Heart Transplant Update

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Relevant Financial Relationship Disclosure Statement

I will discuss off label use and/or investigational medications in my presentation

I have relationship(s) to disclose: Clinical Trial Principal Investigator (Amgen-GALACTIC HF, Biocardia-CardiAMP, Mesoblast-DREAM HF) and Stock Interest (Abbott, Abbvie, Repligen, Portola, Amarin)

Lecture Overview:

- Historical perspective of heart transplantation
- Statistics and survival post heart transplant
- Immunologic compatibility
- Post heart transplant complications
- Conventional and novel methods to detect rejection
- Advances in immunosuppression strategies
- Future directions

Heart Transplantation
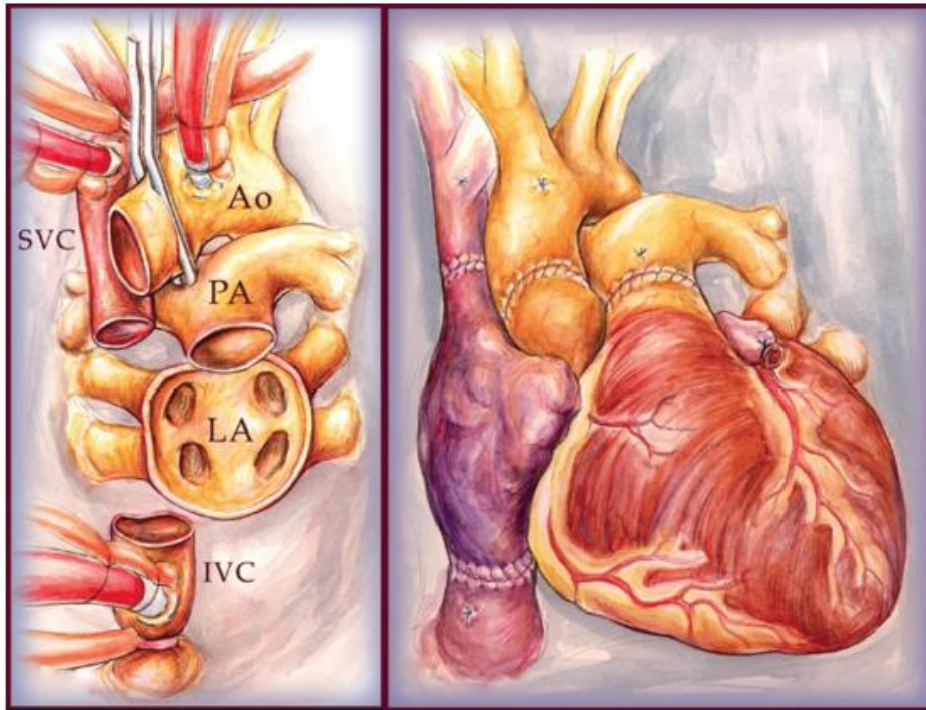
Over 50 years since the first human heart transplant

- **1967**   First human heart transplant performed by Christiaan Barnard in South Africa

- **1968**   Norman Shumway performed first adult human transplant in United States

- **1976**   Cyclosporine discovered, radically improving survival by reducing rates of rejection

The Evolution of Immunosuppressive Therapies

1960's
 Corticosteroids
 Polyclonal antibodies
 Azathioprine
1980's
 Cyclosporine
 Monoclonal antibodies
1990's
 Tacrolimus
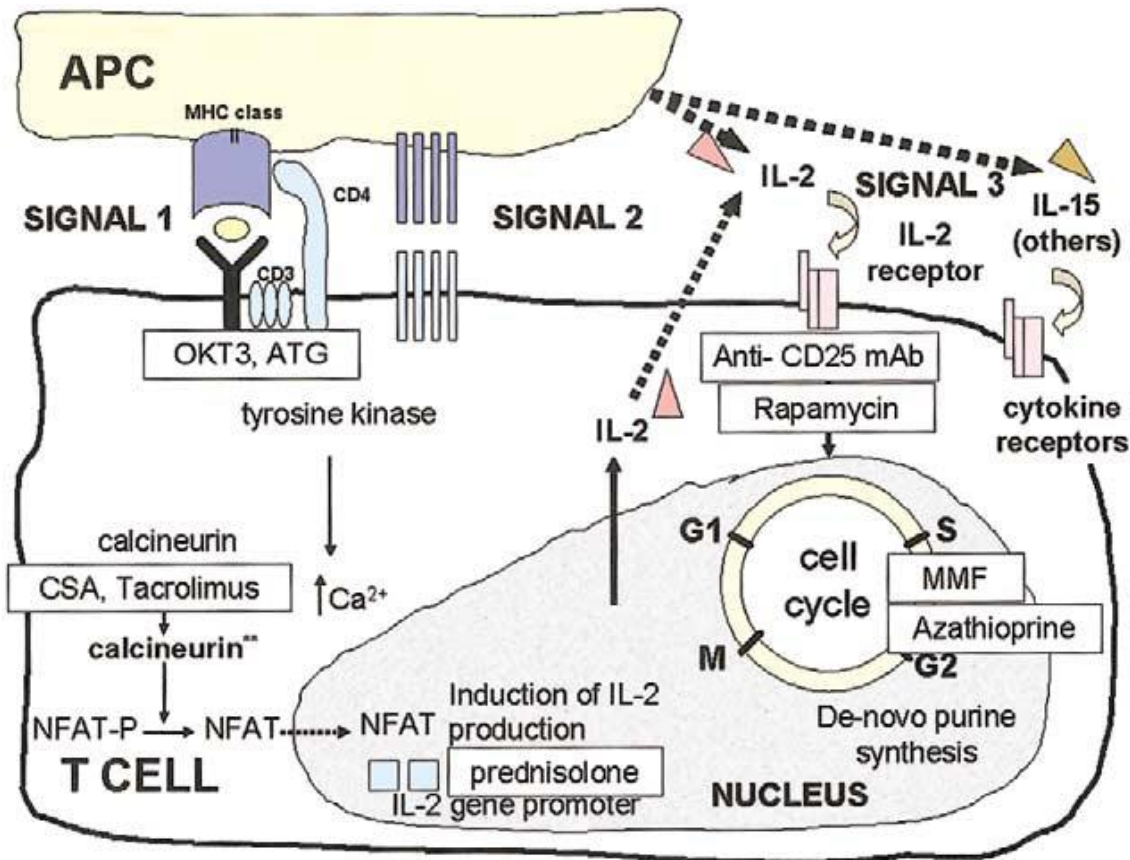 Mycophenolate mofetil
 Sirolimus
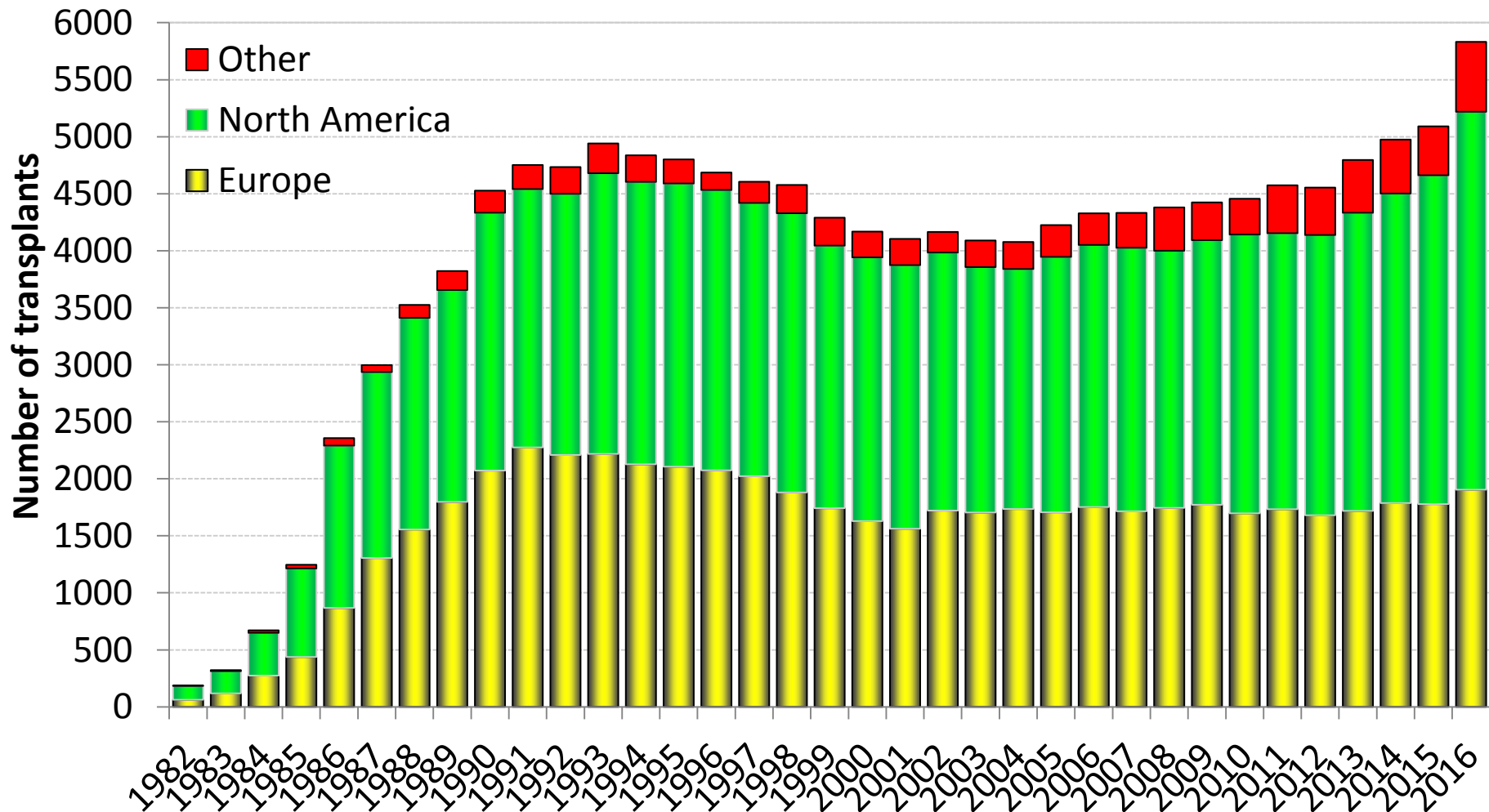 IL2R Blockade
2000's
 Everolimus
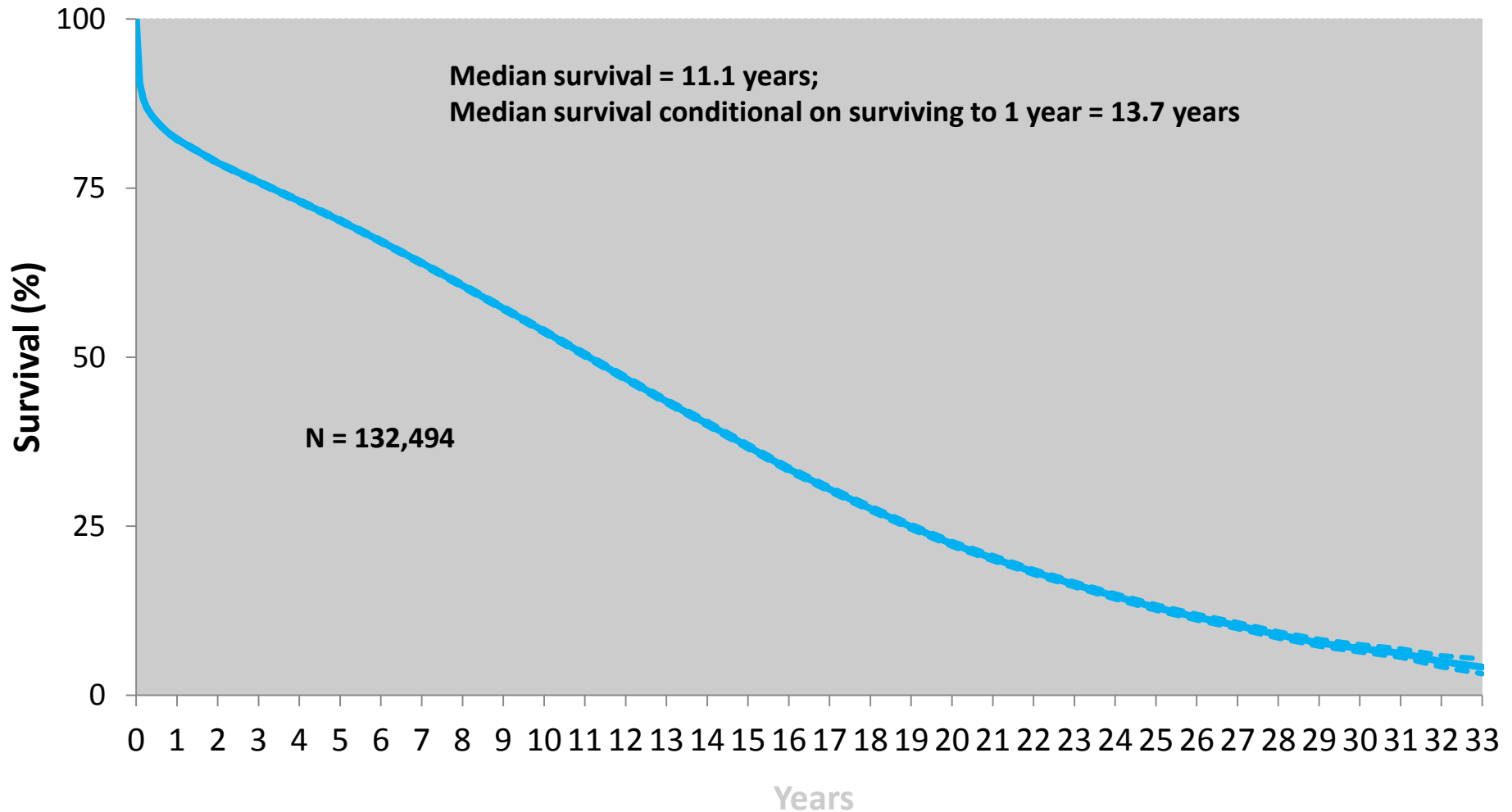
Pathways of maintenance immunosuppression

