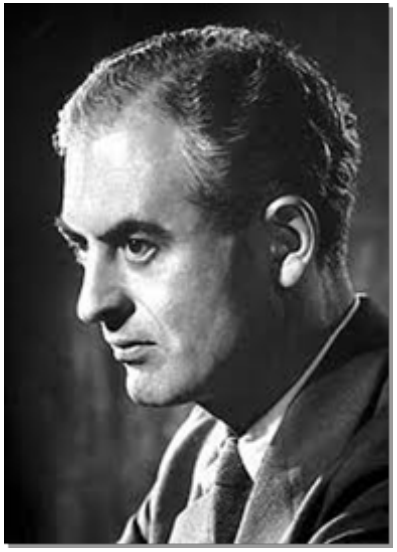
Immune Monitoring in the Current Era

Dr. Paul Keown, Immunology, 2012

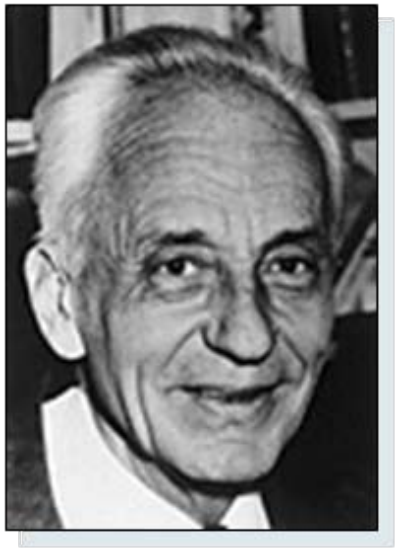
Objectives

- Review history of transplantation immunology and key discoveries leading to our current practice
- Summarize methods of HLA typing, antibody detection and cross-matching for transplantation
- Discuss the emerging role of post-transplant monitoring including new biological concepts

Landmarks in histocompatibility



Peter Medawar
University College
London, UK
b. 1915, d. 1987

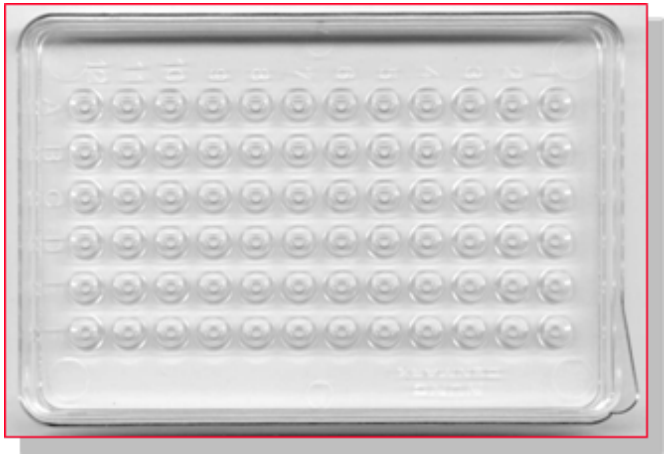


Jean Dausset
Universite de Paris
Paris, France
b. 1916, d. 2009

Jon van Rood
University of Leiden
Leiden, NL
b. 1926

Paul Terasaki
UCLA Medical School
Los Angeles, USA
b. 1929

Serological assays



HLA typing

HLA-A, B and C (class I)

HLA-DR (class II)

Antibody screening

Panel reactive antibodies (PRA)

Specific anti-HLA antibodies

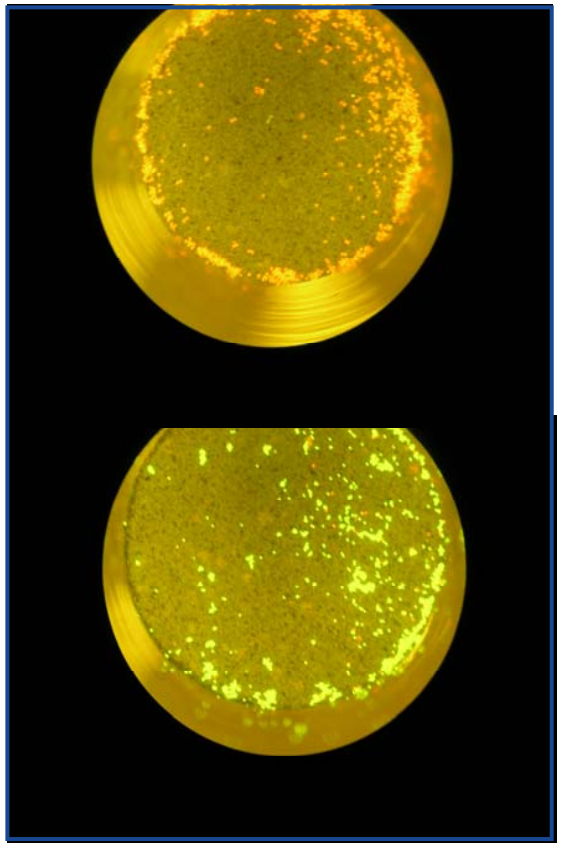
Crossmatching

Routine crossmatch (CDC)

Enhanced crossmatch (AHG-CDC)

The cytotoxic crossmatch

Score	% dead	Interpretation
0	0	N. I.
1	1-10	Negative
2	11-20	Doubtful
4	21-50	Weak +
6	51-80	Intermediate +
8	81-100	Strong +



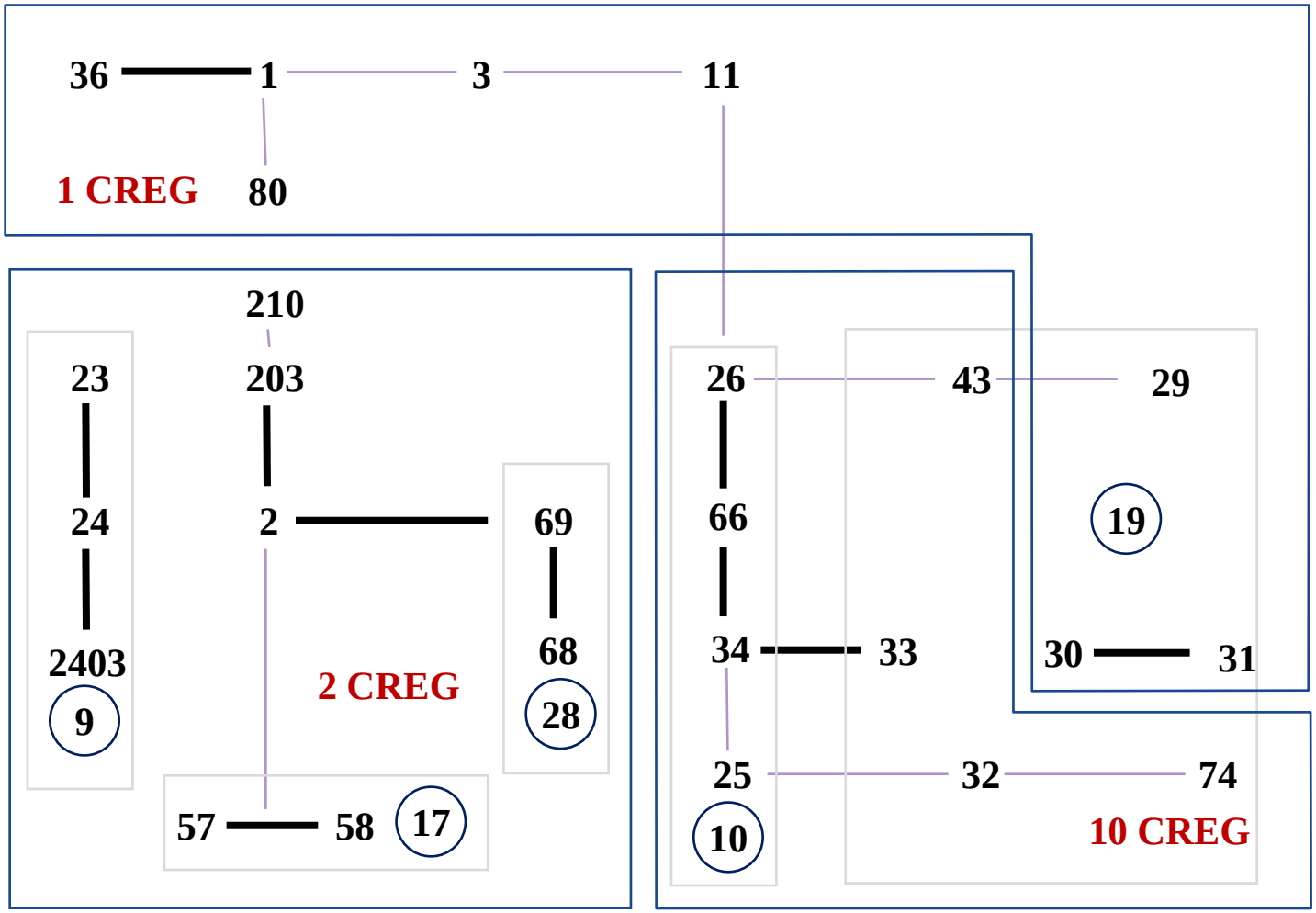
HLA antigens, class I

HLA–A antigens	
1	
2	– 203, 210
3	
9	– 23, 24, 2403
10	– 25, 26, 34, 66
11	
19	– 29, 30, 31, 32, 33, 74
28	– 68, 69
36	
43	
80	

HLA-B antigens			
5	- 51, 5102, 5103, 52,	37	
7	- 703	40	- 60,61
8		41	
12	- 44, 45	42	
13		46	
14	- 64, 65	47	
15	- 62, 63, 75, 76, 77	48	
16	- 38, 39, 3901, 3902	53	
17	- 57, 58	59	
18		67	
21	- 4005, 49, 50	70	- 71, 72
22	- 54, 55, 56	73	
27		78	
2708		81	
35		8201	

HLA–C antigens	
Cw1	
Cw2	
Cw3	- Cw9, Cw10
Cw4	
Cw5	
Cw6	
Cw7	
Cw8	

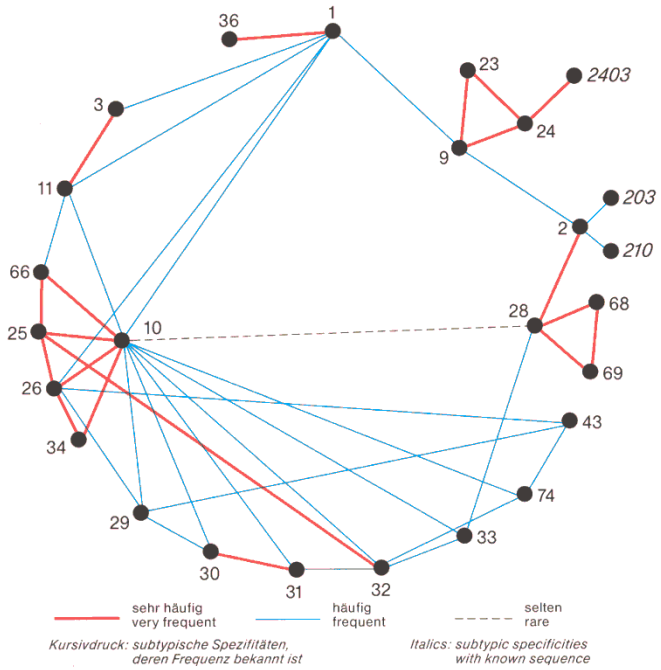
Cross-reactive groups (HLA-A)



Cross-reactive groups

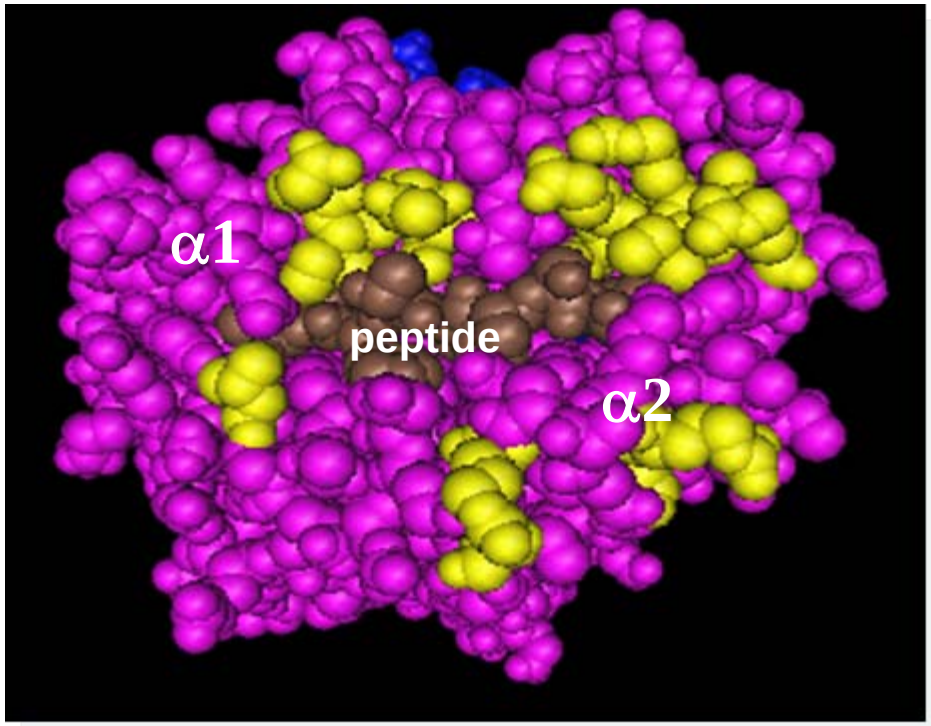
TABLE 40–6 CREGs AND ASSOCIATED ANTIGENS

