Potential endpoints for response to treatment of ABMR in heart transplant recipients

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Disclosure

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Most of the "recommendations" and advices expressed in this lecture are not the result of high grade evidence, but are personal opinions yielded from clinical experience, single center studies, and ongoing research, thus open to active discussion

The ideal endpoint

Relevance

1. Clinically relevant
2. Easy to measure and objective
3. Enough frequent to allow reasonable sized studies
4. Related to the diagnosis of the disease
5. Related to the mechanism of action of the treatment we are testing

Current problems for endpoint definition in cardiac AMR

- Definition of AMR
  - Definition of a target population at high risk of AMR related events
- Identification of the therapeutic target
  - DSA
  - Mechanisms of DSA mediated injury
  - Consequences of DSA mediated injury
- Identification of the clinical target
  - AMR per se (e.g. new onset AMR, relapse AMR)
  - Clinical consequences of AMR
    - Death/graft loss
    - Graft function
    - Graft injury

Conceptual process to develop AMR – related endpoint

- Definition of the disease
  - Recognize disease resolution
- Classification and/or grading of disease severity
  - Define mechanisms or grades with therapeutic implication
- Relevance to the clinical phenotype and prognosis
  - Granularity of the clinical phenotype

Disease phenotyping

What we need	Blood	• Graft Tissue • Blood	• Clinical assessment • Imaging • Functional measures
Methods	• Drug through levels • Immune monitoring • Blood GEP	• Pathology • Tissue GEP • Cell free DNA • Biomarkers	• Symptoms • Cardiac ultrasound • EKG • MRI • RHC

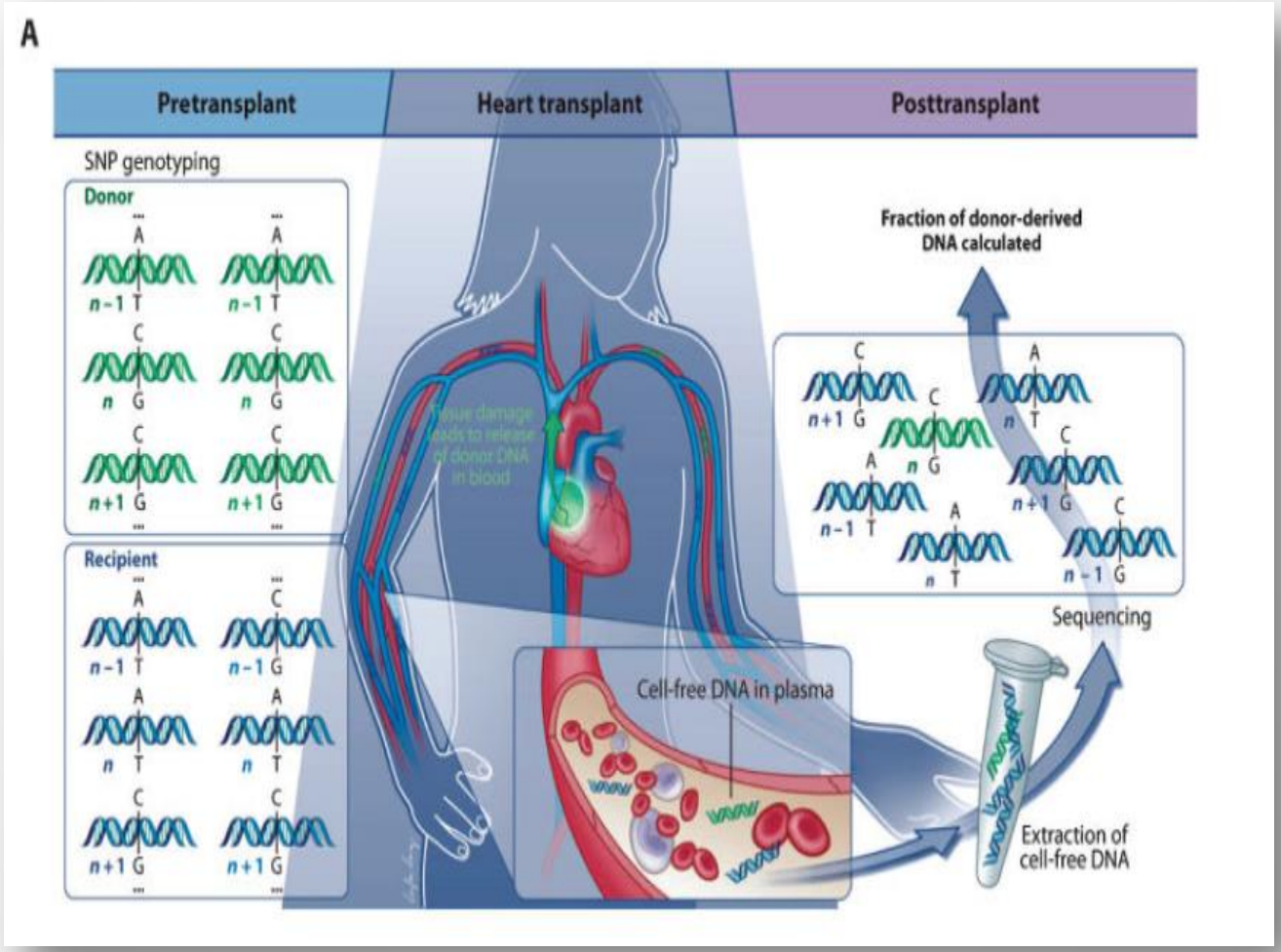
Disease phenotyping

- Precedes the injury
- May be subject to uncontrolled variables
- Good marker of risk but not sufficient for diagnosis

- Is the effect of the disease
- Biomarkers may be diagnostic for injury
- Tissue sampling is required for etiology and grading

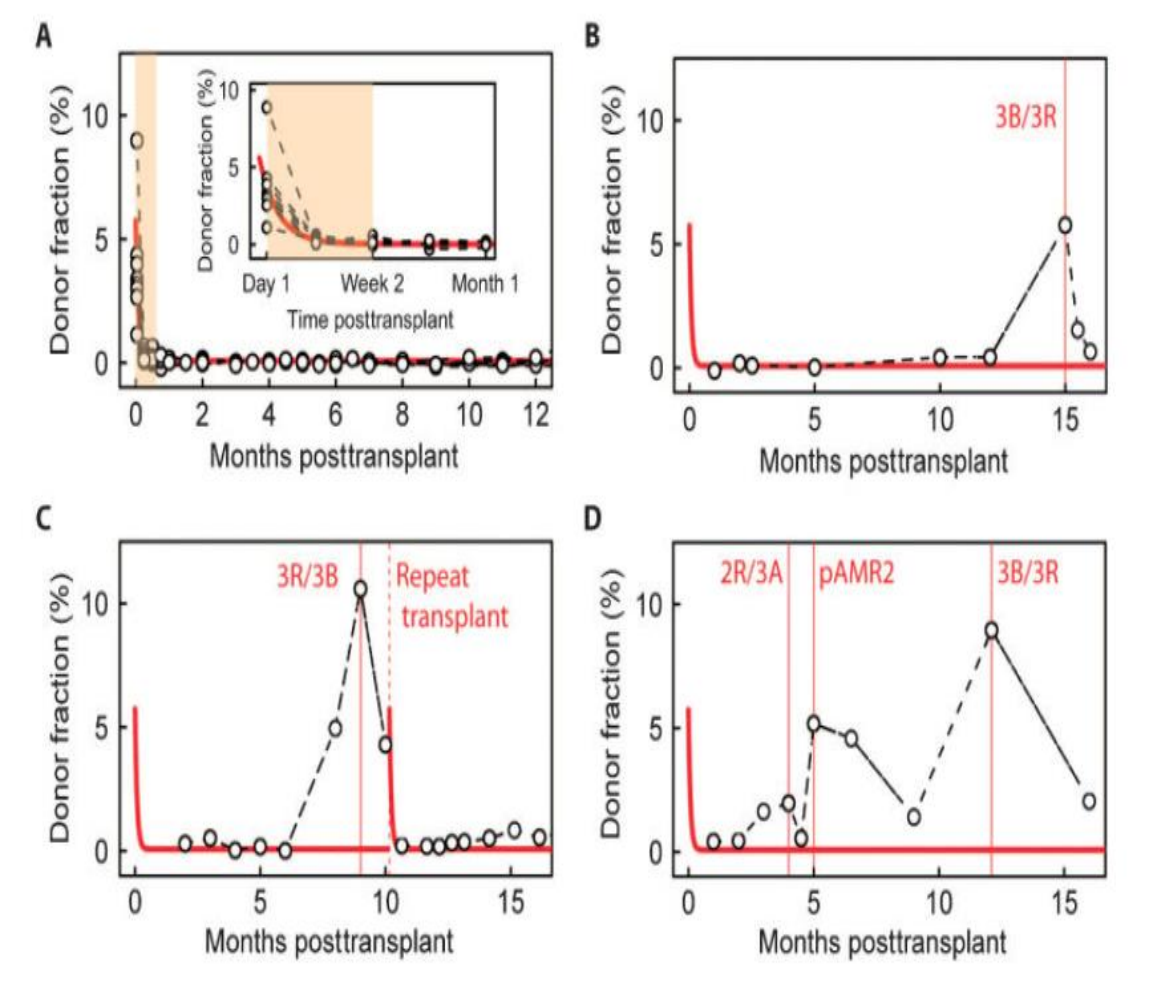
- Is the effect of injury
- May be difficult to capture
- Subclinical injury is missed
- No etiological informations