Categories of Immunosuppressants:

- Calcineurin Inhibitors
  - Tacrolimus
  - Cyclosporin
- Inhibitors of Purine Metabolism
  - Mycophenolate Mofetil
  - Azathioprine
- Proliferation Inhibitors
  - Rapamycin/Sirolimus
  - Everolimus

Number of Adult and Pediatric Heart Transplants by Year and Location

Adult Heart Transplants Kaplan-Meier Survival by Era
(Transplants: January 1982 – June 2016)

Median survival = 11.1 years;
Median survival conditional on surviving to 1 year = 13.7 years

N = 132,494

Survival (%)

Years

Cedars-Sinai Heart Transplant Program

- The Cedars-Sinai Heart Transplant Program began in December 1988 and as of December 2018, more than 1,600 heart transplant surgeries have been performed.

- In the past 5 years:

  - We have broken the prior U.S. record every year in the annual number of adult heart transplant surgeries performed, averaging more than 120 heart transplant surgeries per year.

  - Overall 1-year survival of 91% surpasses the national average.

SRTR Heart Transplant Outcomes (January 2019)

Table C12D. Adult (18+) <mark>1-year patient survival</mark> (deceased donor graft recipients)
Single organ transplants performed between 07/01/2015 and 12/31/2017
Retransplants excluded

	CACS	U.S.
Number of transplants evaluated	240	6,144
Estimated probability of surviving at 1 year (unadjusted for patient and donor characteristics)	91.99%	91.79%

Table C13D. Adult (18+) <mark>3-year patient survival</mark> (deceased donor graft recipients)
Single organ transplants performed between 01/01/2013 and 06/30/2015
Retransplants excluded

	CACS	U.S.
Number of transplants evaluated	249	5,040
Estimated probability of surviving at 3 years (unadjusted for patient and donor characteristics)	86.75%	85.22%

Table C18. Multi-organ transplant patient survival: 07/01/2015 - 12/31/2017
Adult (18+) Transplants

First-Year Outcomes

Transplant Type	Transplants Performed		Patient Deaths		Estimated Patient Survival	
	CACS-TX1	USA	CACS-TX1	USA	CACS-TX1	USA
Heart-Lung	1	49	0	8	100.0%	82.9%
Kidney-Heart	35	397	2	35	94.3%	90.8%
Liver-Heart	6	62	0	5	100.0%	91.4%

Immunologic Compatibility:

- Crossmatch:
  - Virtual
  - Prospective
  - Retrospective
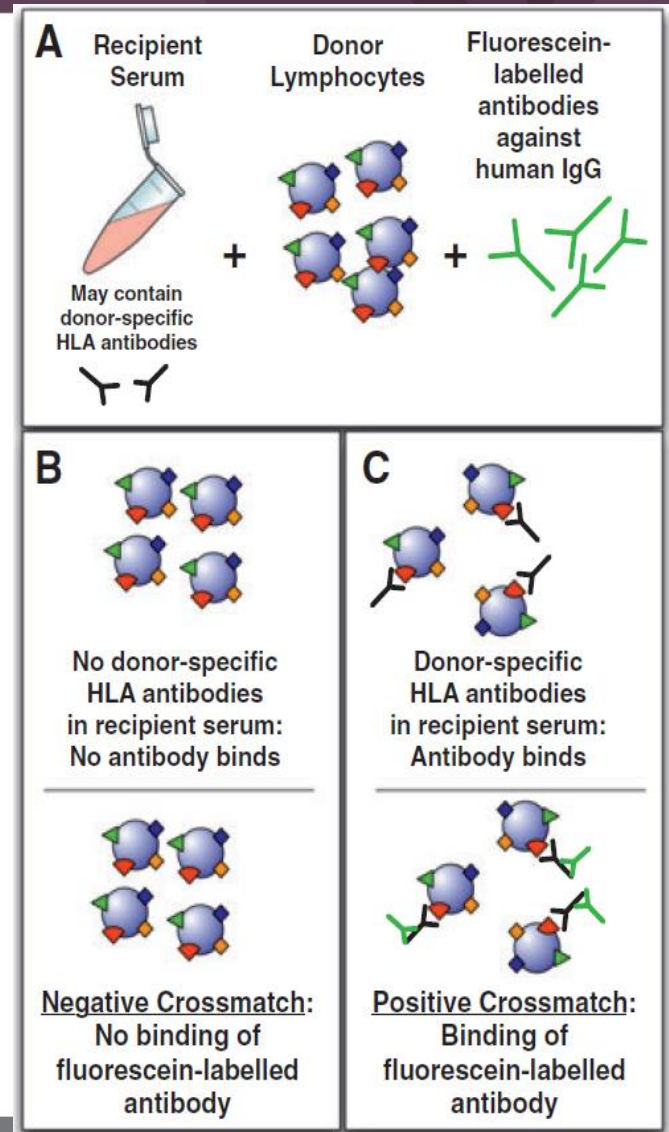
Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation.
N Engl J Med 1969; 280:735–739.

Mulley WR, Kanellis J. Understanding crossmatch testing in organ transplantation. *Nephrology 2010.*

A Recipient Serum — May contain donor-specific HLA antibodies
Donor Lymphocytes
Fluorescein-labelled antibodies against human IgG

B No donor-specific HLA antibodies in recipient serum: No antibody binds
Negative Crossmatch: No binding of fluorescein-labelled antibody

C Donor-specific HLA antibodies in recipient serum: Antibody binds
Positive Crossmatch: Binding of fluorescein-labelled antibody

Panel Reactive Antibody (PRA)

- The serum of a transplant candidate is tested against a "panel" of HLA antigens that is representative of the HLA makeup of the donor population.

- The result of the PRA test is reported as a percentage.

- The "percent PRA" theoretically means that the candidate has HLA antibodies to about that percentage of the potential donor population.

