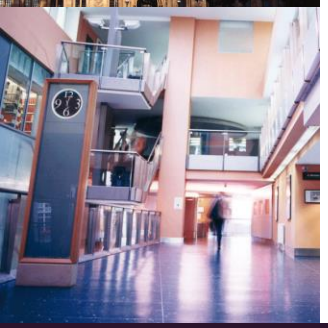
Faculty / Presenter Disclosure

- Faculty: Maria Hernandez Fuentes

- Relationships with commercial interests:
  - Other: Employee of UCB Pharma

  - No off-label (or on-label) use of any product from UCB will be discussed in my presentation.



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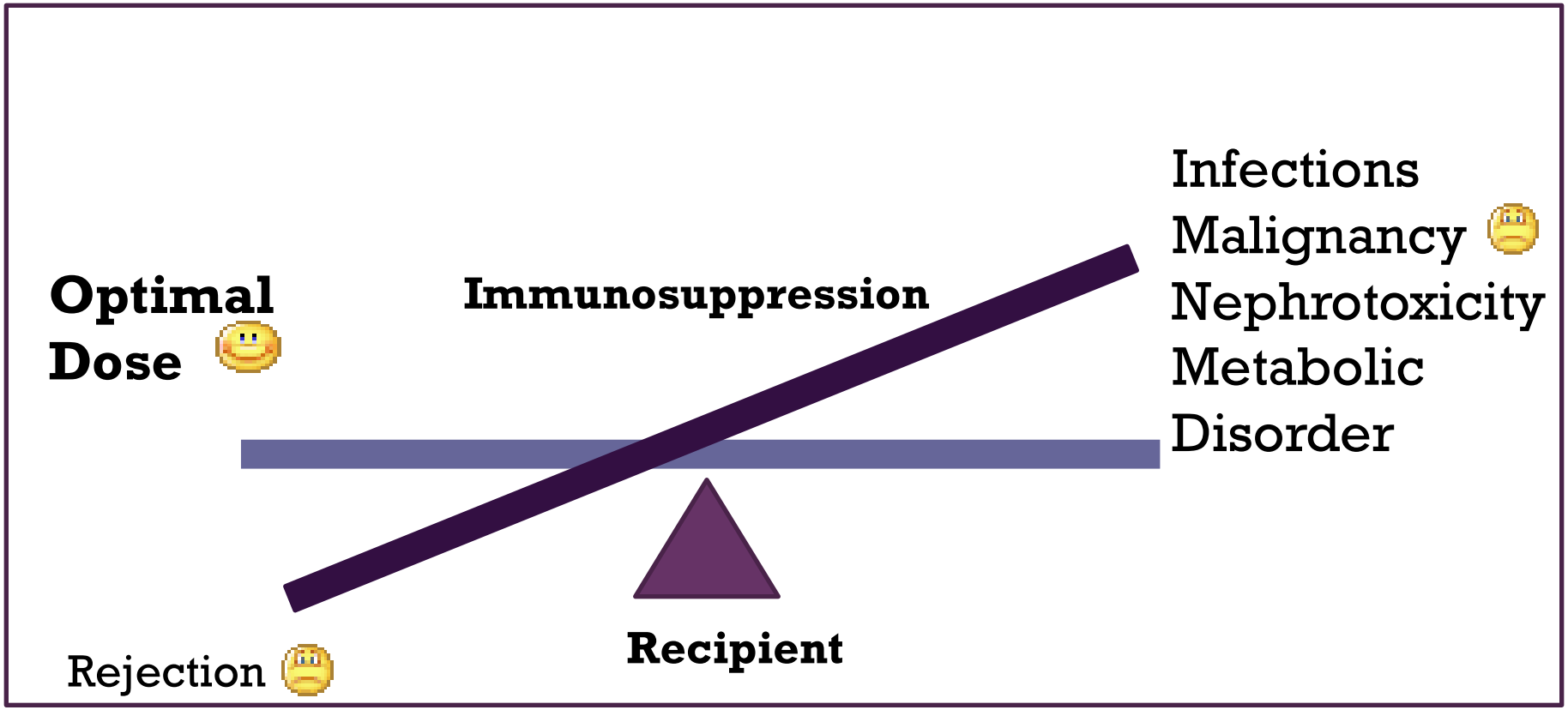
State of the art:

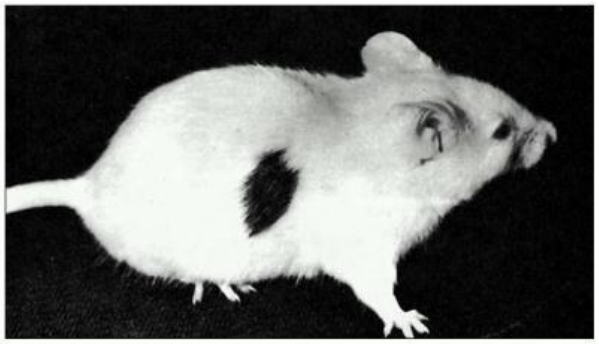
Fingerprints of tolerance

Dr Maria Hernandez-Fuentes

The everlasting Challenge



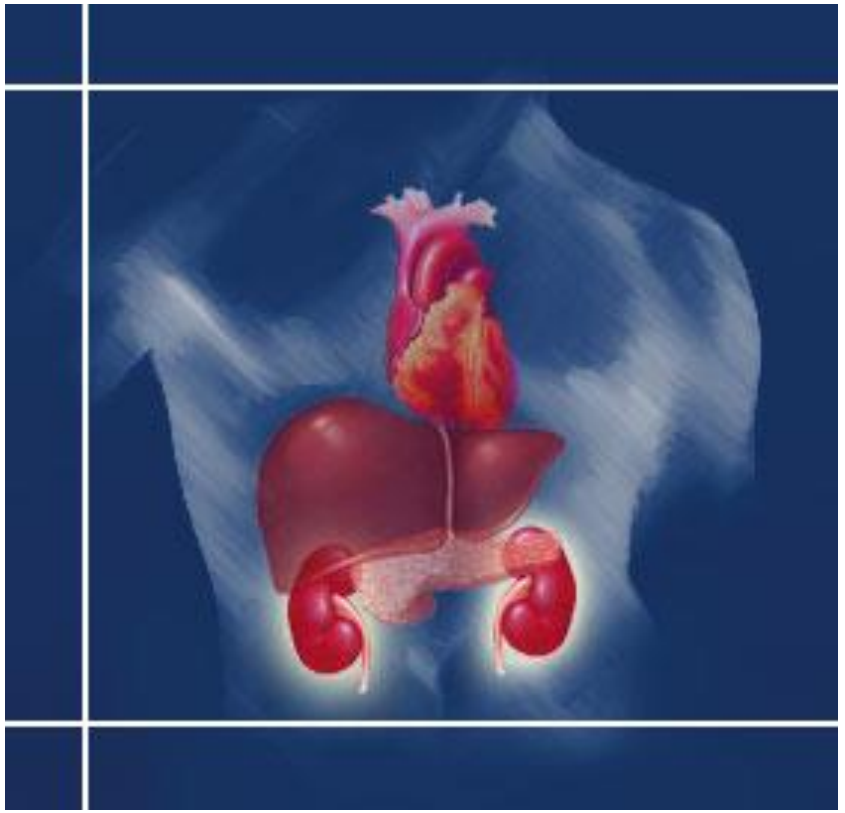
How would finding tolerance help?



Holy Grial in clinical Transplantation:

Tolerance

http://www.ncbi.nlm.nih.gov/books/NBK26921/



Donor-specific unresponsiveness in the context of otherwise normal immune responses

?

Why Biomarkers of Tolerance?

- To identify *spontaneous* "tolerant-phenotype"
  - *Personalised medicine*: could those patients, identified as such, be optimally maintained with less immunosuppression?

- Evaluate novel "tolerance-inducing" therapies (cell therapy, regenerative therapies, etc)
  - would these patients display the same markers as patients with *spontaneous* tolerance?

- Better understand allo-immmune regulation
  - would such markers allow us to understand better mechanisms of tolerance?

2002 – 2010 Indices of Tolerance & ITN

King's College London, UK

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Maria Hernandez Fuentes
Pervinder Sagoo
Esperanza Perucha
Elvira Jimenez
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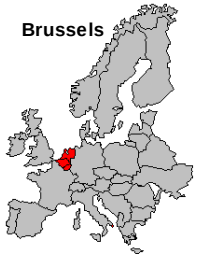

