Genome Canada
Transplant Consortium

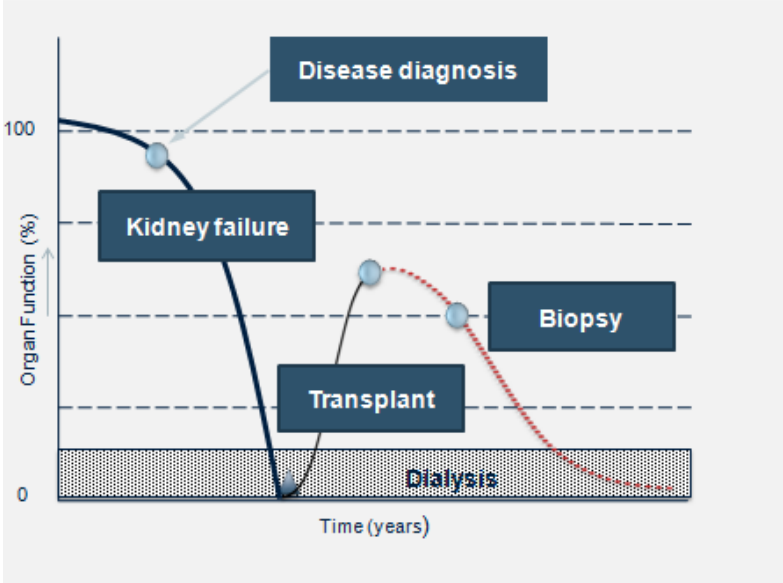
Precision Medicine CanPrevent Antibody-Mediated Rejection

BC Kidney Days
October 2019

Premature transplant failure is a tragic loss of health and resources

Transplant offers superb early success

- Rapid recovery and rehabilitation
- normal growth and development (children)
- lower cost < $20,000 vs $90,000 / year (HD)

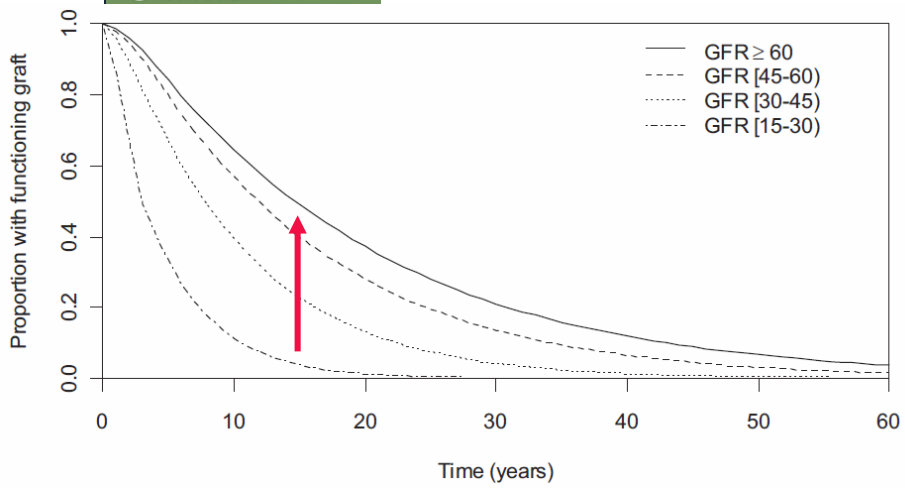


but poor long-term survival

- Few grafts survive beyond 10-20 years
- 500+ patients lose their graft every year
- $1 million incremental lifetime cost of care

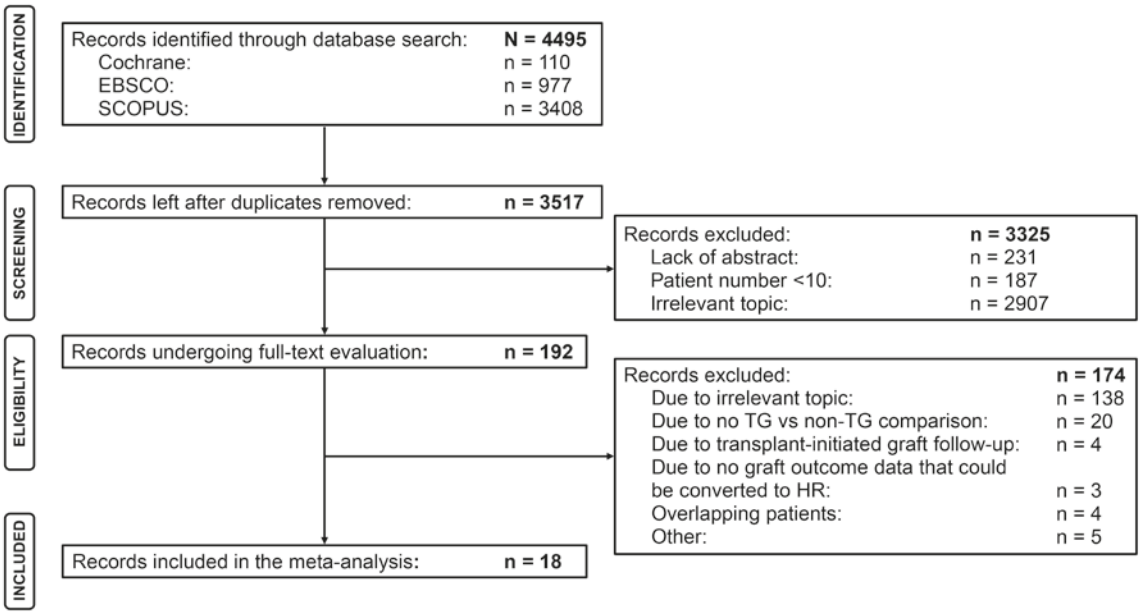
Value in Health

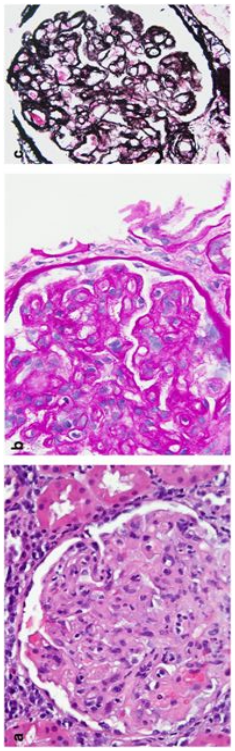
Levy et al, 2014



Association between transplant glomerulopathy and graft outcomes following kidney transplantation: A meta-analysis

Gábor Kovács[1], Giovanna Devercelli[2], Tamás Zelei[1], Ishan Hirji[2], Zoltán Vokó[1,3], Paul A. Keown[4*]





PAS stain

Silver stain

H & E stain

Results:

1. Graft failure is almost 3 times more common with transplant glomerulopathy (HR: 2.85)
2. Median graft survival is reduced by 15 years with transplant glomerulopathy (3.25 vs 18.82 years)

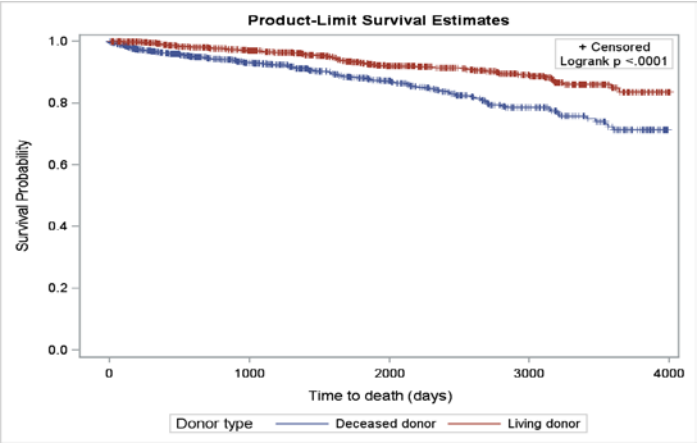
Fillipone et al, Modern Pathology, 2017

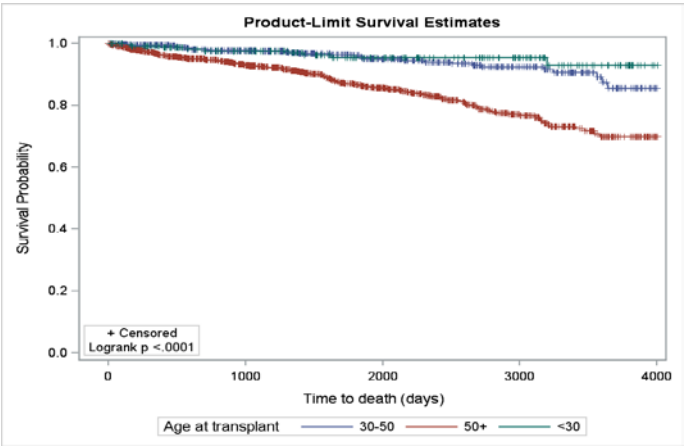
Targeting risk in the BC transplant population

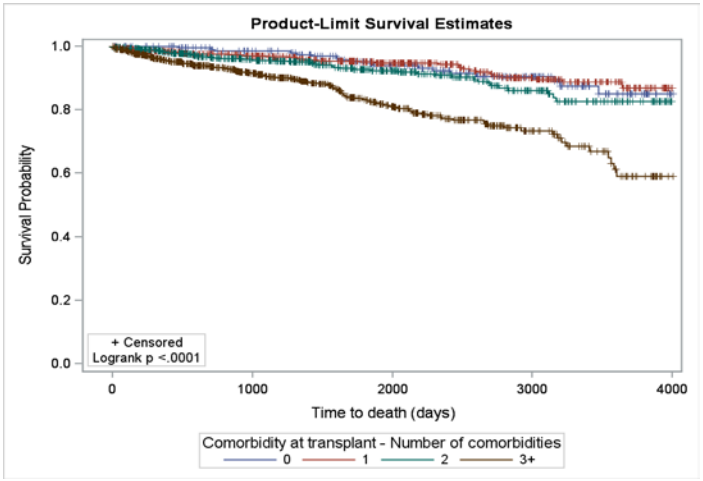
BC Transplant Population, January 1 2008 to December 31 2018

First grafts = 2,325, Died = 277, Graft failure = 159

Analysis by subpopulation







Results:

Patients who die are older, have multiple comorbidities and receive a deceased donor graft

Targeting risk in the BC transplant population

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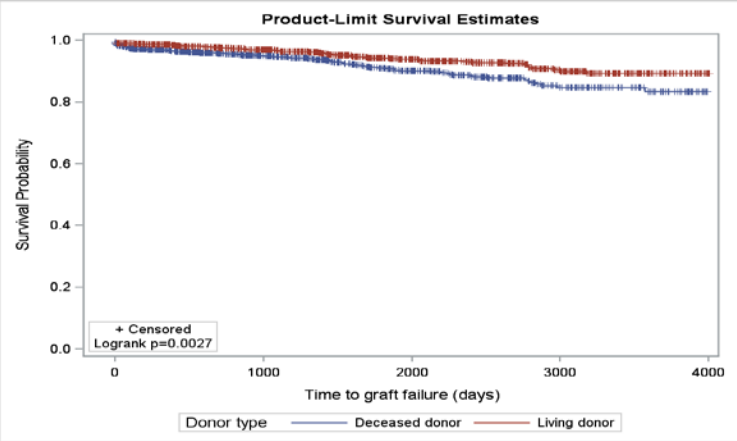
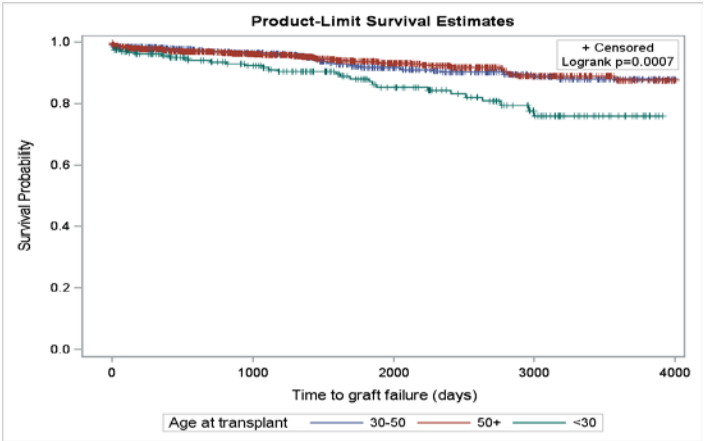
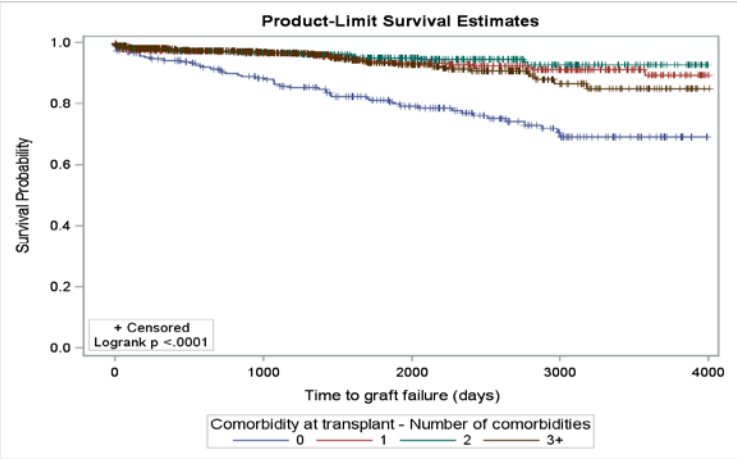
Analysis by subpopulation