Table 2. Multivariate Cox Proportional Hazard Regression Analyses of Patient Survival

Risk Factor[a]	Hazard Ratio	95% Confidence Interval	p Value
PRA (continuous variable)	1.005	1.002–1.009	<0.001
PRA 1%–10%[b]	1.17	0.96–1.42	0.12
PRA 11%–25%[b]	0.94	0.99–1.00	0.73
PRA >25%[b]	1.4	1.09–1.77	0.007

PRA%	30 DAYS	1 YR	3 YRS	5 YRS
<=25 (7720)[+]	94.3[&] (7183)	86.9 (6151)	79.6 (2942)	73.9 (371)
>25 (440)	88.6 (387)	80.3 (325)	72.2 (146)	65.3 (13)

p <0.001

PRA <= 25%   PRA > 25%
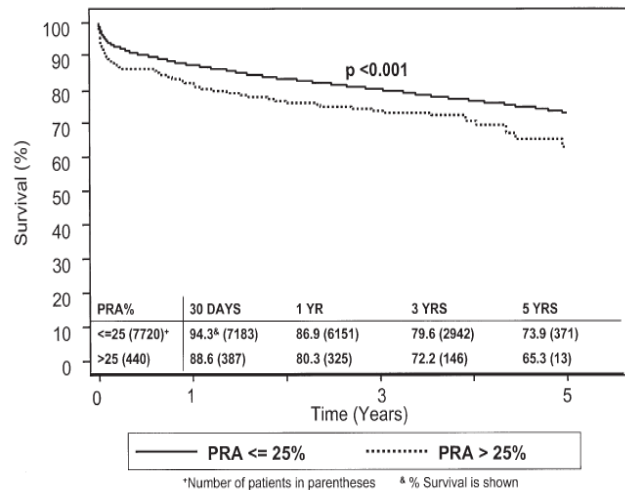
[+]Number of patients in parentheses   [&] % Survival is shown

Fig 3. Kaplan–Meier estimates of mortality stratified by panel-reactive antibody (PRA) ≤ 25% versus panel-reactive antibody > 25%.

Nwakanma NU, et al. Influence of pre-transplant PRA on outcomes in 8,160 heart transplant recipients in recent era. Ann Thorac Surg 2007.

Calculated PRA (cPRA)

- The cPRA is the percentage of donor hearts in a given population to which a heart transplant candidate has significant anti-HLA antibodies.

- The corresponding antigens to these antibodies are deemed unacceptable. Those potential donors with these unacceptable antigens are automatically turned away.

- The threshold to determine when an anti-HLA antibody is significant is defined by the heart transplant program and is usually dependent on the strength of the antibody.

- The higher the cPRA, the harder it is to find a suitable donor (eg 70% cPRA means that 70% of donors will be automatically turned away).

Post-Transplant Complications

- **Rejection**
- Infection
- Cardiac Allograft Vasculopathy (CAV)
- Hypertension
- Nephropathy
- Malignancy

Rejection

- Signs and symptoms
  - Atrial Fibrillation/Atrial Flutter
  - CHF signs and symptoms
  - Systolic dysfunction/Echocardiogram-Stat
  - New heart block and or bradycardia
- Cellular rejection: leukocytes and necrosis
  - Grade 0: no rejection
  - Grade 1R: mild rejection
  - Grade 2R: moderate rejection
  - Grade 3R: severe rejection
- Antibody-Mediated Rejection (AMR)
  - pAMR 0
  - pAMR 1 (H+), pAMR 1 (I+)
  - pAMR 2
  - pAMR 3

Endomyocardial Biopsy (EMB)

- Currently, the gold standard for rejection surveillance is the invasive endomyocardial biopsy.

- Among expert pathologist, there is only 67% concordance to identify rejection in the heart biopsy.

- Therefore, there is an unmet need for additional test(s) to detect heart transplant rejection
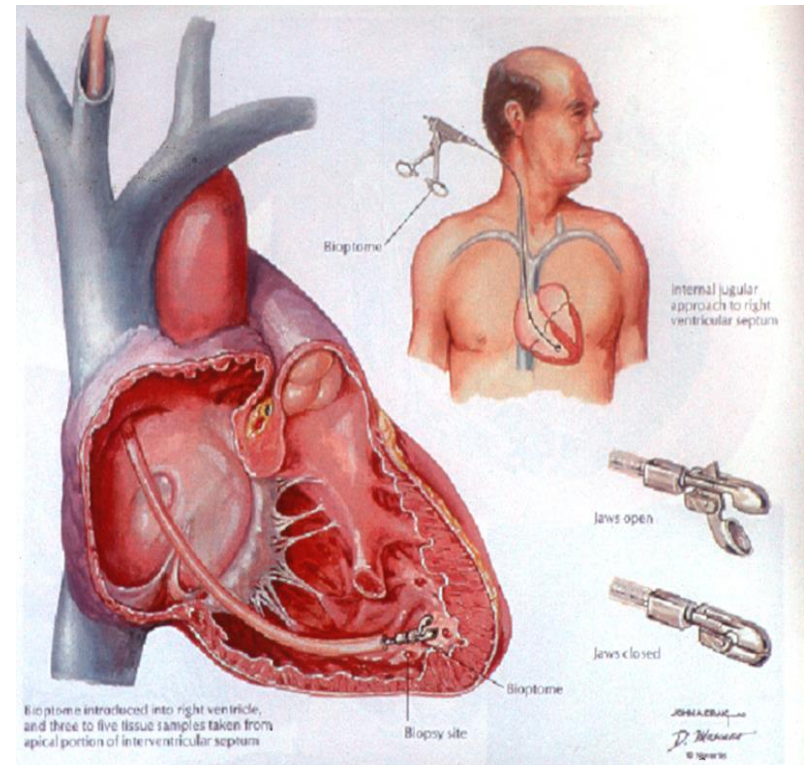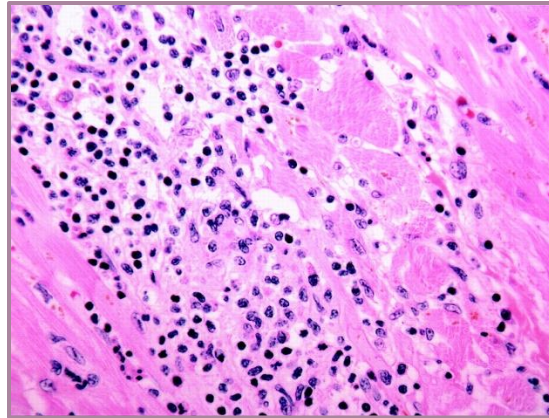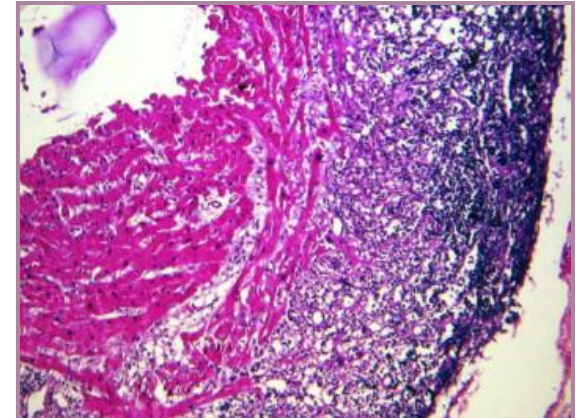
Common Histological Artifacts that Mimic Rejection
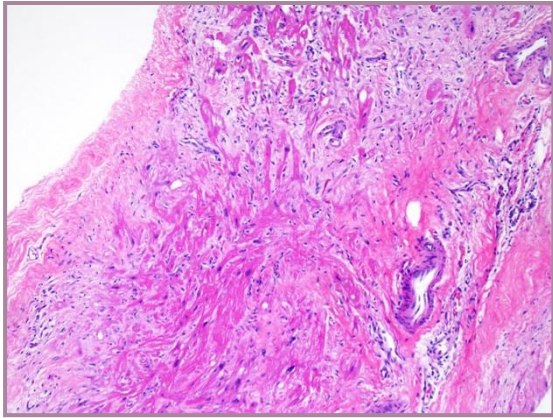
Old biopsy site
- Common for bioptome to be guided to same site as previous biopsies
- Myocytes in disarray
- If recent: B and T cells seen

Infection
- CMV or Toxoplasma will show lymphocytic infiltrates

"Quilty effect"
- First described in 1981 by Billingham et al.
- Occurs in 10% to 20% of EMBs
- Lymphocytic lesion with B and T cell infiltrates
- Considered by some to be a side-effect of cyclosporine

Novel Means to Detect Rejection in Heart Transplantation

- Molecular paradigm in diagnosis of rejection
 - Intragraft mRNA transcripts
- Donor-derived cell free DNA

Intragraft mRNA Assessment of Rejection

- Molecular phenotyping offers the possibility for increased accuracy in diagnosing and treating pathological states

- Traditional histological methods are often subjective and results are qualitative

- By relating gene expression to disease states, a system can be created to diagnose pathologies

- Halloran et al at the University of Alberta devised such a system for identifying rejection and allograft injury in transplant biopsy samples: The Molecular Microscope Diagnostic System (MMDx)

Halloran P et al. *Nat Rev Nephrol* 2016;12:534-48
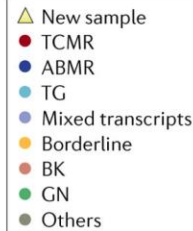
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How Molecular Analysis Works
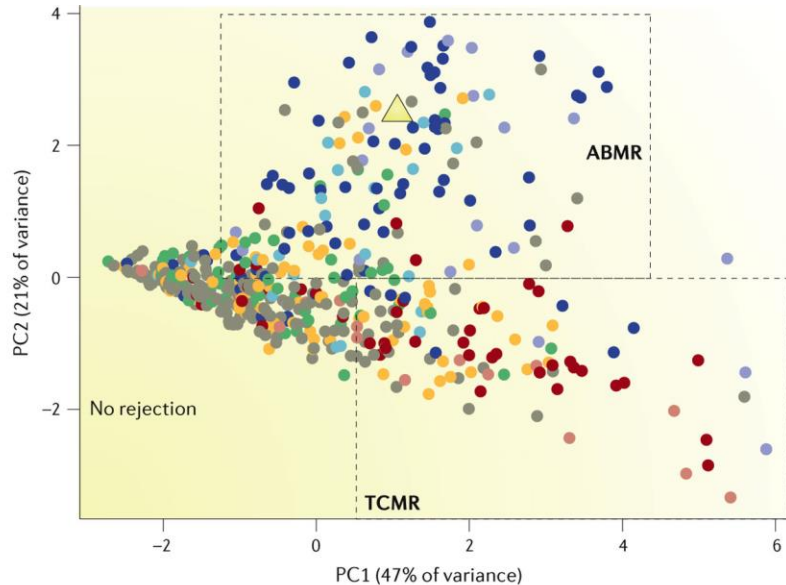
Histology diagnosis of the 50 nearest neighbours
C4d⁻ ABMR: 32%
C4d⁺ ABMR: 16%
Borderline: 12%
TG: 11%
Mixed: 11%
GN: 6%
TCMR: 3%
Other: 3%
TA/IF: 3%
No major abnormalities: 2%
AKI: 1%