CREG	ANTIGENS INCLUDED
A01C	A1, A3, A11, A29, A30, A31, A36, A80
A10C	A10, A11, A19, A25, A26, A32, A33, A34, A43, A66, A74
A02C	A2, A9, A23, A24, A28, A68, A69, A203, A210, A2403, B17, B57, B58
B05C	B5, B18, B35, B51, B52, B53, B78, B5102, B5103
B07C	B7, B8, B13, B22, B27, B40, B41, B42, B47, B48, B54, B55, B56, B59, B60, B61, B67, B81, B82, B703
B08C	B8, B14, B16, B18, B38, B39, B59, B64, B65, B67, B3901, B3902
B12C	B12, B13, B21, B37, B40, B41, B44, B45, B47, B49, B50, B60, B61, B4005
B21C	B5, B15, B17, B21, B35, B46, B49, B50, B51, B52, B53, B57, B58, B62, B63, B70, B71, B72, B73, B75, B76, B77, B78, B4005, B5102, B5103
Bw4	A9, A23, A24, A25, A32, B5, B13, B17, B27, B37, B38, B44, B47, B49, B51, B52, B53, B57, B58, B59, B63, B77, A2403, B5102, B5103
Bw6	B7, B8, B14, B18, B22, B35, B39, B40, B41, B42, B45, B48, B50, B54, B55, B56, B60, B61, B62, B64, B65, B67, B70, B71, B72, B73, B75, B76, B78, B81, B82, B703, B3901, B3902, B4005

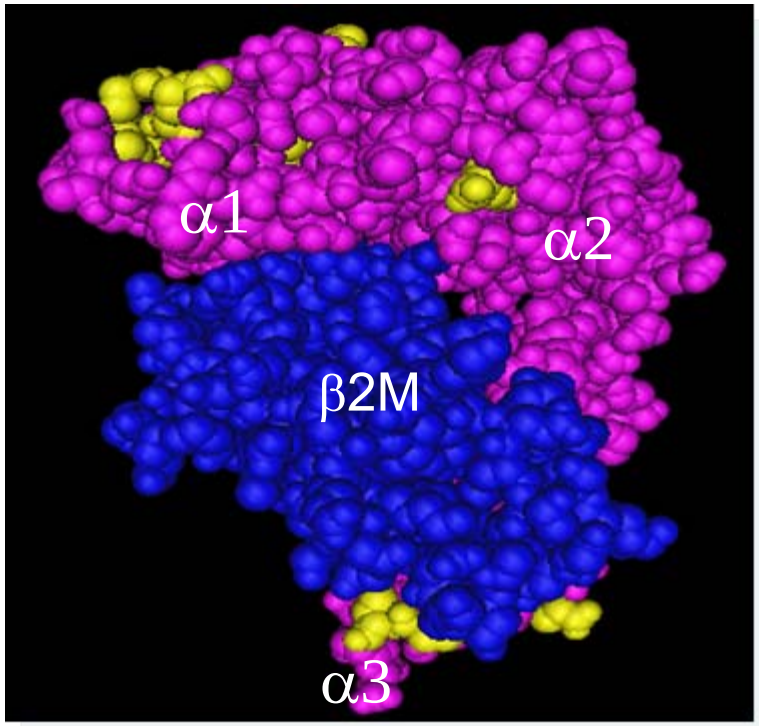


3-D structure of HLA

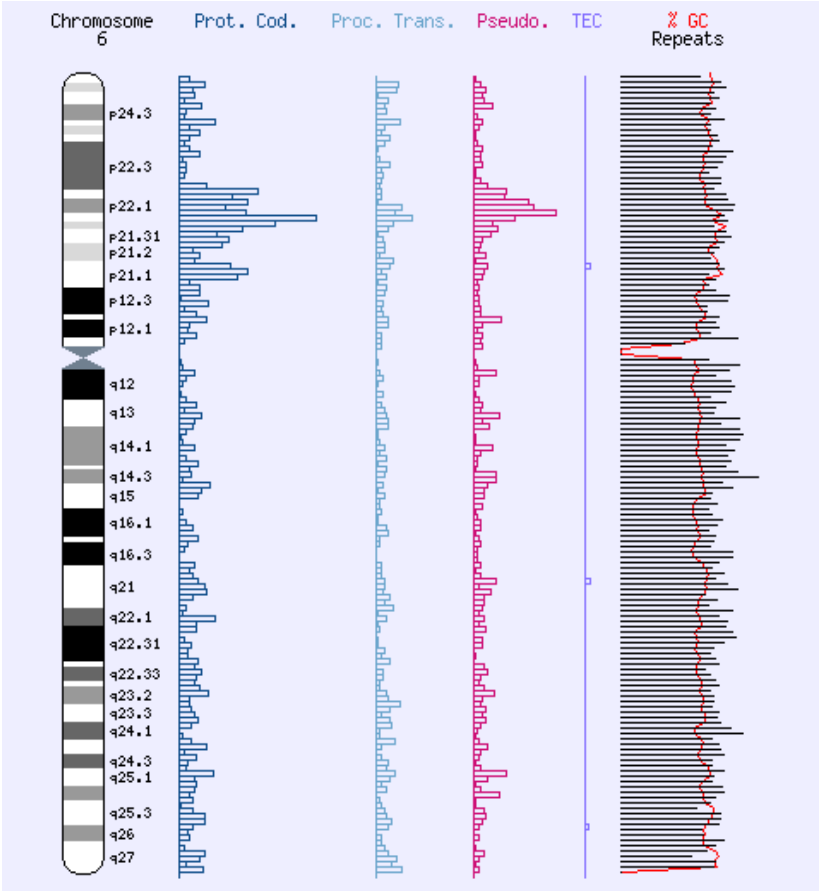
Top view

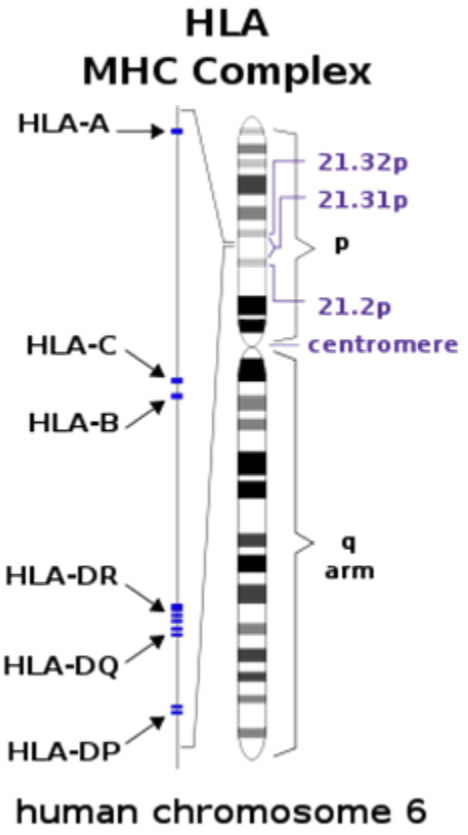


Side view

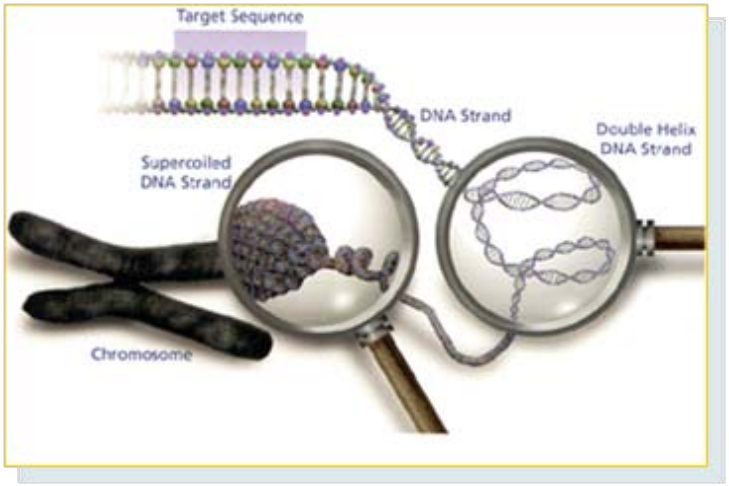


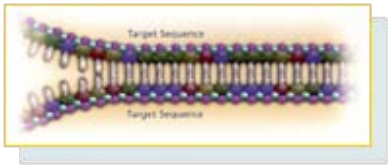
Chromosome 6, home of the MHC

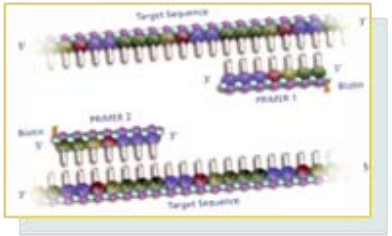


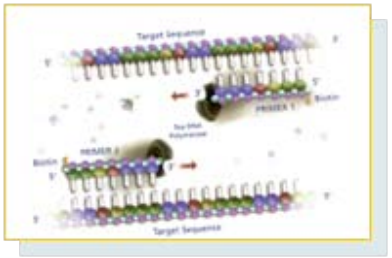


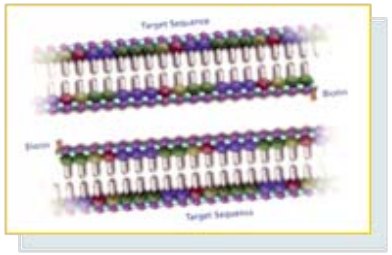
Principles of molecular typing

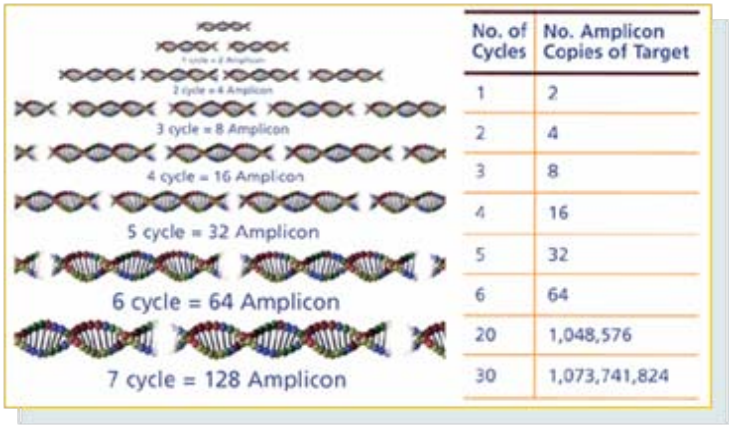






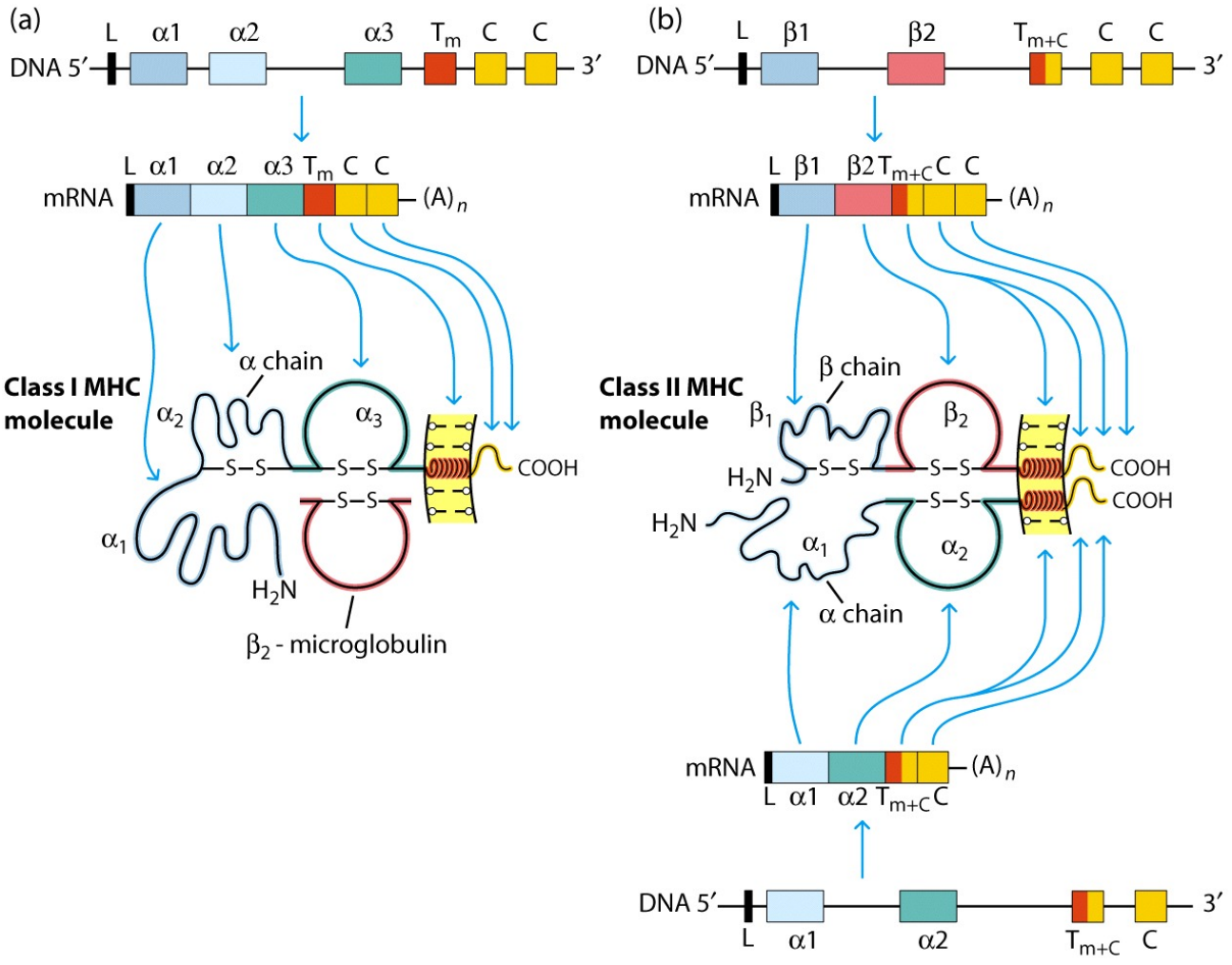






No. of Cycles	No. Amplicon Copies of Target
1	2
2	4
3	8
4	16
5	32
6	64
20	1,048,576
30	1,073,741,824