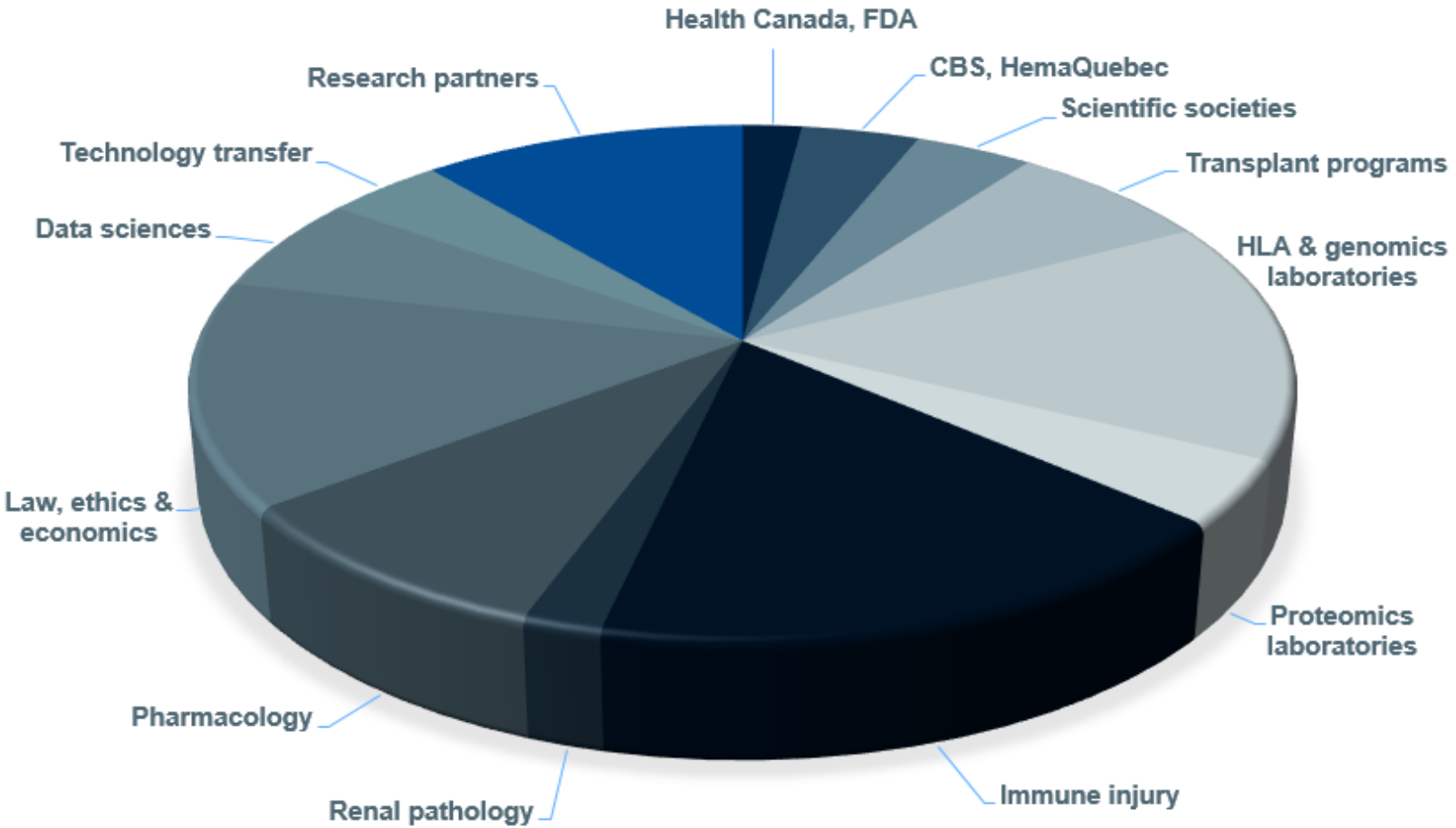
Results:

Patients who lose their grafts are younger, have few comorbidities and receive a deceased donor graft.

Note: 50% of DD recipients under 30 yrs. Lose their graft in 8 yrs.

And an expert and experienced project management team!

With government, academia, healthcare, patients & industry



New National Programs to prevent graft loss due to AMR

validation

2018

2019

2020

2021

Epitope matching 1

- 1a. Programs for national matching:
  - Epitope frequencies
  - Quantitative impact
  - Prediction of risk
- 1b. Methods for rapid epitope definition
  - Hi resolution SSO
  - RT PCR
  - Sequencing

Immune monitoring 2

- 2a. Recipient immune competence
- 2b. Donor-specific T- & B-cell clonal expansion
- 2c. Antibody formation to graft antigens
- 2d. Non-invasive graft injury

Personalized immunotherapy 3

- 3a. Systems pharmacology to guide current therapy
- 3b. Clinical trials for novel treatment options

Evidence-based health policy 4

- 4a. Legal & policy guidelines
- 4b. Communication strategies for policy
- 4c. Patient, public and provider perspectives
- 4e. Economic model for policy decisions

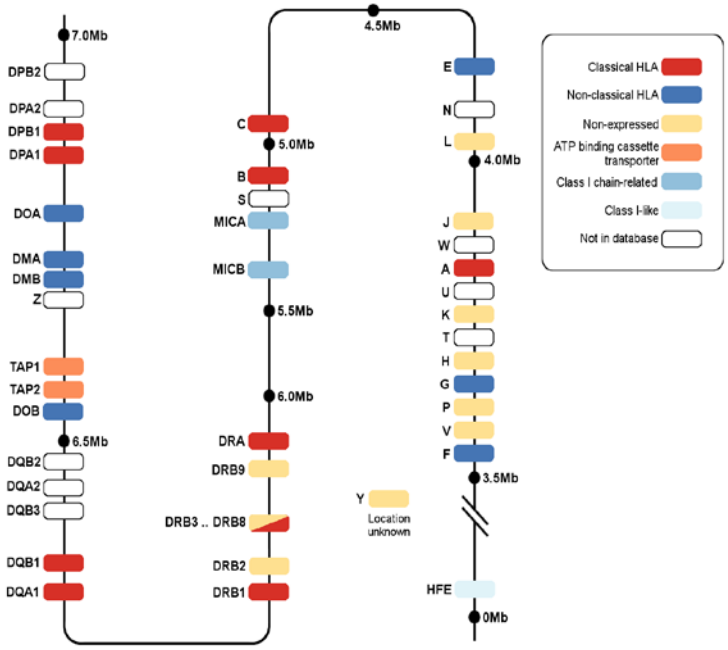
Activity 1a: HLA genes, polymorphisms and nomenclature

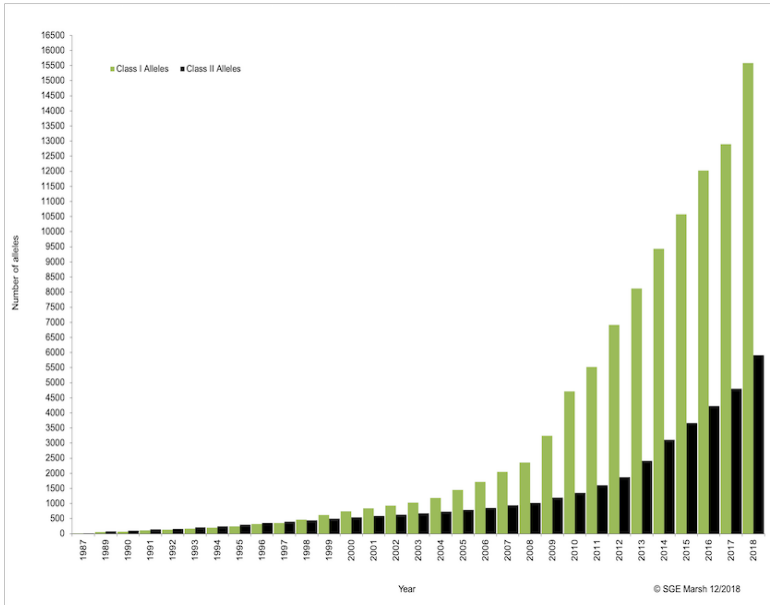
nature REVIEWS GENETICS

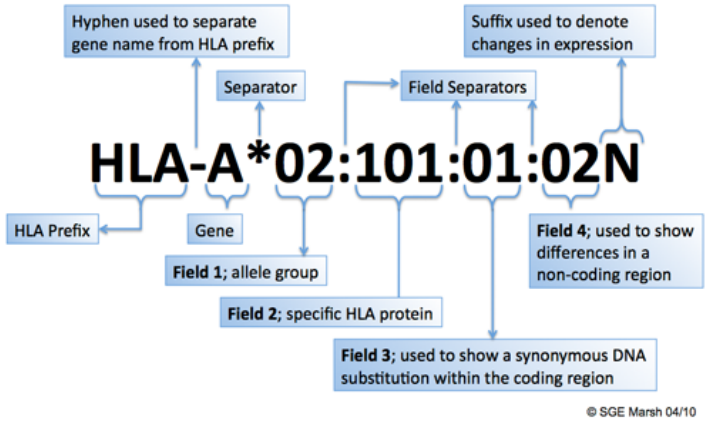
Review Article | Published: 01 December 2004

Gene map of the extended human MHC

Roger Horton, Laurens Wilming, Vikki Rand, Ruth C. Lovering, Elspeth A. Bruford, Varsha K. Khodiyar, Michael J. Lush, Sue Povey, C. Conover Talbot Jr, Mathew W. Wright, Hester M. Wain, John Trowsdale, Andreas Ziegler & Stephan Beck

