Kidney 1:
Significance of i-IFTA, revisiting TCMR & mixed rejection

Treatment of Late and Mixed Rejection

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No conflicts of interest
Some "off label" discussions

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Banff-SCT Joint meeting
Barcelona, Spain
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Lecture overview

Definition, incidence and time course

Clinical scenarios of mixed rejection

- Sensitization
- Iatrogenic under-immunosuppression
- Non-compliance

Anti-rejection treatments

Strategic approach to late rejection

Mixed and late rejection: definitions

1. Mixed rejection:

Defined by pathology

Pathophysiological diagnosis to target treatment

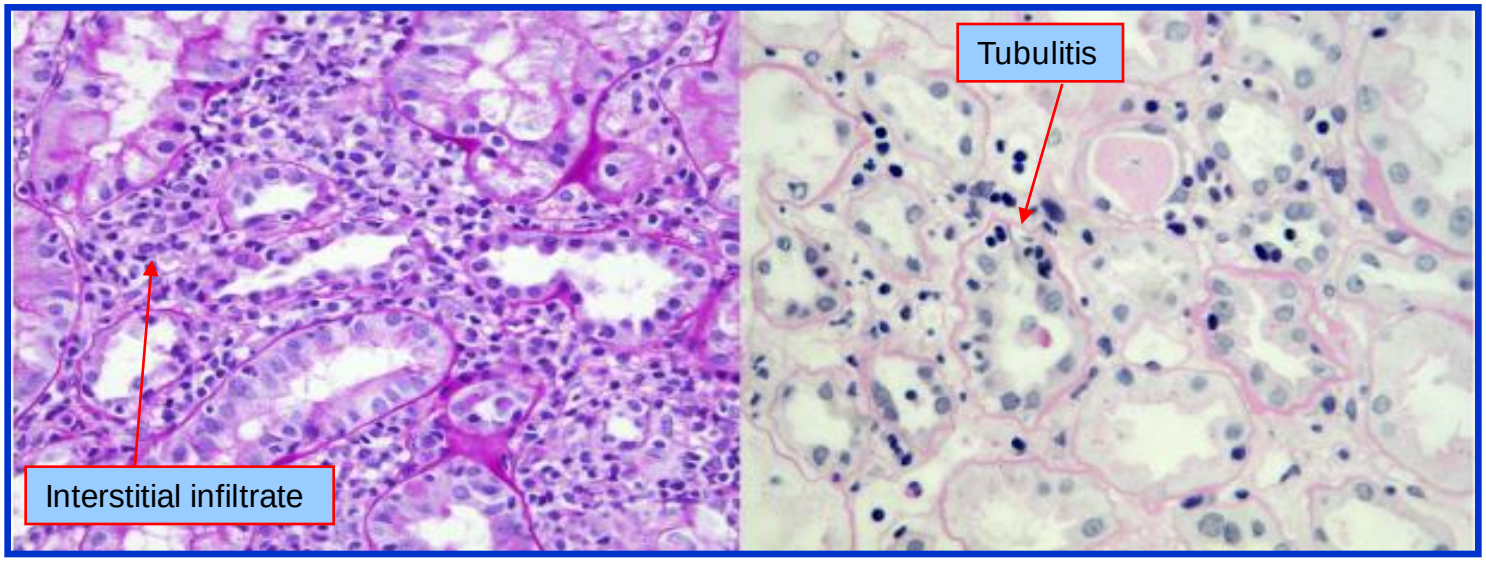
TCMR + AMR = Mixed rejection

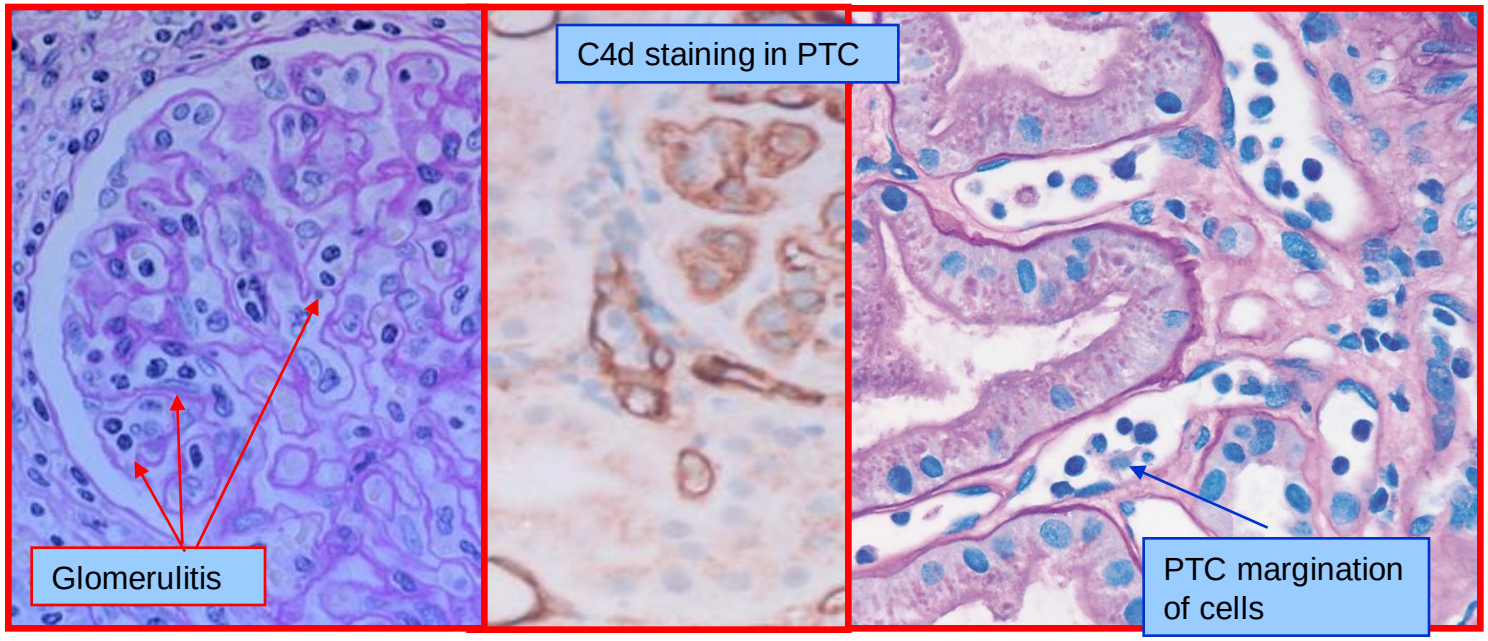
2. Late rejection:

Beyond 3 months

Mixed acute rejection

Infiltrating cells





TCMR

+

AMR

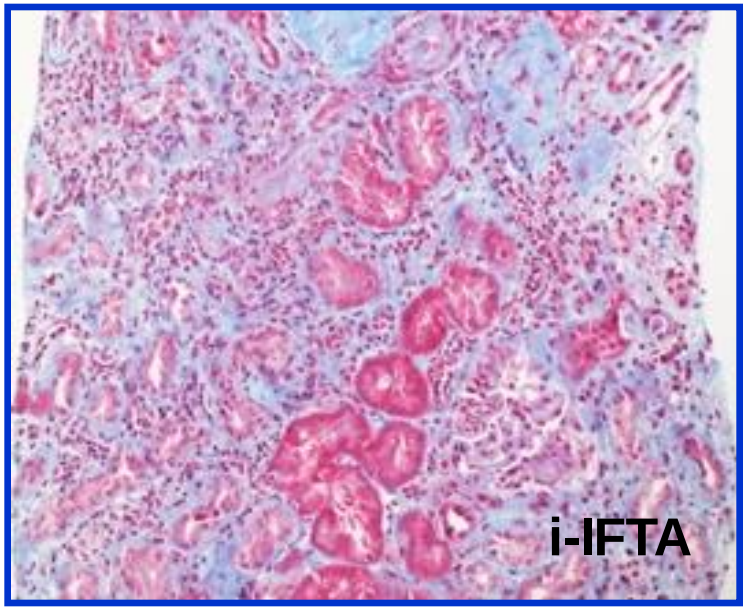
Pictures:
Drs B Colvin
& Trpkov

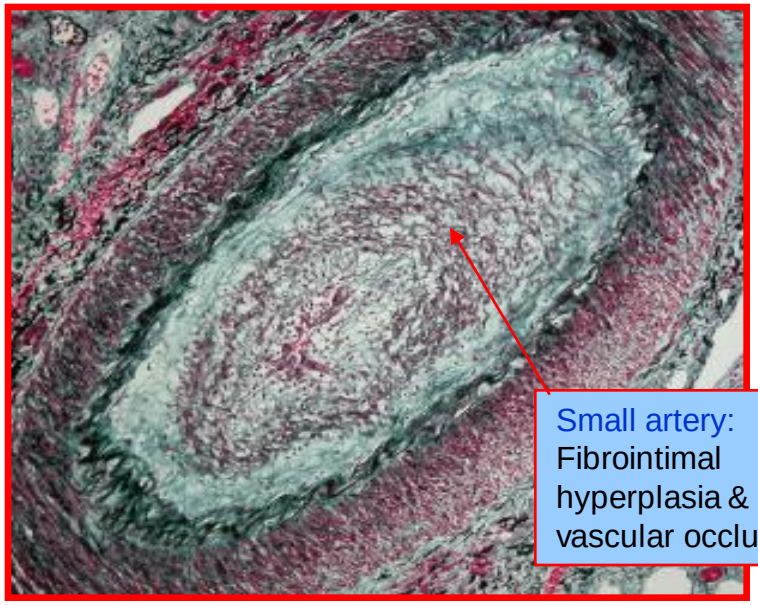
From
Nankivell
& Alexander
NEJM 2010

Mixed subacute / chronic rejection

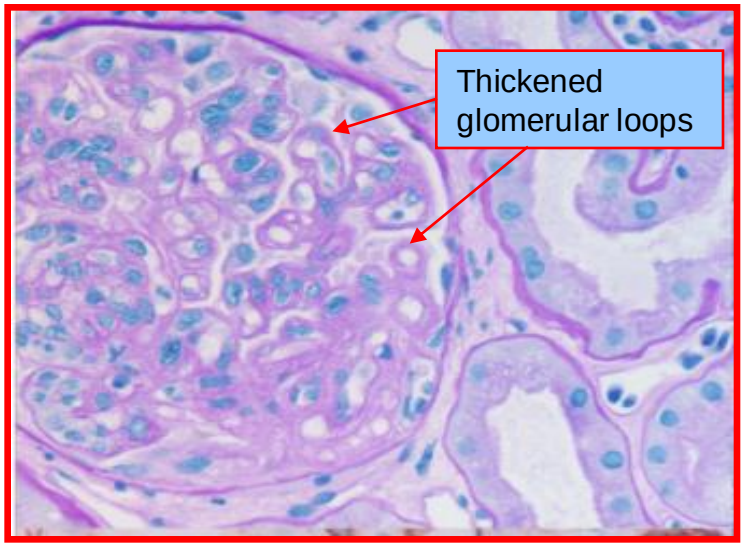
Tissue changes