Molecular HLA typing



Molecular structure of HLA

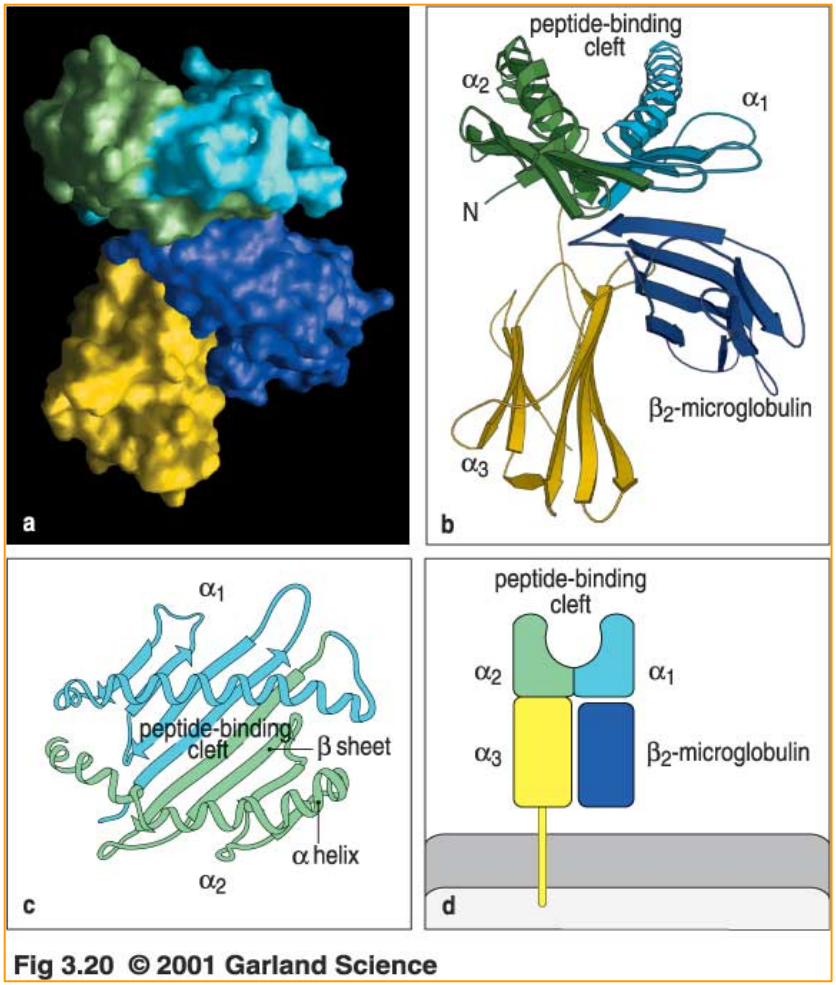


Fig 3.20 © 2001 Garland Science

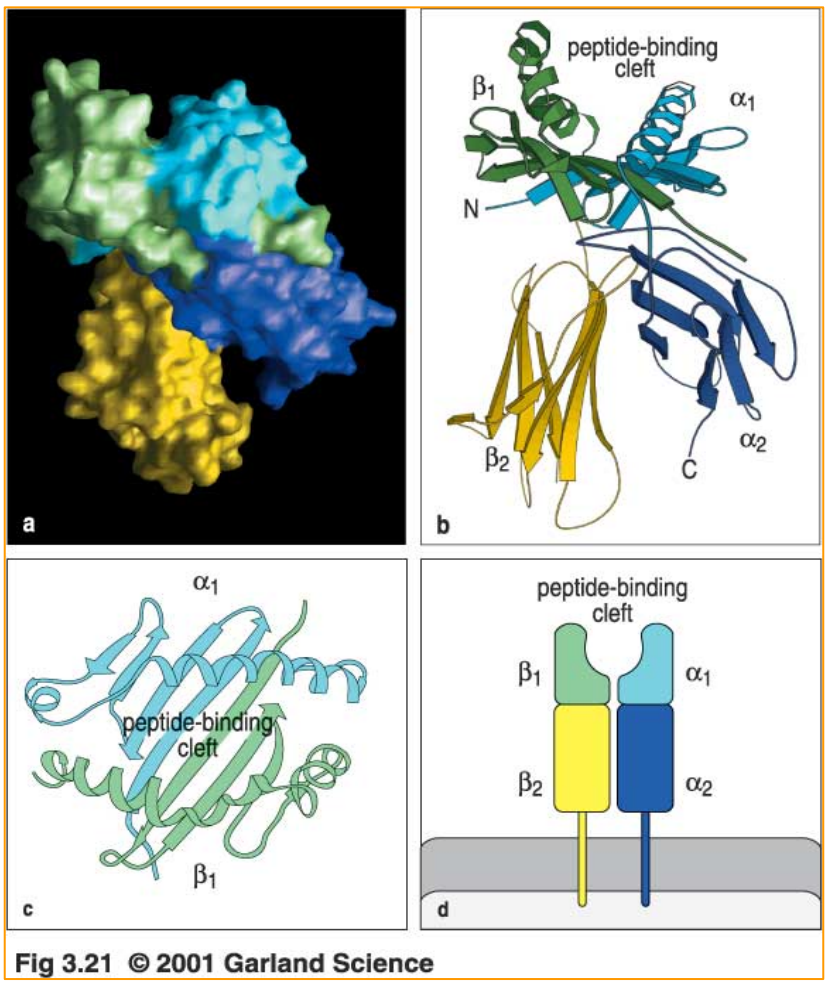
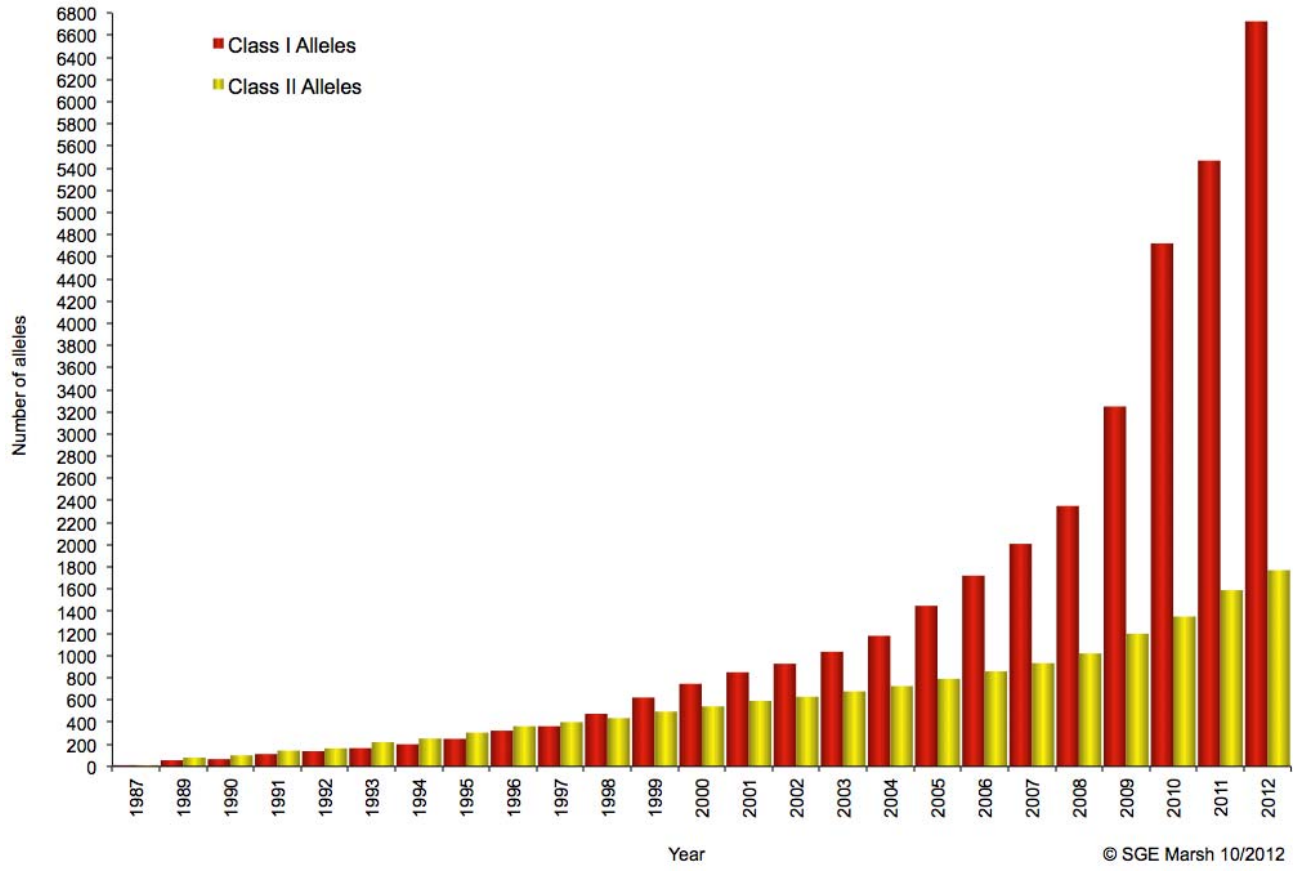
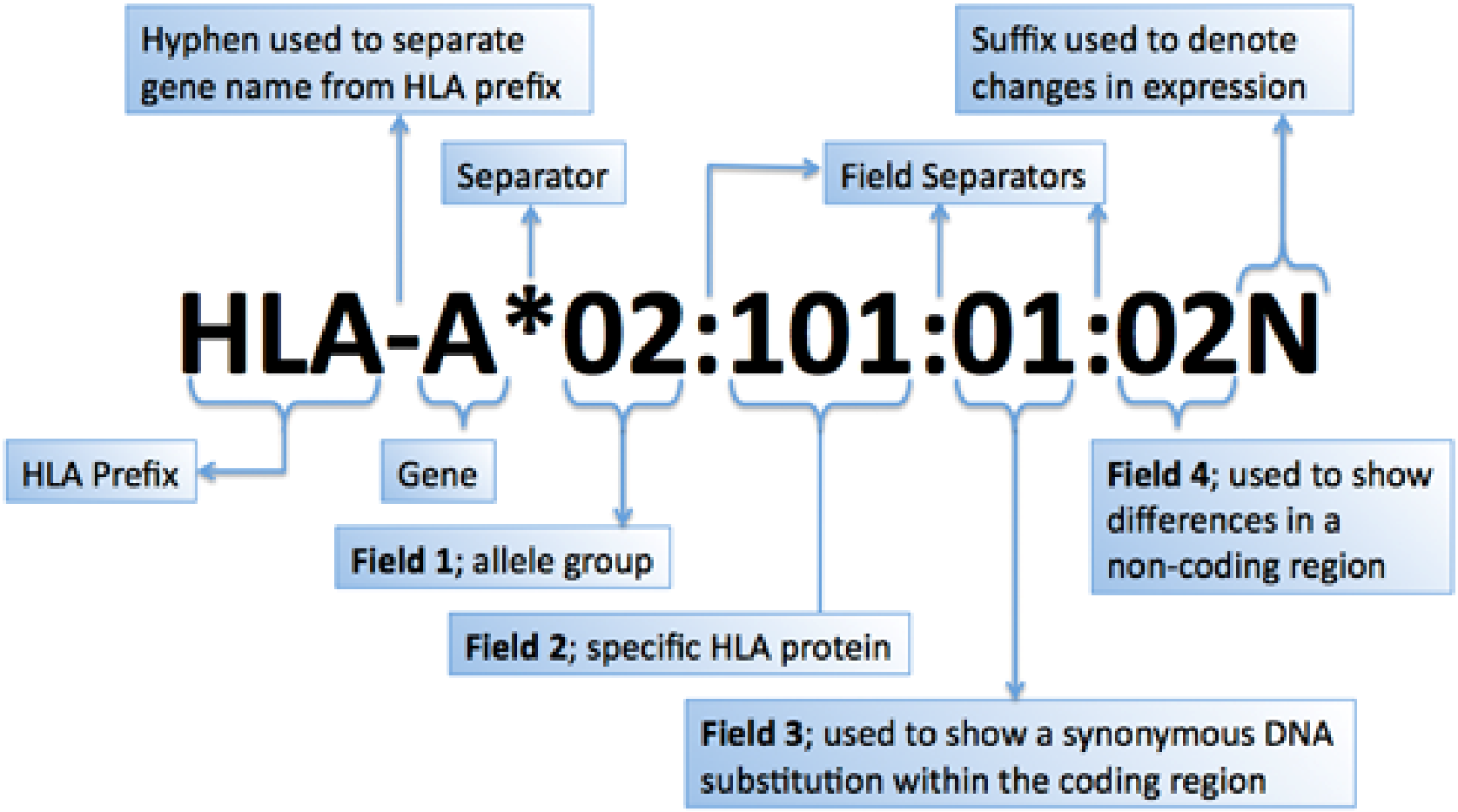