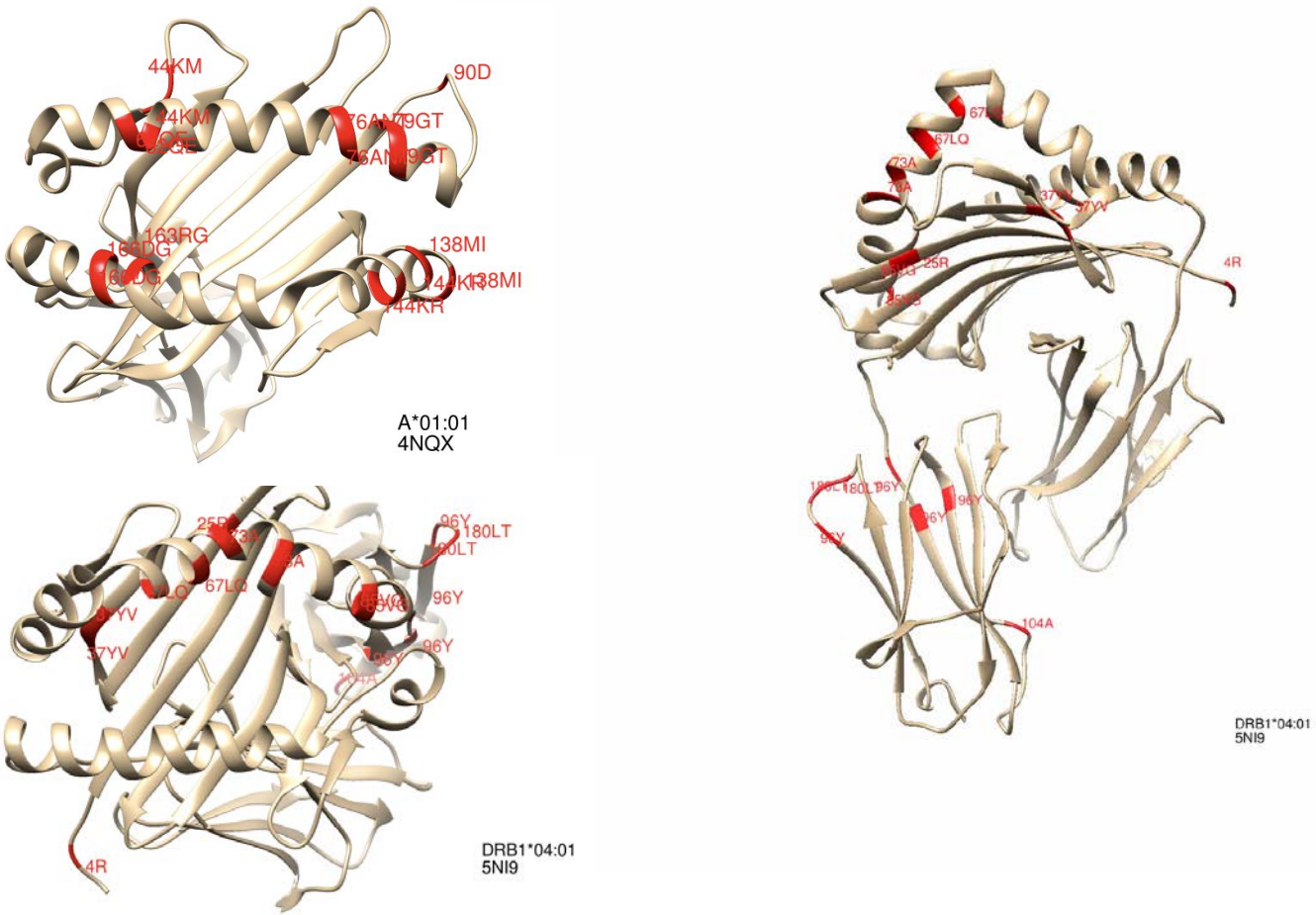






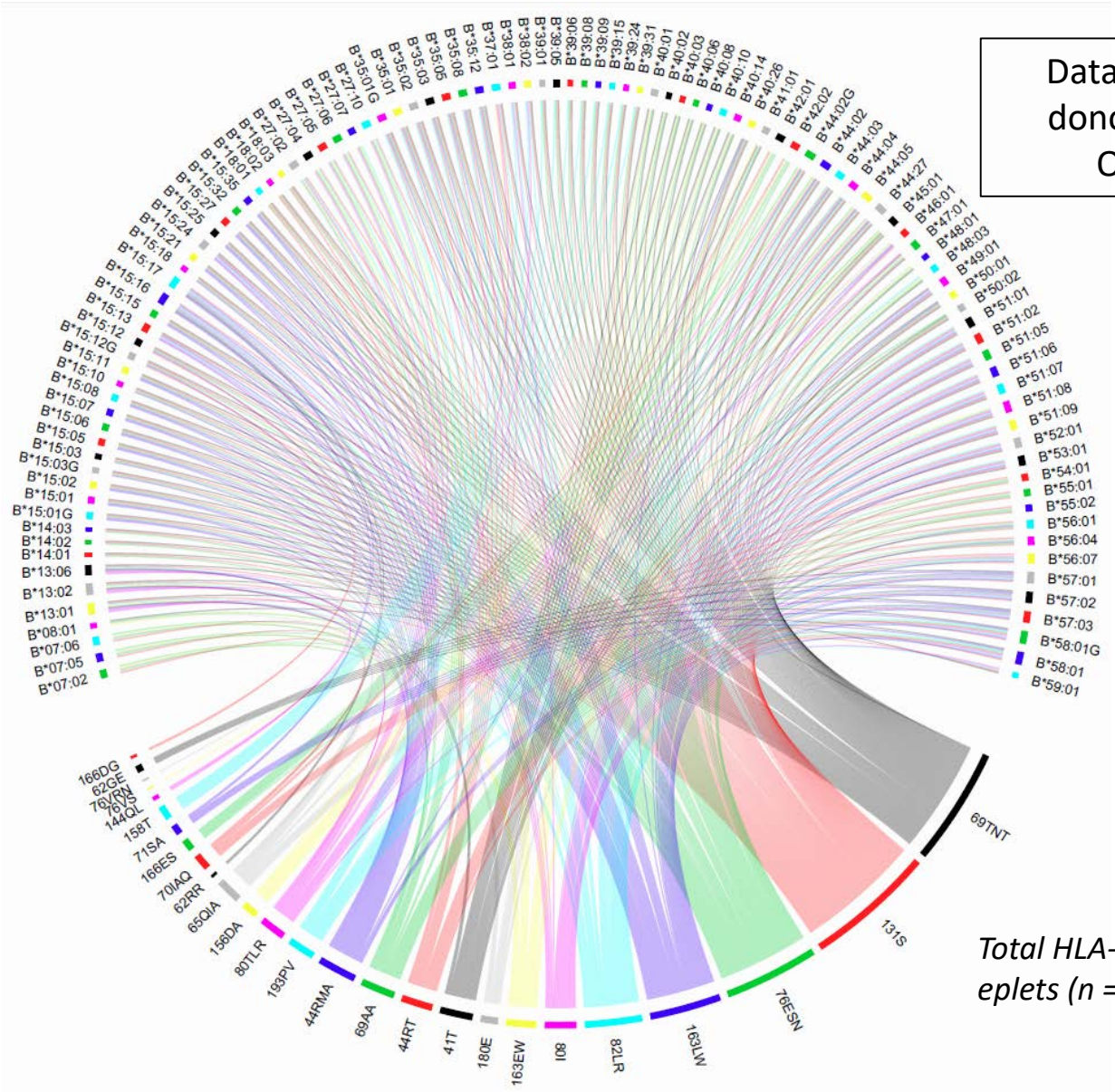
Mapping Relevant Eplets on HLA Protein

Peptide-binding fragment of HLA is engaged by the T-cell receptor. Antibody-defined eplets are highlighted in red. Many eplets occur at the peptide-binding region, but some occur outside this region



Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311.

Results: Conversion of alleles into eplets reduces complexity

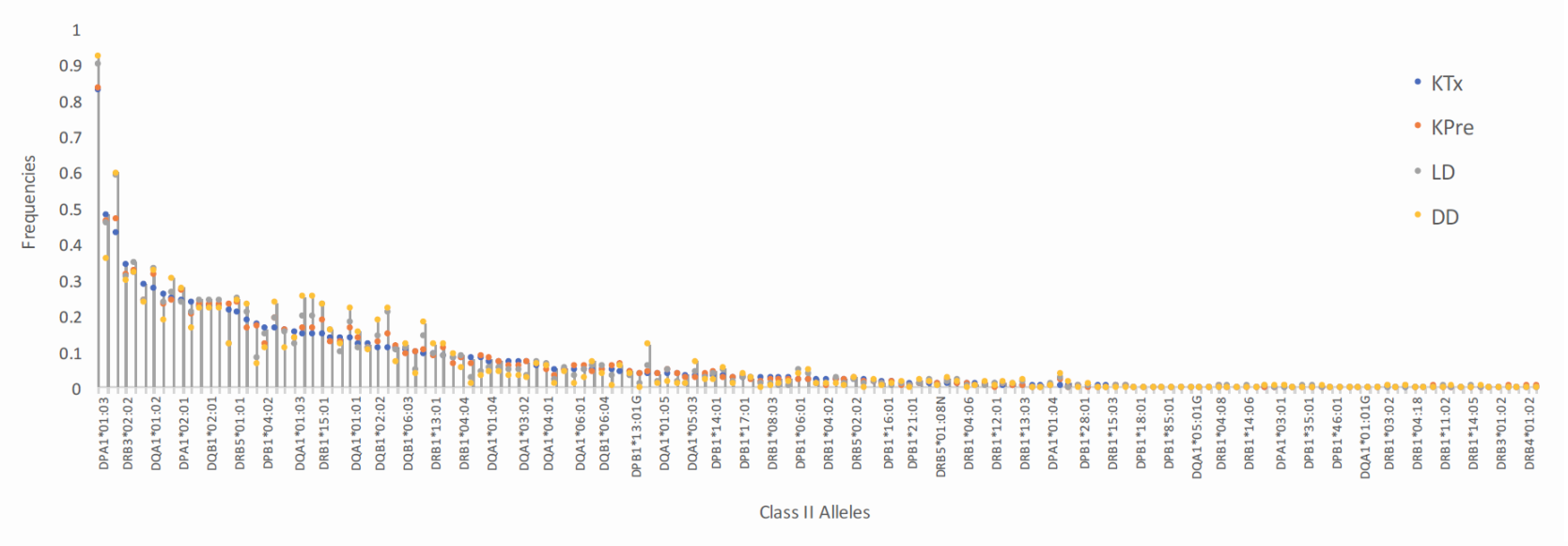


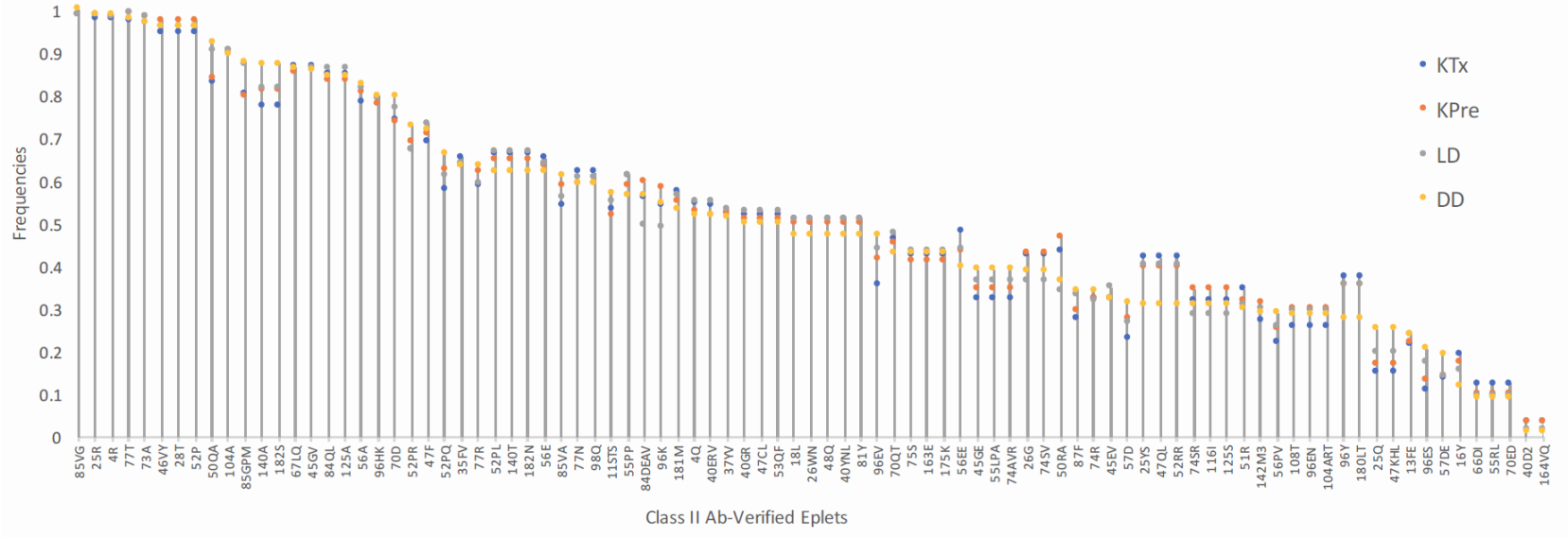
Data from 2216 kidney patients and donors sequenced by NGS between October 2016 – January 2019

- 361 alleles identified translated into 148 eplets
- 206 class I alleles, 59 eplets
- 155 class II alleles, 89 eplets

Total HLA-B alleles identified (n = 107) and all eplets (n = 26) coded by them

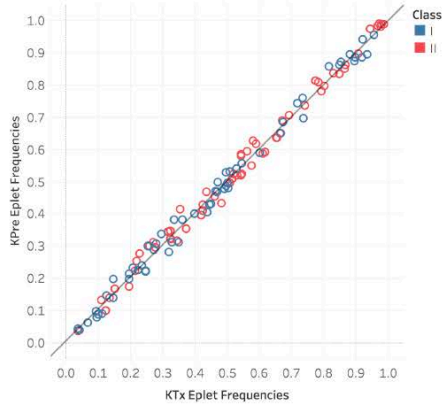
Results: Frequencies across all class I and II eplets were more similar between patient and donor groups than allele frequencies





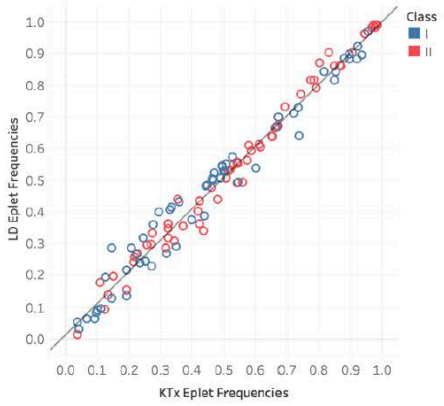
Results: Eplet frequencies in kidney patient and donor groups

KTx vs. KPre



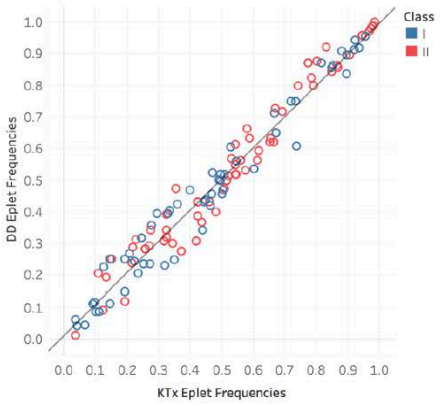
R-Squared: 0.991685
Standard error: 0.0240275
p-value: < 0.0001

KTx vs. LD



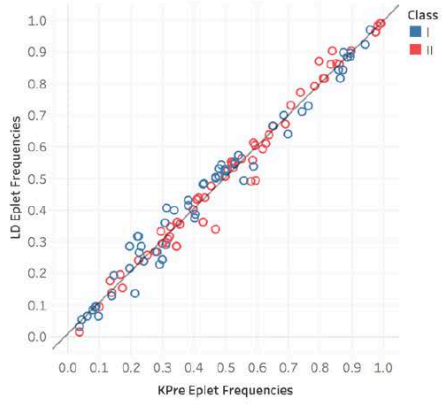
R-Squared: 0.97851
Standard error: 0.0386031
p-value: < 0.0001

KTx vs. DD



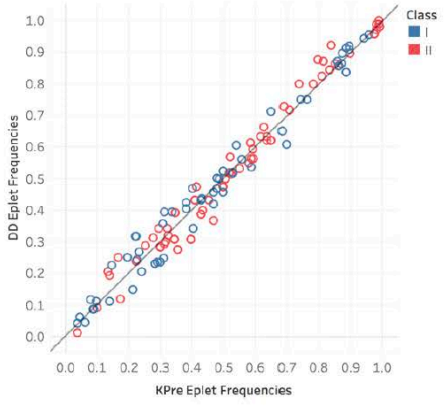
R-Squared: 0.961018
Standard error: 0.0523923
p-value: < 0.0001

KPre vs. LD



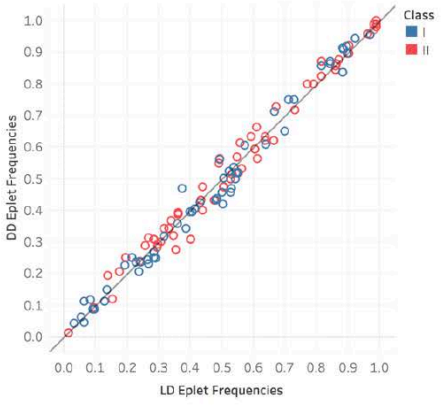
R-Squared: 0.981139
Standard error: 0.0361641
p-value: < 0.0001