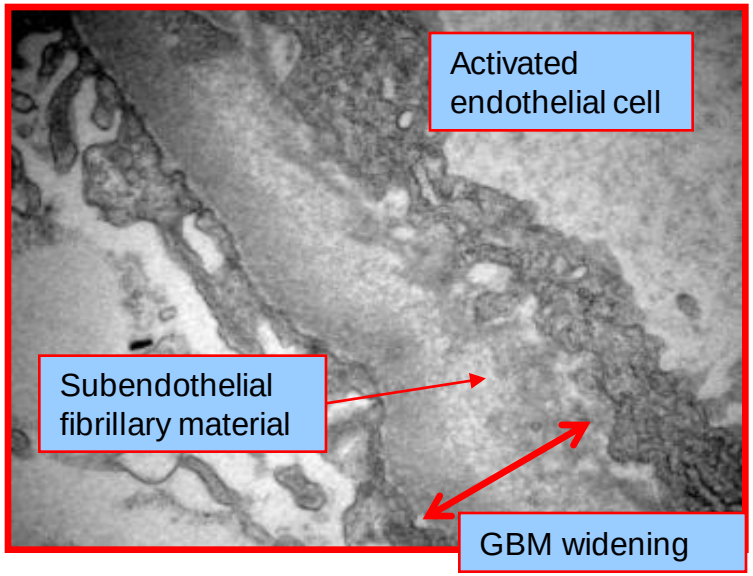
Chronic TCMR





CAMR





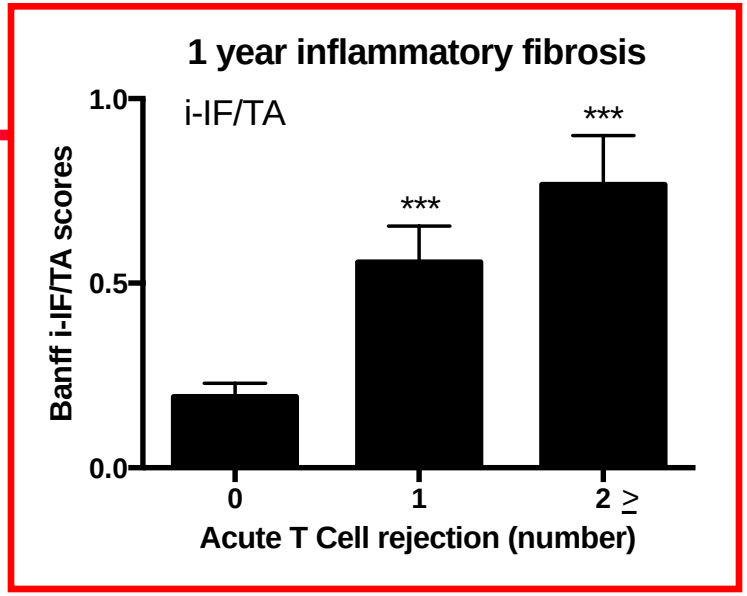
From Nankivell & Alexander NEJM 2010

Pictures: Drs B Colvin M Sheende & R Murugasu

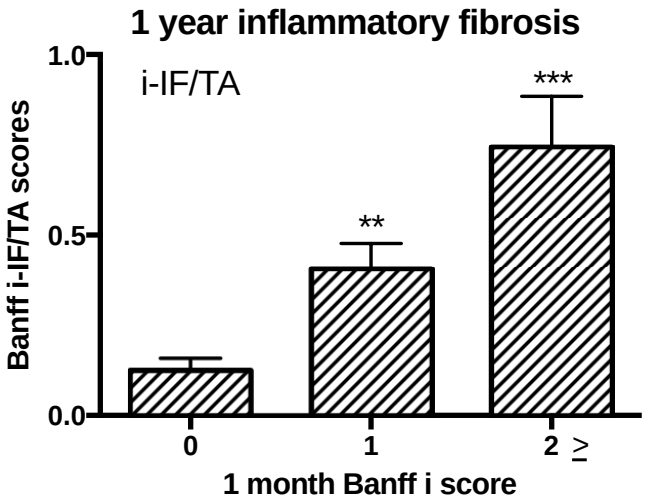
TCMR and late i IFTA

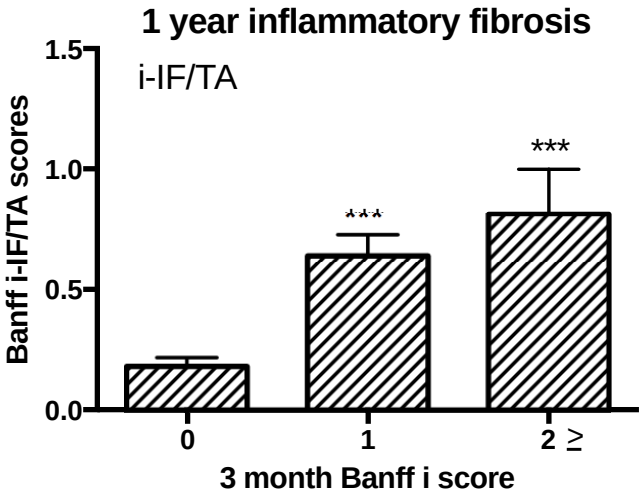
1 year i IFTA

- Acute T cellular rejection