Cell free DNA: a novel intriguing biomarker



De Vlaminck I *Sci Transl Med*. 2014 June 18; 6(241): 241ra77

Donor DNA as marker for graft injury



De Vlaminck I *Sci Transl Med*. 2014 June 18; 6(241): 241ra77

Major etiologies leading to graft injury/dysfunction

- Cellular rejection
  - Interstitial inflammation
  - Graft antigens/APC -> Effector Tcell -> Myeloid activation -> myocyte injury
- Antibody-mediated rejection
  - Intracapillary inflammation triggered by circulating antibodies
- Cardiac Allograft Vaculopathy
  - Chronic vasculitis associated with fibrosis and related to immune-mediated and metabolic-mediated phenotypes

Clin-heart study

- Objective: characterize graft dysfunction and its risk factors after HT
- Prospective enrollment of patients with:
  - Acute/chronic presentation of GD
    - LVEF<55%
    - Angina/fatigue with known CAV grade 2 or greater
    - ACS
    - CHF symptoms with normal EF and unknown CAV
  - Stable short term (<5y)
  - Stable long-term (>10y)
- Endpoint: combined death/hospitalization CV cause

Manfredini V, PhD thesis – manuscript in preparation

Study patients

- Out of a total of 350 screened patients we enrolled:
  - 32 patients with GD (10 % prevalence of GD), 75% of which with previous history of CV events
  - 101 stable patients (61 short term and 41 long term)
- Up to 2 years follow-up

Manfredini V, PhD thesis – manuscript in preparation

Etiology of GD

Heterogeneous presentation of GD patients:

- 66% of patients with GD had symptomatic CAV but no DSA;
- 55% low EF in the context of acute or recurrent rejection – the rest in the context of severe CAV;
- 45% of patients with current or previous rejection had also evidence of CAV
- 10% patients had symptoms of HF with no clear evidence of CAV or rejection

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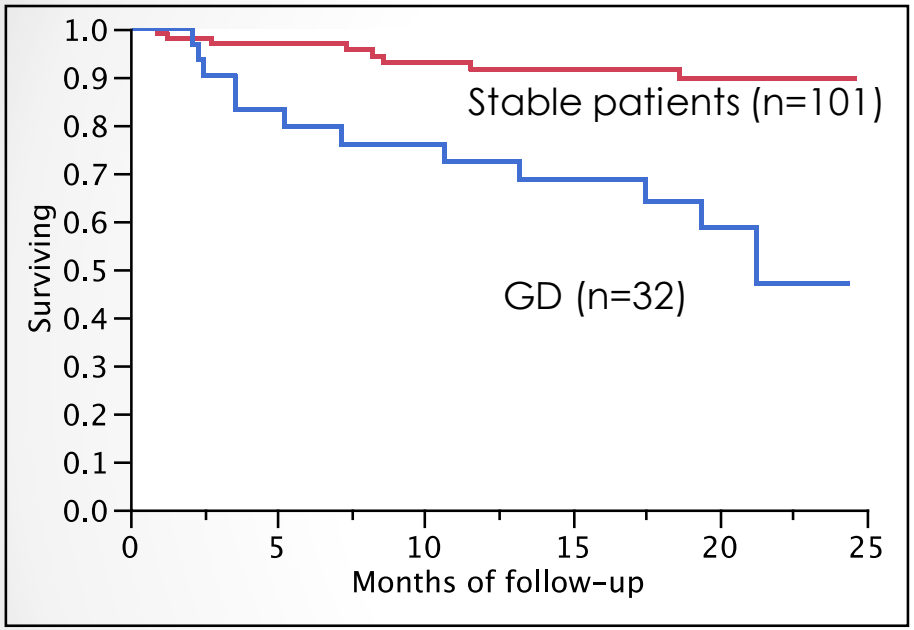
Outcomes



Features of GD patients

- Reduced EF
- CAV
- DSA (22 vs. 7%)
- Wider QRS (131 vs. 104ms)
- Lower BP, higher HR

Prognostic indicators in GD patients:

- LVEF<55%; longer distance from HT; chronic presentation

Manfredini V, PhD thesis – manuscript in preparation

Interpretation

- Graft dysfunction after HT may have a composite definition and heterogeneous etiology
- Reduction in EF and clinical recurrence are relevant prognostic indicators
- AMR may be responsible for less than half of cases of GD, with DSA detectable only in a minor proportion of cases

Role of biomarkers

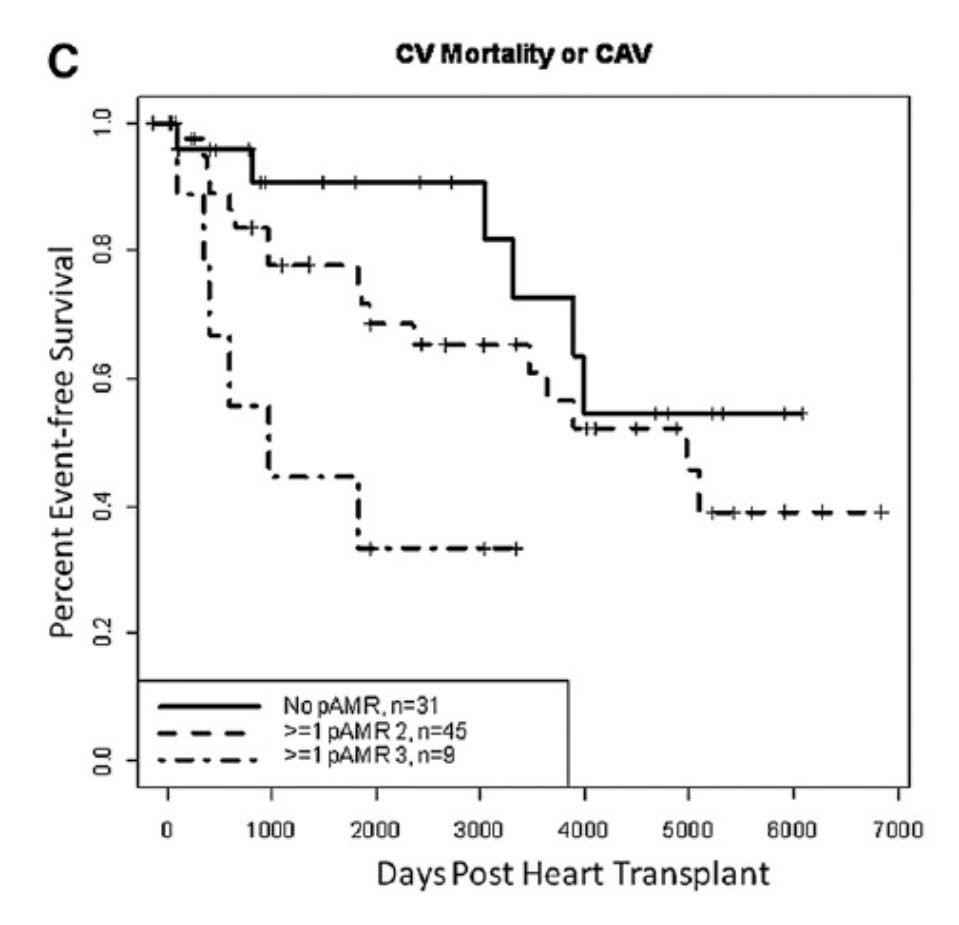
- Blood biomarkers of graft injury or immune activation (including DSAs) cannot be sufficient for the *diagnosis* of the disease
- Diagnosis require direct and comprehensive analysis of the graft (tissue sampling, coronary angio, EKG, echo)
- Biomarkers are promising tools to stratify patients at risk
- Clearance of biomarkers (cfDNA in particular) could represent a reliable surrogate for disease resolution after treatment

Thus, we need tissue: Is the current pAMR grading enough?