ITERT - INSERM, France

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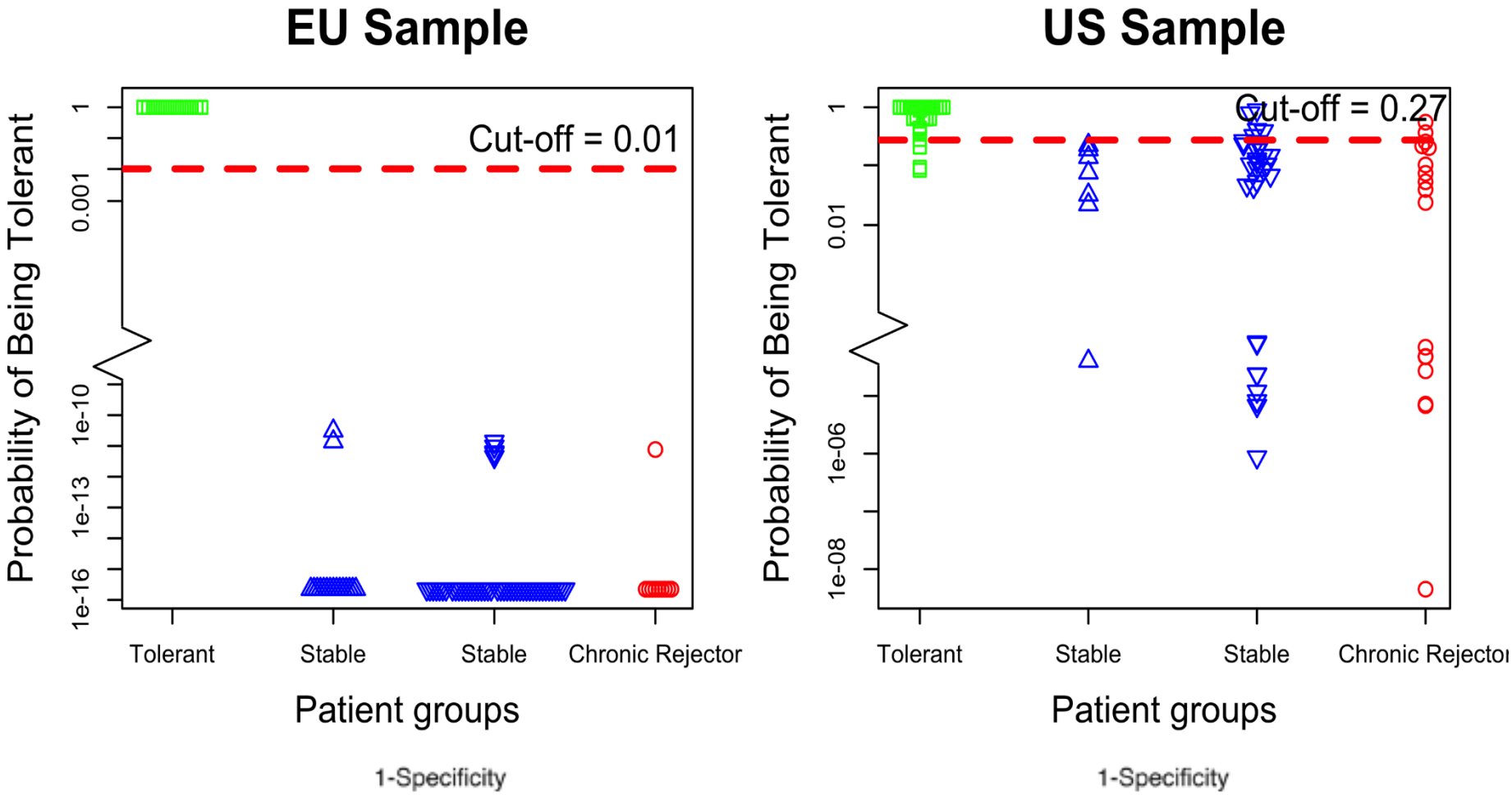
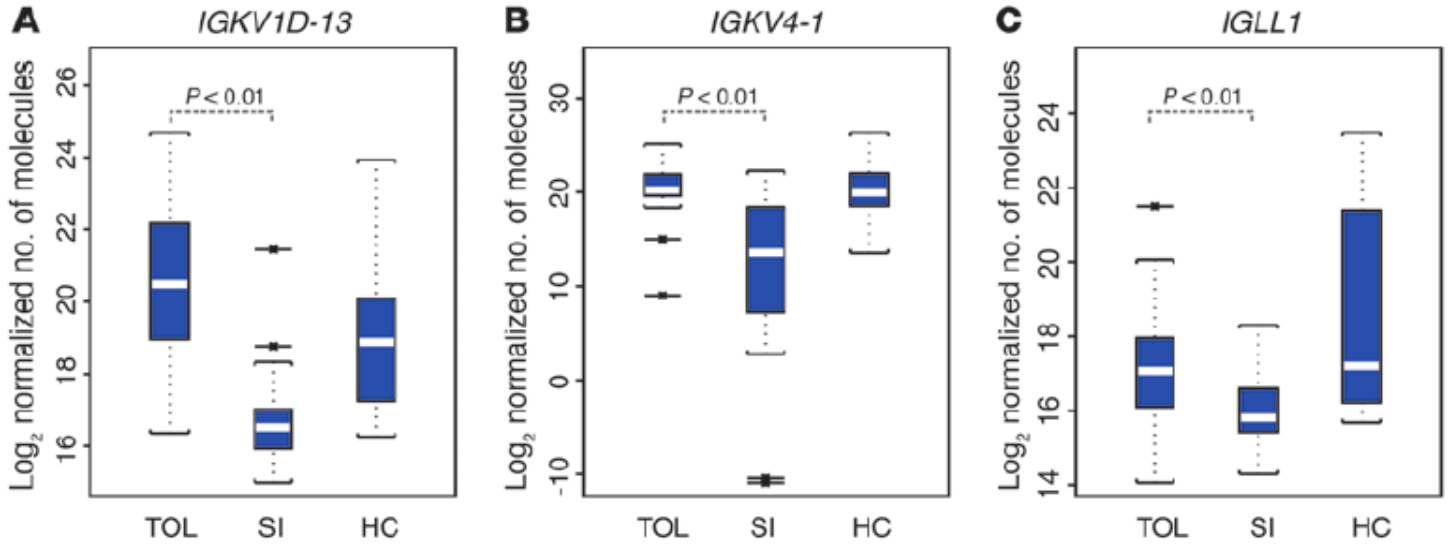
Predicted Probability of being tolerant



Sagoo P*, Perucha E*, www.transplant-tolerance.org.uk, et al. ***J CLIN INVEST*** (2010) 120 (6): 1848-1861

Identification of a B cell signature associated with renal transplant tolerance in humans

Kenneth A. Newell,[1] Adam Asare,[2,3] Allan D. Kirk,[1] Trang D. Gisler,[2,3] Kasia Bourcier,[2,3] Manikkam Suthanthiran,[4] William J. Burlingham,[5] William H. Marks,[6] Ignacio Sanz,[7] Robert I. Lechler,[8,9] Maria P. Hernandez-Fuentes,[8,9] Laurence A. Turka,[3,10] and Vicki L. Seyfert-Margolis,[3,11] for the Immune Tolerance Network ST507 Study Group



Tolerant subjects showed increased expression of multiple B cell differentiation genes.
A set of just 3 of these genes distinguished tolerant from non-tolerant recipients in a unique test set of samples.

Newell K, et al www.immunetolerance.org, et al. *J CLIN INVEST* **(2010)** 120 (6): 1836-47

Identification of a peripheral blood transcriptional biomarker panel associated with operational renal allograft tolerance

Sophie Brouard[a], Elaine Mansfield[b,c], Christophe Braud[a], Li Li[b], Magali Giral[a], Szu-chuan Hsieh[b], Dominique Baeten[a,d], Meizia Zhang[b,e], Joanna Ashton-Chess[a], Cécile Braudeau[a], Frank Hsieh[f], Alexandre Dupont[a], Annaik Pallier[a], Anne Moreau[g], Stéphanie Louis[a], Catherine Ruiz[b], Oscar Salvatierra[b], Jean-Paul Soulillou[a,i], and Minnie Sarwal[b,i]