KPre vs. DD



R-Squared: 0.975104
Standard error: 0.0418702
p-value: < 0.0001

LD vs. DD



R-Squared: 0.981902
Standard error: 0.0356986
p-value: < 0.0001

Results: HLA eplet sub-groups and epitypes

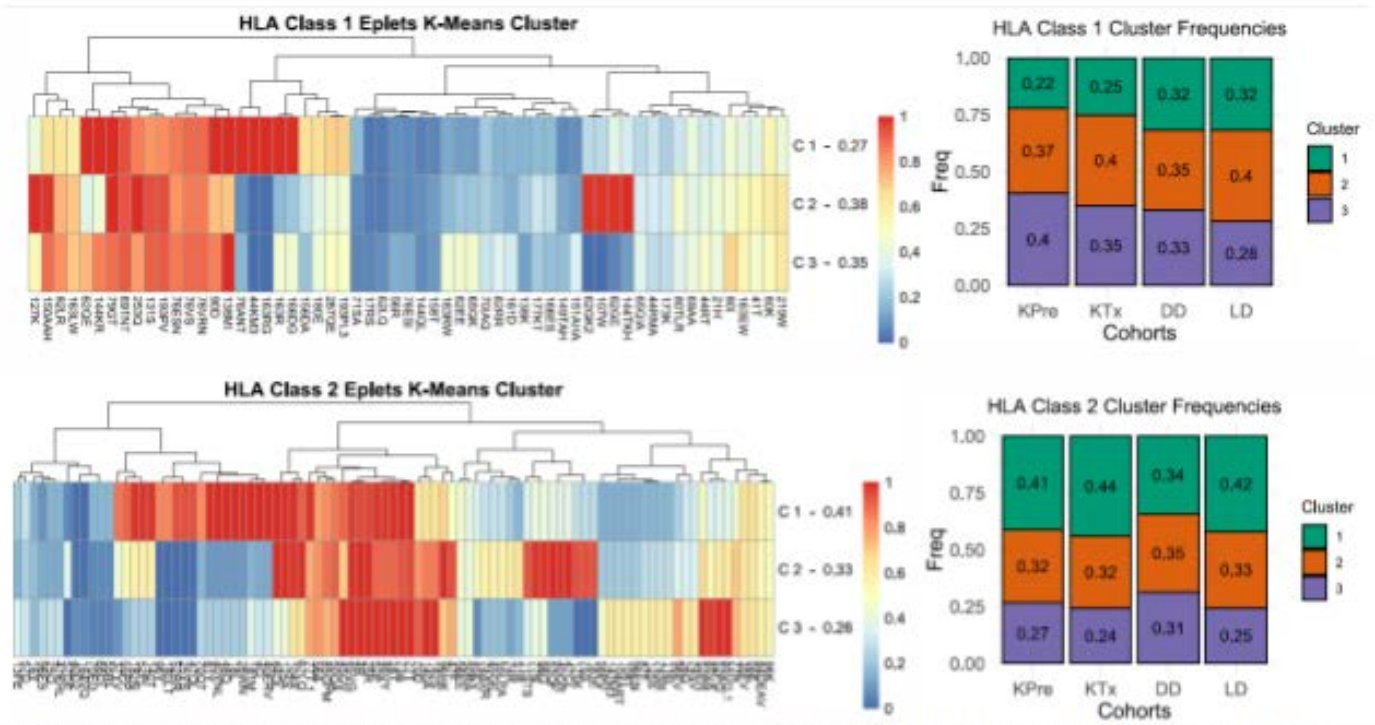


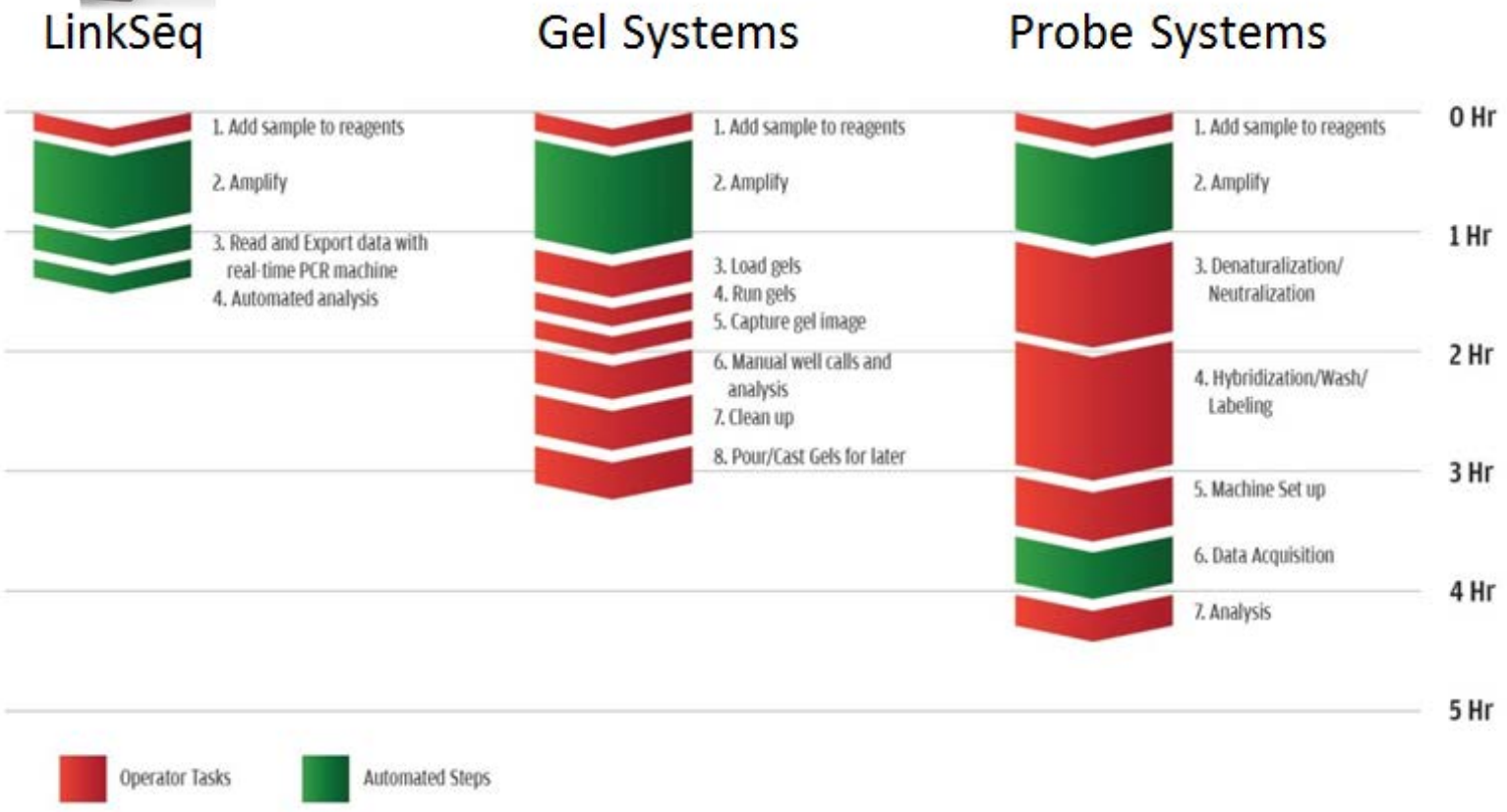
Image 3. Patients were segregated into three clusters, independently for class I and class II, based on their eplet patterns using a k-means algorithm. Frequent eplets are coloured red, while rare eplets are blue. The frequencies of subjects in each cluster are shown for the four different cohorts based on class I and class II eplet patterns, respectively.

Activity 1b: implementing genomic methods for donor epitope typing

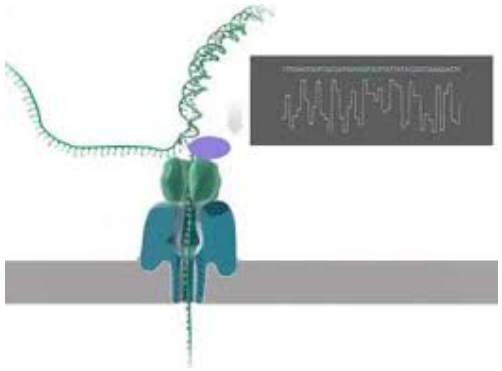
Linkage Biosciences™ **LinkSēq HLA Typing Kits**

ONE LAMBDA **LABType® XR and CWD**

A Thermo Fisher Scientific Brand



Activity 1b: implementing genomic methods for donor epitope typing



Human Immunology
Volume 78, Supplement, September 2017, Page 90

P050 Rapid, high resolution HLA genotyping using nanopore sequencing

Peter M. Clark [a], Deborah Ferriola [a], Dimitri S. Monos [a, b]

nature biotechnology

Article | OPEN | Published: 29 January 2018

Nanopore sequencing and assembly of a human genome with ultra-long reads

Miten Jain, Sergey Koren [...] Matthew Loose

Nature Biotechnology **36**, 338–345 (2018) | Download Citation

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Home / August 2017 / The power of Oxford Nanopore MinION in human leukocyte antigen immunogenetics

Article Abstract

The power of Oxford Nanopore MinION in human leukocyte antigen immunogenetics

Authors: Marcel G. J. Tilanus

Comprehensive haplotyping of the HLA gene family using nanopore sequencing

Sebastian Johansson[1], Szilveszter Juhos[1], David Redin[2], Afshin Ahmadian[2], Max Käller[2,*]

[1] Stockholm University, Department of Biochemistry and Biophysics, Science for Life Laboratory, SE-171 65 Solna, Sweden

the Journal of Molecular Diagnostics

Accurate Typing of Human Leukocyte Antigen Class I Genes by Oxford Nanopore Sequencing

Chang Liu,* Fangzhou Xiao,† Jessica Hoisington-Lopez,† Kathrin Lang,‡ Philipp Quenzel,‡ Brian Duffy,¶ and Robi D. Mitra†

New National Programs to prevent graft loss due to AMR

validation

2018

2019

2020

2021

Epitope matching 1

- 1a. Programs for national matching:
  - Epitope frequencies
  - Quantitative impact
  - Prediction of risk
- 1b. Methods for rapid epitope definition
  - Hi resolution SSO
  - RT PCR
  - Sequencing

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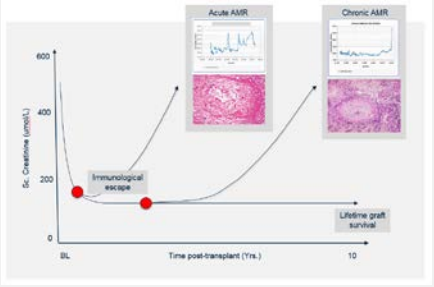
Evidence-based health policy 4

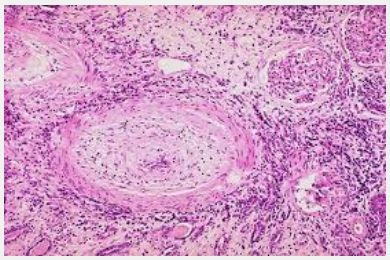
- 4a. Legal & policy guidelines
- 4b. Communication strategies for policy
- 4c. Patient, public and provider perspectives
- 4e. Economic model for policy decisions

Activity 2: Study design, sampling, biobanking and analytical methods

Baseline



Quiescence

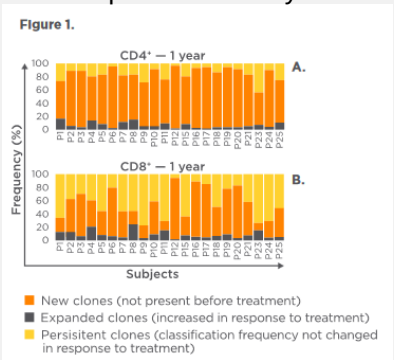


Rejection

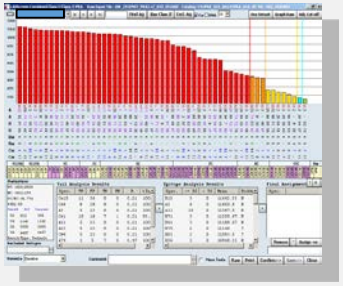
Patient samples
- DNA
- Serum
- Cells
- RNA

Immune competence

Donor-specific immunity



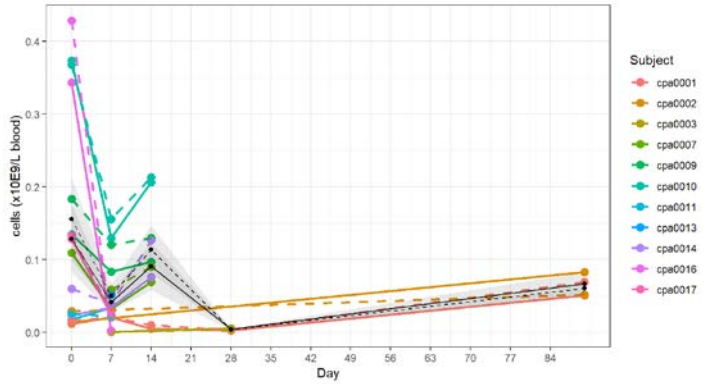
Antibody response



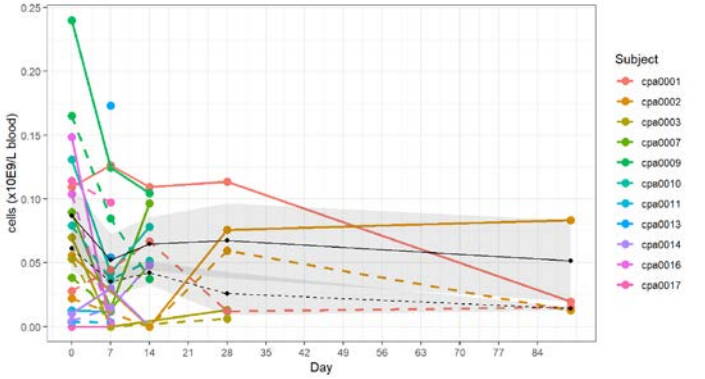
Graft injury

2a. Immune monitoring: recovery of immune competence (phenotype)