- **pAMR0** = Negative for pathologic AMR; histology and immunopathologic. studies both negative
- **pAMR1** = Suspicious for pathologic AMR; histologic findings positive (pAMR 1-H) ***or*** immunopathologic findings positive (pAMR 1-I)
- **pAMR2** = Positive pathologic AMR; ***both*** histologic and immunopathologic findings are positive or CD68+ cells are found in at least 10% of capillaries
- **pAMR3** = Severe pathologic AMR; interstitial hemorrhage or edema, capillary fragmentation, endothelial cell pyknosis and/or karyorrhexis

Kobashigawa, et al. J Heart Lung Transplant 30 (3): 252-269, 2011
Barry, et al., J Heart Lung Transplant 30 (6): 601-611, 2011

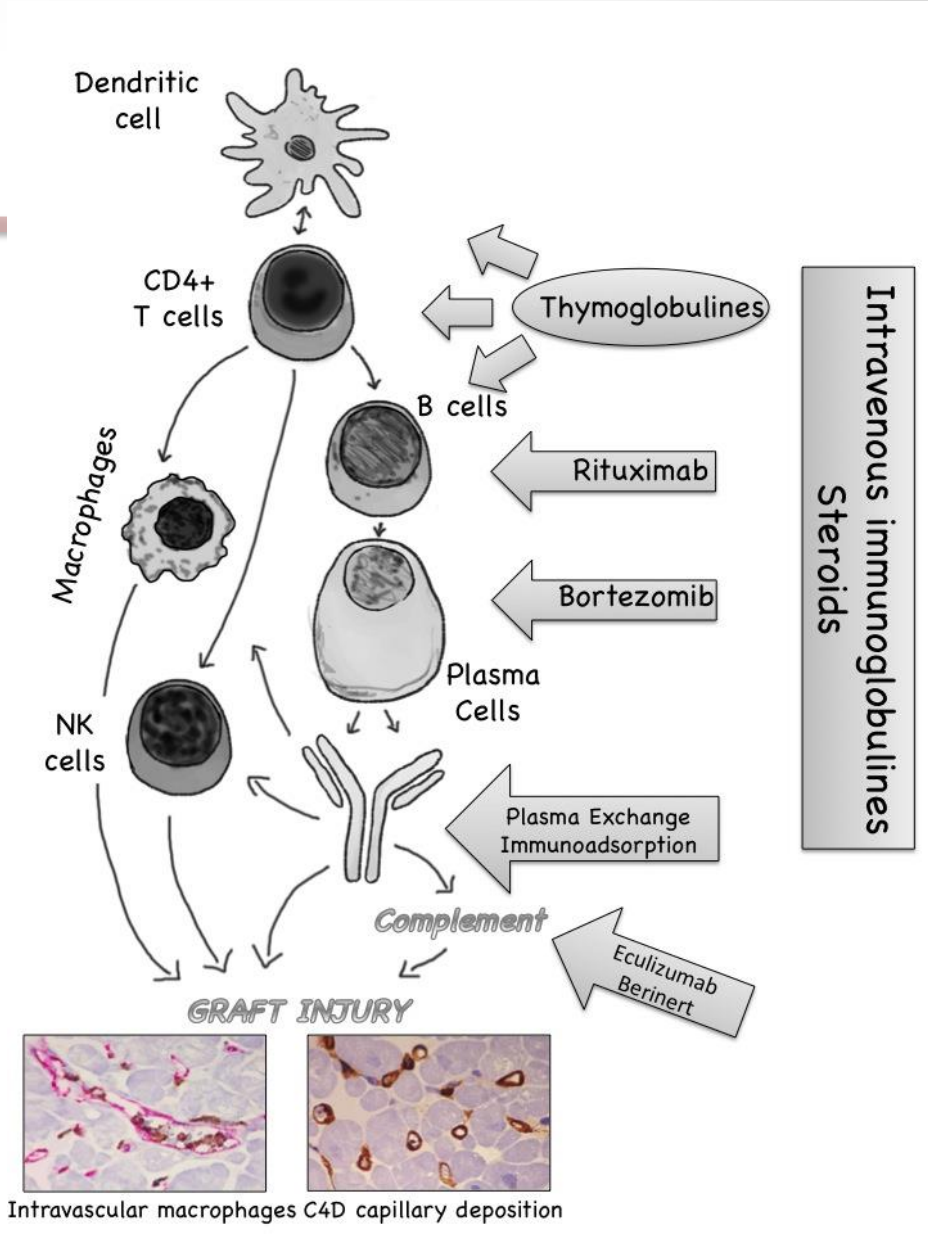
pAMR and prognosis



Everitt; J Heart Lung Transplant 2012;31:686–93

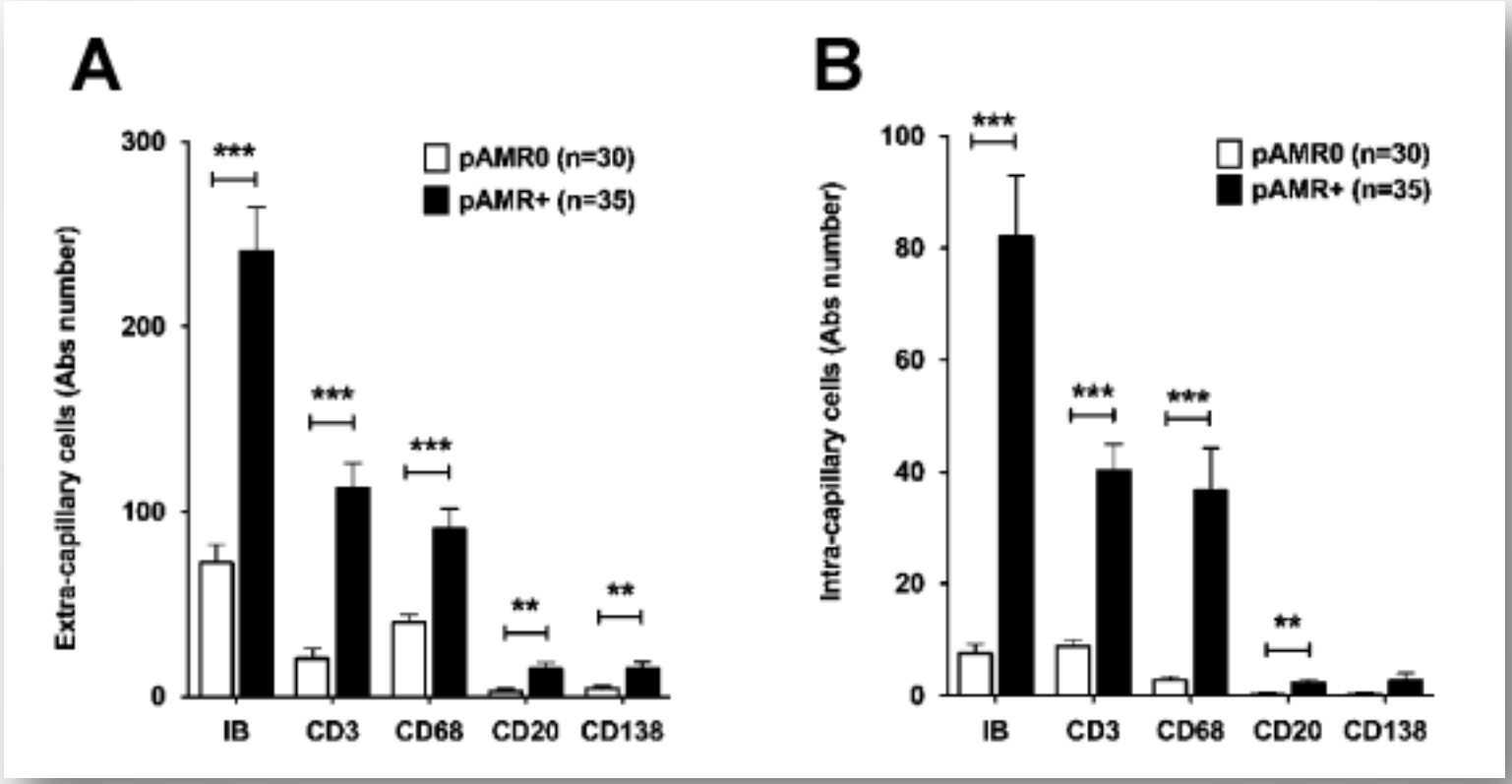
Players in AMR

- Allorecognition mechanisms
- Lymphocytes
- Antibodies
- Complement (not always)
- NK and Macrophages
- Inflammatory cytokines



Manfredini V Curr Opin Organ Transplant 2017, 22:

Current pAMR classification does not account for cellular infiltrate



Fedrigo M et al. Am J of Transplant 2015; 15: 526–534

Current pAMR classification does not account for cellular infiltrate

Table 2 Cardiovascular Mortality Hazard Rates (95% CI) and Severity of MR[a]

	1R	2R	3R
pAMR 1 (H^+) or (I^+)	1.67 (1.42 to 1.96)	1.91 (1.29 to 2.83)	2.18 (1.15 to 4.14)
pAMR 2	2.57 (1.92 to 3.44)	3.08 (1.74 to 5.44)	3.70 (1.49 to 9.16)
pAMR 3	3.95 (2.42 to 6.46)	4.97 (1.97 to 12.56)	6.26 (1.45 to 26.91)

1R, mild cellular rejection; 2R, moderate cellular rejection; 3R, severe cellular rejection; AMR, antibody-mediated rejection; pAMR 1(H^+), histopathologic AMR alone; pAMR 1(I^+), immunopathologic AMR alone; pAMR 2, pathologic AMR; pAMR 3, severe pathologic AMR.