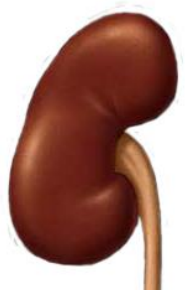
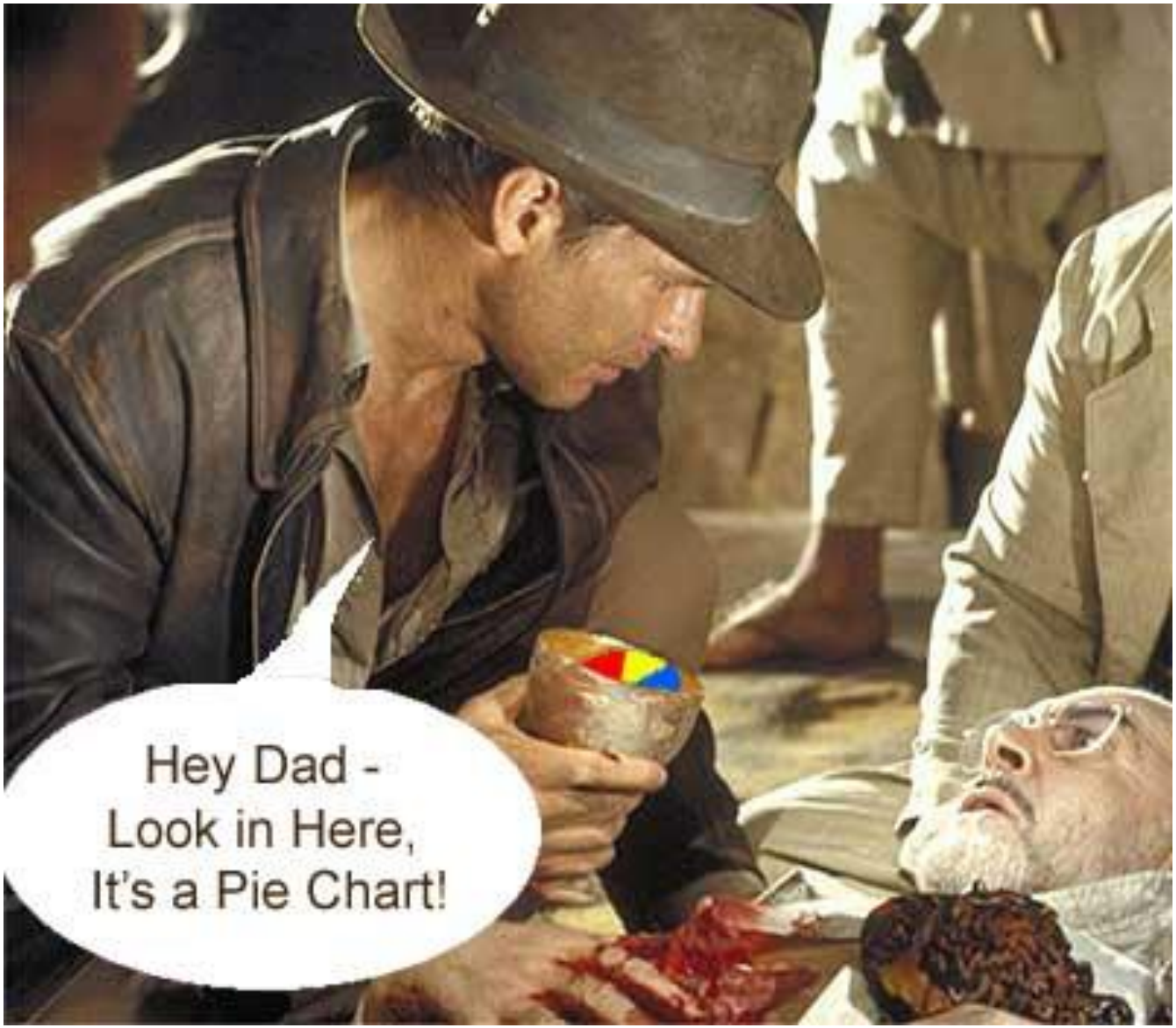


Table 1. Demographic summary of patient groups (median and range)

	Training groups			Test groups					
	TOL	CR	N	TOL-test	CR-test	MIS	STA	AR	N-test
Number	5	11	8	12	11	10	12	14	8
Age, years	67	56	23	37.5	52	55.5	49	20	46
	58–73	28–75	11–27	20–87	10–59	28–83	31–67	16–24	30–66
% Male	80	63.60	37.5	75	63.6	54.50	58	64.20	0
Time post-transplant, months									
Mean	178	59	NA	137	48	139.5	172	12	NA
Range	108–360	20–158		86–372	11–158	47–262	48–269	0.5–108	
Serum creatinine, μM/liter									
Mean	122	244	NA	115	244	98.5	107	152	NA
Range	82–139	127–492		70.4–149.6	100–686	64–161	63–147	110–704	

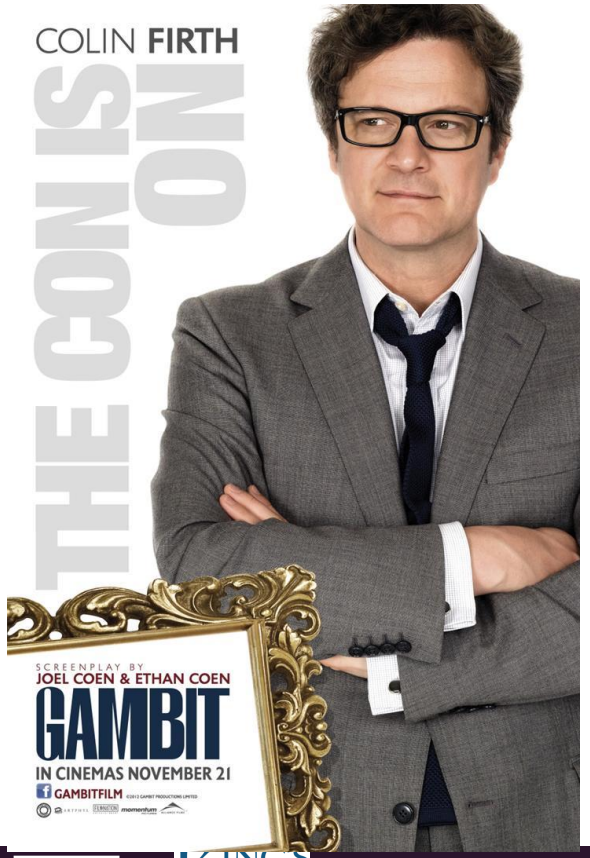
From microarray results a "tolerant footprint" of 49 genes.
These biomarkers were applied for prediction of operational tolerance by microarray and real-time PCR in independent test groups.
33 of 49 genes correctly segregated tolerance and chronic rejection phenotypes with 99% and 86% specificity.
The expression signature suggests that **TGF- might contribute to this process**, possibly by regulating specific phenotypes of peripheral regulatory T cells or altering the threshold for T cell activation

Proc Natl Acad Sci USA. **2007** 25;104(39):15448-53


Hey Dad -
Look in Here,
It's a Pie Chart!