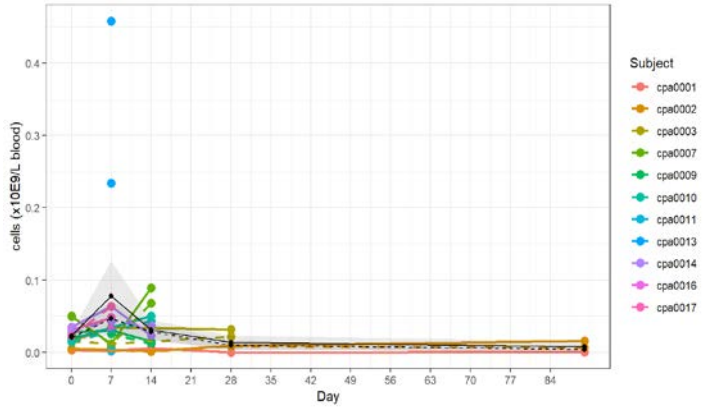
CD3, CD4 Cells



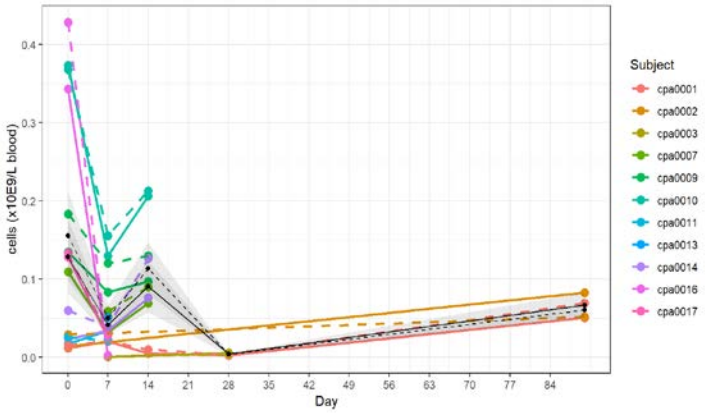
Memory CD8 Cells



CD19 B Cells

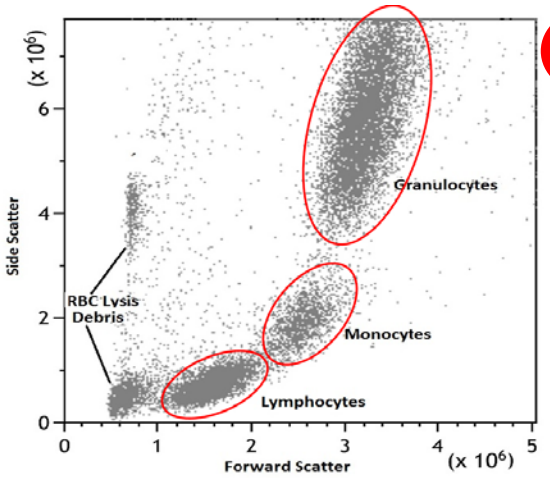


CD56, NK Cells

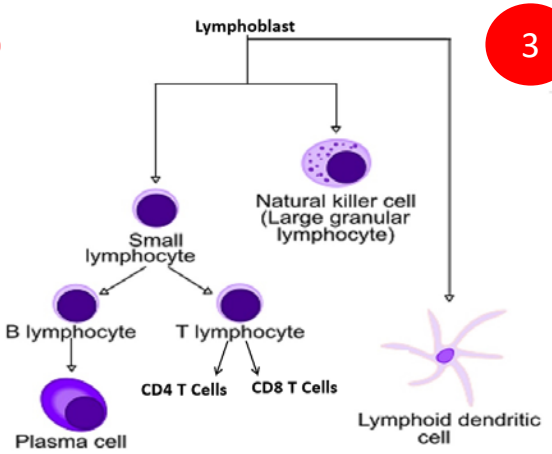


2a. Immune monitoring: recovery of immune competence (phenotype)

1



2



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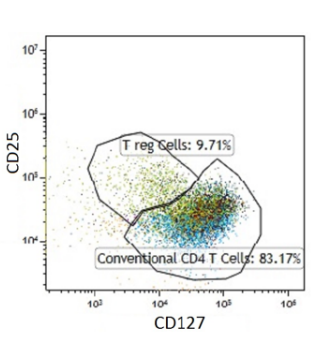
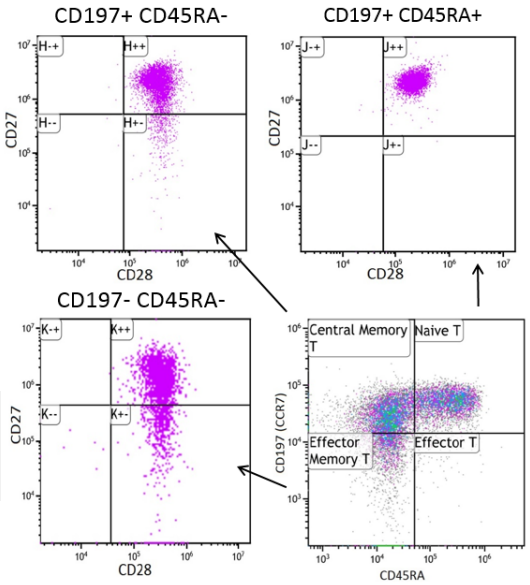
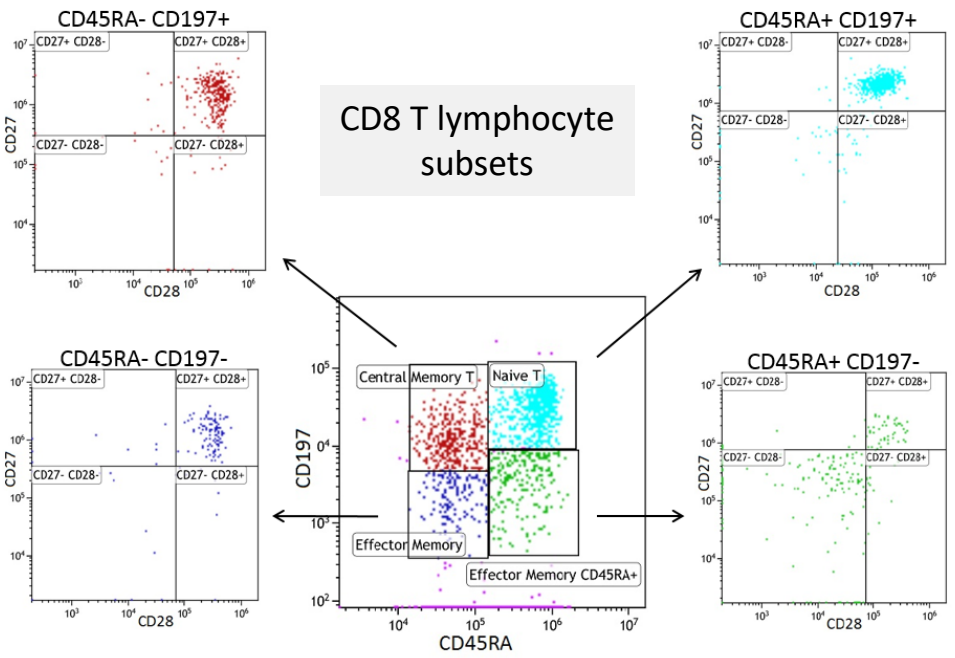




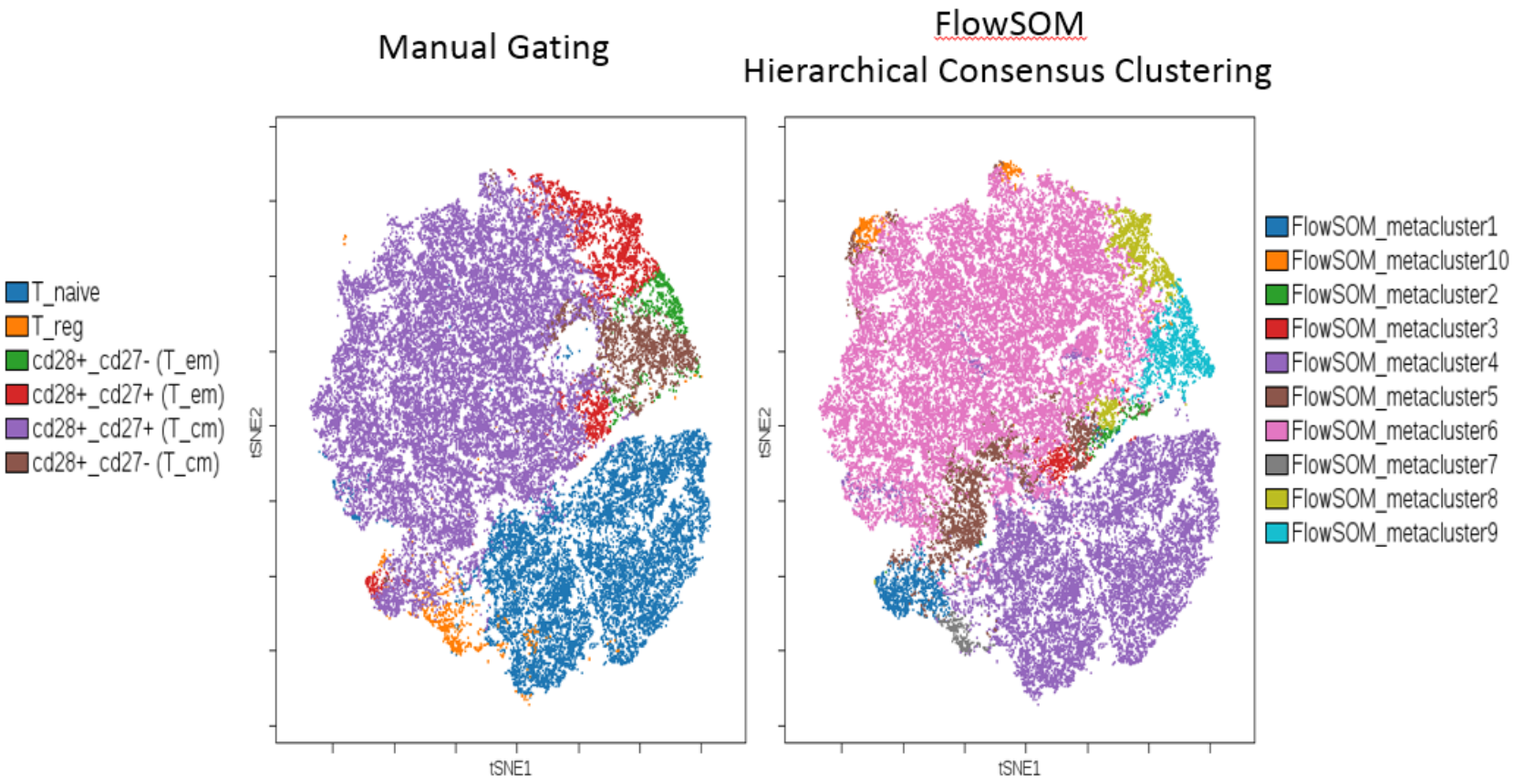
CD4 T lymphocyte subsets



CD8 T lymphocyte subsets

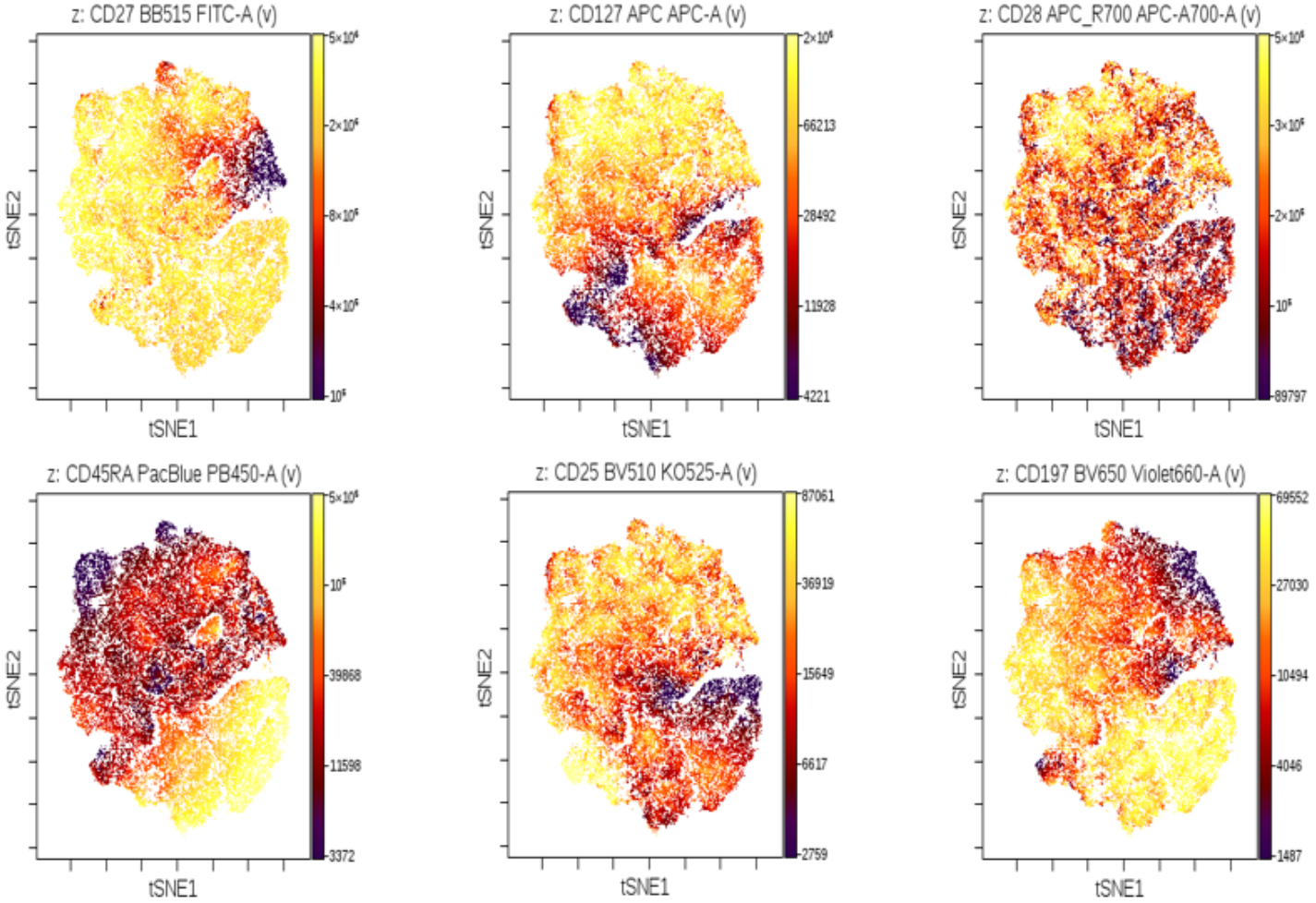
2a. Immune monitoring: recovery of immune competence (phenotype)

T-distributed Stochastic Neighbor Embedding (tSNE) representation, CD4 T cell panel