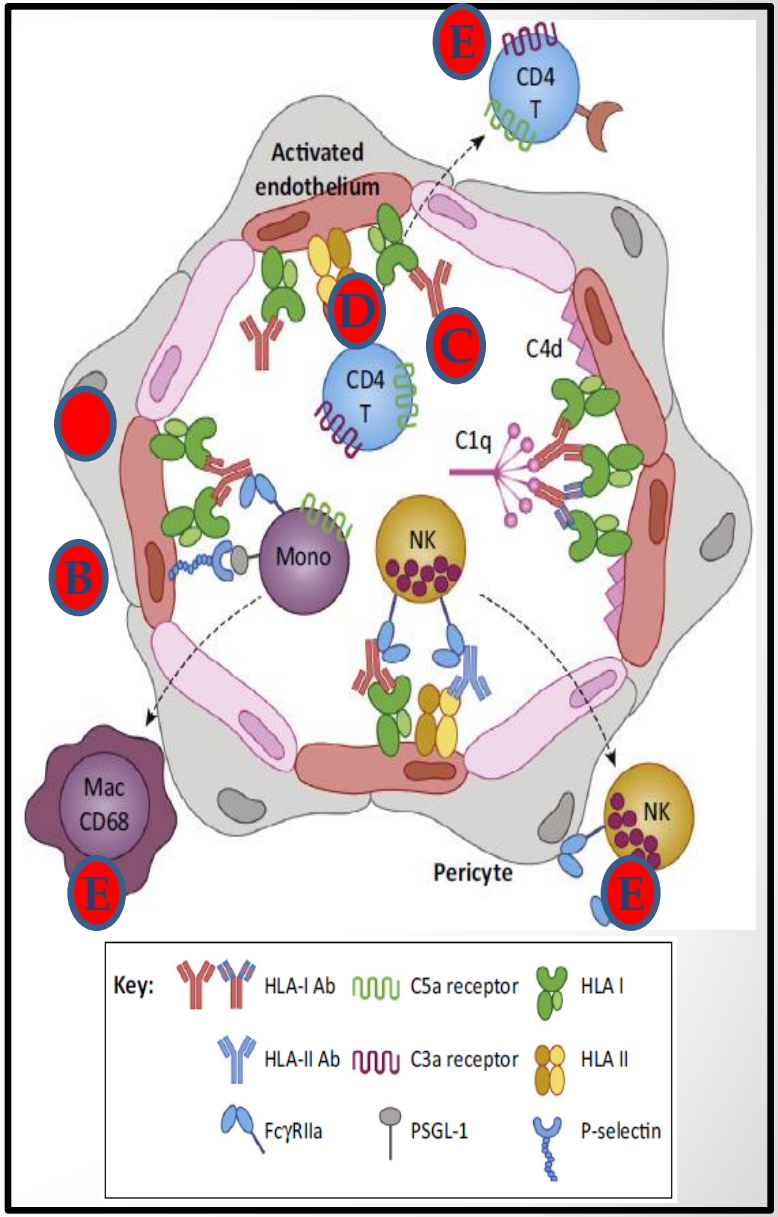
[a]Referent group is "no rejection."

Kfoury A et al. J HeartLungTransplant2016;35:335–341

The Perfect Storm: HLA Antibodies, Complement, FcgRs and Endothelium in AMR

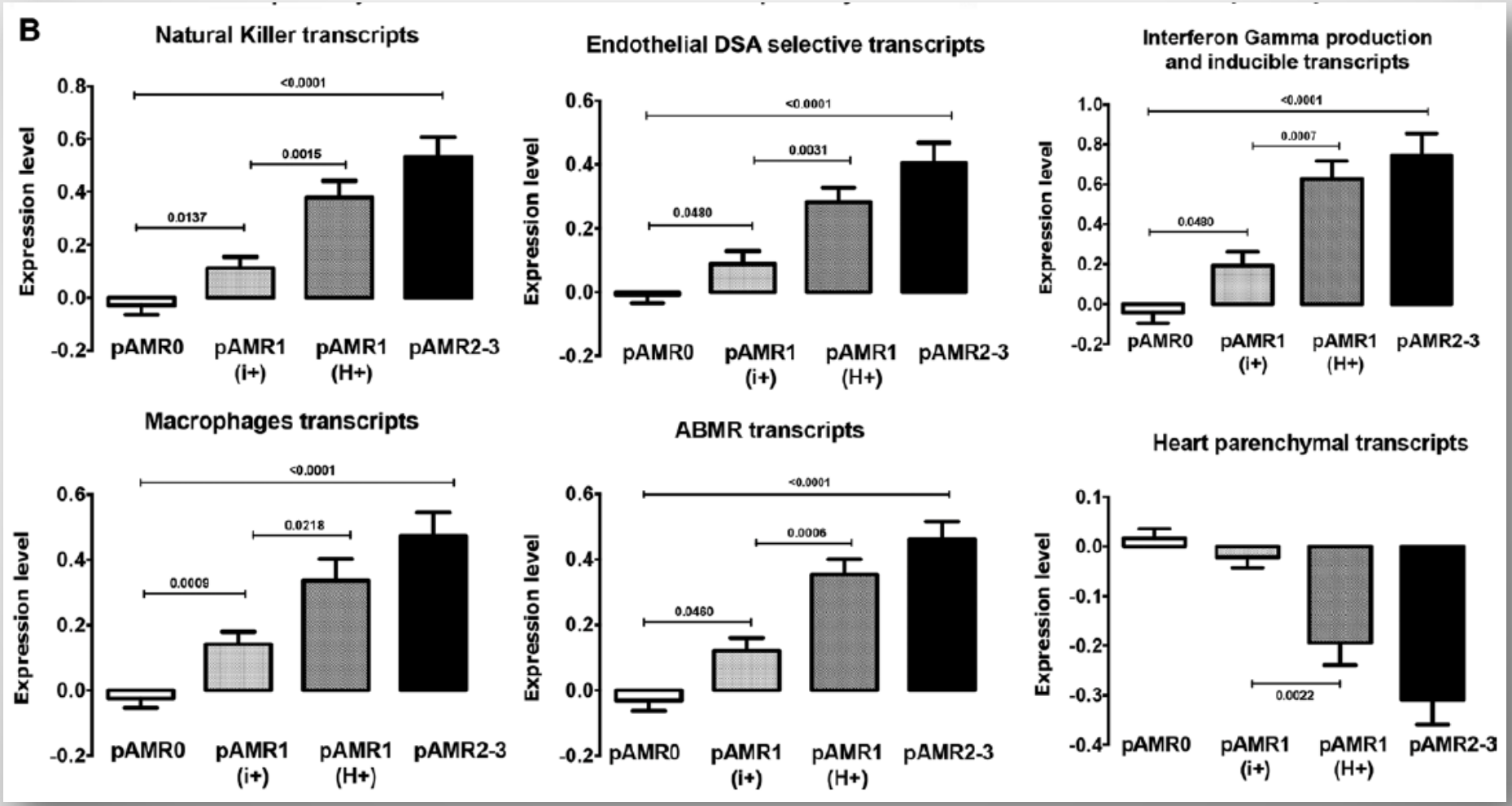
In the pathophysiological process of AMR, ligation of HLA antibodies to the HLA antigens on endothelial cells (EC) of the allograft microvascular can:

- cause EC activation by triggering intracellular signaling. (Jin et al., *AJT*. 2014; Jindra et al., *J Immunol*. 2008).
- **B** increase P-selectin expression on EC surface. (Valenzuela et al., *AJT*. 2013; Valenzuela et al., *J Immunol*. 2013)
- **C** trigger the classical complement cascade.
- **D** enhances EC immunogenicity to recipient CD4 T cells.
- **E** The expression of P-selectin and the activation of classical complement pathway augment leukocytes recruitment and infiltration.
- **F** induce EC to secret cytokines, such as IL-6, IL-8, CXCL10, CCL2,and CCL5. (Naemi et al., *Transplantation*. 2013)



Thomas, Reed *et. al,. Trends Mol Med. 2015.*

Is pathology grading reflecting what it is happening in the tissue ?