Fig 3.21 © 2001 Garland Science

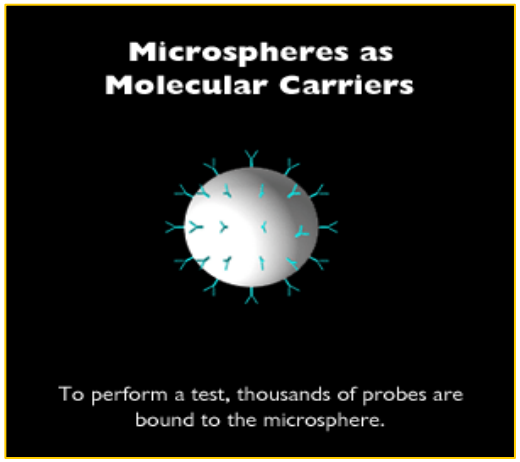
Increasing number of HLA alleles

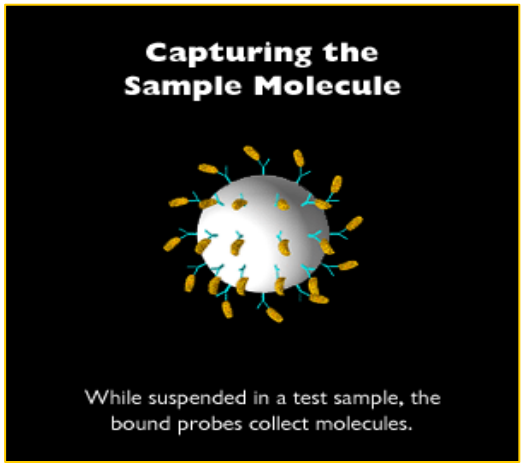


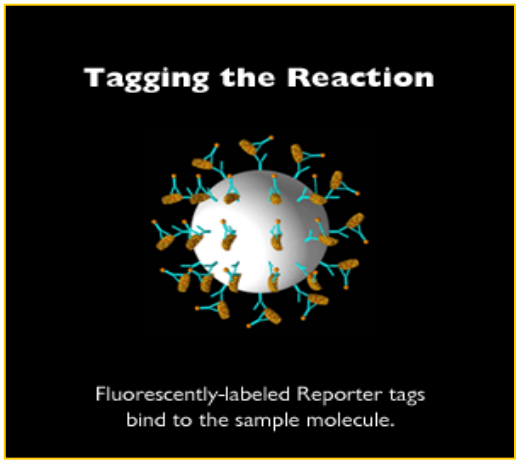
Current nomenclature of HLA

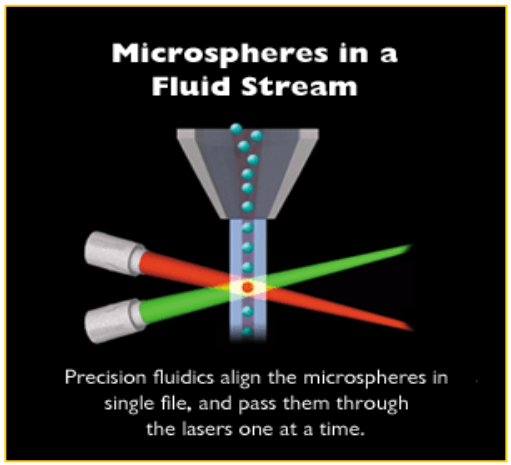


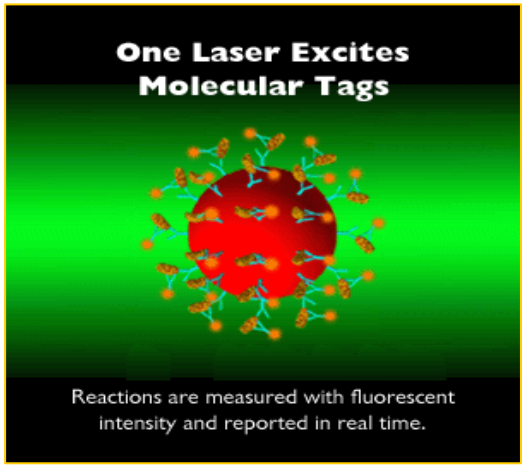
Luminex X-map technology

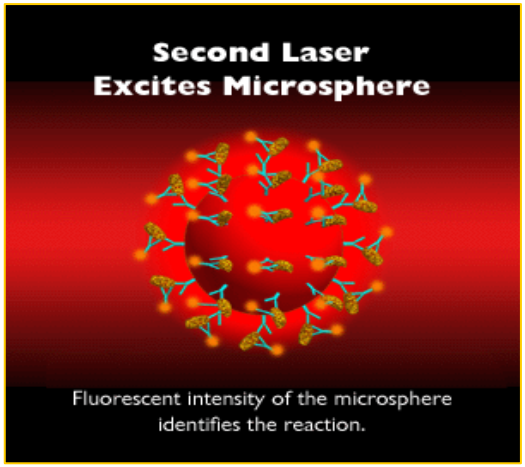




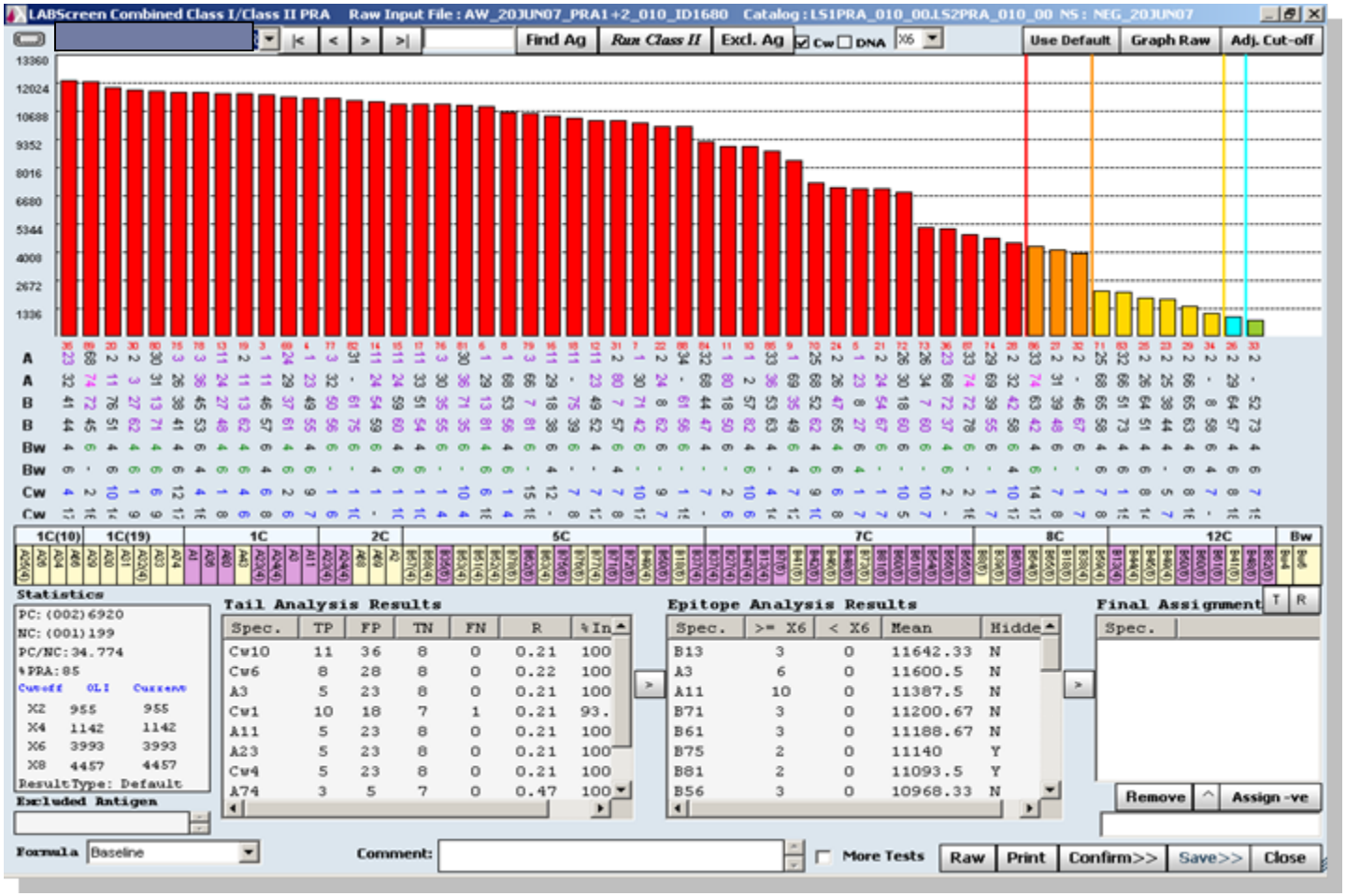




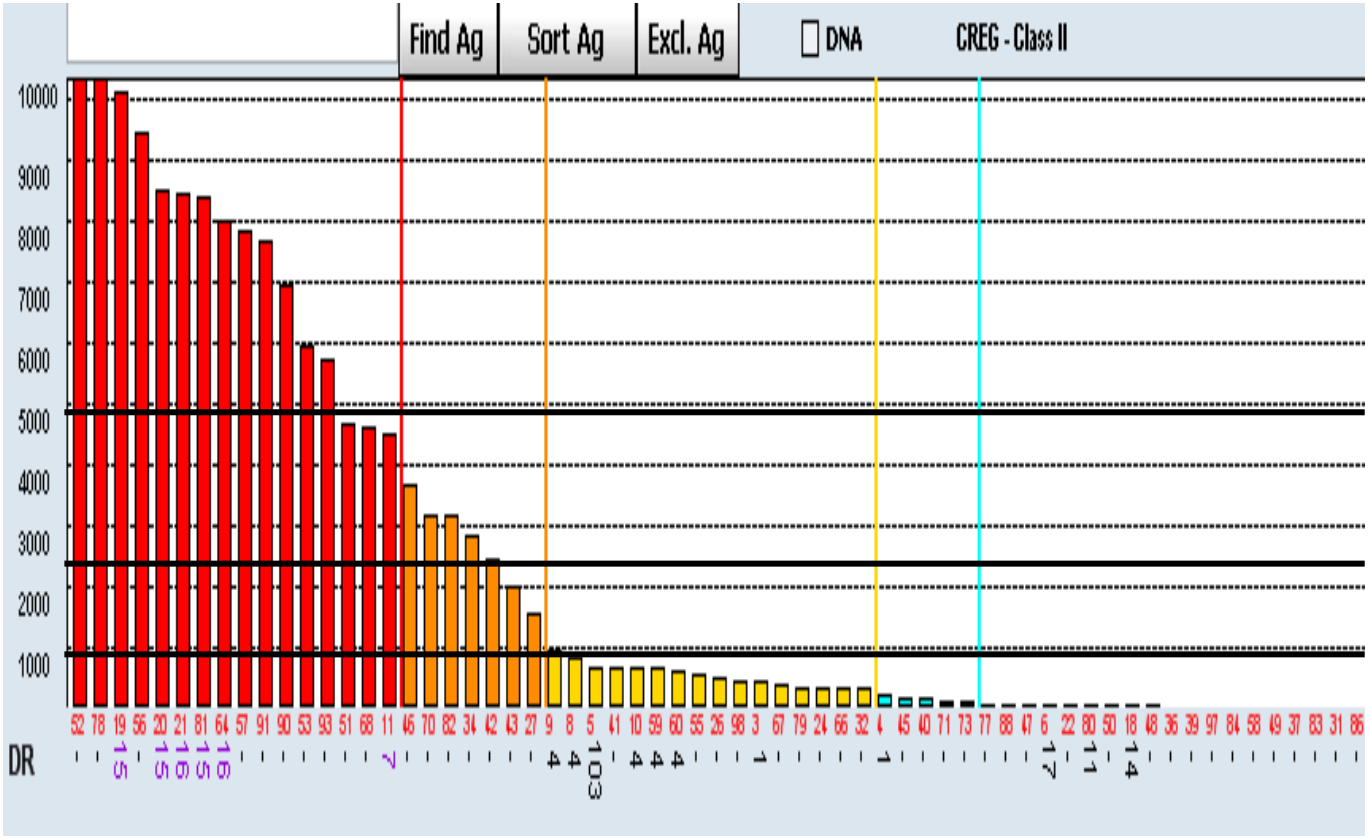




Luminex HLA PRA beads



HLA antibody: risk estimation



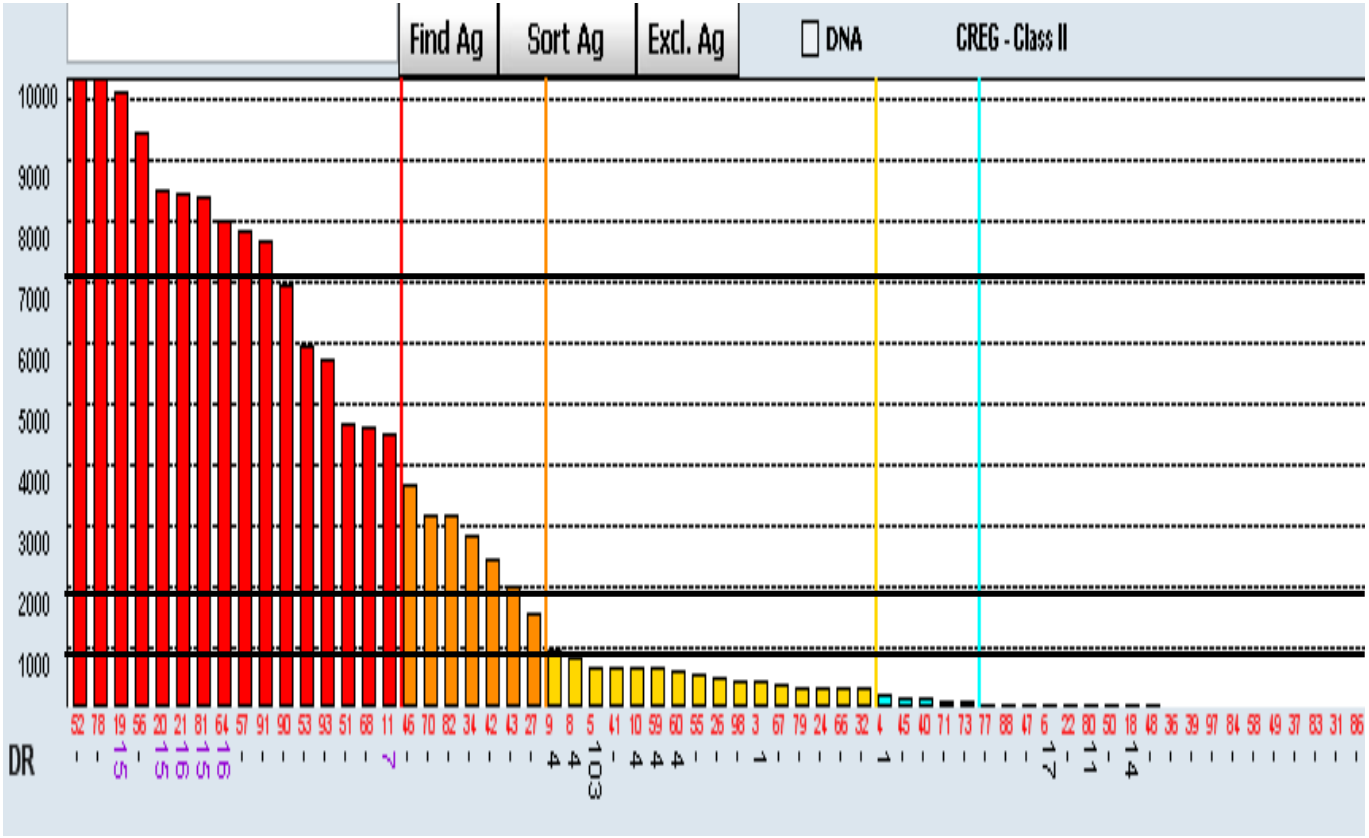
High risk

Mod. risk

Low risk

Very weak

HLA antibody: risk estimation



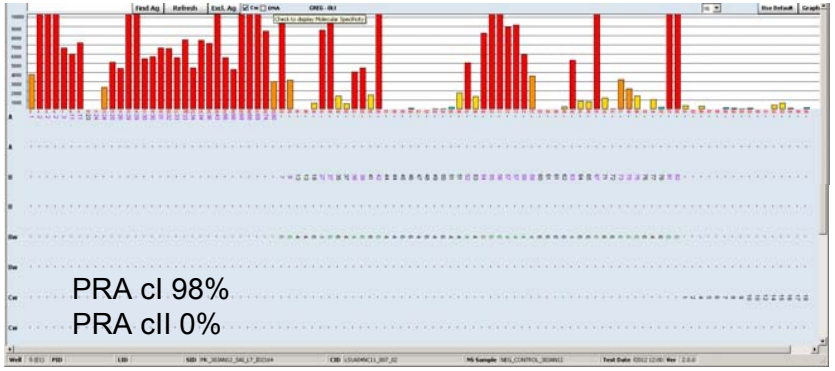
High risk

Mod. risk

Low risk

Very weak

Patterns of antibody reactivity

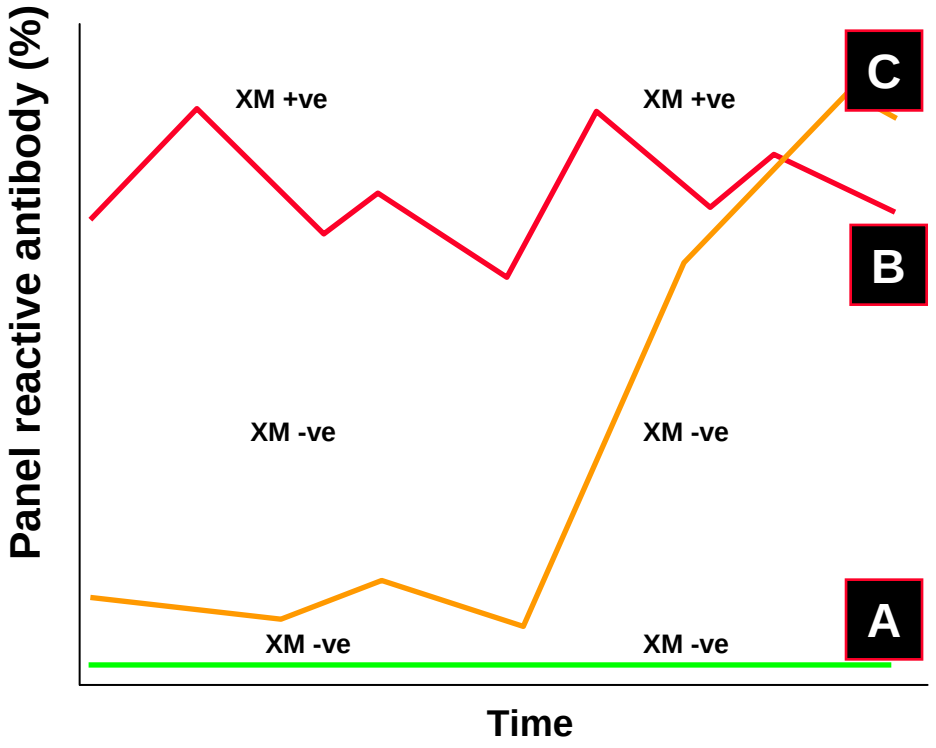


Virtual crossmatching

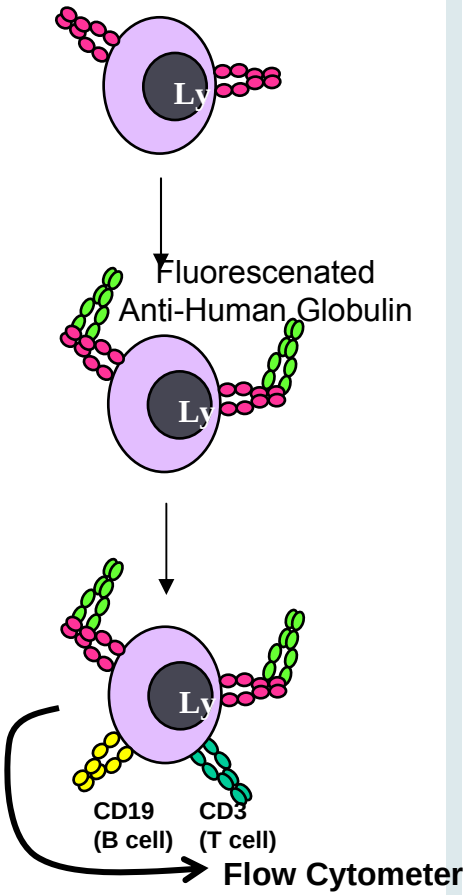
Virtual Cross-match is a simple comparison of antibody expression in the patient's serum with the HLA type of the potential donor.