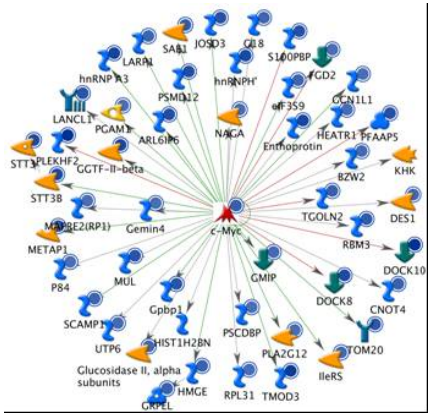


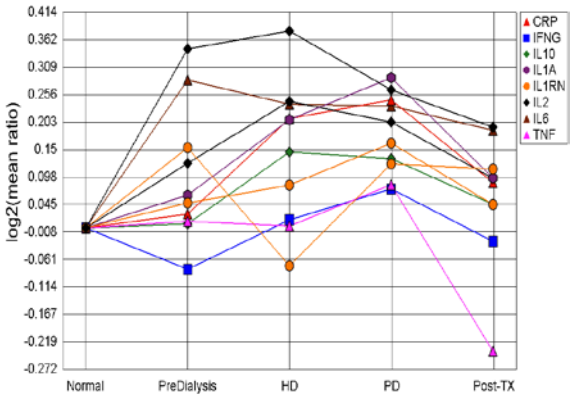
2a. Immune monitoring: recovery of immune competence (phenotype)

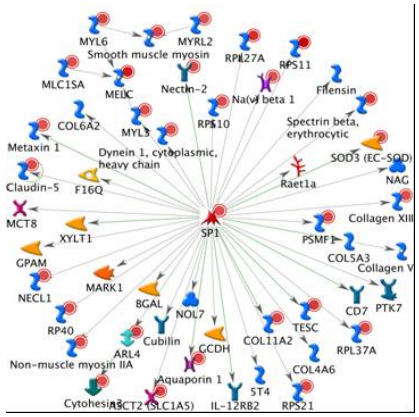
T-distributed Stochastic Neighbor Embedding (tSNE) representation, CD4 T cell panel, expression levels of clustering channels

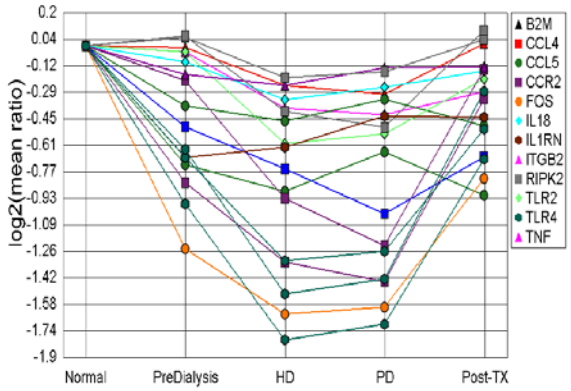


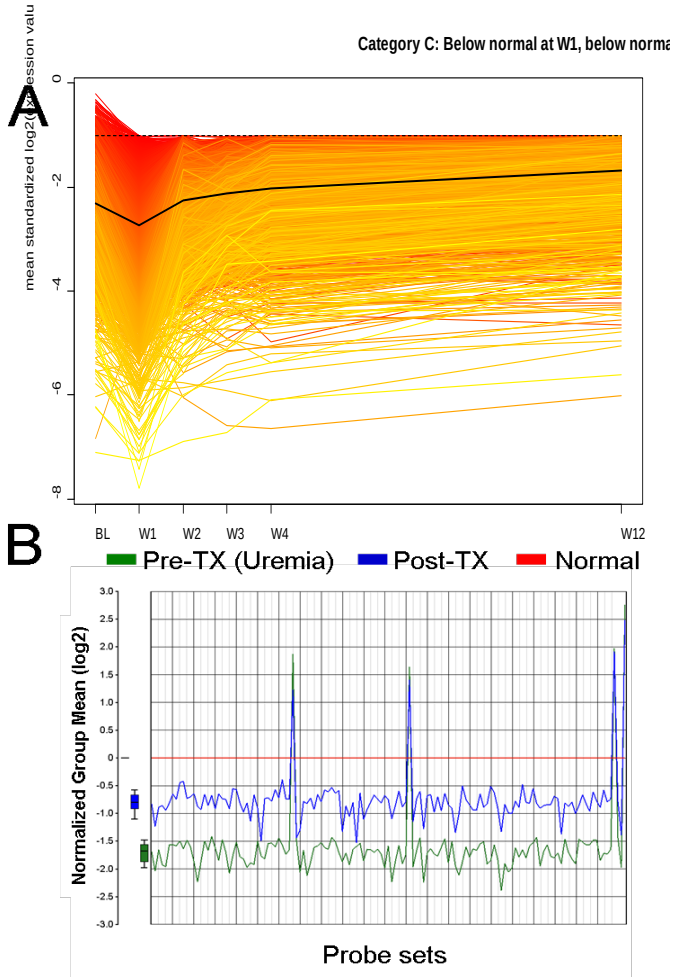
2a. Immune monitoring: recovery of immune competence (genotype)











Transcripts for many key cytokines are elevated or suppressed in chronic renal failure, HD and PD but expression levels return towards normal after transplantation

2b: Immune monitoring of the donor-specific TCR repertoire

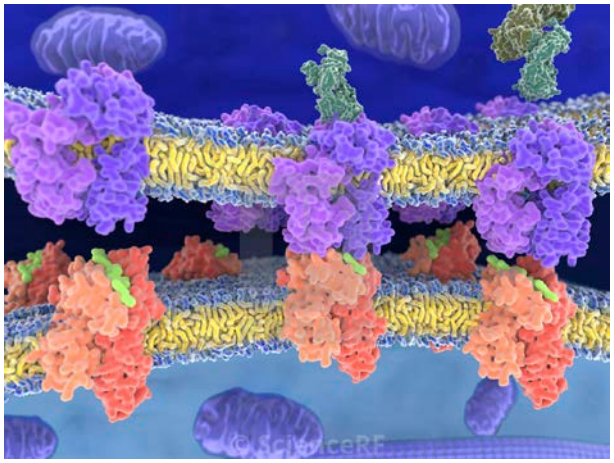
Science Translational Medicine

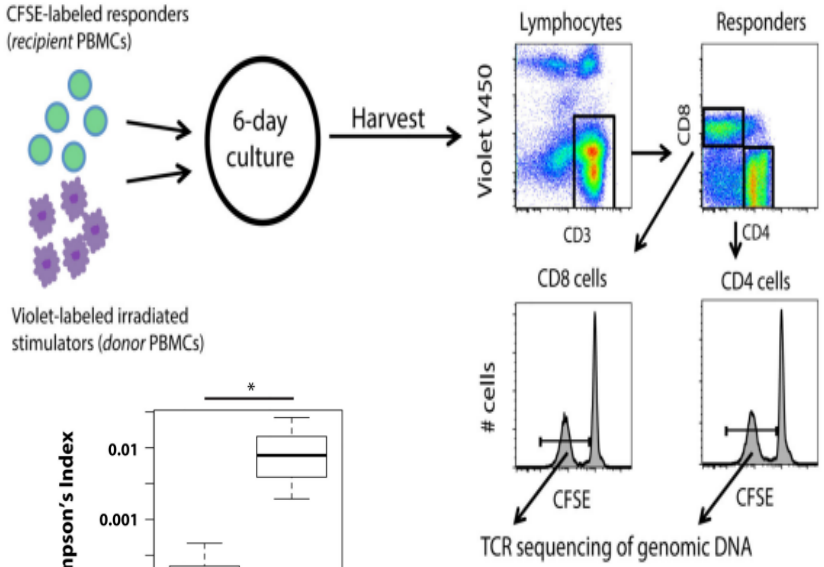
RESEARCH ARTICLES

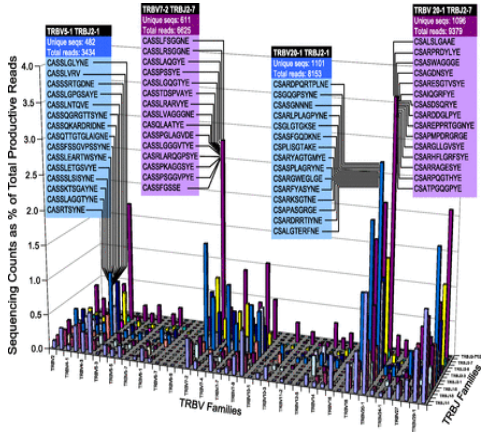
Tracking donor-reactive T cells: Evidence for clonal deletion in tolerant kidney transplant patients

BY HEATHER MORRIS, SUSAN DEWOLF, HARLAN ROBINS, BEN SPRANGERS, SAMUEL A. LOCASCIO, BRITTANY A. SHONTS, TATSUO KAWAI, WAICHI WONG, SUXIAO YANG, JULIEN ZUBER, YUFENG SHEN, MEGAN SYKES

SCIENCE TRANSLATIONAL MEDICINE | 28 JAN 2015 : 272RA10 |







Activity 2c: Health technology assessment of HLA antibody testing

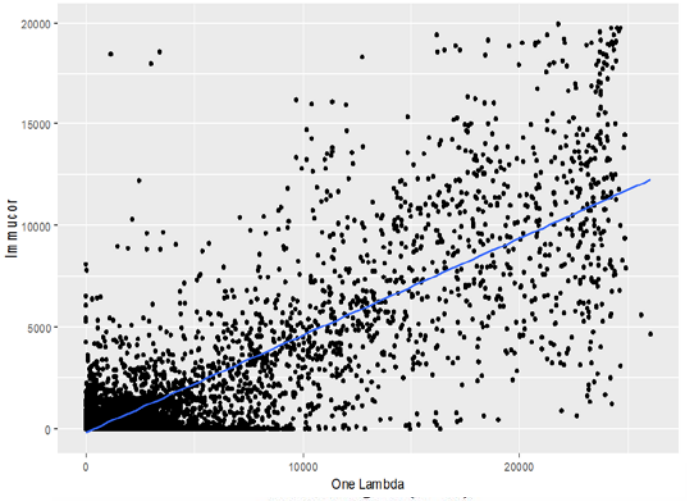
ONE LAMBDA
A Thermo Fisher Scientific Brand

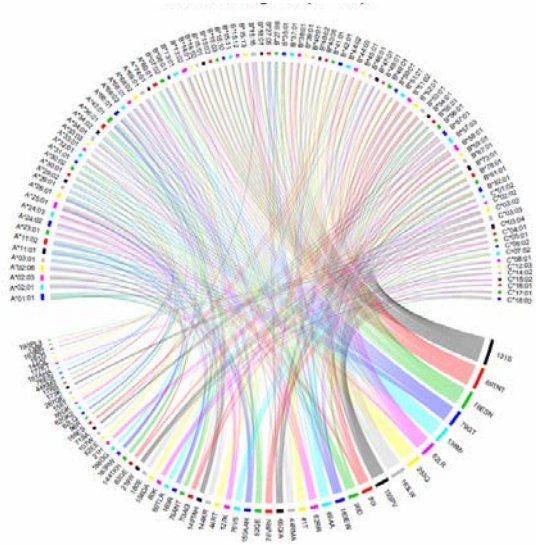
LS1A04	LABScreen Single Antigen HLA Class I - Combi Full Specification	HLA-A, HLA-B, HLA-C
LS2A01	LABScreen Single Antigen HLA Class II - Group 1 Full Specification	HLA-DRB, HLA-DQB

IMMUCOR

LIFECODES LSA Class I Kit
Single antigens for the detection of the HLA Class I IgG antibodies.

LIFECODES LSA Class II Kit
Single antigens for the detection of the HLA Class II IgG antibodies.

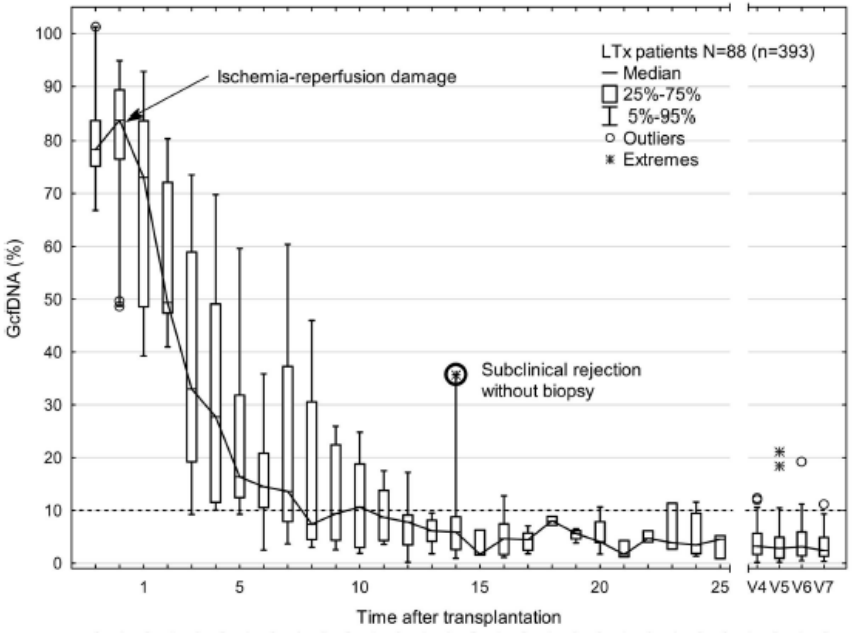


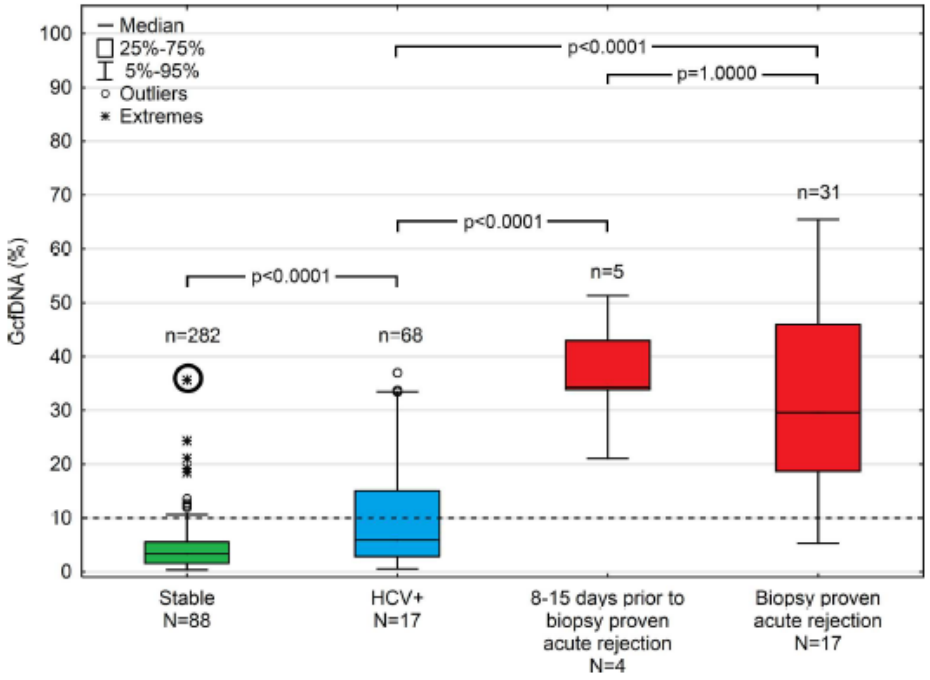


Activity 2d: non-invasive monitoring of graft injury

Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study

Ekkehard Schütz[1], Anna Fischer[2], Julia Beck[1], Markus Harden[3], Martina Koch[4], Tilo Wuensch[5], Martin Stockmann[5], Björn Nashan[4], Otto Kollmar[6], Johannes Matthaei[2], Philipp Kanzow[2], Philip D. Walson[2], Jürgen Brockmöller[2], Michael Oellerich[2] =