Proportion of the 50 nearest neighbours surviving
1 year: 0.74
3 years: 0.43

△ New sample
● TCMR
● ABMR
● TG
● Mixed transcripts
● Borderline
● BK
● GN
● Others

PC2 (21% of variance)
PC1 (47% of variance)
ABMR
No rejection
TCMR

Nature Reviews | Nephrology

- Microarrays analyze mRNA in biopsy samples suspended in a RNA reagent e.g. RNALater

- Machine learning builds algorithms that assign probability of disease state: Known as "classifiers"

- A "classifier" algorithm uses multiple gene-expression values in contrast to traditional simple gene sets

- New biopsy results are compared to 50 nearest neighbors in a reference set

The Molecular Landscape of Rejection:
Using rejection associated transcripts as classifiers

Nature Reviews | Nephrology
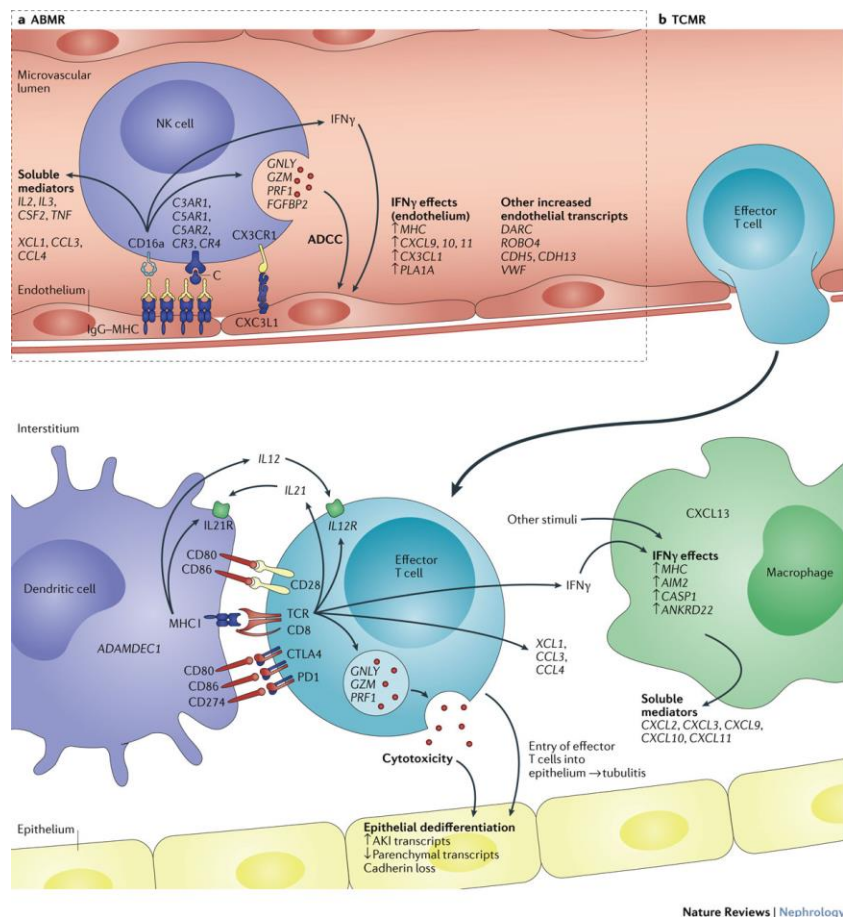
TCMR Landscape

- CTLA4 signaling in cytotoxic T cell
- T cell receptor signaling
- Dendritic cell differentiation
- IFNγ-mediated effects

AMR Landscape

- NK cell signaling
- Endothelium activation
- Leukocyte-EC interaction
- IFNγ-mediated effects

Halloran P et al. *Nat Rev Nephrol* 2016;12:534-48

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Archetype Analysis

Archetype analysis estimates probability of:
- No rejection
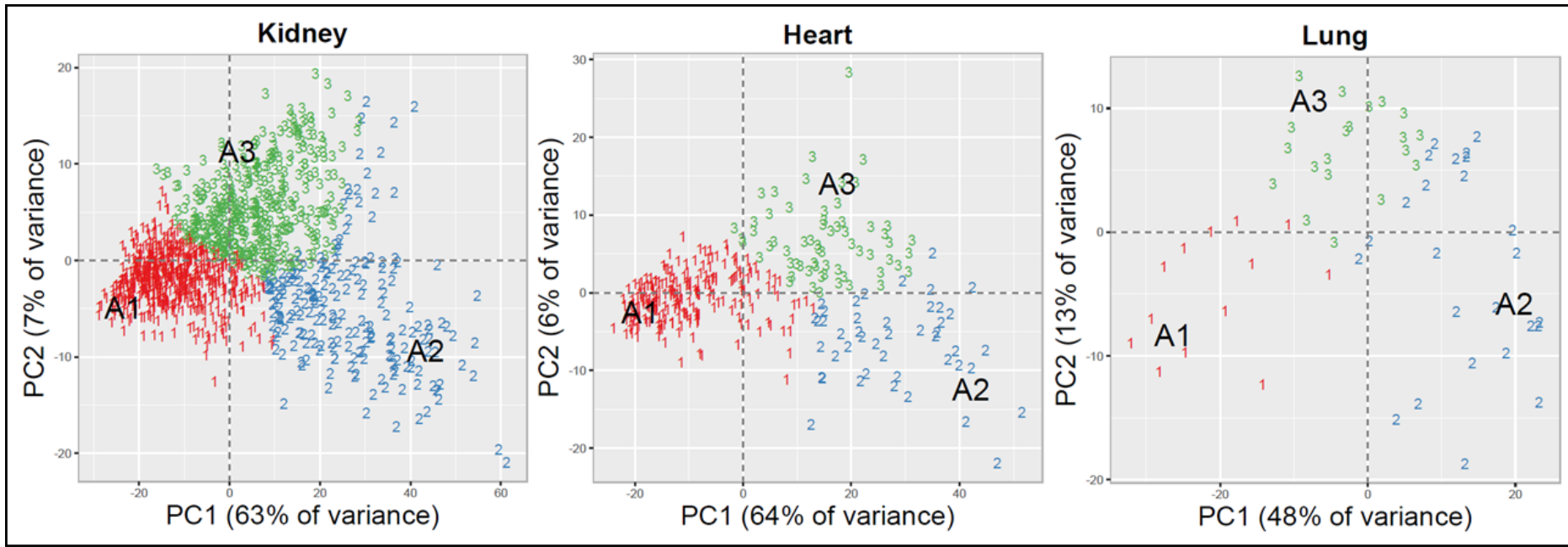- TCMR
- ABMR

in a single EMB bite

Archetype analysis is an unsupervised method that assigns each biopsy a score based on its similarity to cases in the reference set: scores add up to 1.0

Comparing the A1,2,3 Archetypes for kidney, heart, and lung as estimated by expression of rejection-associated transcripts (RATs)
A1: no rejection; A2:TCMR; A3: ABMR (or ABMR-like)

- A1=no rejection; A2=TCMR or TCMR-like; A3=ABMR or ABMR-like

Figure 1. Archetype analysis based on expression of 453 kidney-derived rejection-associated transcripts (RATs) separated biopsies with non-rejection (A1), TCMR-like (A2), and ABMR-like (A3) features. 1208 kidney, 331 heart, and 58 transbronchial lung biopsies were used.

The INTERHEART Study

- Prospective validation of the MMDx in heart transplantation

- Assign molecular scores (probability) of T cell mediated rejection and antibody mediated rejection in heart transplant biopsies, in a reference set of 200 biopsies

- Create molecular classifiers that predict antibody mediated and T cell mediated rejection

# Examples of Heart Transplant Endomyocardial Biopsy MMDx-Heart Reports

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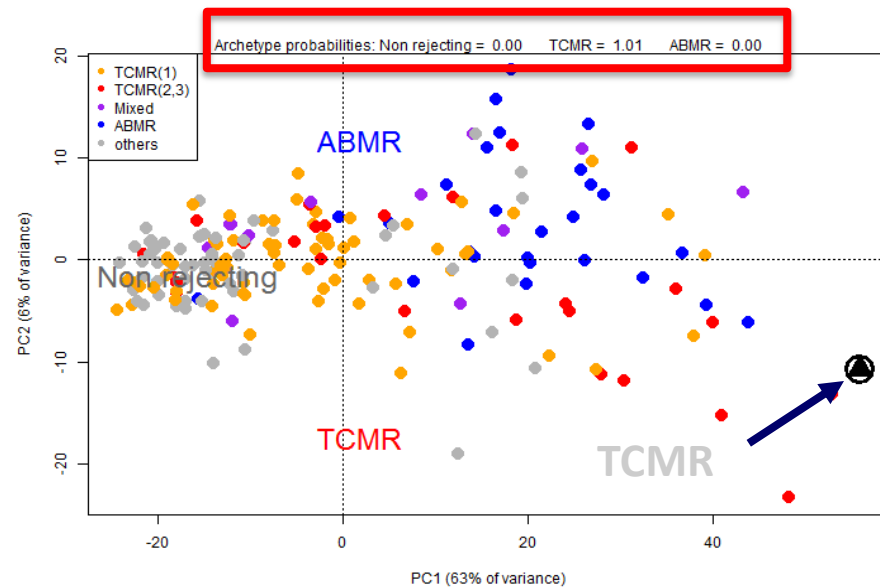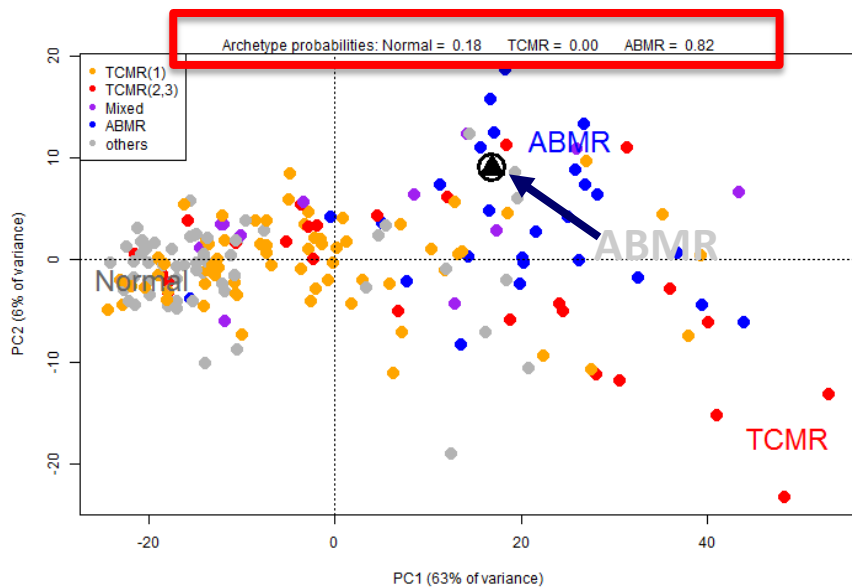
DNA the Molecule of Life