Genetic Analysis & Monitoring of Biomarkers of Immunological Tolerance

GAMBIT study



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Biomarkers of Tolerance:

Patient groups in retrospective cohort; aim to find the *prevalence of "tolerance"*

Tolerant recipients

Stable function
No immunosuppression

n=14

Stable recipients

Stable function

Little immunosuppression
Medium i-sup
High i-sup

n=190

Chronic Rejection

Graft Dysfunction

Medium i-sup
High i-sup

n=36

Index Group

Control Groups

Immunosuppression in GAMBIT

CNI	Azathioprine vs MMF	Prednisone	N	Percentage #
Cyclosporine	Azathioprine	No	25	13.2
Cyclosporine	Azathioprine	Yes	7	3.7
Cyclosporine	MMF	No	29	15.3
Cyclosporine	MMF	Yes	12	6.3
Cyclosporine	None	No	9	4.7
Cyclosporine	None	Yes	6	3.2
Tacrolimus	Azathioprine	No	11	5.8
Tacrolimus	Azathioprine	Yes	1	0.5
Tacrolimus	MMF	No	24	12.6
Tacrolimus	MMF	Yes	8	4.2
Tacrolimus	None	No	3	1.6
Tacrolimus	None	Yes	4	2.1
None	Azathioprine	No	1	0.5
None	Azathioprine	Yes	17	8.9
None	MMF	Yes	12	6.3
None	None	Yes	12	6.3
None	None	No	1 *	0.5

Percentage from all stable patients
* Patient on Sirolimus single therapy
Note: Information of drug regimen was missing for 8 of the stable patients (4.2%)

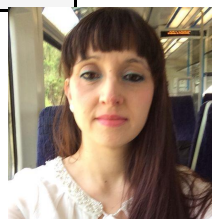
Retrospective Study

17 therapy groups

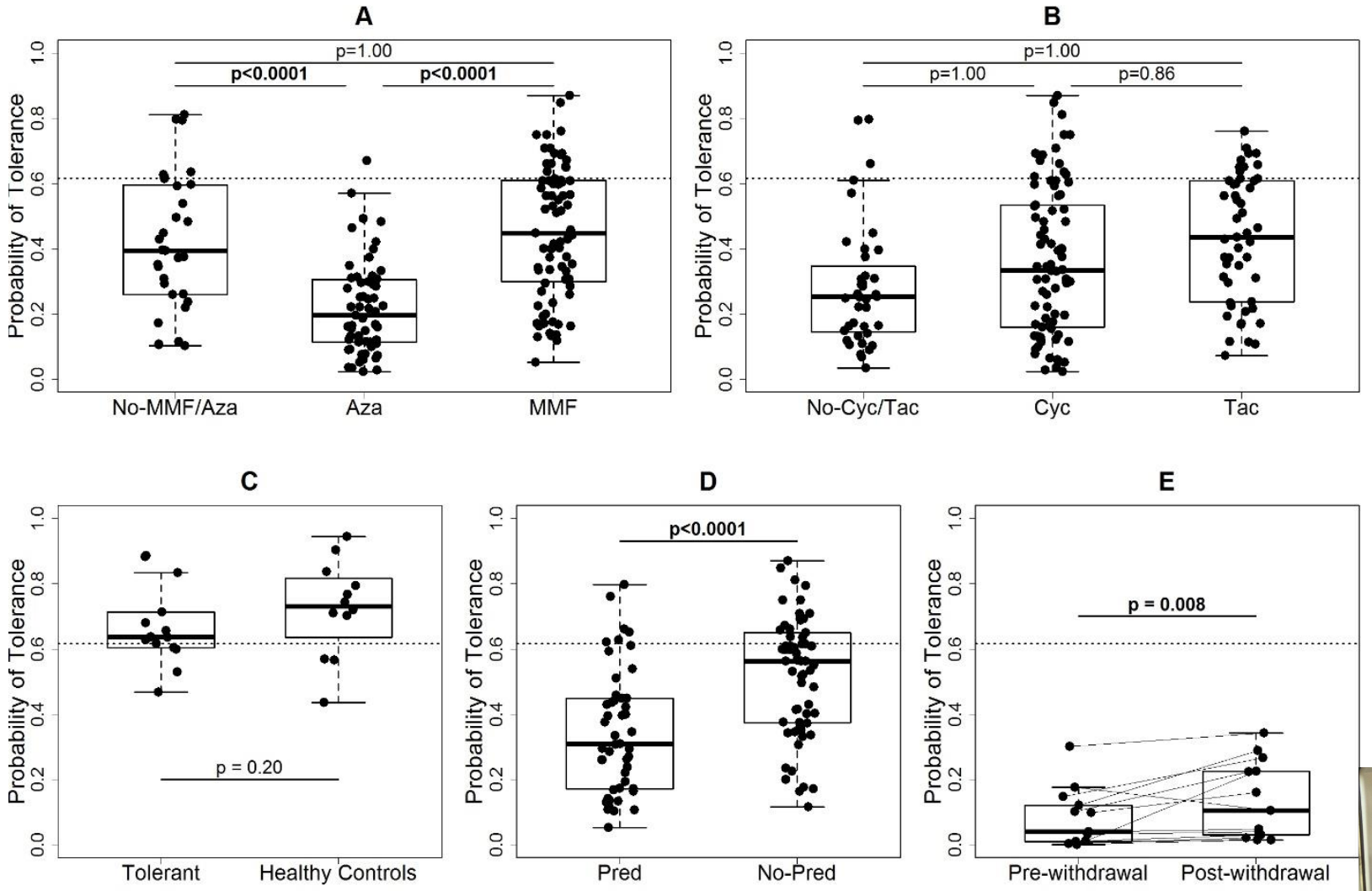
Unadjusted effect of immunosuppression in gene expression