Loupy A et al. Circulation 2017 135:917–935

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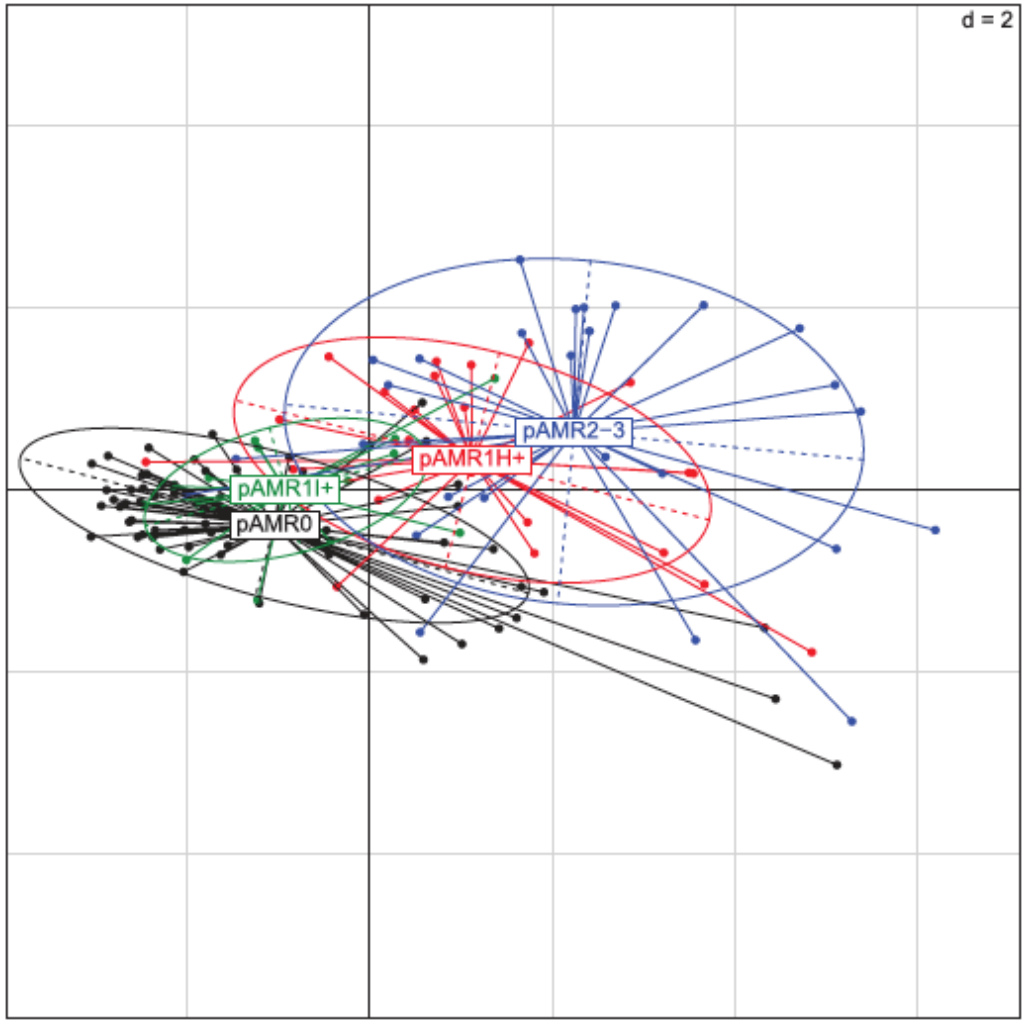
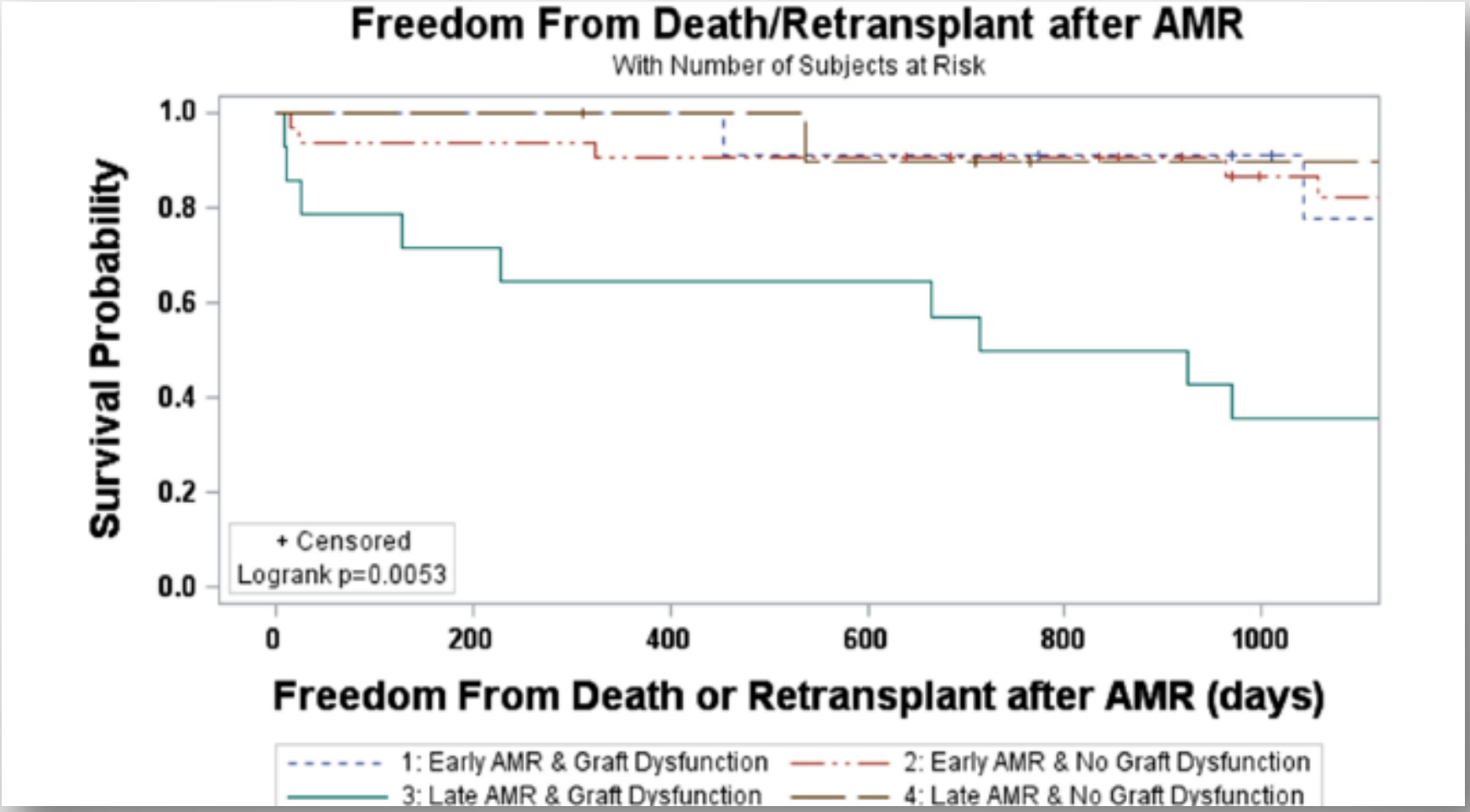


Loupy A et al. Circulation 2017 135:917–935

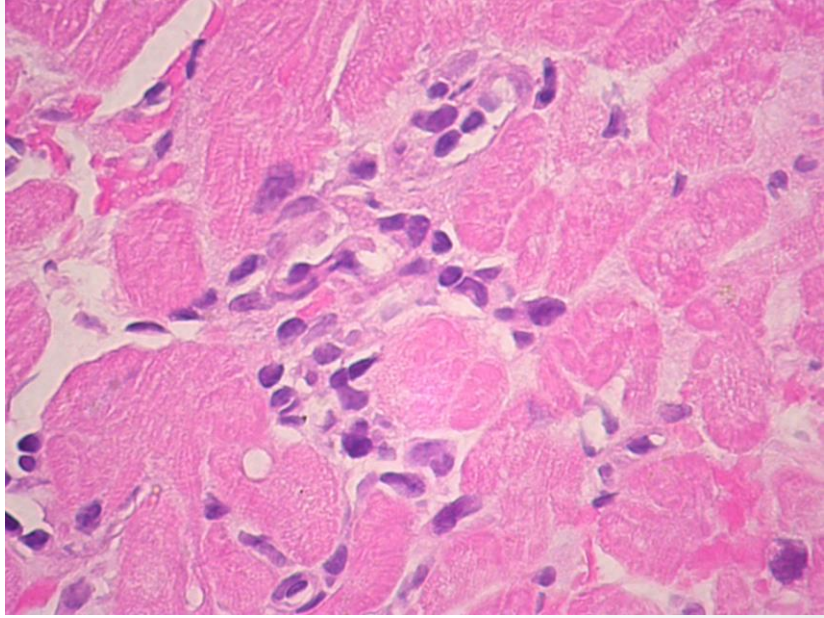
Is pAMR enough to identify patients at risk?