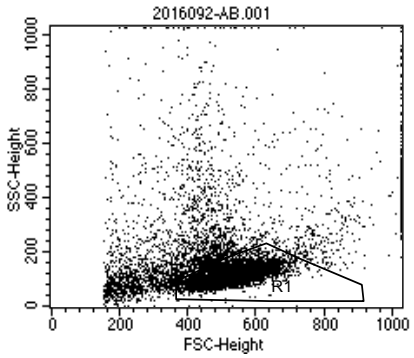
Antibodies are measured routinely for all patients on the transplant waiting list and records stored. Any new antibodies detected are added to a growing list of "cumulative antibodies" that determine the prior exposure of the patient.

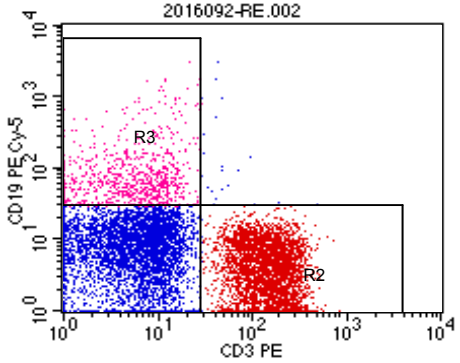
At the time of potential transplantation, the list of "cumulative antibodies" is compared with the HLA type of the donor, and reported. Patients with known antibodies to the donor are routinely excluded from DD transplantation in BC and Canada.

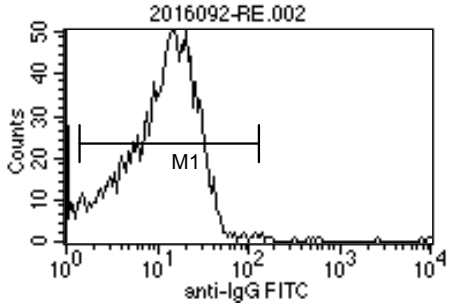


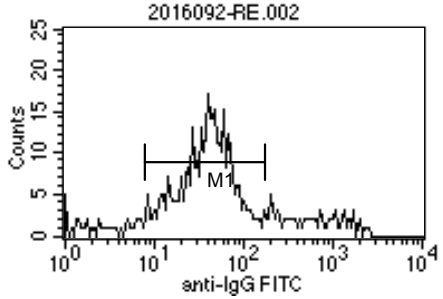
Flow crossmatch (FCXM)









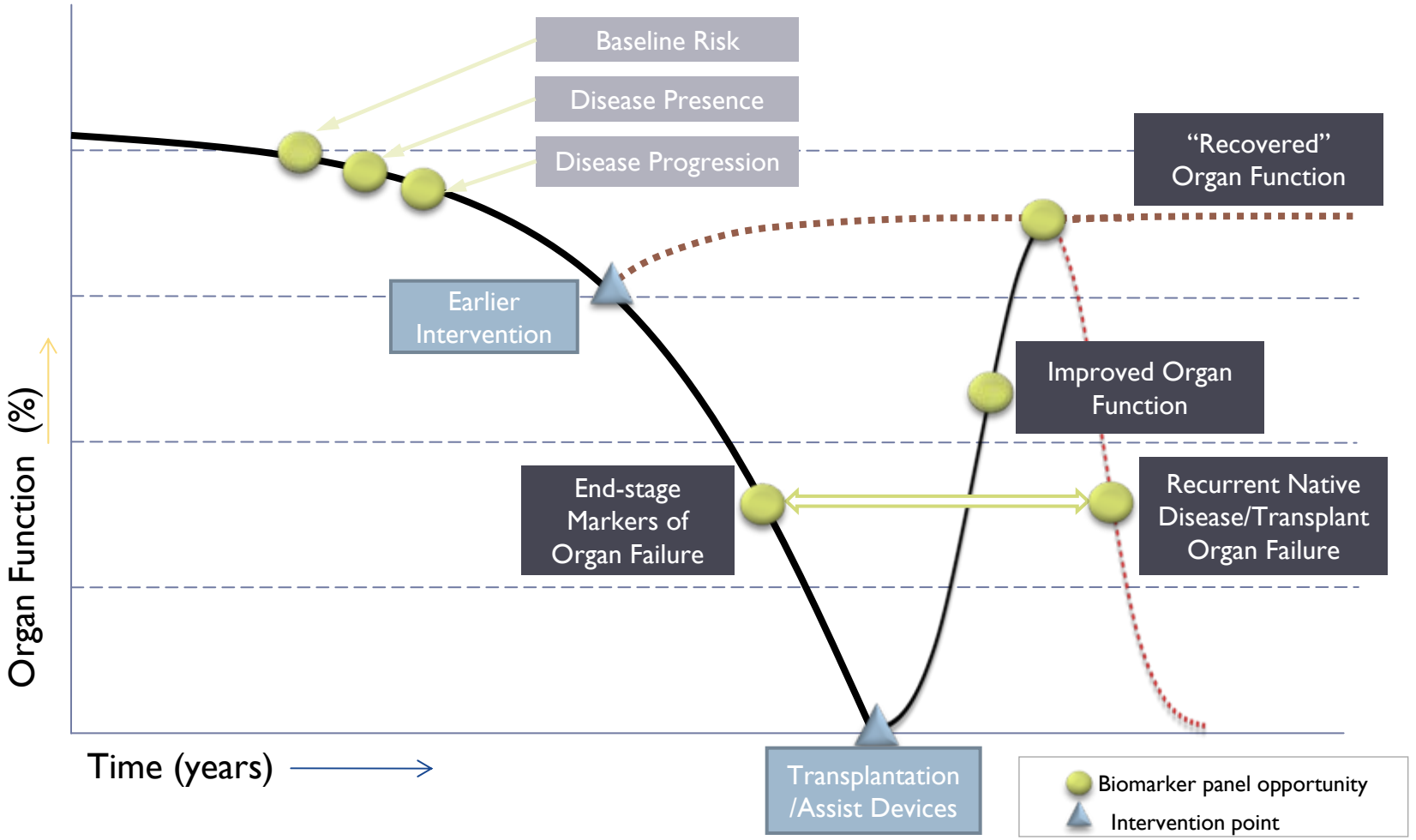


Interpretation of crossmatch

DSA	FCXM	AHG-CDC	RR of AMR
+ve	+ve	+ve	Extreme
+ve	+ve	-ve	High
+ve	-ve	-ve	Moderate
-ve	+ve	-ve	Minor
-ve	-ve	-ve	Normal