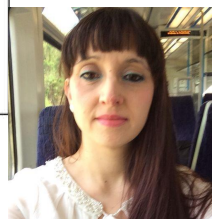
	Pred	Cyc	Tac	Aza	MMF
PNOC	0.11	0.10	0.041	0.76	1.00
CD79b	2.1×10^{-04}	1.00	0.12	8.1×10^{-04}	0.94
TCL1A	1.9×10^{-06}	0.17	0.020	6.7×10^{-16}	1.00
H3ST1	1.3×10^{-04}	0.30	0.14	3.6×10^{-05}	0.20
SH2DB1	0.42	1.00	1.00	$< 2.0 \times 10^{-16}$	0.11
TLR5	4.0×10^{-03}	1.00	0.095	1.00	1.00
MS4A1	3.0×10^{-03}	0.73	0.18	1.1×10^{-04}	1.00
FCRL1	1.7×10^{-04}	1.00	0.73	1.1×10^{-10}	1.00
FCRL2	5.7×10^{-04}	1.00	0.15	1.6×10^{-05}	1.00
FoxP3_AMann	0.69	0.16	9.0×10^{-03}	1.00	1.00

Rebollo-Mesa, et al AJTx 2016



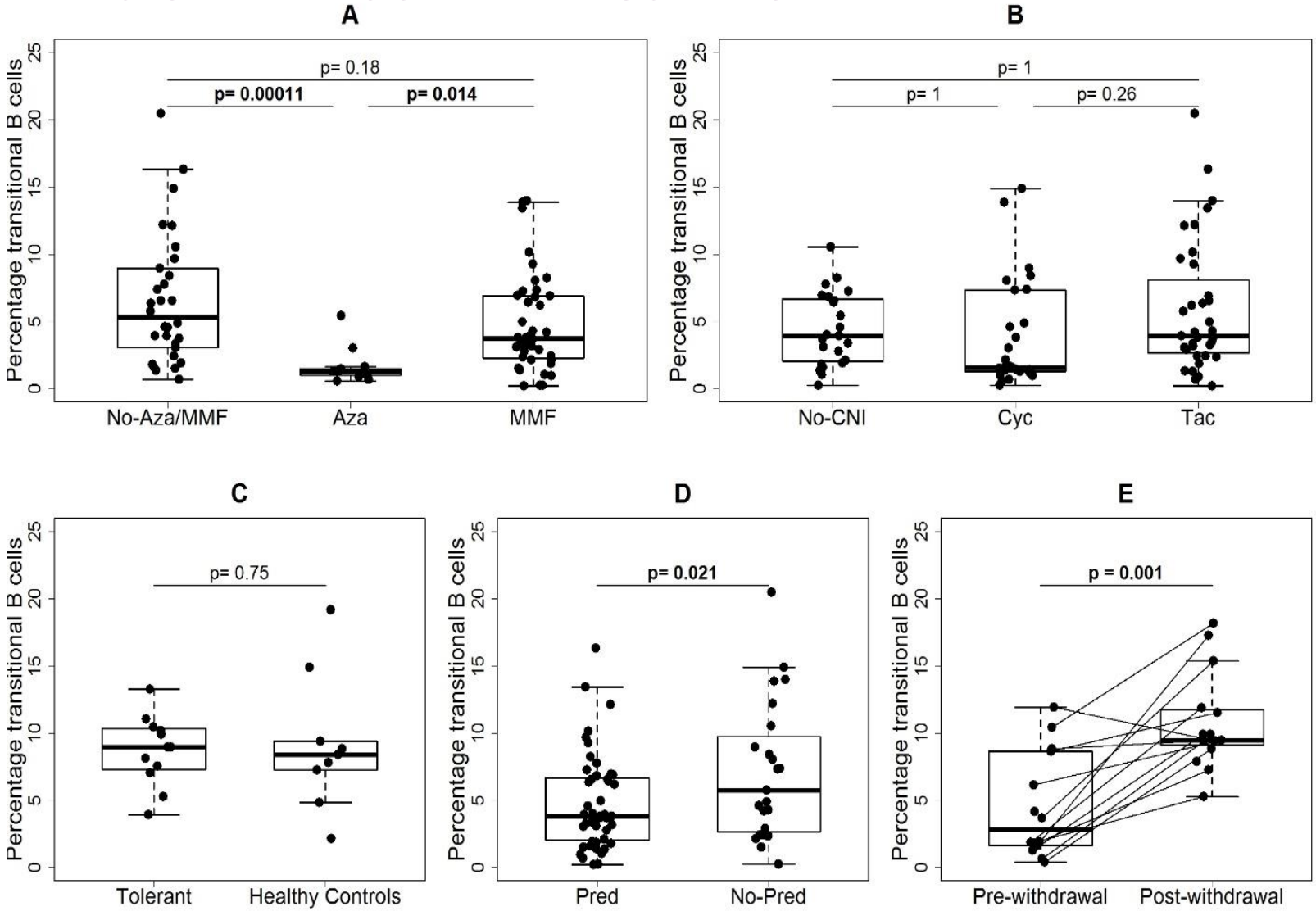
Immunosuppressants had a confounding effect on the expression of IoT gene set. RT-PCR





Rebollo-Mesa, et al AJTx 2016

Immunosuppressants affected the transitional B cell subset size.