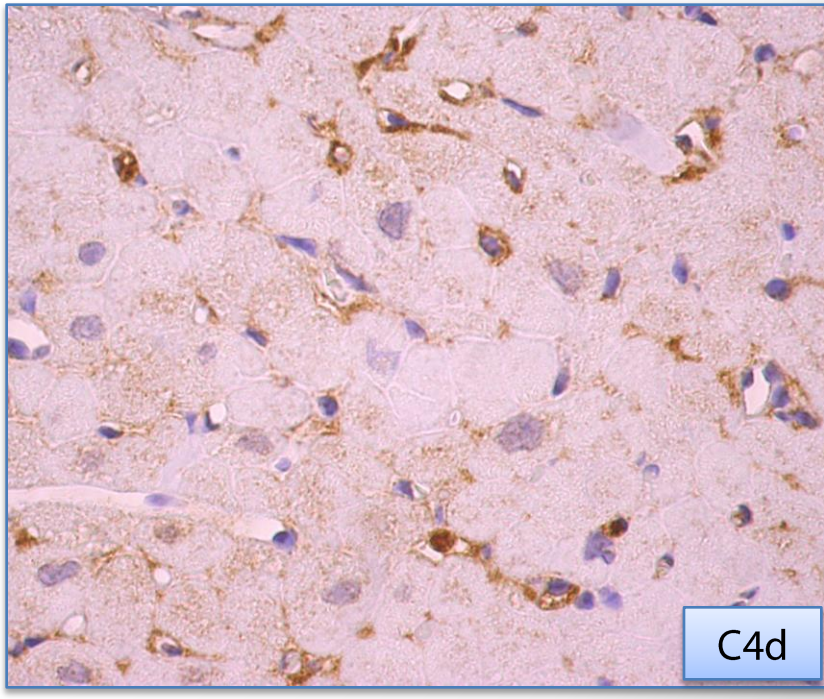


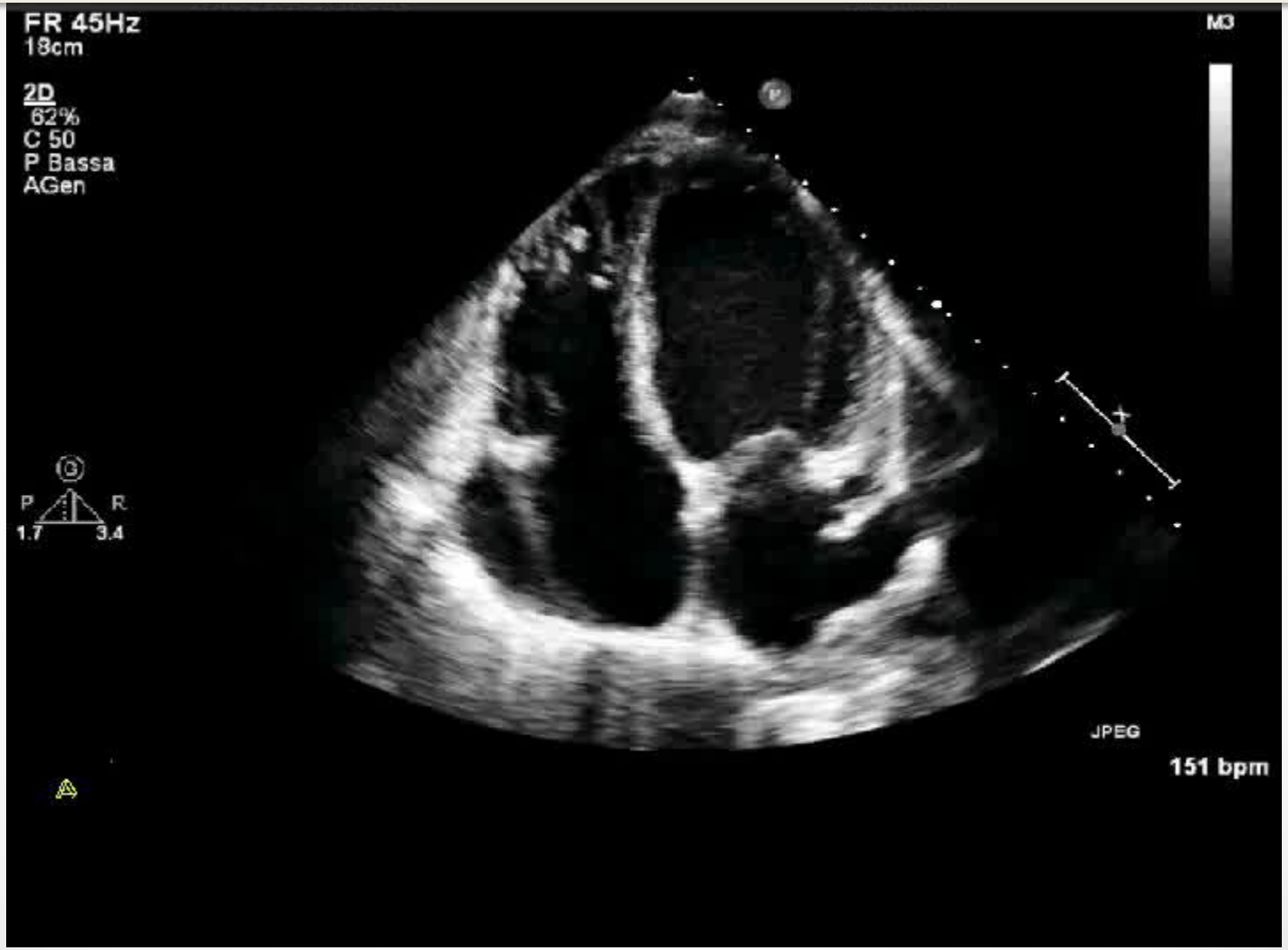
Clerkin KJ et al. J HeartLungTransplant2016;35:1059–1066

- CR: grade 1R (1A)
- Histology for AMR: negative
- Immunohistochemistry:
  - C4d distribution: focal positive (>10% <50%)
  - C4d intensity: strong
  - CD68 intravascular macrophage: negative (<10%)
- Anti HLA
  - class I antigens A31, B35
  - class II antigens DR16, DR15, DQ6
  - MFI 3600