Influenced by Transplant, Tissue, Target, Titre, Type, and Timing

Monitoring organ injury



Acute antibody-mediated rejection

Conventional sequence of ABR

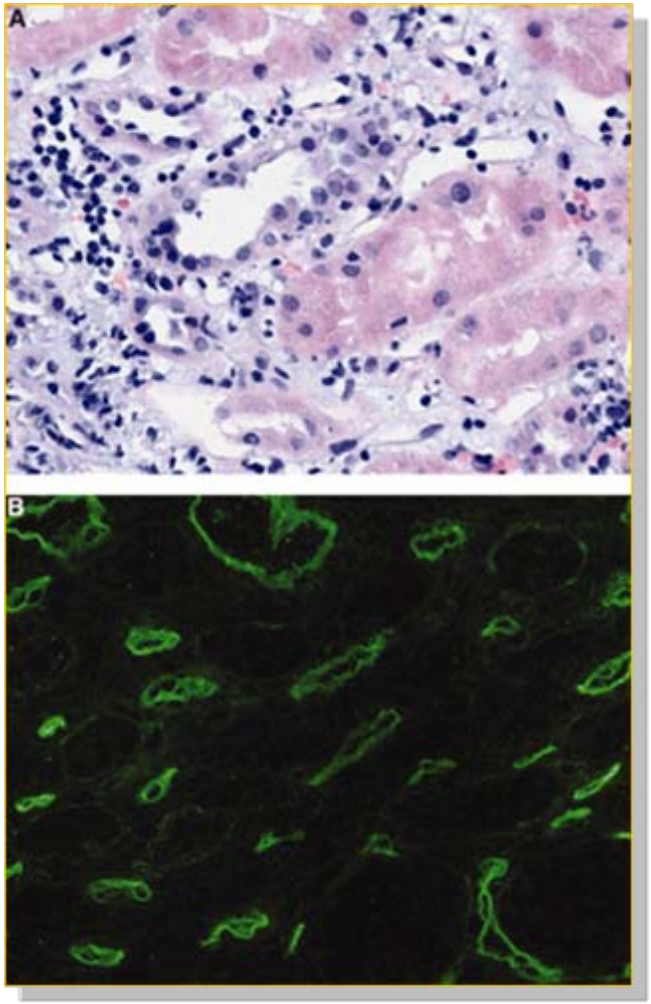
Stage 1: Alloantibody in circulation

Stage 2: C4d deposited in graft

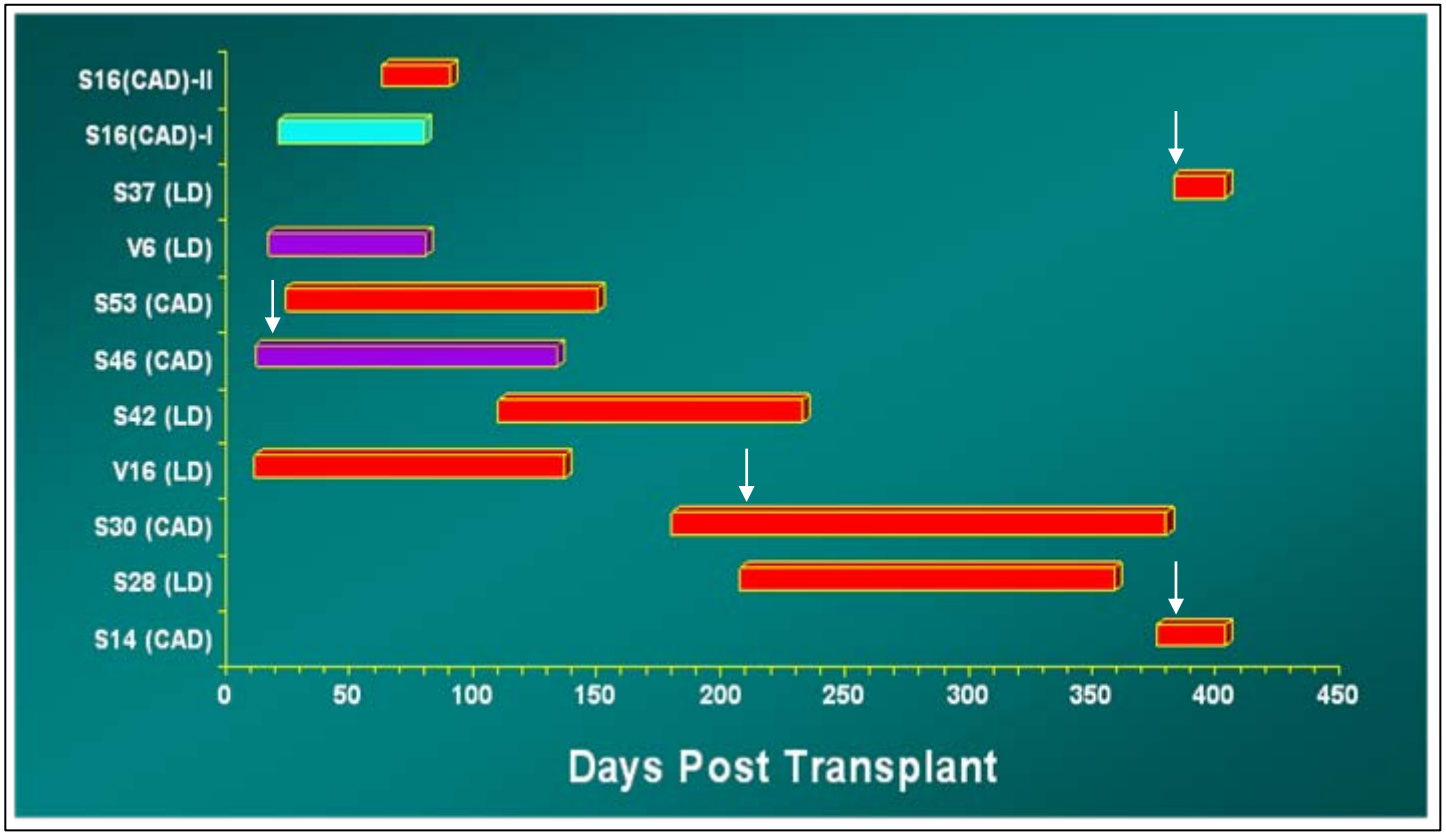
Stage 3: Histological injury to graft

Stage 4: Physiological dysfunction

Stage 5: Graft failure



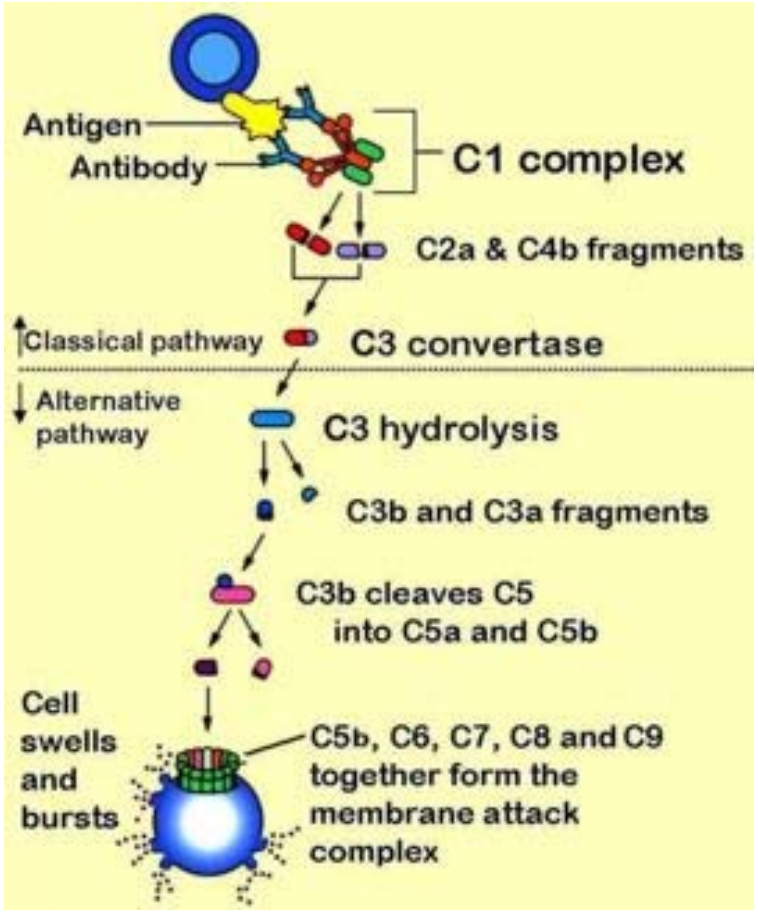
HLA antibodies post-transplant

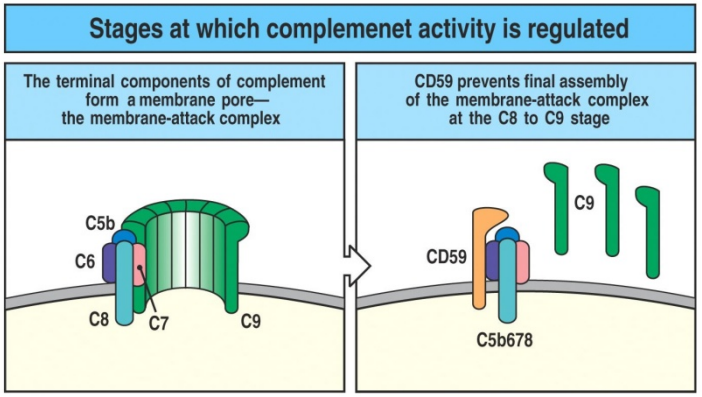


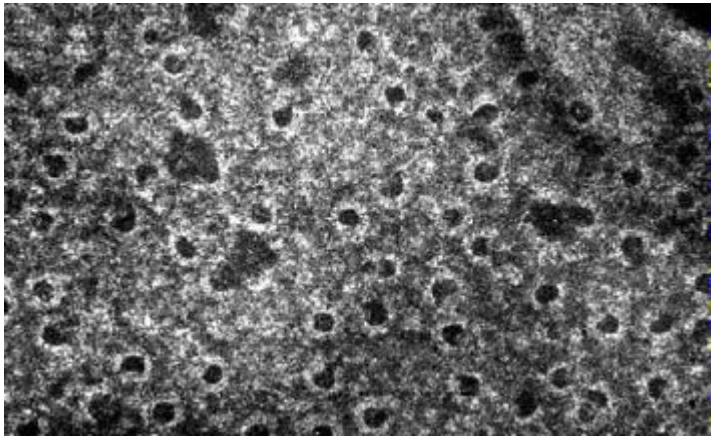
Class I, Non DSA Class II, DSA Class II, Non-DSA

Gill et al: Transplantation 2010

Complement activation







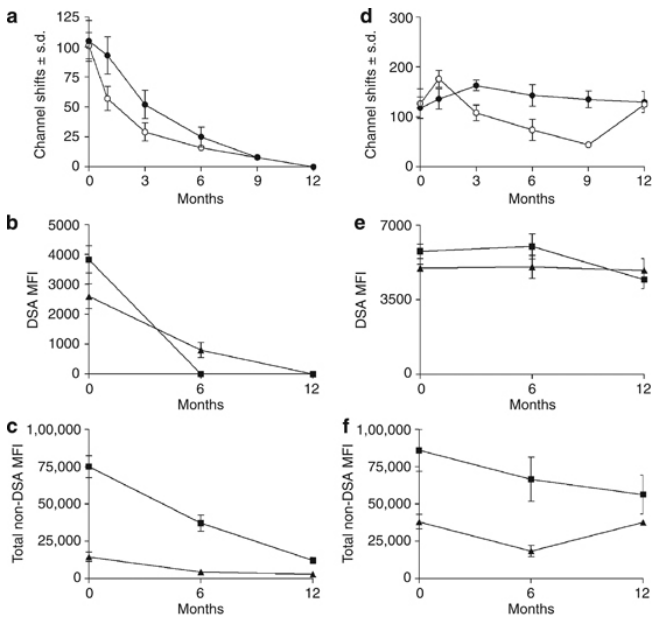
C1Q fixing antibodies

C1q-Fixing Human Leukocyte Antigen Antibodies Are Specific for Predicting Transplant Glomerulopathy and Late Graft Failure After Kidney Transplantation
Yabu, Julie M.1,5; Higgins, John P.2; Chen, Ge2,3; Sequeira, Flavia2,3; Busque, Stephan4; Tyan, Dolly B.2,3
Transplantation: 15 February 2011