Rebollo-Mesa, et al AJTx 2016

Conclusions 1

- Immunosuppression drugs the patients are taking (Aza + Pred)
    - have a major effect on the expression of the chosen genes
    - major effect on the size of the Transitional B cell compartment

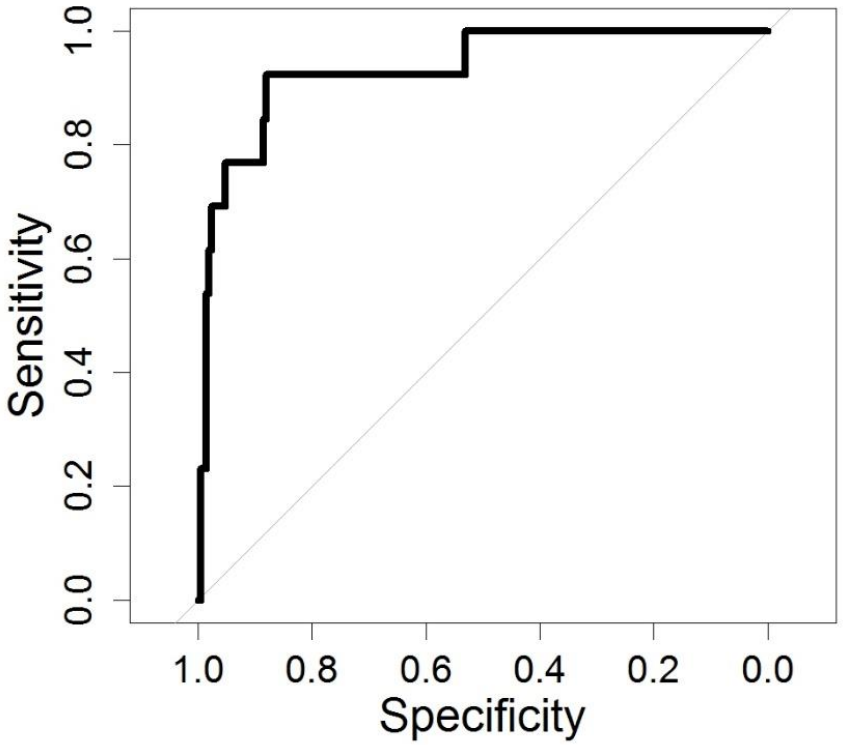
Rederived IS-independent gene-signature : IoT arrays

Validation using Fluidigm platform on GAMBIT samples.

EXPRES.	Molecular Function	Biological Processes	Documented protein expression in
↓ ATXN3	Ubiquitin-specific protease activity	Protein metabolism	Caudate Nucelus, Cerebellum Frontal Cortex, Pons, Ubiquitous
↓ BCL2A1	Receptor signaling complex scaffold activity	Apoptosis	B cell, Bone Marrow, Colon, Intestine, Leucocyte, Lymph node, Ovary, Spleen, T cell
↓ EFF1A1	Transcription regulator activity	Regulation of cell cycle	B cell, Islets of Langerhans, Lacrimal gland, Leukocyte, Monocyte, Neutrophil, Plasma, Saliva, Semen, Skeletal muscle, Tear
↓ TNFAIP3	Transcription regulator activity	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	Macrophages
↓ NFKBIA	Transcription regulator activity	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	Neutrophil, T cell
↑ GEMN7	Ribonucleoprotein	Regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	Spinal cord tissues
↑ IGLC1	Antigen binding	Immune response	B lymphocytes
↑ MS4A4	Unknown	Unknown	Intestine and colon
↑ RAB40	GTPase activity	Cell communication Signal transduction	Platelets, Liver, Heart, Kidney, Plasma

Rebollo-Mesa, et al AJTx 2016

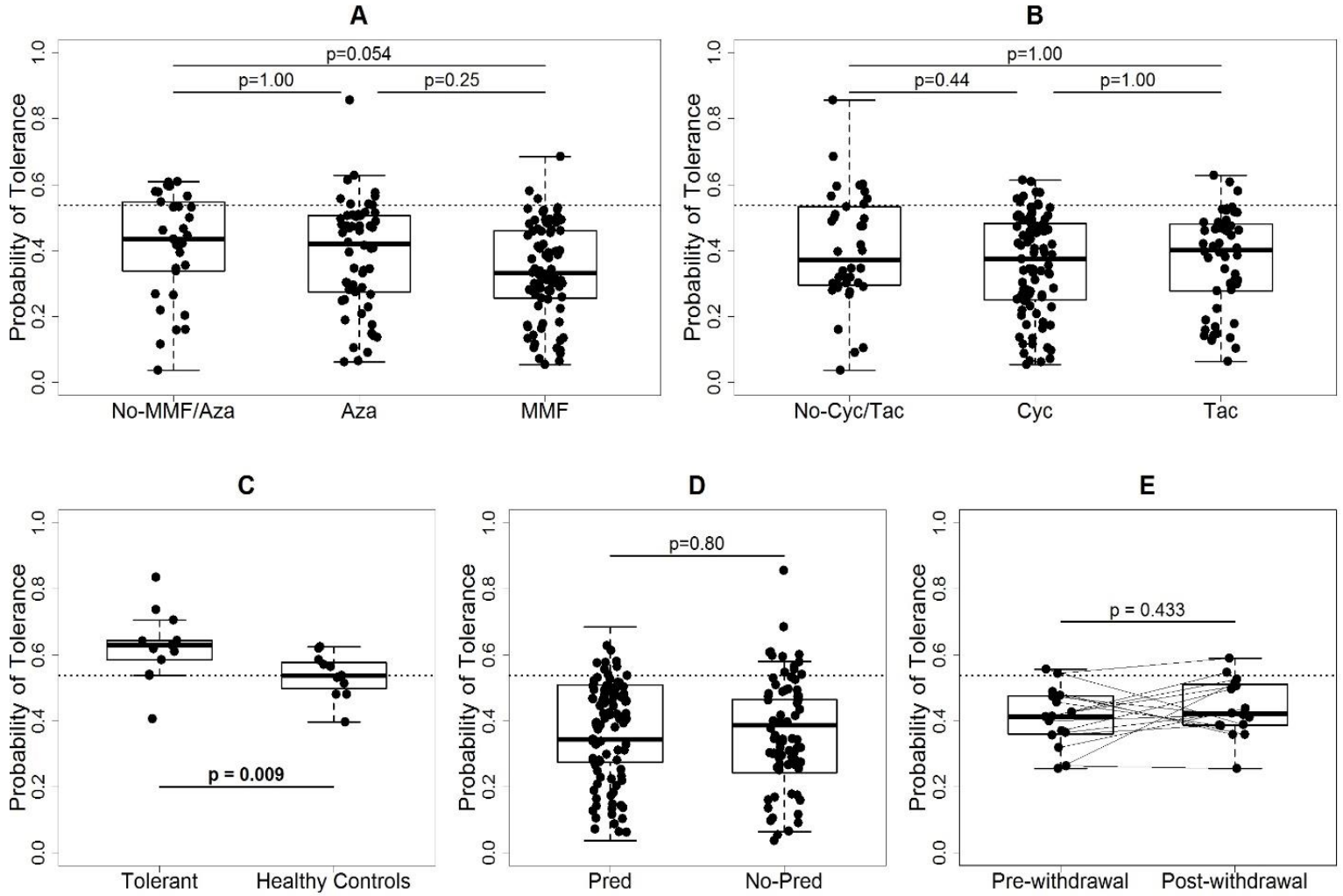
Diagnostic characteristics of IS-independent signature of tolerance



