Indices of Tolerance

A retrospective clinical study aimed at developing bioassays which examine aspects & biomarkers of the immune system, which may then be used to predict the presence of transplantation tolerance in kidney transplant patients, allowing the informed and safe withdrawal of immunosuppression

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Study Supported by: **Immune Tolerance Network, European Union FP V, RISET Consortium**

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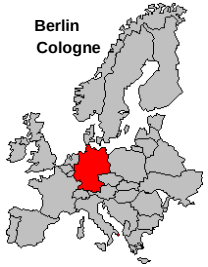

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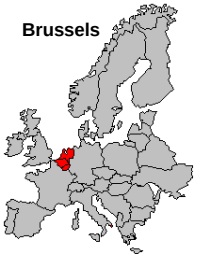

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The Indices of Tolerance study was a multi-centre collaborative effort, drawing on the scientific expertise of a number of research teams across Europe, and with the assistance of clinicians across the continent in recruiting kidney transplant patients. We have also collaborated extensively with the Immune Tolerance Network in the USA.

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Patient Recruitment

Group	Clinical definition	N
"Tolerant" Drug Free **(Tol-df)**	Stable kidney function > 1 year Serum creatinine < 160μmol/L and < 10 % rise NO immunosuppressive drugs	11
Low Pred **(S-LP)**	Stable kidney function > 1 year; Serum creatinine < 160μmol/L and < 10 % rise < 10 mg/day prednisone	12
Stable non CNI **(S-nCNI)**	Stable kidney function > 1 year; Serum creatinine < 160μmol/L and < 10 % rise never on Calcineurin Inhibitors	10
Stable CNI **(S-CNI)**	Stable kidney function > 1 year; Serum creatinine < 160μmol/L and < 10 % rise Triple therapy with Calcineurin Inhibitors post-transplant	30
Chronic rejection **(CR)**	evidence of immunologically driven chronic allograft nephropathy on adequate immunosuppression	9
		71

In order to identify the specific characteristics of the immune system presented in tolerant transplant patients, this study recruited & compared groups of kidney transplant patients based on their transplant function and immunosuppressive drug use, with the key patient group, Tolerant-Drug Free patients, whom display good graft function in the absence of any immunosuppressive drugs

Tolerant Drug-free patients

Gender	2  and 9 
Age	34 – 60 years old
Time Post-Transplantation	4 – 30 years
Time immuno-suppression free	1.5 – 13 years
Kidney function	75 – 140 μmol/L serum Creatinine 50 - 84 mL/min/1.73 m^2 Glomerular Filtration Rate
Donor Type	7 Cadaveric, 4 Live Related donor
Country of Origin	1 Czech Republic, 1 Italy 1 Switzerland, 3 France, 5 UK

Tolerant-Drug Free patients are incredibly rare and over the course of this study 11 patients were identified across Europe – their clinical characteristics are detailed above

Identifying Tolerance
studying the immune system

MICRO ARRAY

Donor-specific immune reactivity & regulation ELISPOT

Regulatory T cell suppression assays

RT-PCR of Tolerance-associated genes

ALLO-ANTIBODY detection

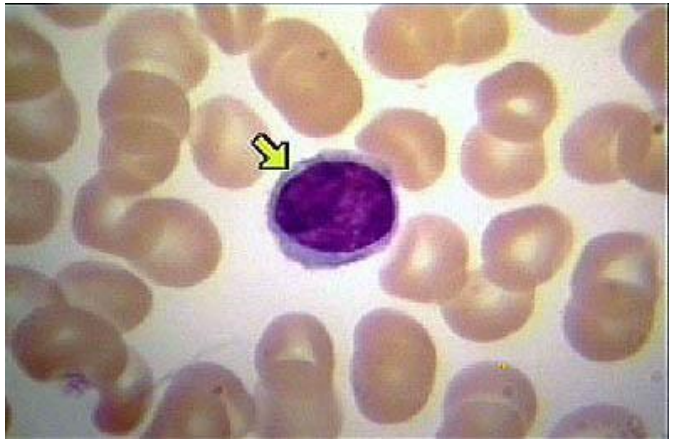
TRANS-VIVO delayed type hypersensitivity response