cell

chromosomes

gene
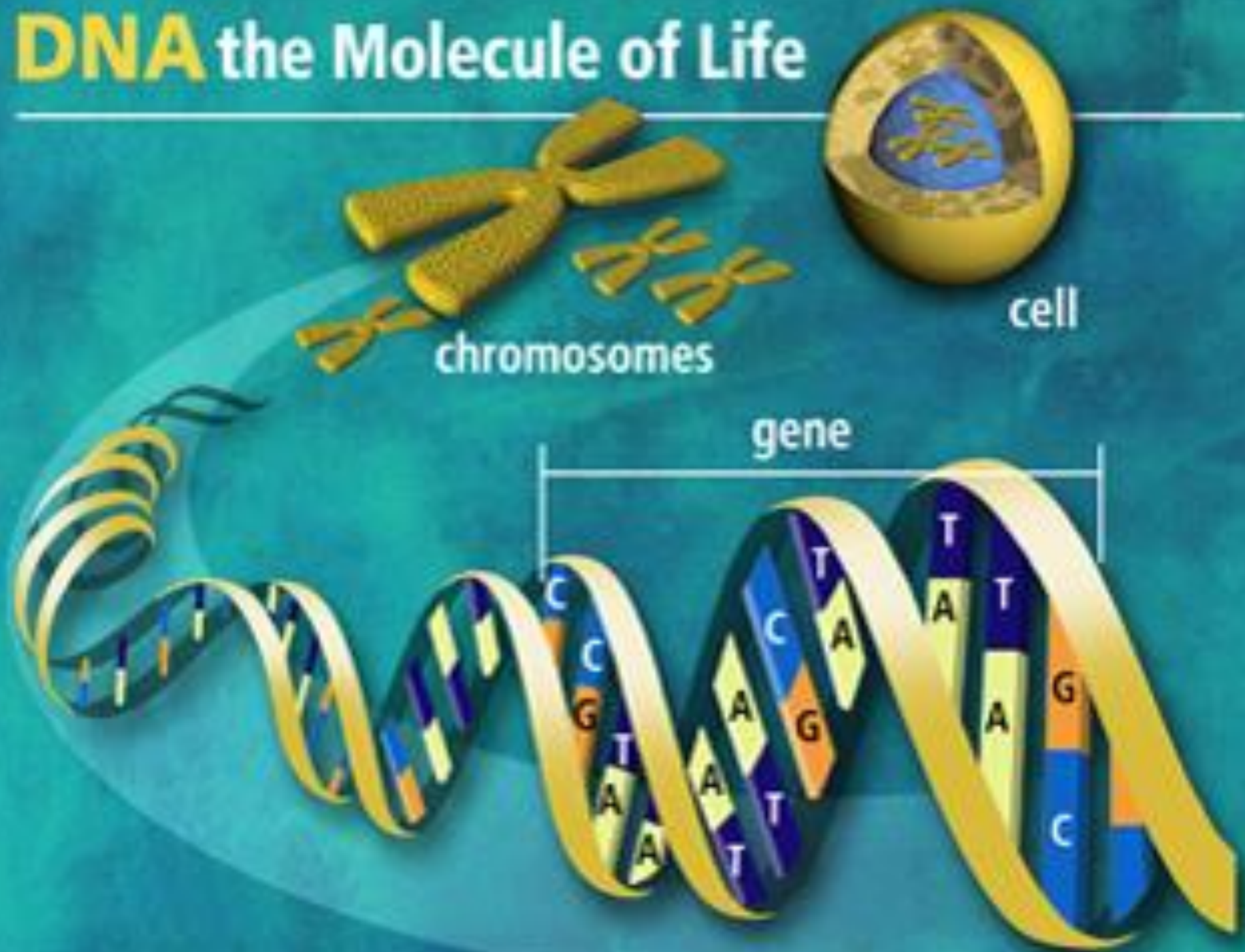
# Cell-Free DNA (cfDNA) is Found in Circulating Blood

- cfDNA is released from healthy, inflamed or diseased tissue from cells undergoing apoptosis or necrosis.
- cfDNA can be extracted from a blood sample and analyzed.
  - Steady state level due to basal rate of cellular turnover
    - 6 ng/mL plasma (1000 genomes/mL)
  - Higher levels with increased cell damage
    - Trauma
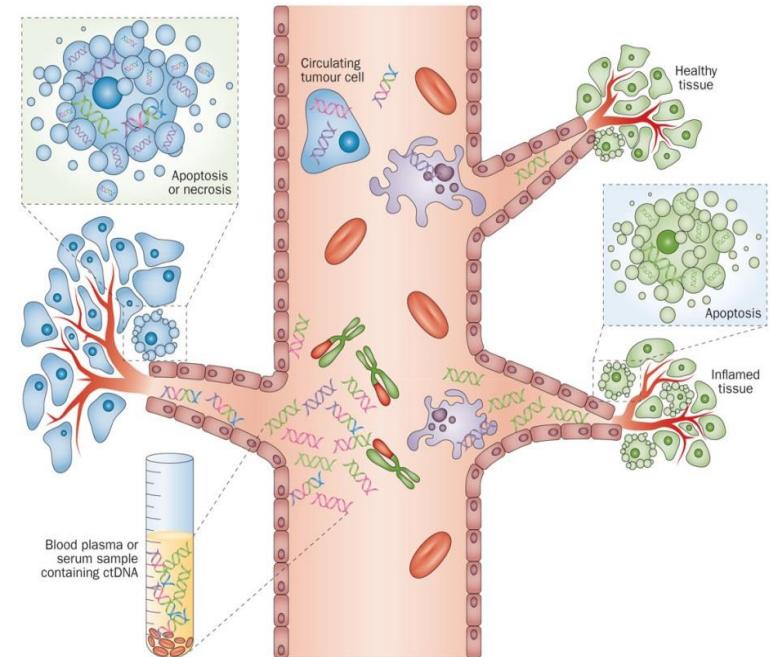
Crowley, E. *et al.* (2013) *Nat. Rev. Clin. Oncol.* doi:10.1038/nrclinonc.2013.110

Transplantation:
A New Application for cfDNA

Donor-derived cell-free DNA (dd-cfDNA) may be useful for non-invasive surveillance of allograft status

Non-rejection (Quiescent)
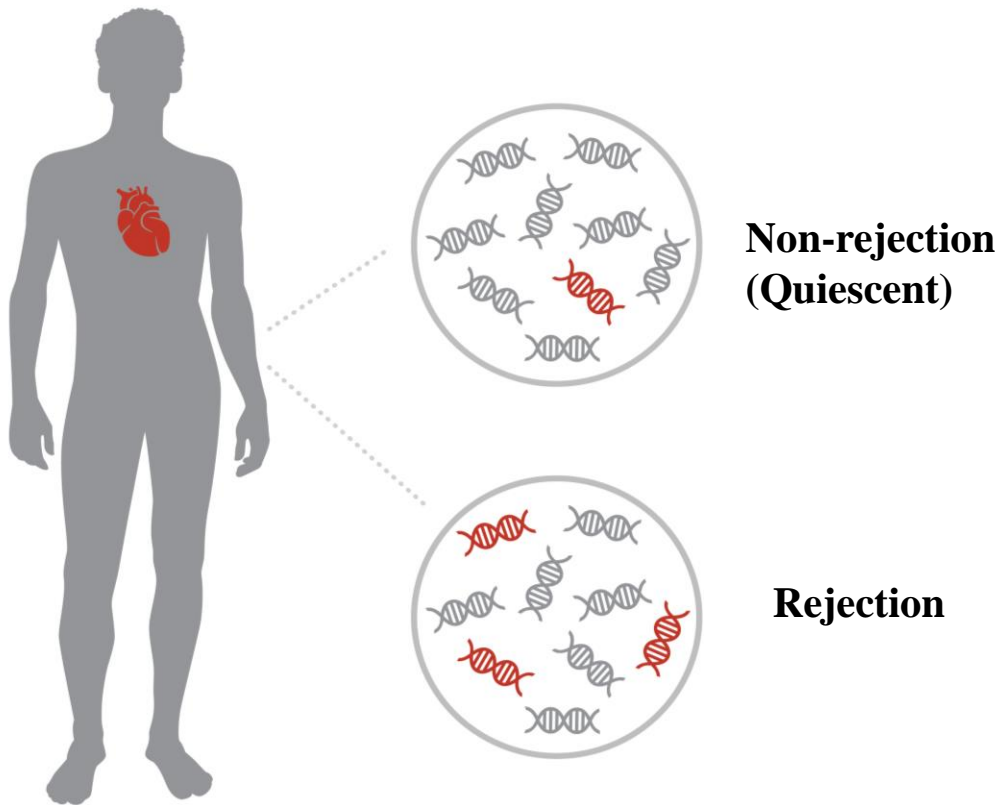
Rejection

Hypothesis:
dd-cfDNA increases with allograft rejection and decreases following effective rejection treatment
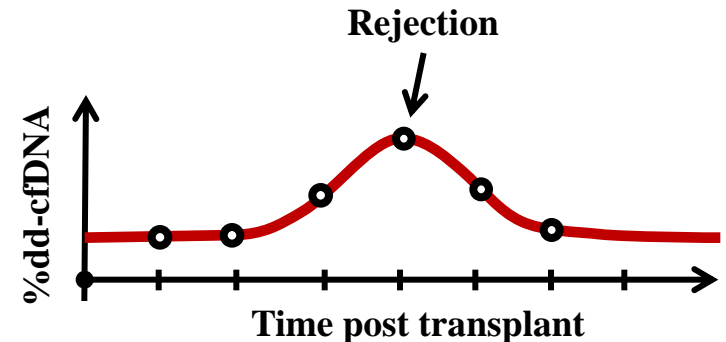
DART Study Design