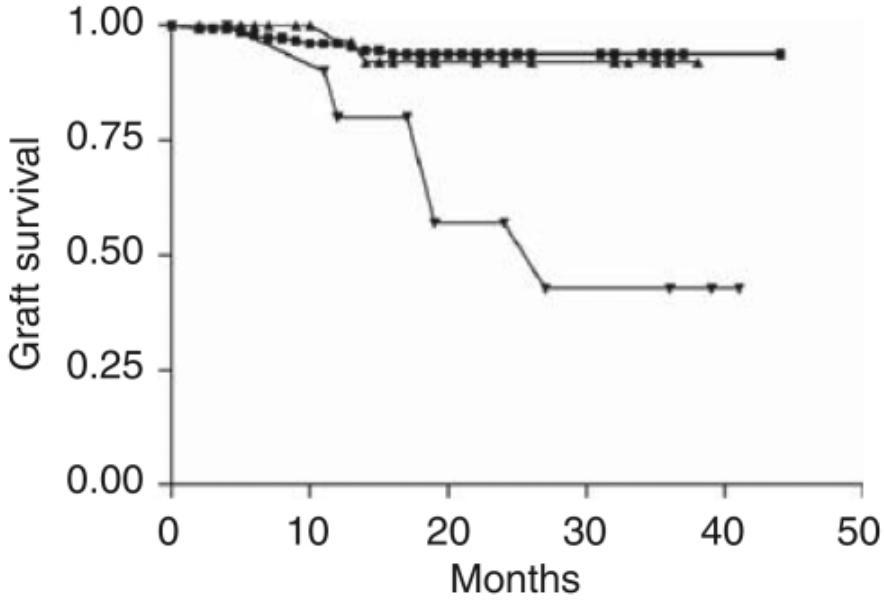
Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads
G. Chen A, F. Sequeira A, D.B. Tyan A,*
Histocompatibility, Immunogenetics, and Disease Profiling Laboratory, Department of Pathology, Stanford University School of Medicine, Palo Alto, CA 94304, USA
Human Immunology, 2011

Complement (C1q) fixing solid-phase screening for HLA antibodies increases the availability of compatible platelet components for refractory patients.
Fontaine MJ, Kuo J, Chen G, Galel SA, Miller E, Sequeira F, Viele M, Goodnought LT, Tyan DB.
Transfusion 2011 Department of Pathology, Stanford Hospital and Clinics, Stanford University, Stanford, California 94305-5626, USA. magalif@stanford.edu

HLA antibodies post-transplant



FCXM, DSA, and non-DSA levels during the first year post-transplant. Group I (*n*=33) eliminated FCXM, DSA, and non-DSA within 12 months. Group II (*n*=15) maintained FCXM, DSA, and non-DSA levels against class I and II throughout the study interval. ○ T-FCXM; • B-FCXM. ▪ anti-class I (MFI); ▲ anti-class II MFI.



Actuarial graft survival. Deaths with functioning grafts were censored. ▪ Patients with preoperatively negative flow cytometric crossmatch (FCXM; *n*=239); ▲ Group I; ▼ Group II. *P*<0.001, graft survival among group II versus group I.

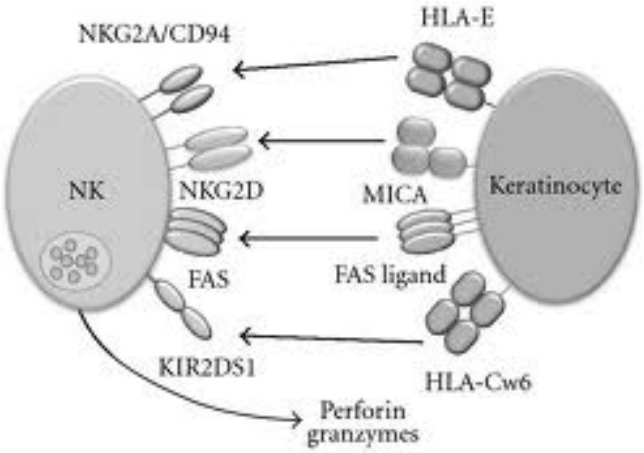
MHC class I polypeptide (A:B)

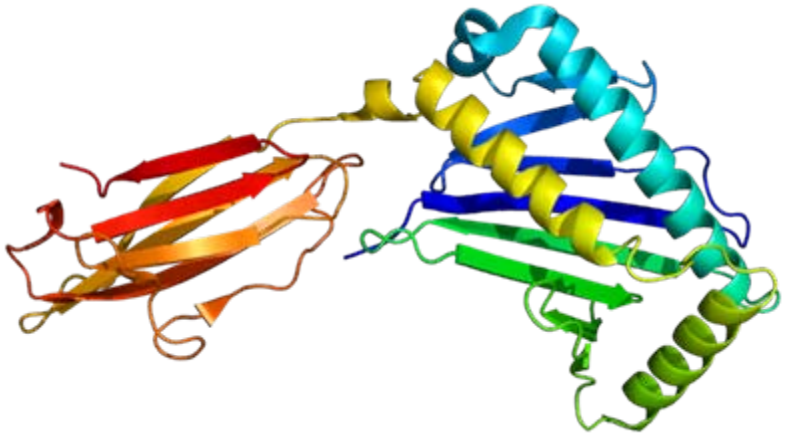
MHC class I polypeptide-related sequence A(B) is a protein that in humans is encoded by the polymorphic *MICA(B)* gene in the MHC at 6p21.3.

The protein product is expressed on the cell surface, although unlike canonical class I molecules does not seem to associate with beta-2-microglobulin.

It is thought that MICA(B) functions as a stress-induced antigen that is broadly recognized by NK cells, NKT cells, and most of the subtypes of T cells.

MICA is the ligand for NK cell activating receptor NKG2D(II). There are 80 MICA and 30 MICB alleles.





MICA in clinical transplantation

Detection of anti-MICA antibodies in patients awaiting kidney transplantation, during the post-transplant course, and in eluates from rejected kidney allografts by Luminex flow cytometry.
Zou Y, Heinemann FM, Grosse-Wilde H, Sireci G, Wang Z, Lavingia B, Stasny P.
Human Immunology 2006 Mar;67(3):230-7. Epub 2006 Mar 30.
Transplantation of Immunology Division, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390-8886, USA.

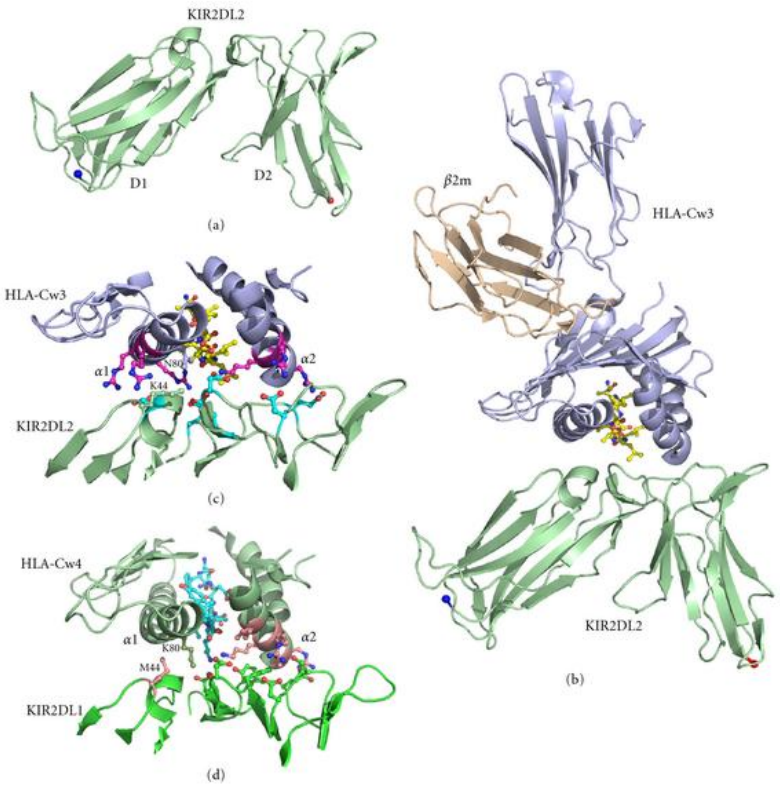
Acute rejection associated with donor-specific anti-MICA antibody in a highly sensitized pediatric renal transplant recipient
Shoba Narayan[1], Eileen W. Tsai[1], Qiuheng Zhang[2], William D. Wallace[3], Elaine F. Reed[2], Robert B. Ettenger[1]
Pediatric Transplantation. 27 DEC 2010

HLA and MICA: Targets of Antibody-Mediated Rejection in Heart Transplantation
Zhang, Qiuheng1,2,6; Cecka, J. Michael1,2; Gjertson, David W.1; Ge, Ping1,2; Rose, Marlene L.3; Patel, Jignesh K.4; Ardehali, Abbas4; Kobashigawa, Jon A.5; Fishbein, Michael C.2; Reed, Elaine F.1,2
Transplantation: 27 May 2011

KIR molecules

Killer-cell immunoglobulin-like receptors (KIRs), are a family of cell surface proteins found on NK cells. They regulate the killing function of these cells by interacting with MHC class I molecules, which are expressed on all cell types. This interaction allows them to detect virally infected cells or tumor cells that have a characteristic low level of Class I MHC on their surface.

Most KIRs are inhibitory, meaning that their recognition of MHC suppresses the cytotoxic activity of their NK cell. Only a limited number of KIRs have the ability to activate cells. KIR molecules are highly polymorphic. To date, 14 distinct KIR have been identified: eight are inhibitory types and six are activating.



Anti-AT1R type 1 antibodies

Anti-Angiotensin Type 1 Receptor Antibodies Associated With Antibody Mediated Rejection in Donor HLA Antibody Negative Patients

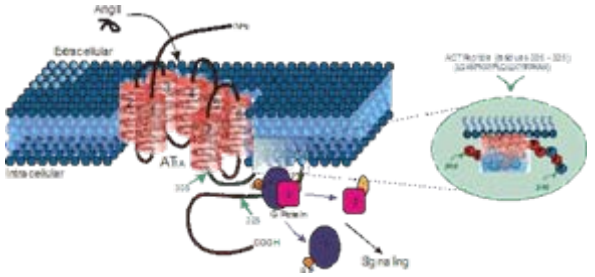
Nancy L. Reinsmoen,[1,7] Chih-Hung Lai,[1] Harald Heidecke,[2] Mark Haas,[3] Kai Cao,[1] Geraldine Ong,[1] Mehrnoush Naim,[1] Qi Wang,[1] James Mirocha,[4] Joseph Kahwaji,[5] Ashley A. Vo,[5] Stanley C. Jordan,[5] and Duska Dragun[6]

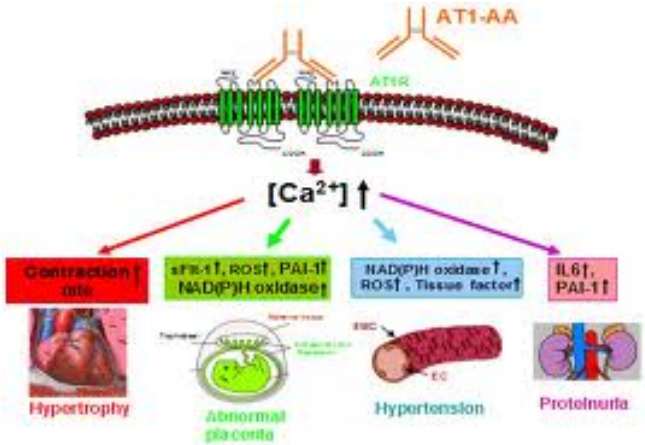
Background. Angiotensin type 1 receptor (AT_1R) mediates most physiologic and pathophysiologic actions of its endogenous ligand, angiotensin II, with overactivity leading to vascular remodeling and hypertension. Antibodies to AT_1R are implicated in several vascular pathologies. The aim of our study was to determine the impact of antibody to AT_1R on clinical outcomes including antibody mediated rejection (AMR), with or without C4d deposition, in patients whose sera contained no donor human leukocyte antigen (HLA)-specific antibody (HLA-DSA).

Methods. Pretransplant sera from 97 recipients and sera obtained at the time of acute rejection (AR) were tested by Luminex-based single-antigen bead assays to determine HLA-DSA and antibodies to major histocompatibility class I chain-related gene A (MICA). The presence of antibody to AT_1R was determined by a cell-based ELISA method using a cutoff of 17 units to distinguish high from low binding.

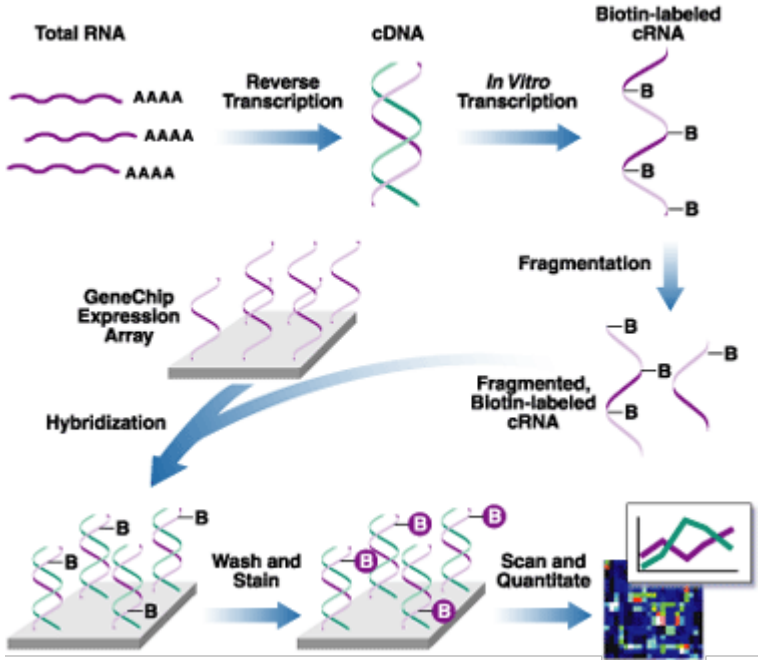
Results. Sera from 63 recipients were determined to have no HLA-DSA and no donor-specific MICA antibodies pretransplant and at the time of AR, and 16 of these recipients were diagnosed with AR including 7 with AMR and 9 with cellular AR (cell-mediated rejection). High-binding AT_1R antibodies were identified for six of seven in the AMR+ group and zero of nine in the cell-mediated rejection+ group ($P=0.0009$).