TCR LANDSCAPE analysis

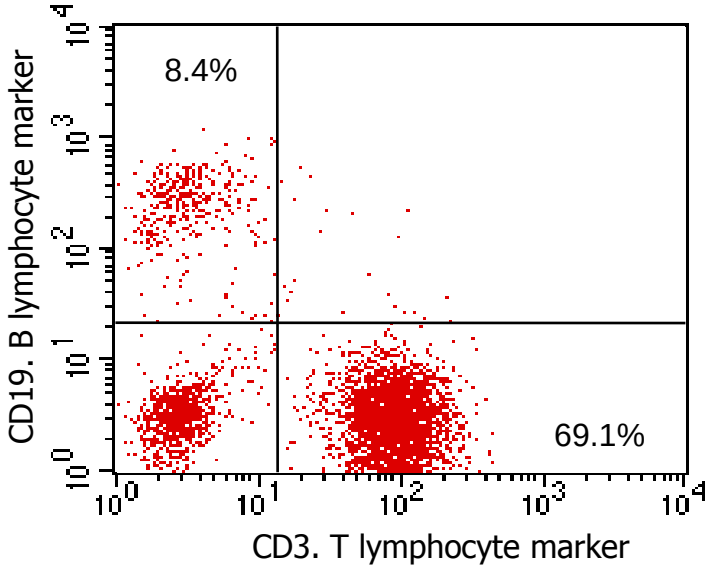
Peripheral Blood phenotyping

Several bioassays were developed to study various molecular and functional aspects of the immune system – These assays were performed in parallel on blood samples taken from the transplant patient groups previously described. The aim was to identify which tests could be used to specifically identify the tolerant transplant patients from the other patients groups

Biomarker
Ratio of lymphocyte subsets and activation markers



A treated blood sample looked under the microscope. Cell with arrow is a lymphocyte the others are red blood cells

