mycophenolate sodium (MPS) or P and everolimus (EVL). Prior to withdrawal, a transplant biopsy was done to ensure that no subclinical rejection was present. Drug levels were closely monitored. An interim analysis was performed after enrollment of half of the intended number of patients (n = 264). Mean follow-up was 14 ± 5 months. After conversion, acute rejection percentages were 3% in the P/CsA group, 22% in the P/MPS group and 0% in the P/EVL group ($p < 0.009$). We conclude that switching immunosuppressive therapy from a calcineurin containing triple regimen to double therapy with P and CsA or EVL at 6 months after renal transplantation, is effective in preventing late rejection. Double therapy with prednisolone and MPS after withdrawal of CsA resulted in an increase in severe acute rejection episodes. Renal function at the latest follow-up, i.e.at 9 ± 3 months after conversion in the EVL group was significantly better than in the P/CsA group.

Paris&Amsterdam

Focus on kidney transplantation studies:

Prof. S.FLORQUIN (AMC, Amsterdam) &
E.RONDEAU (Hopital Tenon, Paris)

Both units working in collaboration intend to develop validated analyses of renal transplant biopsies to identify local markers of tolerance and to predict the development of fibrosis.

Cellular infiltrates in renal biopsies are the hallmark of rejection but are also frequently observed in biopsies of good functioning grafts. The significance of these infiltrates is still a matter of debate. Their characterization may give insight in the function of these cells and provide additive surrogate markers for immune reactivity of a renal transplant recipient towards his graft, and for renal outcome.

The teams would like to develop predictive diagnostic tools to assess the fibrogenic properties of the infiltrates. More specifically, they propose to investigate for the presence of epithelial to mesenchymal transition (EMT) markers expressed by tubular epithelial cells in the vicinity of the infiltrating cells. Recently, there has been evidence that tubular epithelial cells could not only be the victim, but also actively participate to the development of fibrotic lesions: if injured, epithelial cells may either die or undergo profound phenotypic changes characteristic of mesenchymal cells and act as fibroblasts, a process called EMT. The group has recently demonstrated that some epithelial phenotypic changes (such as the *de novo* expression of vimentin and the translocation of beta catenin into the cytoplasm), compatible with the first steps of EMT, were frequently detected in well-functionning renal grafts at an early time point post-Tx, and all the more when the infiltration was severe. More importantly, we found that the early expression of these markers (at three months) was predictive of late interstitial fibrosis and tubular atrophy (at one year), the main features of chronic allograft nephropathy.

The aim is to confirm that, in a prospective study and in a population of kidney transplanted patients, the type of cellular infiltrates and the epithelial behavior mirror the degree of graft acceptance, and that the biomarkers we have selected will be relevant tools to identify the tolerant or near-tolerant patients in whom the immunosuppressive regimen could be minimized.

Renal biopsies obtained from patients included in 1. ten Berge's proposal (protocol 6 and 24 months after transplantation as well as upon indication, total biopsies = 200) as well as protocol renal biopsies from patients included in Tenon (protocolar 3 and 12 months after transplantation as well as upon indication, total: 100 patients, 250 biopsies) will be:

- Scored according to the Banff'07 classification by two independent pathologists (SF and PC).
- Picro-sirius red staining on 4 µm sections will be analyzed using computer-assisted image analysis (AMC).
- Immunostainings will be performed on 4 µm paraffin sections using standard techniques. Positive cells will be counted/mm2 and ratio will be calculated. Conventional markers for T cells (CD3), B cells (CD20), NK cells (CD56), monocytes/macrophages (CD68), mast cells (cKIT), regulatory T cells (FoxP3) will be used to identify cellular infiltrates. To characterize the epithelial phenotype, and detect EMT: vimentin, localization of □-catenin (basal or cytoplasmic), and CD44 (a receptor for hyaluronan).
- C4d staining on paraffin sections is added as a marker for humoral immunoreactivity. For C4d staining, the percentage of positive peritubular capillaries will be estimated (AMC).

These data will be correlated with data obtained from Professor Tenthe RISET consortium and with long-term renal outcome data

Picture of the RISET consortium at the last meeting of the group, held in Brussels (Belgium) in March 2009.

Newsletter edited by ONT ORGANIZACIÓN NACIONAL DE TRASPLANTES

www.risetfp6.org

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Organización Nacional de Trasplantes - ONT	
Universität Bern, Department of Clinical Research, Cardiology	Switzerland
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Academic Medical Centre, Amsterdam, Renal Transplant Unit	
Leiden University Medical Center, Dpt of Nephrology	
King's College London, Dpt of Nephrology and Transplantation, Thomas Guy House	United Kingdom
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University of Oxford, Nuffield Department of Surgery, John Radcliffe Hospital	
University of Oxford, Sir William Dunn School of Pathology	

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