pAMR=0



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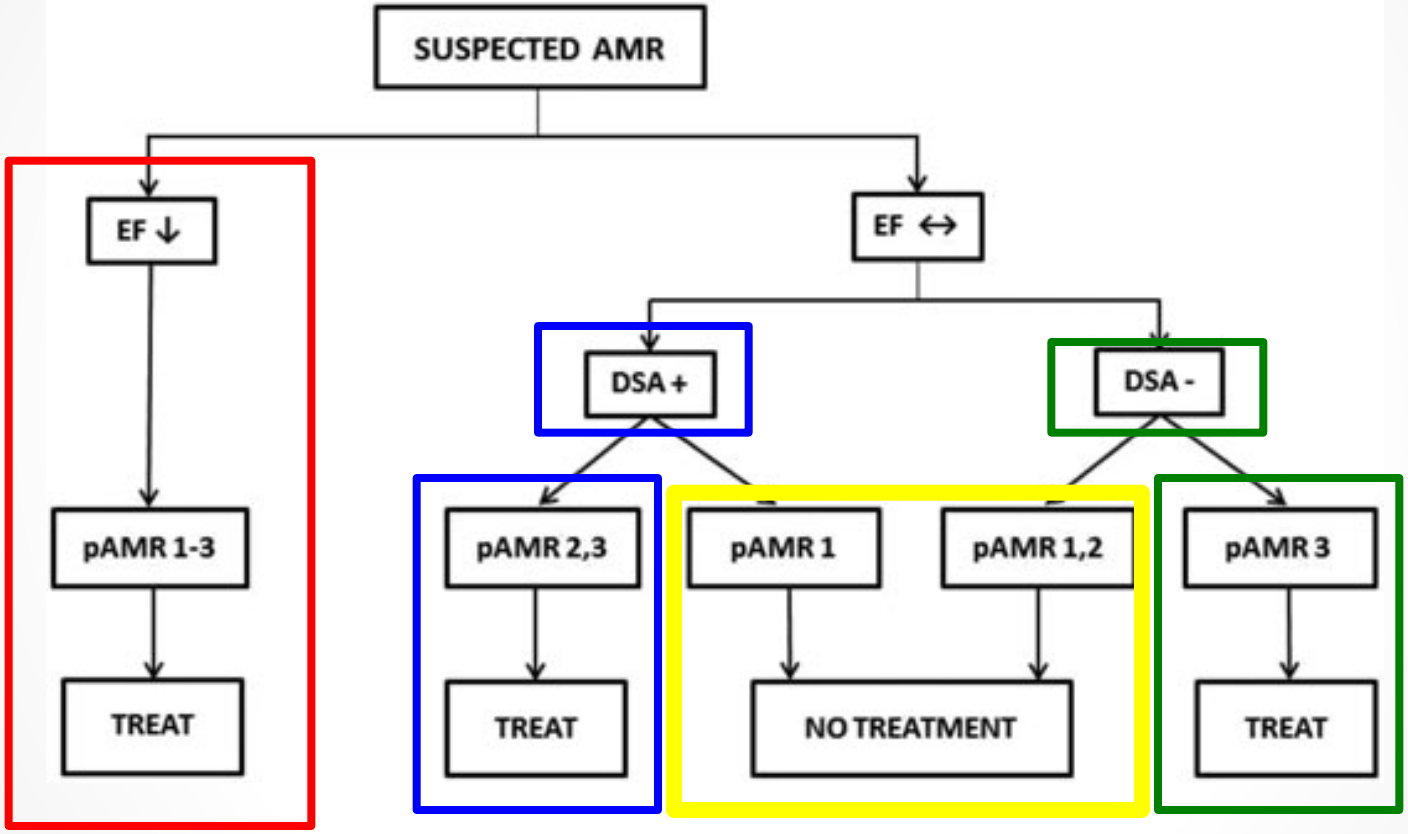
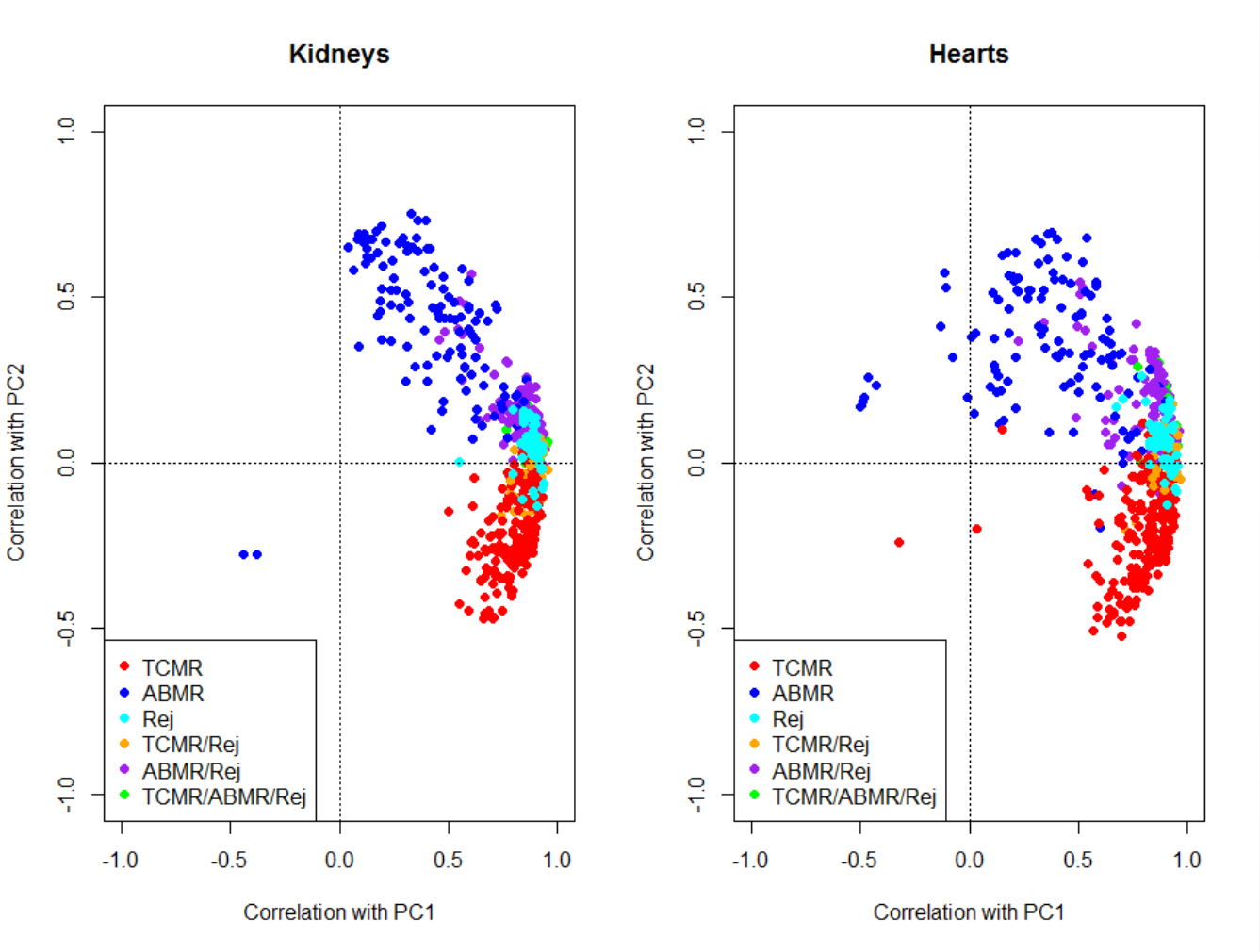
When pAMR need treatment?



Chih Am J Transplant 2013

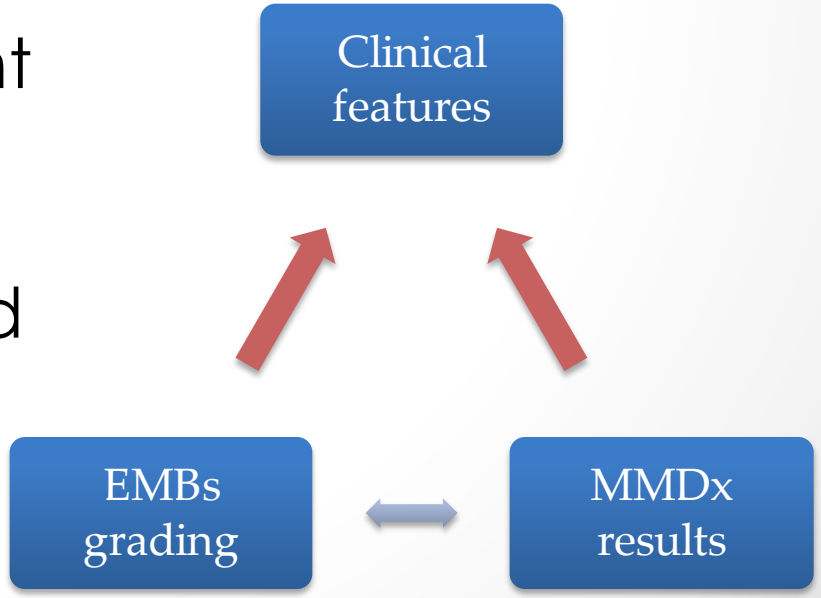
Molecular profiling and graft function



Borgese L et al Transplant Int 2016

Aim & Methods

- Prospective study
- 37 EMBs from 32 heart transplant patients
- Patients also underwent right heart catheterization and cardiac ultrasound



Borgese L et al Transplant Int 2016

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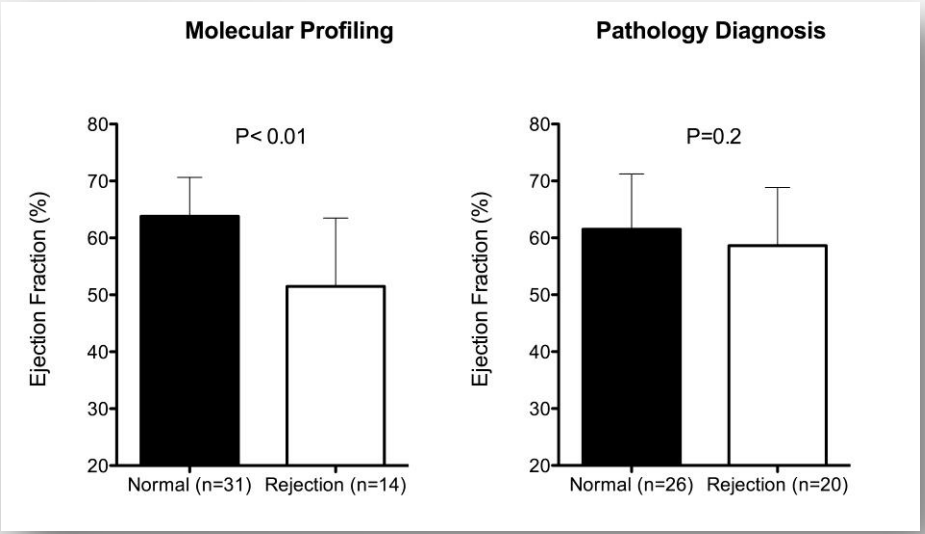
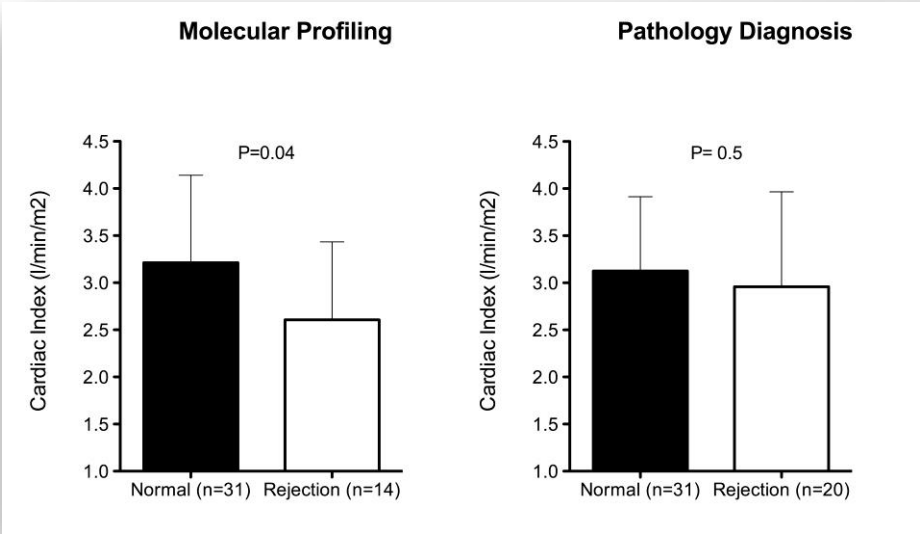