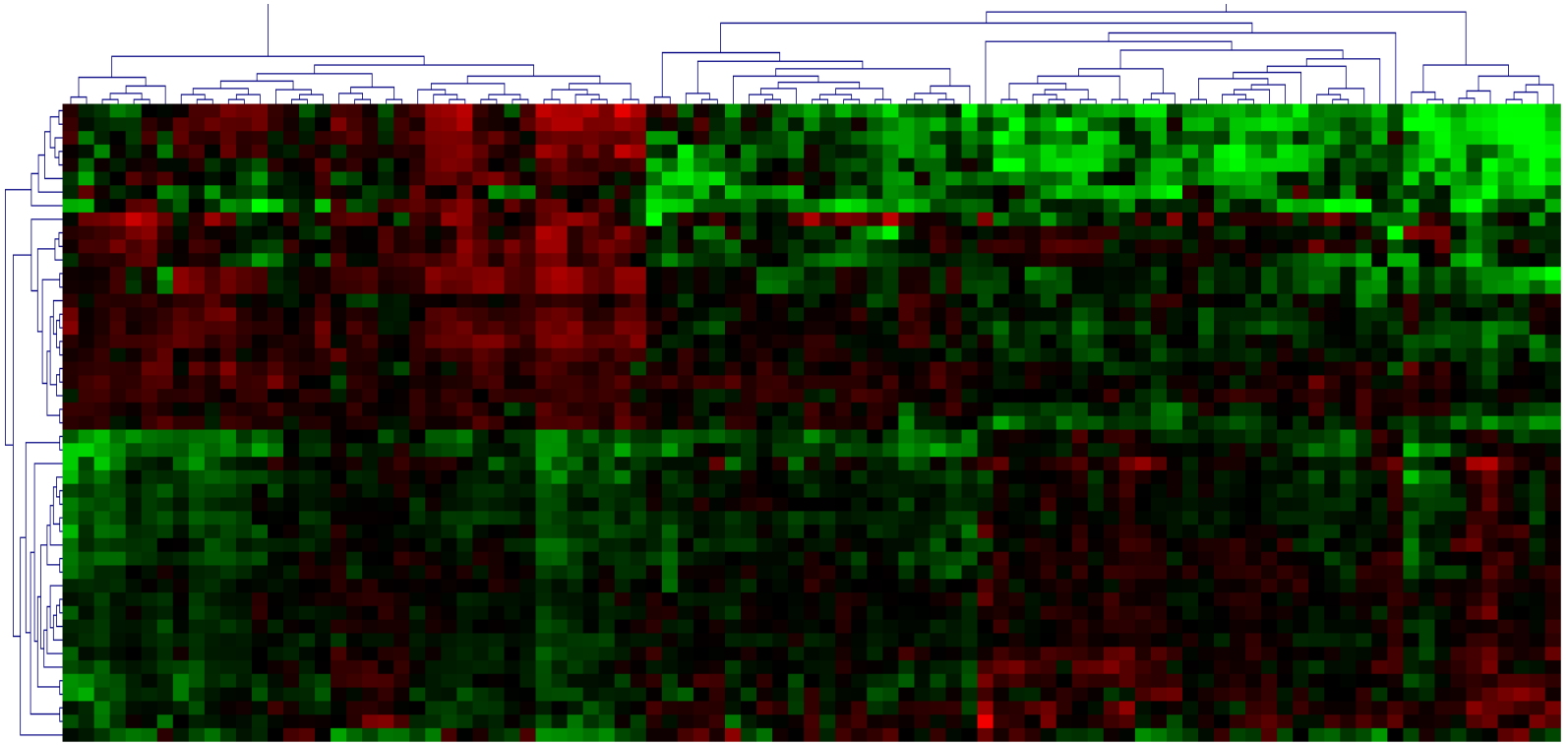


To study percentages of different set of white cells we use flow cytometry. This is an example of the results we get

By examining the percentages of different white cells (B & T lymphocyte subsets) within peripheral blood samples, the study identified that Tolerant-Drug free patients can be distinguish from the other patient groups using their ratio.

By examining some activation markers on a set of white cells (level of CD25 expression on CD4+ T cells) within peripheral blood samples, the

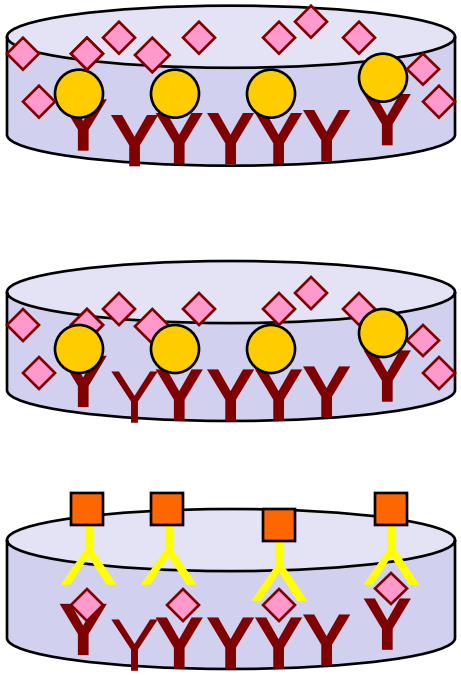
Biomarker
Gene expression analysis. Microarray on whole blood



Microarray test results are shown as "Gene expression maps" that look like this one

A set of genes were found to be differentially expressed specifically by the Tolerant Drug free patient group, compared to the other transplant groups, when examined by microarrary gene analysis of whole blood.