New National Programs to prevent graft loss due to AMR

validation

2018

2019

2020

2021

Epitope matching 1

- 1a. Programs for national matching:
  - Epitope frequencies
  - Quantitative impact
  - Prediction of risk
- 1b. Methods for rapid epitope definition
  - Hi resolution SSO
  - RT PCR
  - Sequencing

Immune monitoring 2

- 2a. Recipient immune competence
- 2b. Donor-specific T- & B-cell clonal expansion
- 2c. Antibody formation to graft antigens
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Personalized immunotherapy 3

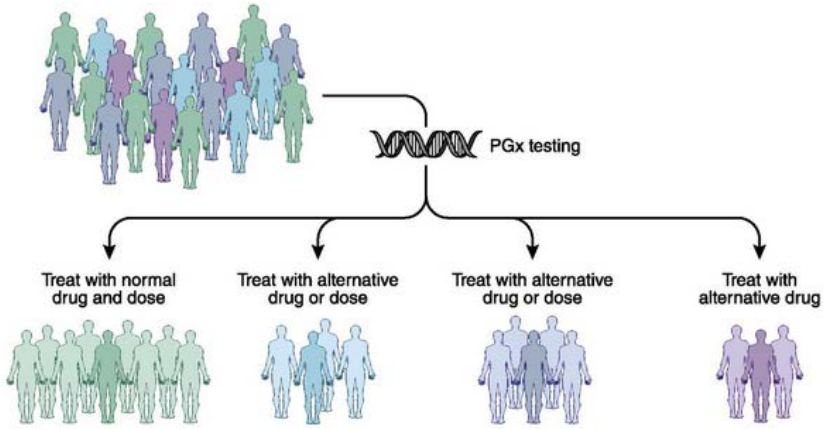
- 3a. Systems pharmacology to guide current therapy
- 3b. Clinical trials for novel treatment options

Evidence-based health policy 4

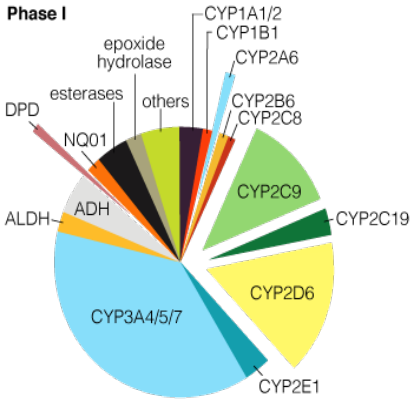
- 4a. Legal & policy guidelines
- 4b. Communication strategies for policy
- 4c. Patient, public and provider perspectives
- 4e. Economic model for policy decisions

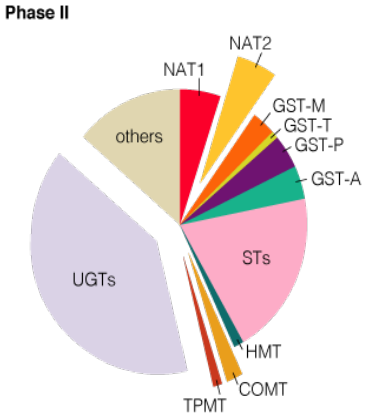
Activity 3a: To develop and test a systems pharmacology model

Systems pharmacology model

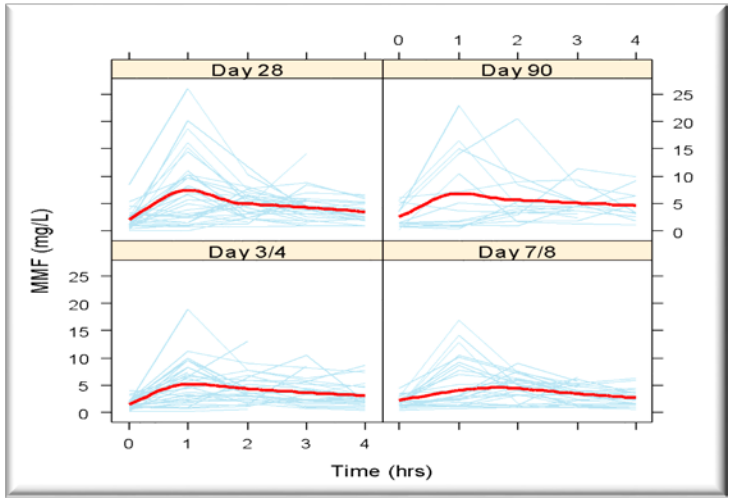


Pharmacogenomics

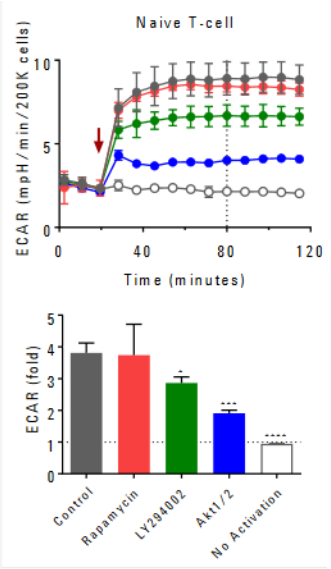




Pharmacokinetics



Pharmacodynamics



Risk-based model of the graft response

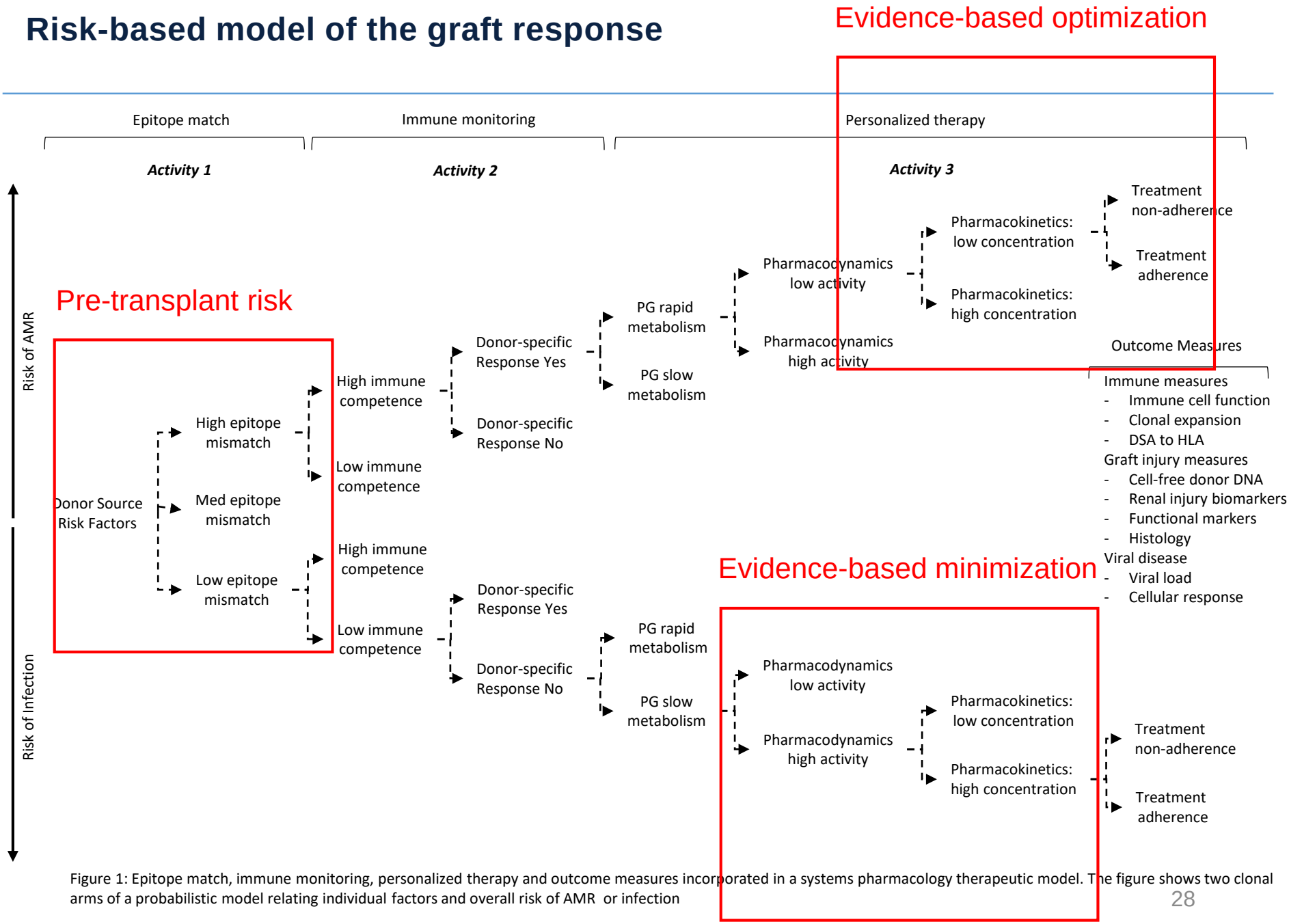
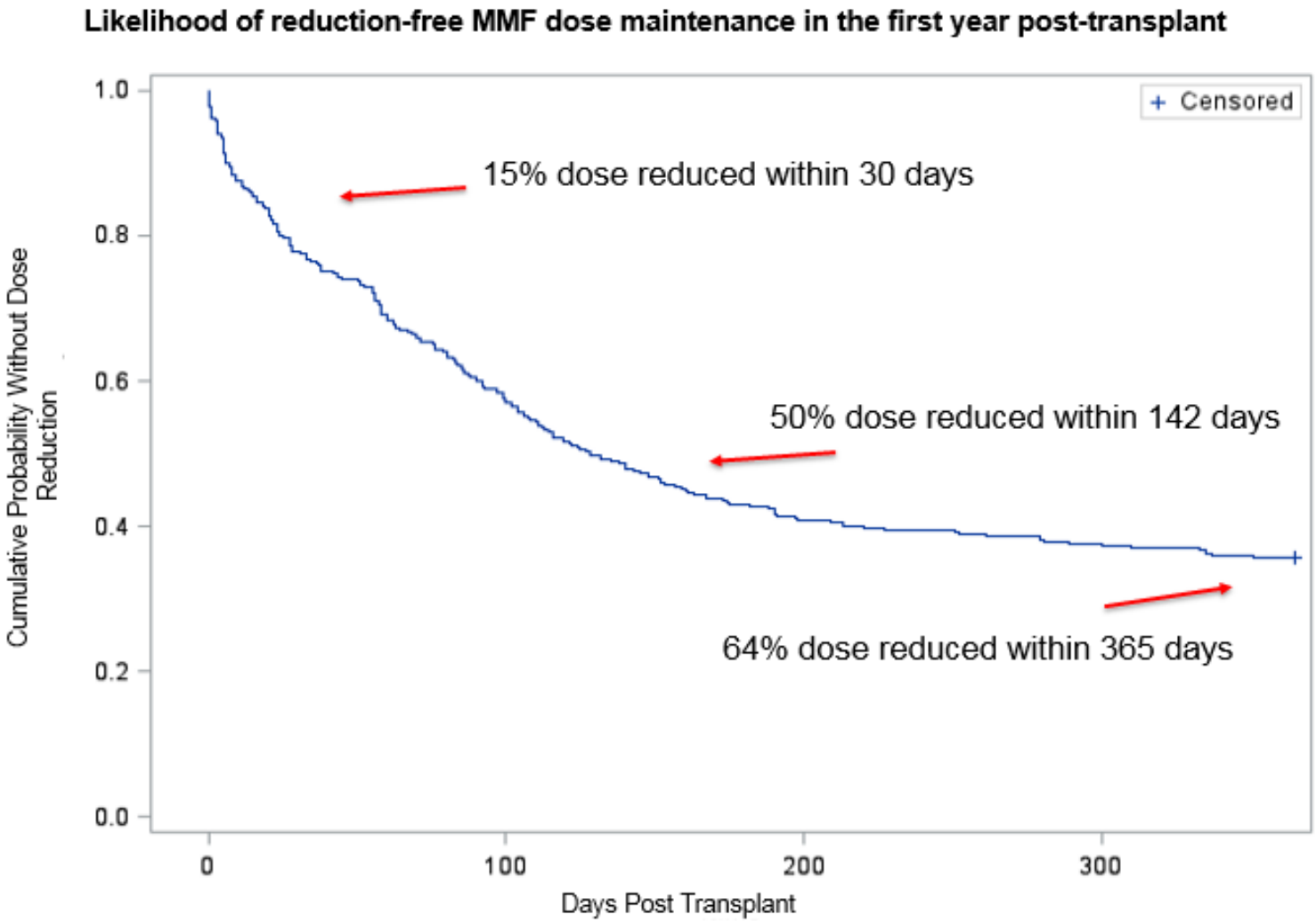
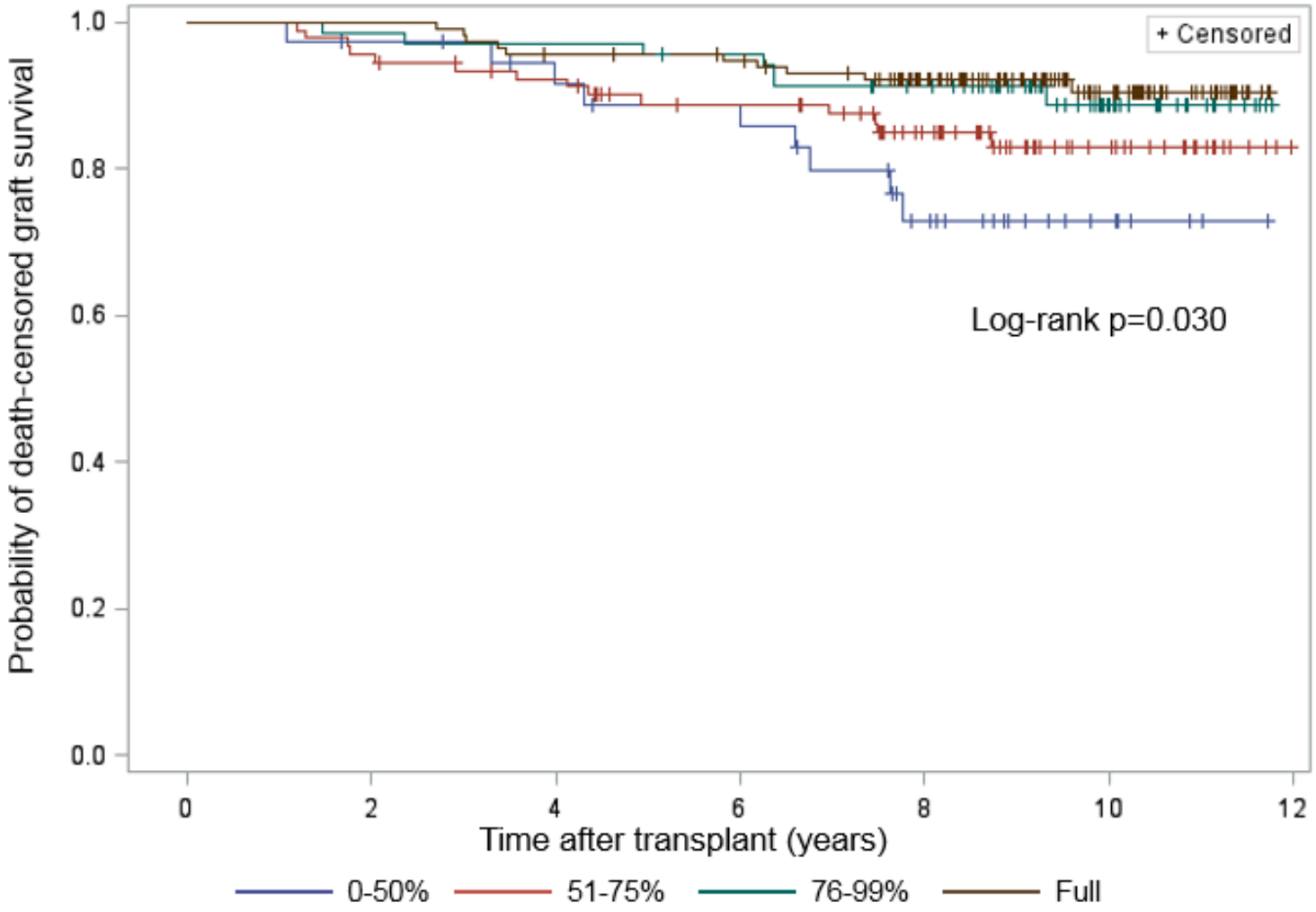