- The Circulating Donor-Derived Cell-Free **D**NA (dd-cfDNA) in Blood for Diagnosing **A**cute **R**ejection in Kidney **T**ransplant Recipients (DART)

- 14 centers, 384 patients
  - Enrolled at time of transplant and followed for 2 years

    OR

  - Enroll at time of clinical suspicion of rejection; blood draw at time of enrollment and 2 years follow-up

- Allograft rejection reference cases met biopsy-based, histologic Banff WG 2013 criteria for acute or chronic active ABMR
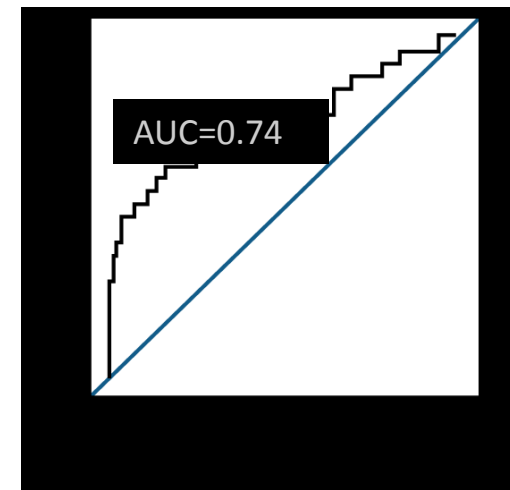
Bloom RD et al. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034.

dd-cfDNA Discriminates Active Rejection from No Active Rejection in Clinical-Suspicion Setting

dd-cfDNA

Median 5.3-fold higher in active rejection vs no active rejection. Receiver-Operator characteristics curve shows dd-cfDNA discriminates active rejection
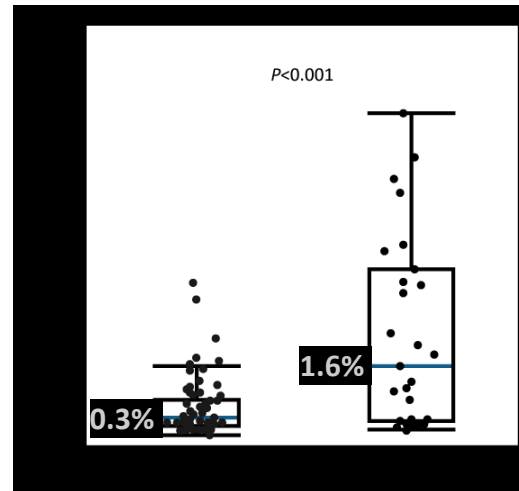
Serum creatinine

Does not discriminate active rejection from no active rejection.

- Active rejection = Acute, active ABMR; Chronic/active ABMR; and TCMR, n=27 samples from 27 patients
- No active rejection, n=80 samples from 75 patients

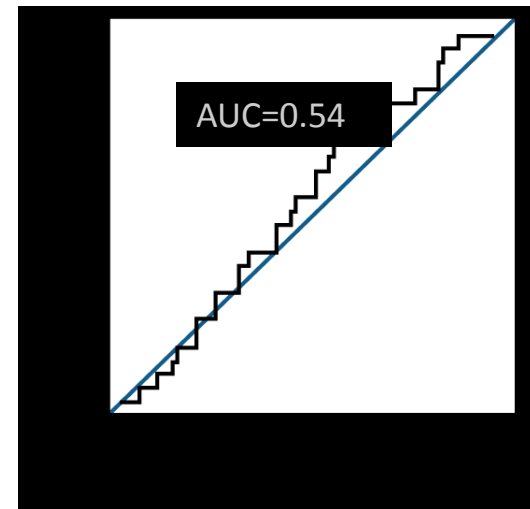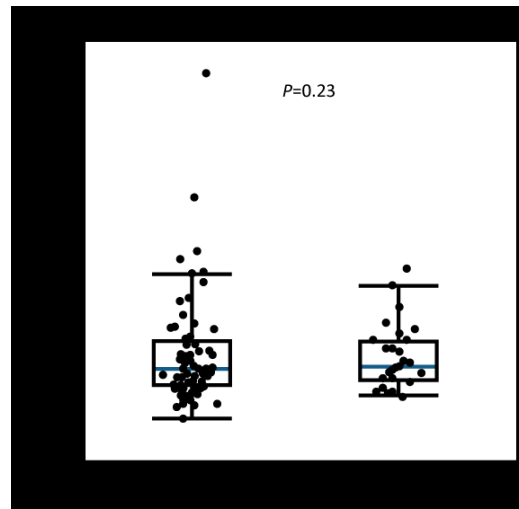# dd-cfDNA Provides Stratification With Higher Probability of Active Rejection at 1% dd-cfDNA Cutoff

Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.74 (95% CI 0.61-0.86)
Sensitivity	85%
Specificity	59%
NPV	84%
PPV	61%

Bloom RD et al. *J Am Soc Nephrol.* 2017. doi:10.1681/ASN.2016091034

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Example Case with Longitudinal Collection in DART
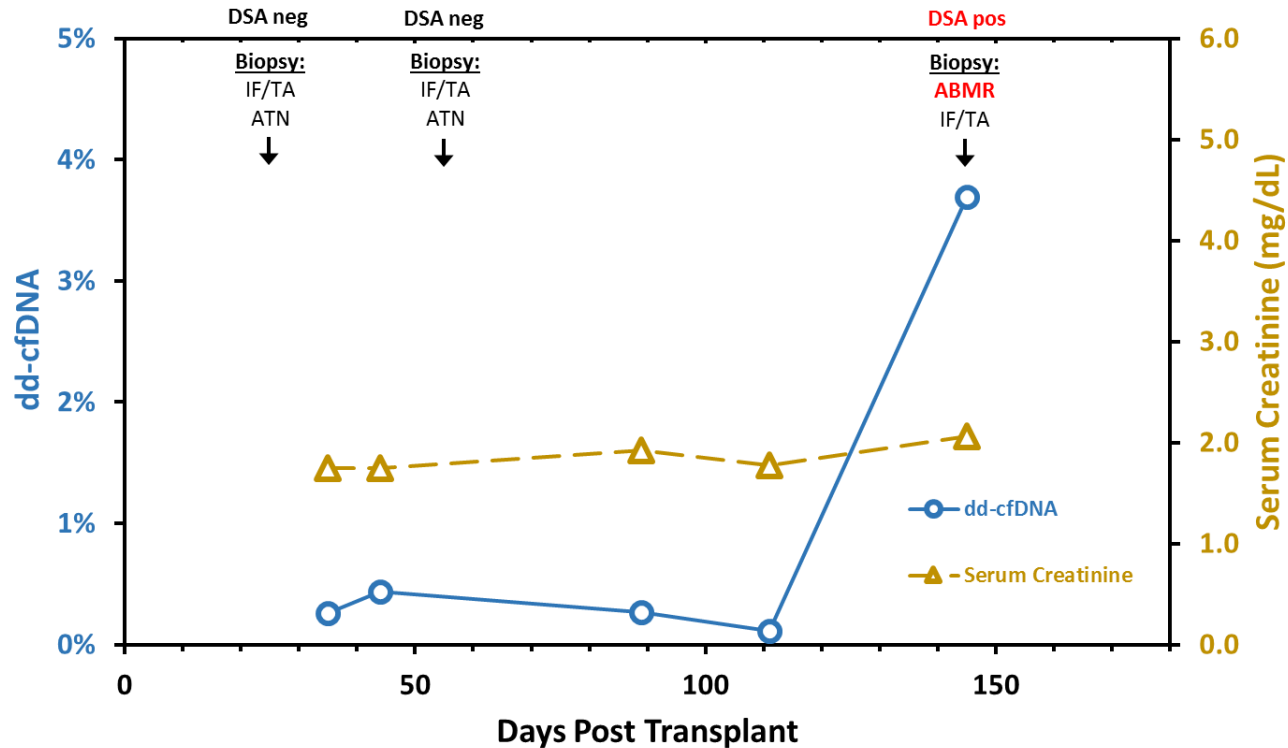
Serial dd-cfDNA rises significantly with rejection
Potential to detect rejection earlier than biopsy or serum creatinine

Bromberg et al., J Assoc Lab Med, 2017

Study Design: dd-cfDNA in Heart Transplantation

CARGO II observational study:
Heart transplant recipients from 17 centers;
737 patients, 7977 samples

Clinical status, including endomyocardial biopsy grades (graded by four independent pathologists) and blood were collected at routine surveillance visits for up to two years.