Bioassay
Direct pathway ELISPOT

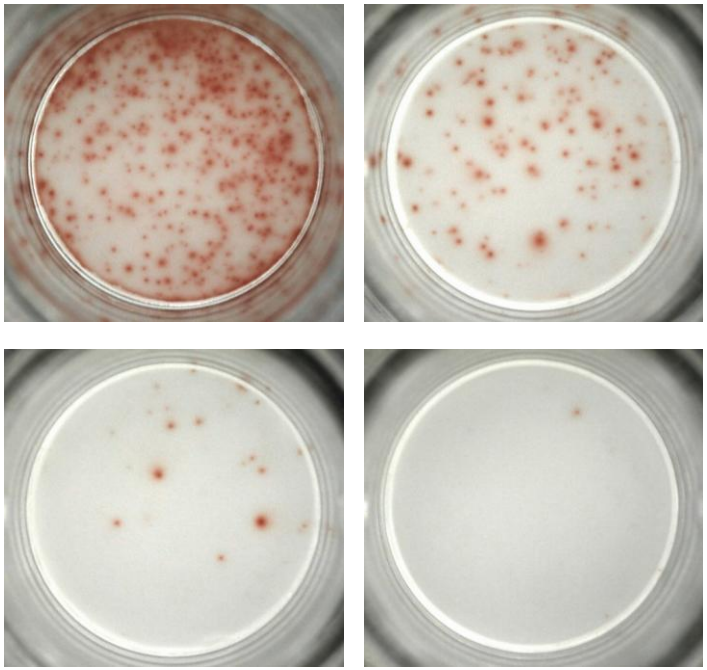


To study how white cells from recipients respond to donors, we culture recipient cells on plastic plates with donor material for 1 day.

We use different reagents that make responding cells become a red spot (as shown on right).

Then we have a machine that counts the red spots.

On the right you can see examples of very intense responses (top left) and negative responses (bottom left)



The ELISpot bioassay assesses the specific immunological responses of a set of white cells from (CD4+ T cells) isolated from peripheral blood, induced against either Donor cells or 3rd party (unrelated) cells.

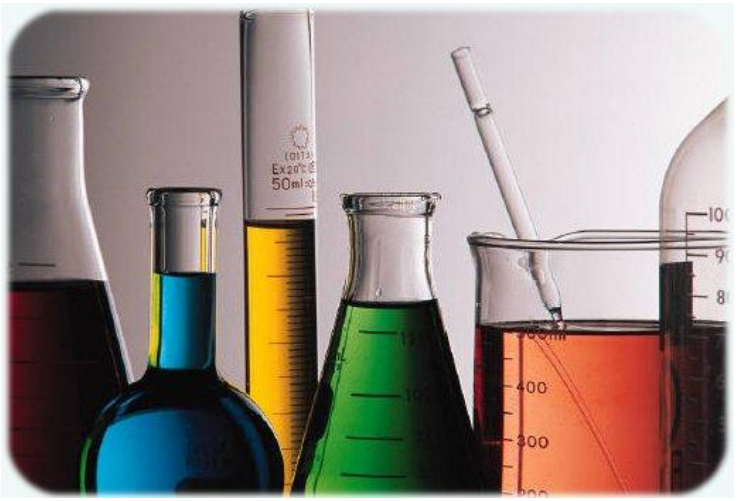
Tolerant patients were found have the lowest ratio of donor: 3rd party responses compared to other patient groups, suggesting that Tolerant patients have a significantly lower immunological response against donor cells compared to the other kidney transplant patient groups

Summary of Findings

This study concludes that by using the bioassays & biomarkers listed above, the presence of transplantation tolerance in kidney transplant patients may be clearly identified (high specificity & sensitivity).

This tentative "fingerprint" of clinical transplantation tolerance now needs to be tested and validated with more patient samples.

If substantiated, these characteristics could be used to select patients for deliberate and carefully monitored immunsuppressive drug weaning or withdrawal.



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many of their donors

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GP surgeries in UK

& many clinicians and nurses

contributing to this study,

without ALL of whom this research would not have been possible !!!!