Performance measure	**IS-free signature**
AUC	0.93
95% CI of AUC	0.86-1.00
CV AUC	0.81
Cutoff	0.54
Sensitivity	0.92
Specificity	0.88
AUC Timepoint 2	0.83
95% CI of AUC Timepoint 2	0.67 - 0.99

Rebollo-Mesa, AJTx et 2016

Robust immunosuppression independence of IS-independent set



Rebollo-Mesa, et al AJTx 2016

Conclusions 3

- P(tolerance) unchanged after steroid withdrawal: New-signature highlights natural counter-regulatory mechanisms, and excludes the alterations of the immune effector pathways transiently activated or inhibited by IS drugs.
- This signature is "tolerance –specific" as it is significantly different from Healthy Controls
- The use of robust statistical methods that prevent false-positive results, and control confounding is essential prior to translation of clinical prediction models.

clinical investigation
http://www.kidney-international.org

OPEN

see commentary on page 875

A common gene signature across multiple studies relate biomarkers and functional regulation in tolerance to renal allograft

Daniel Baron[1,2,3], Gérard Ramstein[4], Mélanie Chesneau[1,2,3], Yann Echasseriau[1,2,3], Annaick Pallier[1,2,3], Chloé Paul[1,2,3], Nicolas Degauque[1,2,3], Maria P. Hernandez-Fuentes[5], Alberto Sanchez-Fueyo[6], Kenneth A. Newell[7], Magali Giral[1,2,3], Jean-Paul Soulillou[1,2,3], Rémi Houlgatte[8,9,10] and Sophie Brouard[1,2,3,10]

CLINICAL RESEARCH www.jasn.org

A Three-Gene Assay for Monitoring Immune Quiescence in Kidney Transplantation

Silke Roedder,* Li Li,† Michael N. Alonso,‡ Szu-Chuan Hsieh,* Minh Thien Vu,* Hong Dai,* Tara K. Sigdel,* Ian Bostock,§ Camila Macedo,‖ Diana Metes,‖ Adrianna Zeevi,‖ Ron Shapiro,‖ Oscar Salvatierra,‡ John Scandling,‡ Josefina Alberu,§ Edgar Engleman,‡ and Minnie M. Sarwal*

The questions …..

- Are any of these found in tolerance inducing strategies?
  - Newell KA, et al AJTx 2015; 15: 2908–2920 .
- Have we learned novel mechanisms of tolerance?

…. Transitional B cells / Role of steroid pathway

- Increased CD40 Ligation and Reduced BCR Signalling Leads to Higher IL-10 Production in B Cells From Tolerant Kidney Transplant Patients. Nova-Lamperti E, et al Transplantation. 2017 Mar;101(3):541-547
- IL-10-produced by human transitional B-cells down-regulates CD86 expression on B-cells leading to inhibition of CD4+T-cell responses. Nova-Lamperti E, et al . Sci Rep. 2016 Jan 22;6:20044.

- Are any of these "true" biomarkers of tolerance?.
  - Clinical trials of weaning are needed = controversial

Guy's and St Thomas' NHS Foundation Trust and King's College London's **Comprehensive Biomedical Research Centre**

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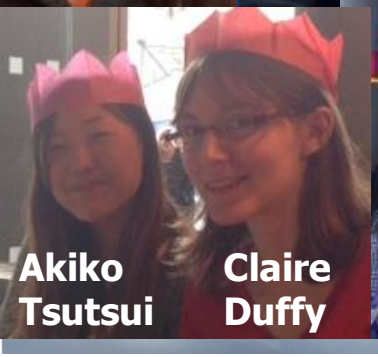

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Dr Rachel Hilton

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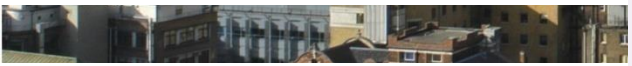

Drs Irene Rebollo-Mesa & Sofia Christakoudi

Dr Jean Harrington

Prof Myfanwy Morgan

Tissue typing at Guy's Hospital:
Dr Robert Vaughn, Livvy Shaw & Team