Rejection (R) cohort

-2/4 pathologists graded sample as 2R or 3R
N=58 patients

Quiescent (Q) cohort

-4/4 pathologists graded sample as 0R
N=249 patients

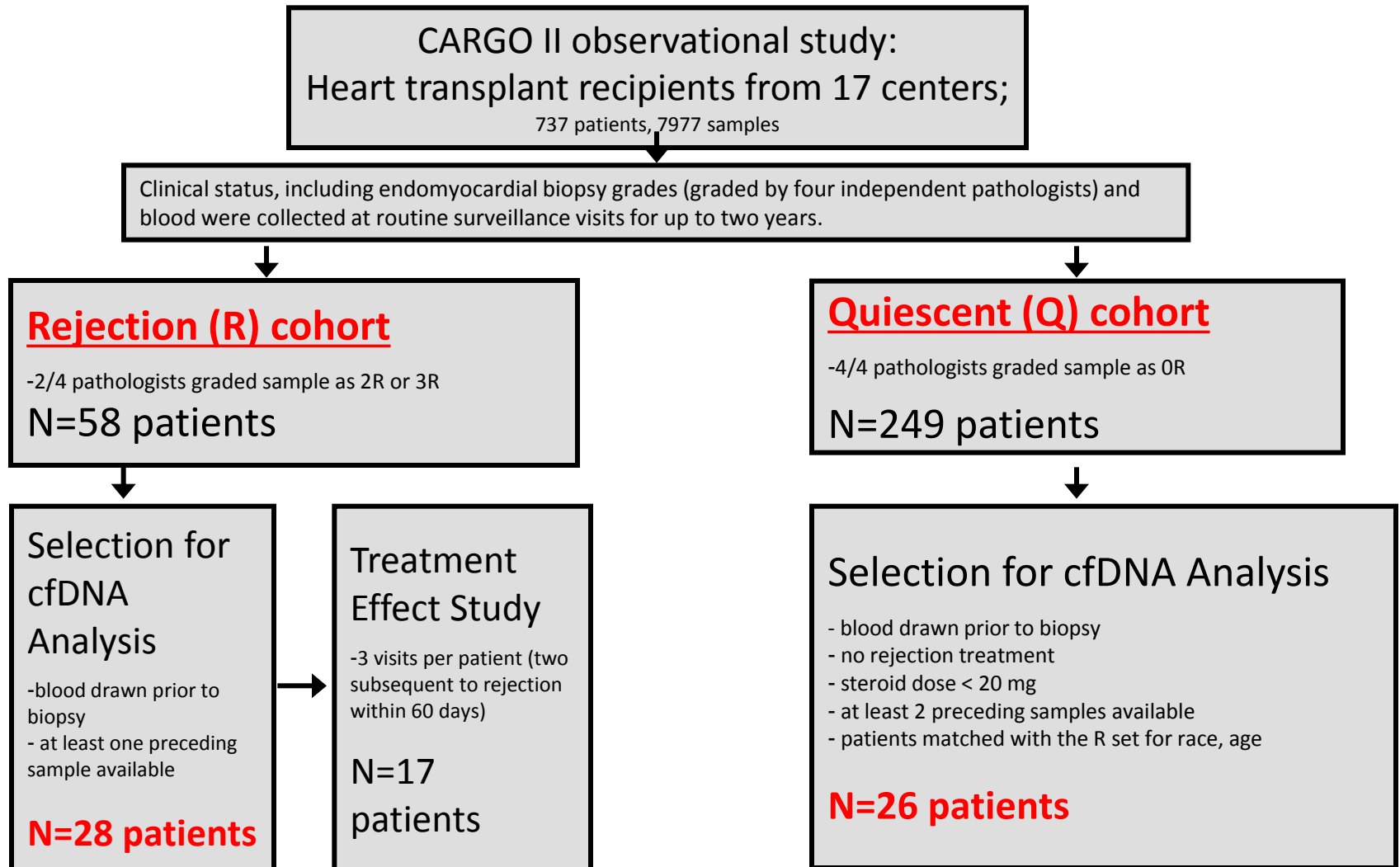### Selection for cfDNA Analysis

-blood drawn prior to biopsy
- at least one preceding sample available

N=28 patients

Treatment Effect Study

-3 visits per patient (two subsequent to rejection within 60 days)

N=17 patients

Selection for cfDNA Analysis

- blood drawn prior to biopsy
- no rejection treatment
- steroid dose < 20 mg
- at least 2 preceding samples available
- patients matched with the R set for race, age

N=26 patients

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dd-cfDNA results from the CARGO II Study

Increased dd-cfDNA Correlates with Biopsy-Proven Rejection in Heart Transplant Recipients
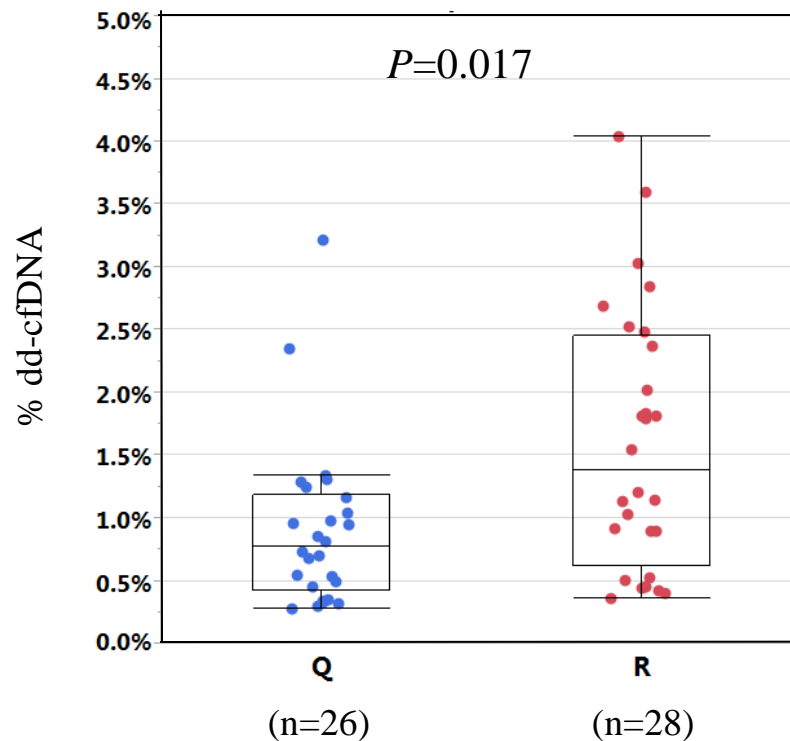
dd-cfDNA from quiescent (Q) and rejection (R) samples, expressed as percent dd-cfDNA in recipient.

Mean Rejection group is 1.9-fold higher than mean Quiescent group

Grskovic M, Kobashigawa J, *Am J Transplant.* 2015; 15 (suppl 3):122.

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dd-cfDNA is Validated to Detect Rejection, Combined ACR and AMR

- Samples from recipients with either ACR or AMR had elevated dd-cfDNA compared to no rejection
- Mixed rejection (biopsy diagnosed with both ACR and AMR, n=2) had the highest dd-cfDNA
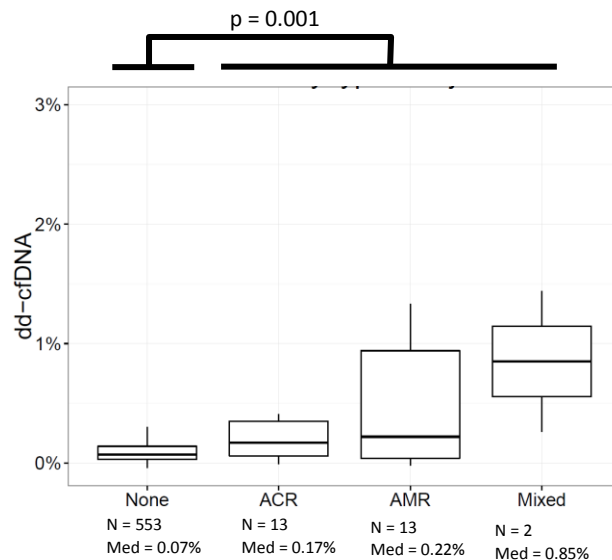

- No Rejection vs All Rejection, p=0.001
- No Rejection vs ACR, p=0.05
- No Rejection vs AMR, p=0.047
- ACR=Grade 2R and Grade 3R
  - Grades 0R and 1R are in the No Rejection group
- AMR=pAMR1 and pAMR2

dd-cfDNA is Validated to Detect AMR

- AMR (combined pAMR1 and pAMR2) vs No AMR, p=0.047

- Biopsy-negative for AMR: 0.07%

- Median pAMR1 & pAMR2: 0.22%, more than three-fold higher

These data suggest use of a 0.2% threshold for discrimination of AMR from No Rejection.
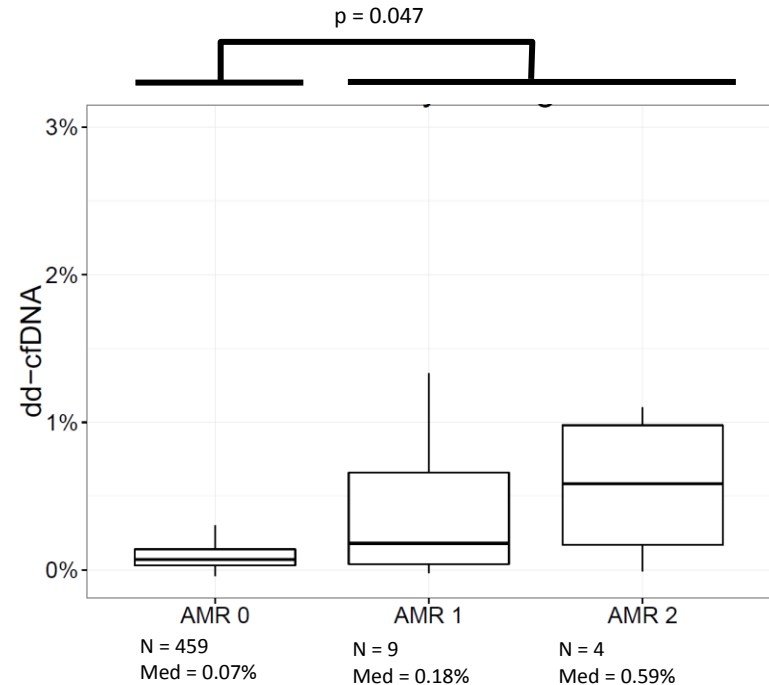
Kobashigawa, presented at the ISHLT Scientific Sessions 2018

Novel testing to detect rejection

- There is an unmet need in transplantation to create an objective diagnostic test for allograft rejection

- The pathology reads of EMB rejection are not consistent

- Gene expression profiling and dd-cfDNA appear to be reliable non-invasive methods to detect rejection

- The intragraft mRNA transcripts (MMDx) may pave the way to a new gold standard for rejection and even improve our understanding of the pathology of rejection

Induction Immunosuppression:
To induce or not to induce?

Common indications

- High risk of acute rejection.
- Impaired renal function (renal sparing protocol).

Common agents

- rATG = Thymoglobulin.
- Basiliximab = Simulect

Safety concerns

- Infection.
- Malignancy.

Frequency of use

- About 50% of patients.
- 30% Simulect and 20% ATG.