Conclusions. A strong association was observed between the presence of high binding to AT_1R and AMR in recipients whose sera contained no antibody to donor HLA or MICA. Assessing the AT_1R antibody status along with the HLA-DSA provides additional information to determine the immunologic risk for recipients.

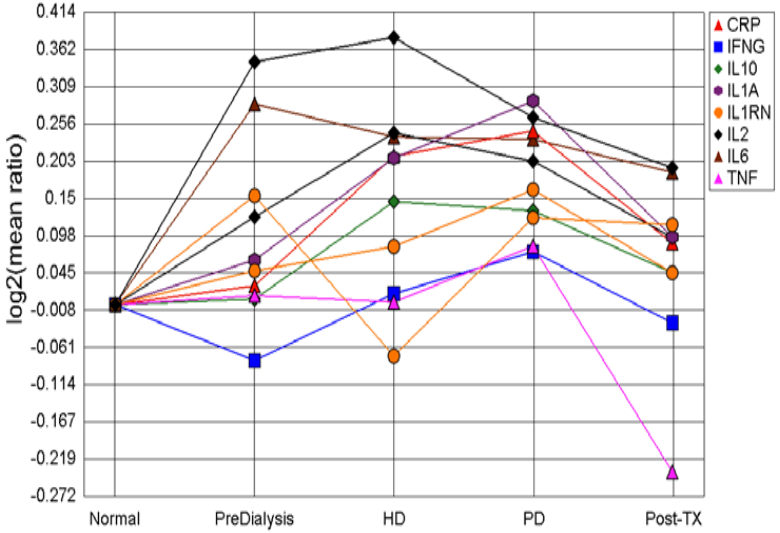




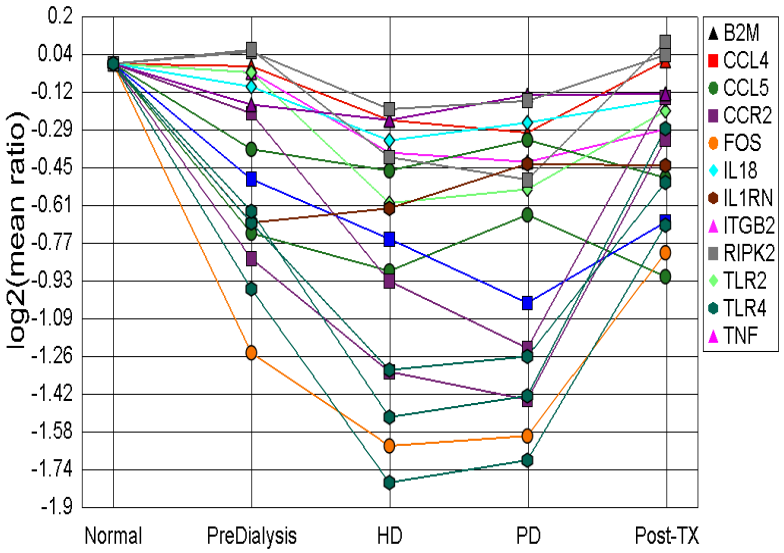
Gene expression - transcriptome



Immunity and inflammation



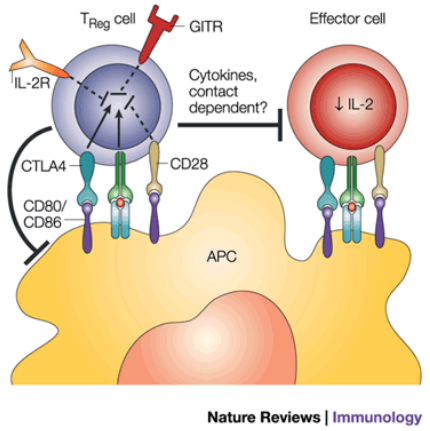
A. Transcripts for many key cytokines are elevated in chronic renal failure, HD and PD (many peaking in PD), but expression levels return towards normal after transplantation



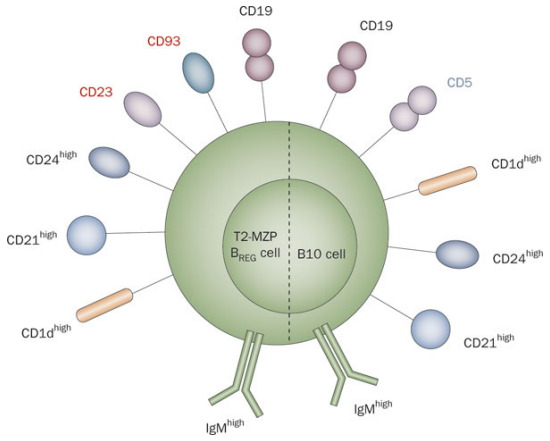
B. Transcripts for many key chemokines are suppressed in chronic renal failure, HD and PD (many reaching a nadir in HD and PD), but expression levels return towards normal after transplantation

Regulatory cells in immunity

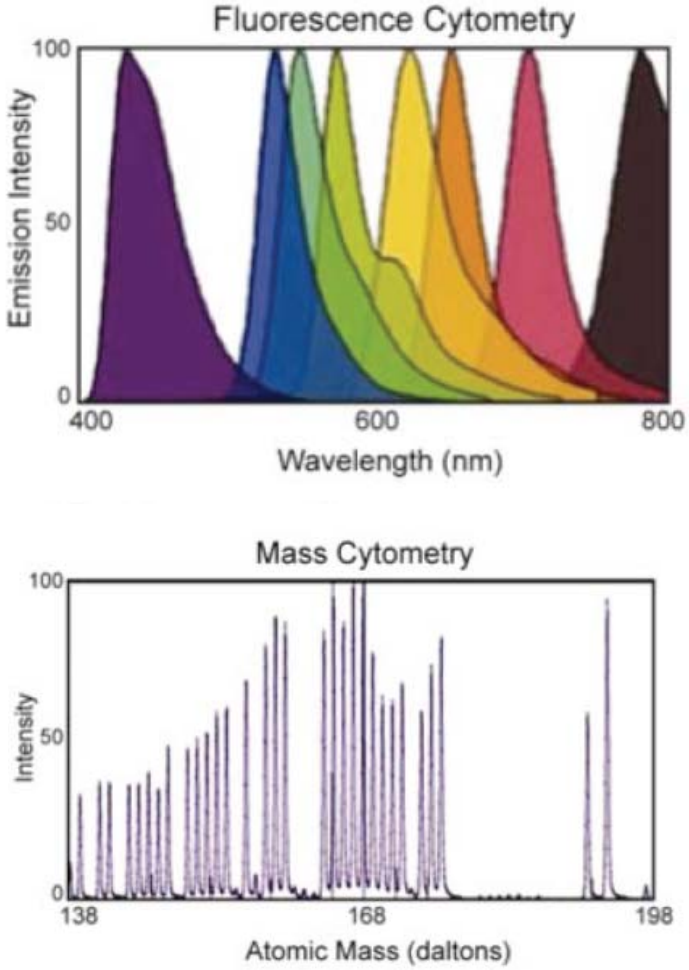
T regulatory cells are a component of the immune system that suppress responses of other cells to prevent excessive reactions. The most well-categorized express CD4, CD25 and Foxp3, and secrete TGF-B and IL-10. These cells are involved in regulating the response to infection, transplantation, and autoimmunity.

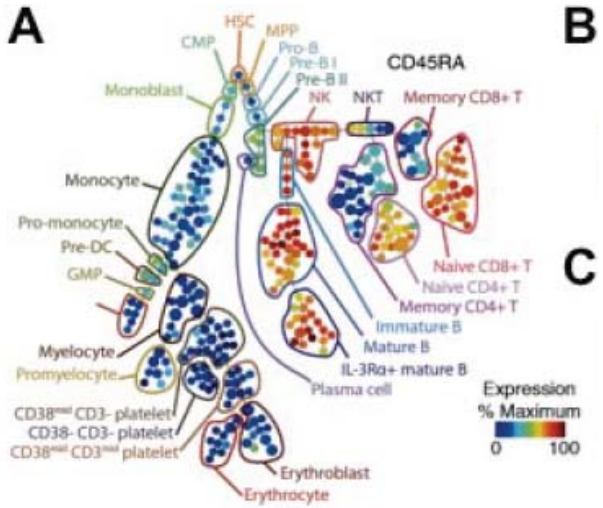


B regulatory cells exist in several forms, marginal zone B cells, transitional type 2-like B cells, or CD5(+) B cells. Regulatory activity is induced following cell activation through a B-cell receptor, CD40, and/or TLR9. Regulatory effects are then mediated by a soluble agent, such as IL-10, and/or direct cell-to-cell contacts that involve CD40 or B7 co-stimulatory molecules.



The Application of Mass Cytometry



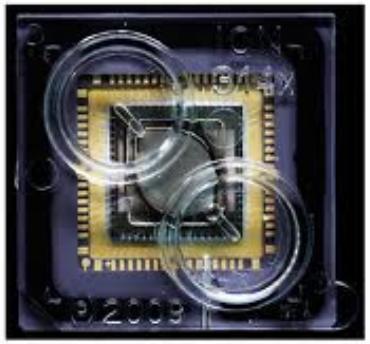


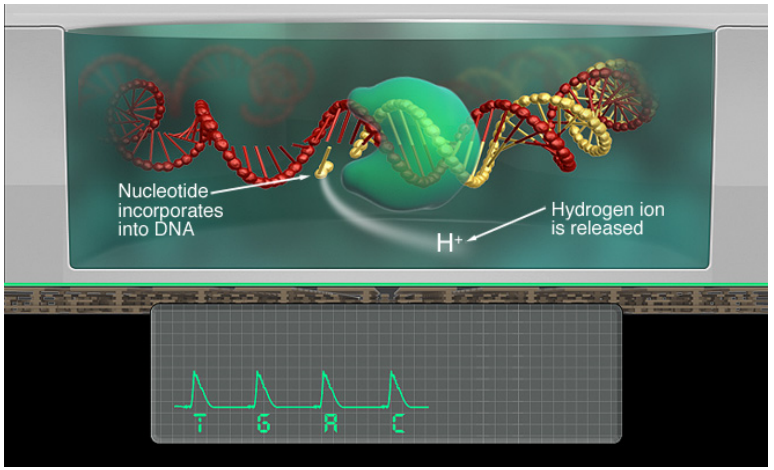
Uses rare elemental isotopes as reporters to perform mass spectrometric analysis of single cells in the human immune system. Permits compensation-free, 34 parameter, single cell analysis for proteomic dissection of immune function at the single cell level

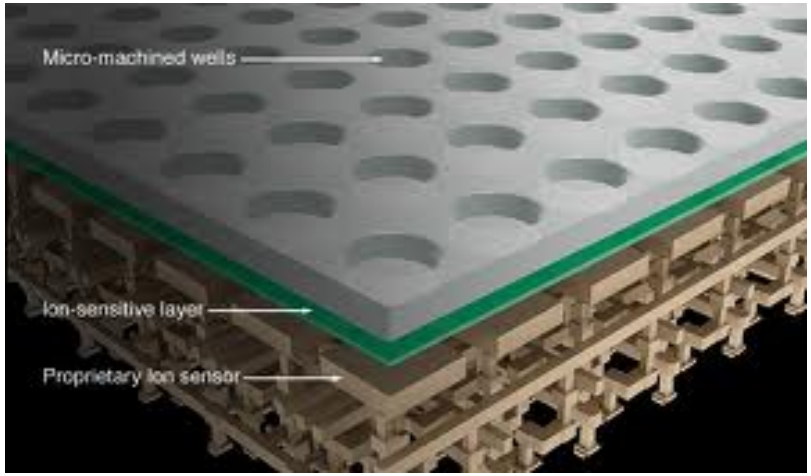
Sean Bendall, Garry Nolan, Stanford University

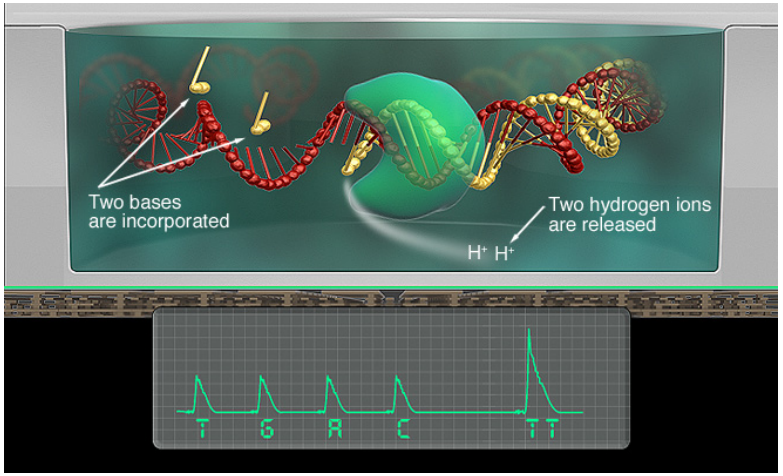
In affiliation with:
THE UNIVERSITY OF BRITISH COLUMBIA

Next generation sequencing









Recognition

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