Figure 1: Epitope match, immune monitoring, personalized therapy and outcome measures incorporated in a systems pharmacology therapeutic model. The figure shows two clonal arms of a probabilistic model relating individual factors and overall risk of AMR or infection

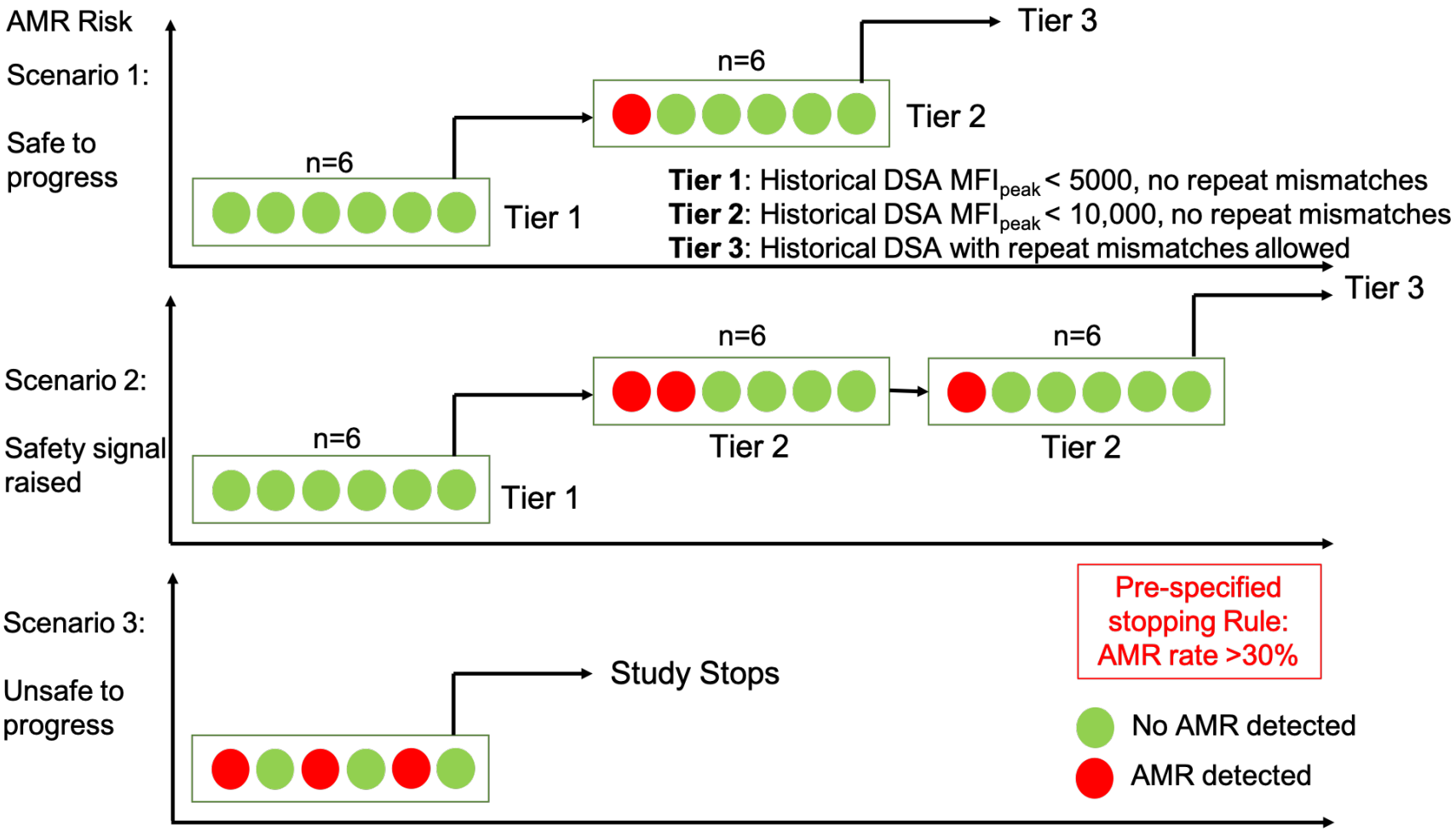
Activity 3a: Impact of therapeutic dose reduction on graft loss



Activity 3a: Impact of therapeutic dose reduction on graft loss



Activity 3b: Implement risk-based stratification in clinical trials

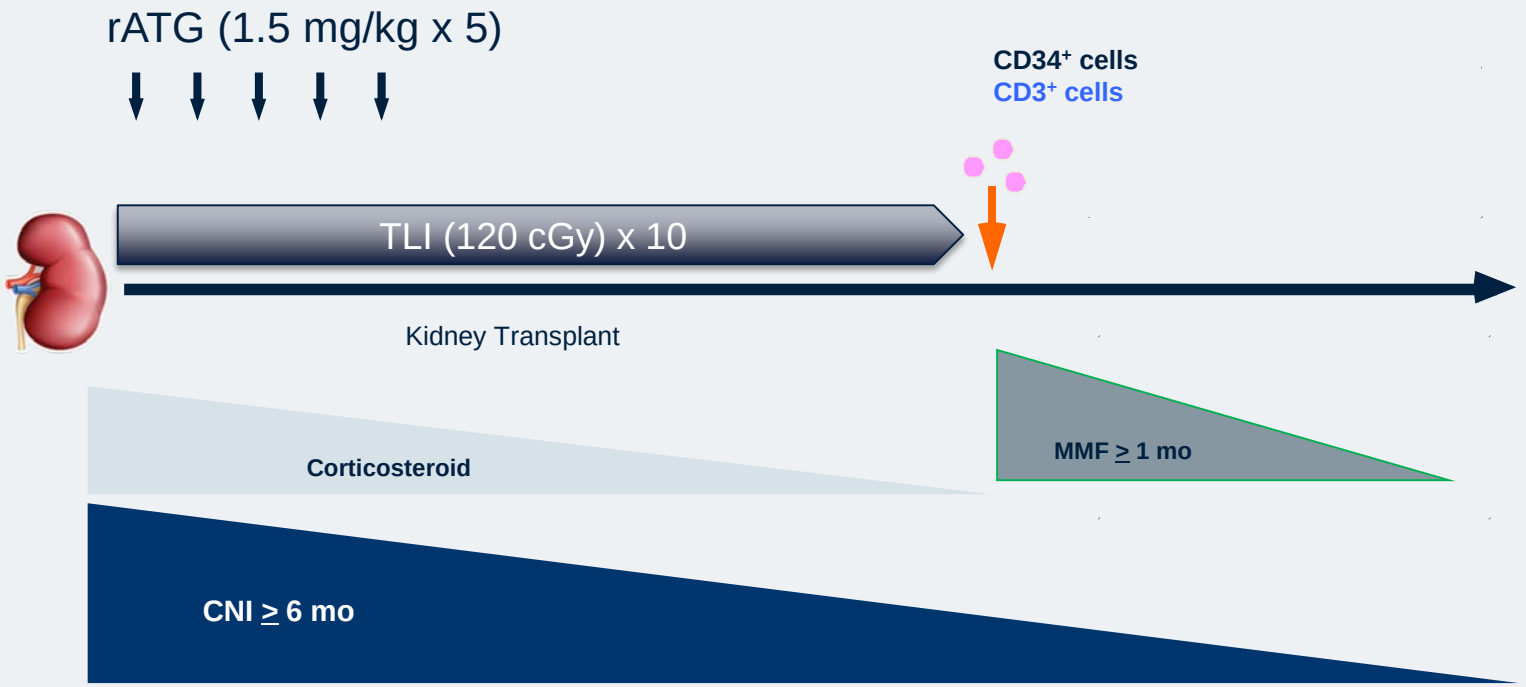


Activity 3: Strategies to induce tolerance: mixed chimerism

American Journal of Transplantation
AST AMERICAN SOCIETY OF TRANSPLANTATION ASTS

J. D. Scandling, S. Busque, J. A. Shizuru, R. Lowsky, R. Hoppe, S. Dejbakhsh-Jones, K. Jensen, A. Shori, J. A. Strober, P. Lavori, B. B. Turnbull, E. G. Engleman, S. Strober

Chimerism, Graft Survival, and Withdrawal of Immunosuppressive Drugs in HLA Matched and Mismatched Patients After Living Donor Kidney and Hematopoietic Cell Transplantation



Withdraw immunosuppression if: Mixed chimerism > 6 months, No evidence of rejection, No GvHD

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G C
T C

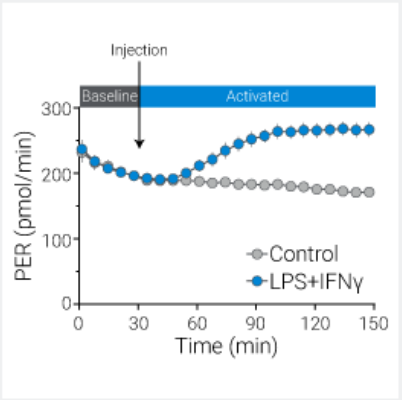
Genome Canada
Transplant Consortium

- prevent AMR, optimize expensive therapy and prolong graft survival
- restore healthy and productive life for patients and their families
- reduce the need for re-transplantation and make more organs available
- extend these benefits to other countries through our global partnerships

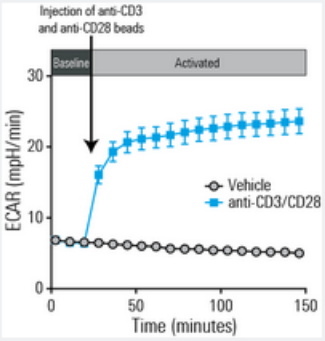
I love my new kidney and I can keep it for

all my

2a. Immune monitoring: recovery of immune competence (function)



Based on recent discoveries, glycolytic pathway dependency for energy production is a robust measure of macrophage activation. Conventionally, progression of macrophage activation is measured in terms of changes in cytokine expression and other end-point (not kinetic) data. The Seahorse XFp analyzer can detect macrophage activation response, stimulated with LPS using our integrated injection ports, by measuring the proton efflux rate (PER) in real time providing an early window of functional information to discriminate activation responses.



Based on recent discoveries, glycolytic pathway dependency for energy production is a robust measure of T cell activation. Conventionally, progression of T cell activation is measured in terms of changes in cell size/morphology, interleukin/interferon expression and cell surface markers, methods which can be time and labor intensive, often involve end-point (not kinetic) data, and a time scale of hours to days. The Seahorse XFp analyzer can detect T cell activation response within several minutes of stimulation with CD3/CD28 immunogenic beads by measuring glycolytic extracellular acidification rate (ECAR) in real time providing an early window of functional information to discriminate activation responses.