# Prevention of Complement Activation and Antibody Development

- Many patients demonstrate elevated levels of antibodies which represent a barrier to heart transplantation.

- Antibody-mediated allograft injury is predominantly mediated through complement activation.

- Complement inhibition may allow highly sensitized patients to successfully undergo heart transplantation.

- Eculizumab is a potentially promising agent which through terminal complement inhibition may allow transplantation across an antibody barrier and may even promote accommodation.

Eculizumab

- Eculizumab is a humanized monoclonal antibody that binds to and subsequently prevents activation of complement component C5 by the amplified C3 convertase molecules.

- Eculizumab is approved by the US Food and Drug Administration for treating paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (HUS).
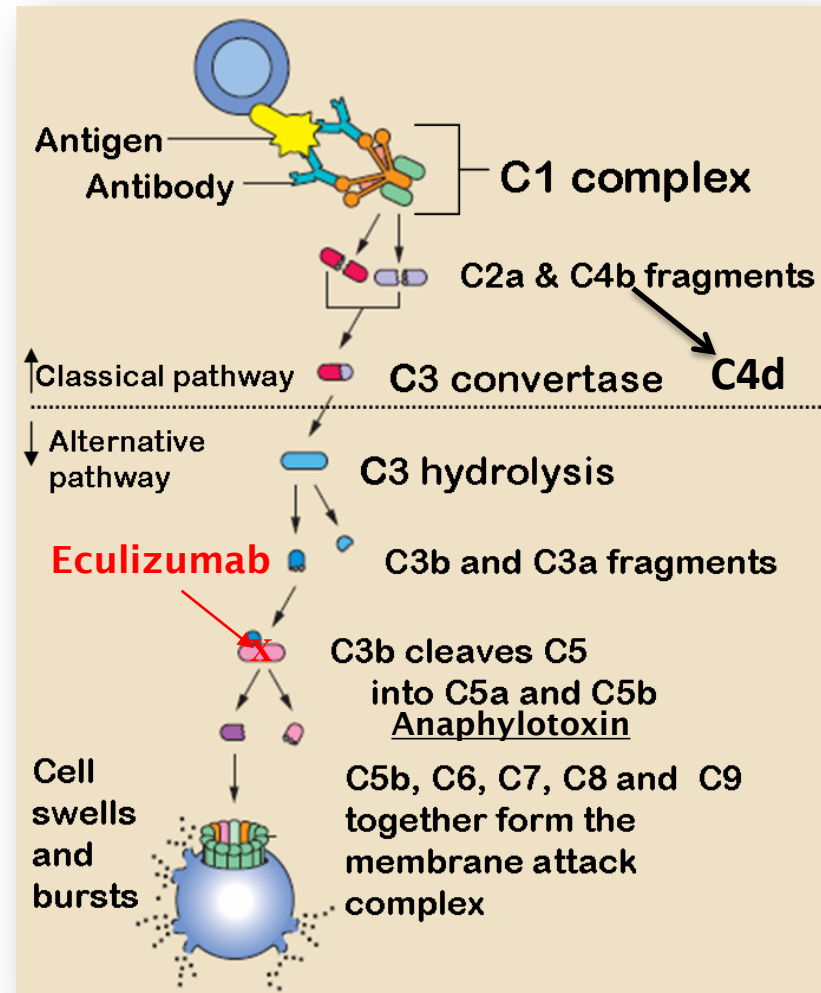
Accommodation

- Accommodation is the absence of humoral-mediated injury and continued function of a graft, despite the presence of anti-donor antibodies in the circulation.

- The difference between accommodation and antibody mediated rejection (AMR) appears to be the level of complement activation[1].

[1]Williams et al. Transplantation. 78(10):1471-1478, November 27, 2004.

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Activation of Complement in AMR and Accommodation

- In an experimental model of cardiac xeno-transplantation[1], grafts with AMR showed deposition of all complement components, including C4d and C5b-C9 MAC.

- However, xenografts demonstrating accommodation showed C4d deposits only.

- As eculizumab has the ability to inhibit C5b-C9 MAC and C5a generation, it could potentially act as a strong promoter of accommodation.

[1]Williams et al. Transplantation. 78(10):1471-1478, 2004.
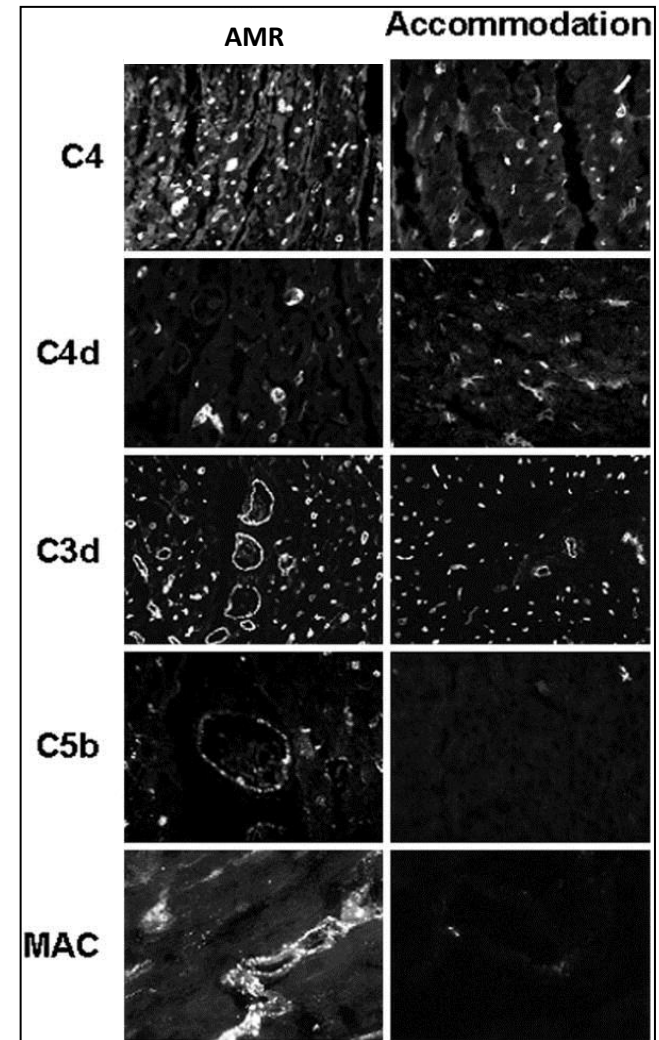
De-novo Use of Eculizumab in Highly Sensitized Patients Undergoing Cardiac Transplantation (DUET Trial)

- Pilot study using eculizumab immediately after heart transplant for the highly sensitized patient (mean cPRA = 83.8 ± 22.6, n=14).
- Study endpoints:
  - Assess efficacy to prevent post-transplant antibodies and AMR.
- Eculizumab Protocol:
  - Eculizumab
    - Day 0: 1200 mg / Day 1,7,14,21: 900 mg / Day 28,42,56: 1200 mg
    - Thymoglobulin 1.5 mg/kg x 5days followed by IVIg 1 gm/kg x 2days

Patel J - unpublished data, ongoing trial

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Demographics (N=14)

Mean recipient Age, Year ± SD	49.5 ± 12.3
Mean Donor Age, Years ± SD	31.9 ± 11.4
BMI, Mean ± SD	25.3 ± 3.7
Female (%)	85.7% (12/14)
Previous Pregnancy in Females (%)	91.7% (11/12)
Ischemic Time, Mean Mins ± SD	130.4 ± 52.1
Primary Reason for Tx, Underlying Diagnosis of CAD (%)	14.3% (2/14)
Status 1 at Transplant (%)	100.0% (14/14)
CMV Mismatch (%)	14.3% (2/14)
Diabetes Mellitus (%)	42.9% (6/14)
Treated Hypertension (%)	57.1% (8/14)
Prior Blood Transfusion (%)	66.7% (8/12)
Pre-Transplant cPRA, Mean ± SD	83.8 ± 22.6
Pre-Transplant Creatinine Mean ± SD	1.5 ± 0.6
Insertion of MCS Device	50.0% (7/14)

Prospective Donor-Specific Crossmatch Results at Transplant

Crossmatch Type	Results, N=14
T-Flow Cytometry Crossmatch	93.1 ± 122.8 MCS
B-Flow Cytometry Crossmatch	228.8 ± 120.6 MCS
T-Cell Complement-Dependent Cytotoxicity Crossmatch	All negative
B-Cell Complement-Dependent Cytotoxicity Crossmatch	All negative

Positive T-Flow >50 MCS   Positive B-Flow >100 MCS

Preliminary Outcomes

Endpoints	N=14
1-Year Actuarial Survival	92.8%
1-Year Actuarial Freedom from Cellular Rejection (ISHLT ≥2R)	100.0%
1-Year Actuarial Freedom from Antibody-Mediated Rejection (AMR ≥2)	76.8%
1-Year Actuarial Freedom from Any Treated Rejection	86.9%
Average 6-Month Left Ventricular Ejection Fraction (%)*	64.4 ± 8.1
% of Patients with DSA at 1 Month Post-Transplant	71.4% (10/14)
1-Year Freedom from Treated Infection	58.6%

* No patient with reduced LVEF

Patel J - unpublished data, ongoing trial

Current Investigations and Future Directions:

- Clinical trials in heart transplantation
  - Optimal testing for heart transplant rejection (Gene expression profile with Allomap, role of Interheart, cellfree DNA, microRNA)
  - Anti-Thymocyte Globulin (rATG) induction therapy compared to standard triple drug therapy in immunologically low risk patients
    - Single center, currently enrolling
  - Tocilizumab with or without standard triple drug therapy
    - Multi-center, currently enrolling
- Possible future trials
  - Optimization of induction therapies for heart transpant
    - IDES induction therapy in highly sensitized patients
    - C1 esterase inhibition in highly sensitized patients
  - Mechanistic studies and optimal treatment strategies for treatment of primary graft dysfunction (early graft loss)
  - Mechanistic studies to understand cardiac allograft vasculopathy (late graft loss)
  - Expansion of the repertoire of agents for maintenance immunosuppression (moving beyond T cell specific therapies)
  - Developing immunotherapies in oncology treatment may yield specific/targeted immunosuppression (to minimize non-cardiac complications of solid organ transplant)

Thank You