Borgese L et al Transplant Int 2016

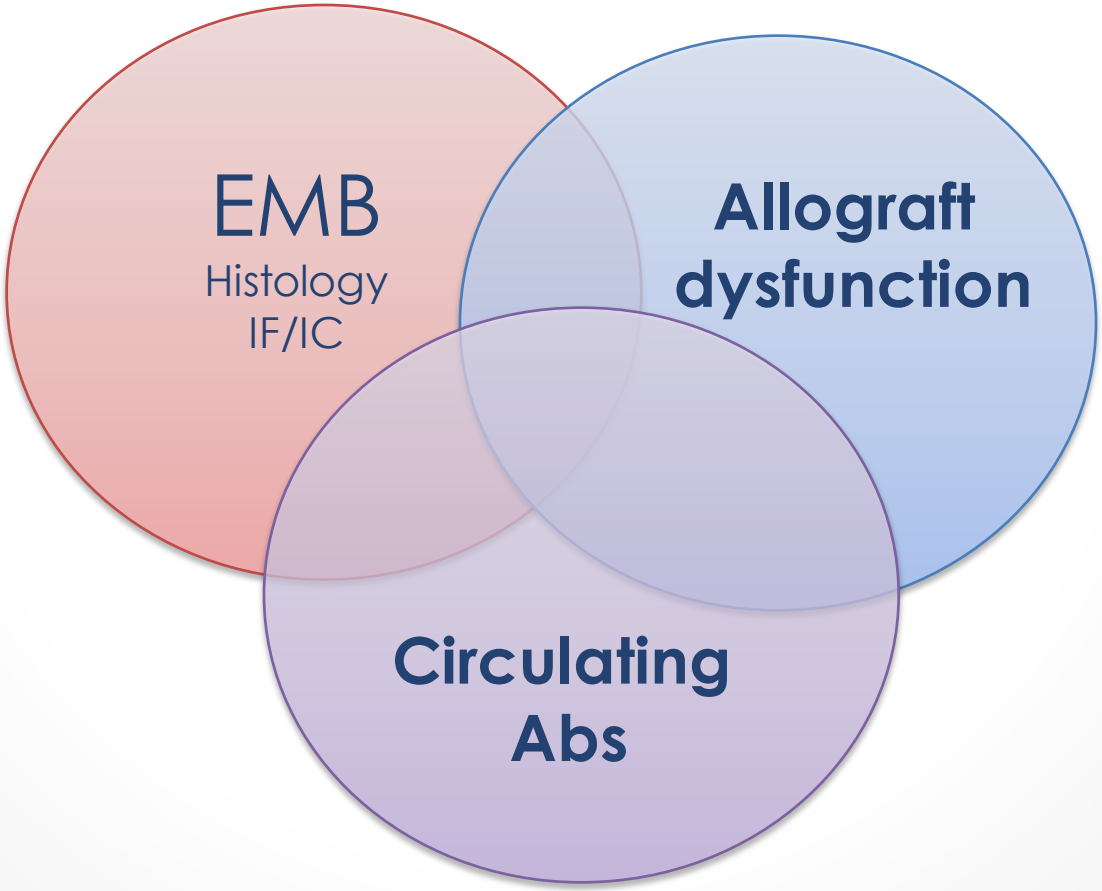
Role of pAMR as endpoint

- pAMR grading is reasonably reproducible and predicts prognosis
- Current grading is missing additional info embedded in the tissue morphology
- pAMR describes pathology findings: graft function need to be taken into account for disease definition
- Molecular profiling may reconciliate most of these pitfalls by identifying tissue molecular signature which is related to graft function

The ideal endpoint

	pAMR	cfDNA	DSA	Graft function	Molecular profiling
Clinically relevant	+/-	+/-	+/-	+++	+ ? ?
Easy to Measure	+	+/-	+	++/-	-
Objective	+/-	++	+/-	+	+
Enough frequent	+	?	-	+/-	?
Related to diagnosis	+/-	--/+	+	-- /+	++
Related to mechanism of action of the treatment	+/-	- /+	+	--	++

Multidisciplinary diagnosis

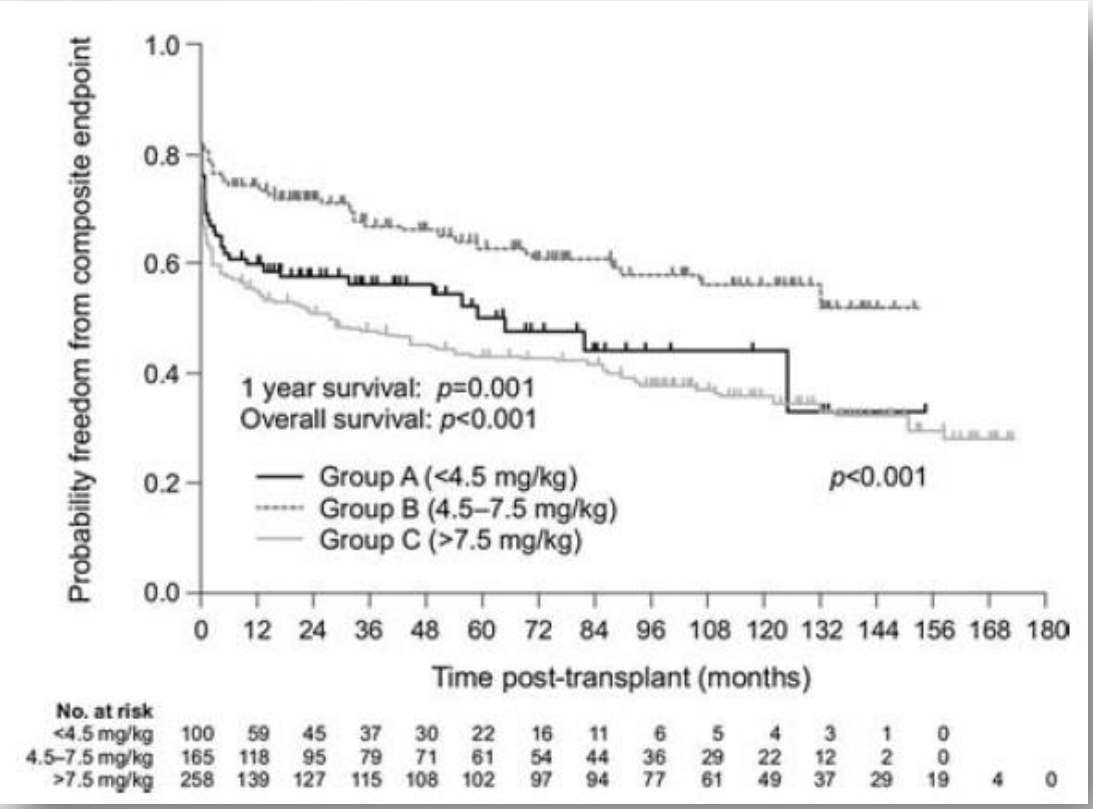


Proposals for AMR trial in HT

- Definition of AMR:
  - Injury biomarkers or signs of graft dysfunction + Histology and/or molecular profile

- Endpoints – short term
  1. Function recovery
  2. Biomarker clearance
  3. Molecular profile/histology clearance
- Endpoints – long term
  1. Recurrence of AMR/CHF symptoms
  2. Changes in EKG
  3. Death/graft loss

… but efficacy is only one side of the coin



Composite Endpoint:

- Death
- Treated rejection
- Treated Infection

Aliabadi et al. Transplantation 2016;100: 685–693

Take home message