- Subclinical rejection



Mean+/-SEM



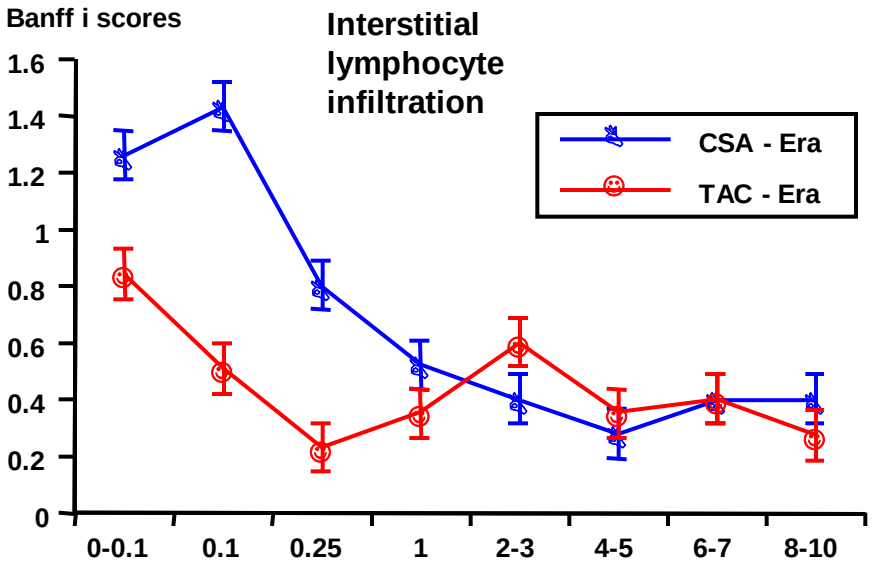


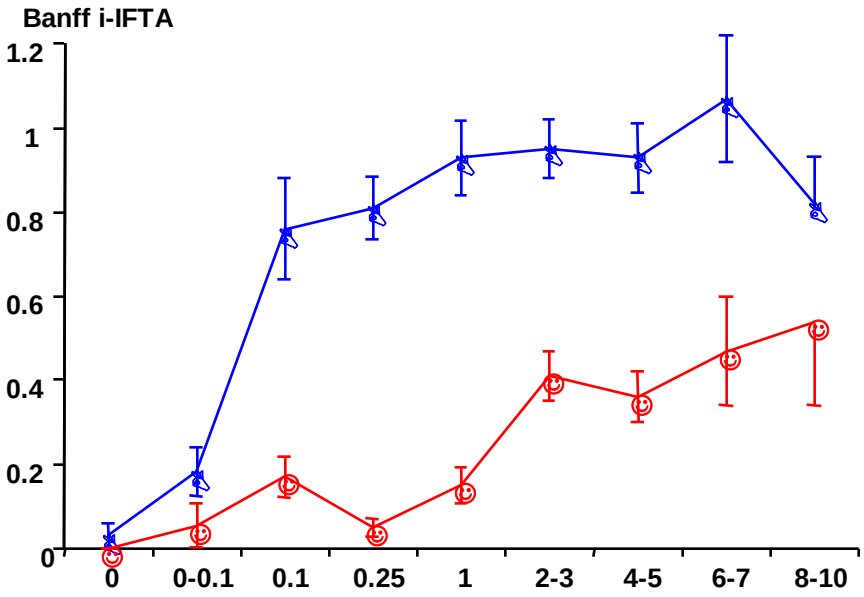
Single centre
Prospective cohort
Protocol biopsy
N=300 SPK
N=3,000 biopsy

Nankivell unpublished

Effects of era on inflammation

Potent TAC-based immunosuppression reduces i-IFTA





Concept:

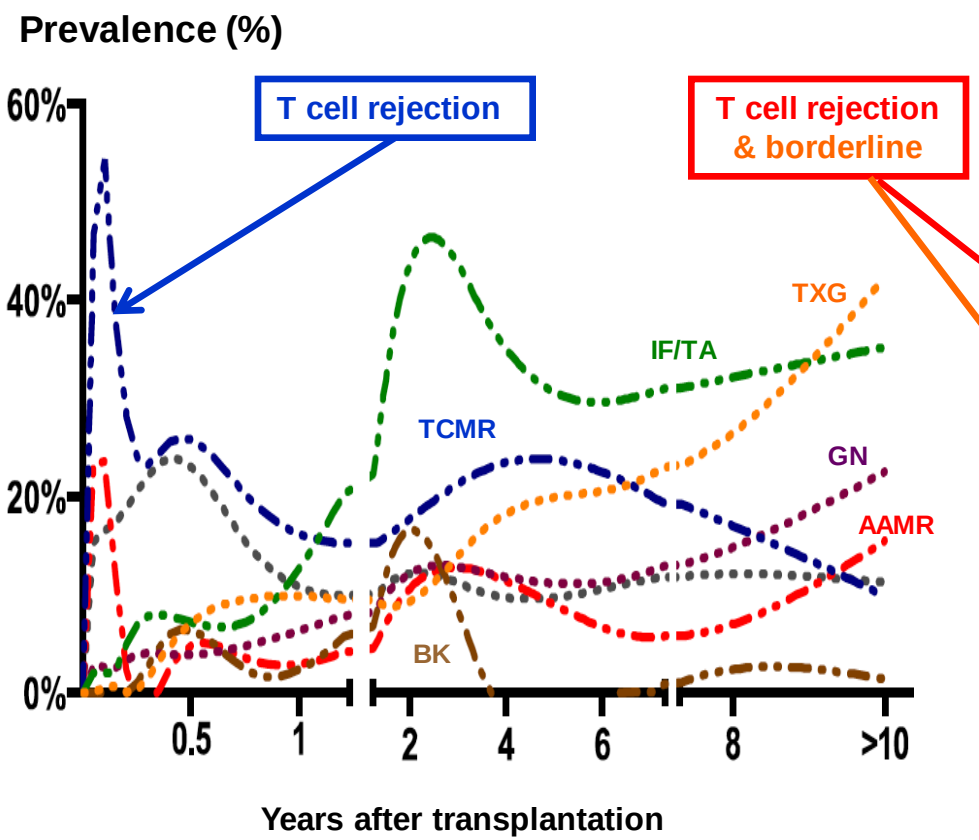
- i IFTA is the histological expression of chronic active TCMR

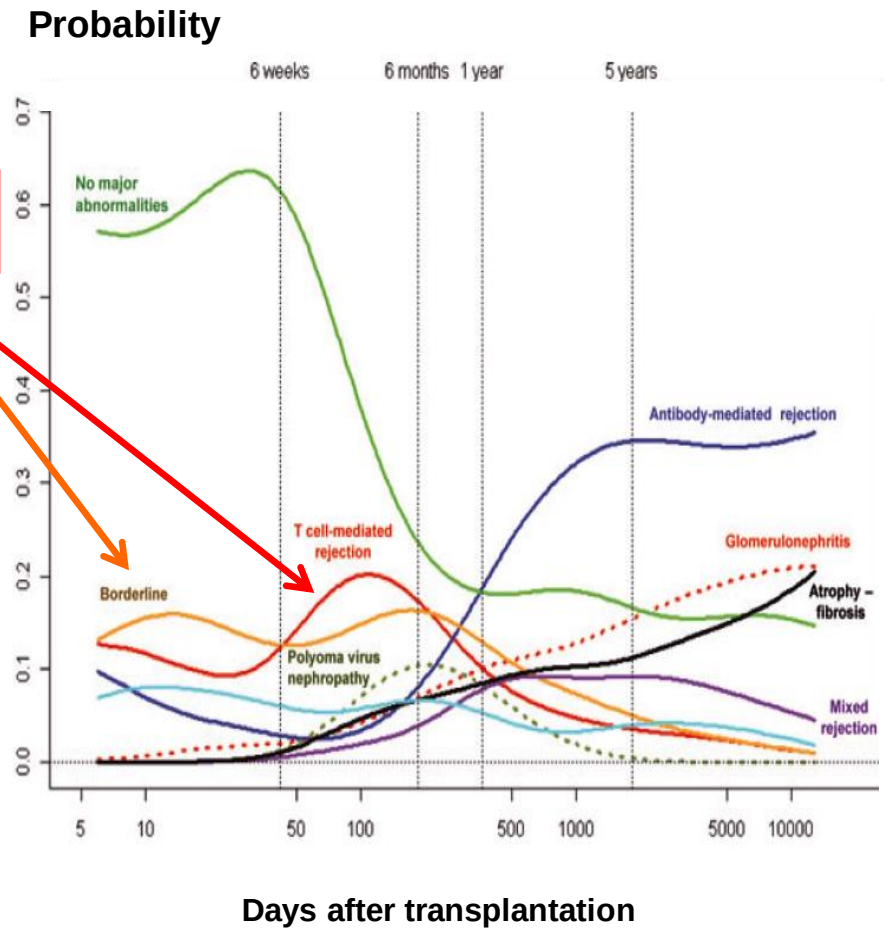
Single centre
Prospective cohort
Protocol biopsy
N=300 SPK
N=3,000 biopsy

Nankivell unpublished

Incidence & time course of mixed rejection

T cell mediated rejection





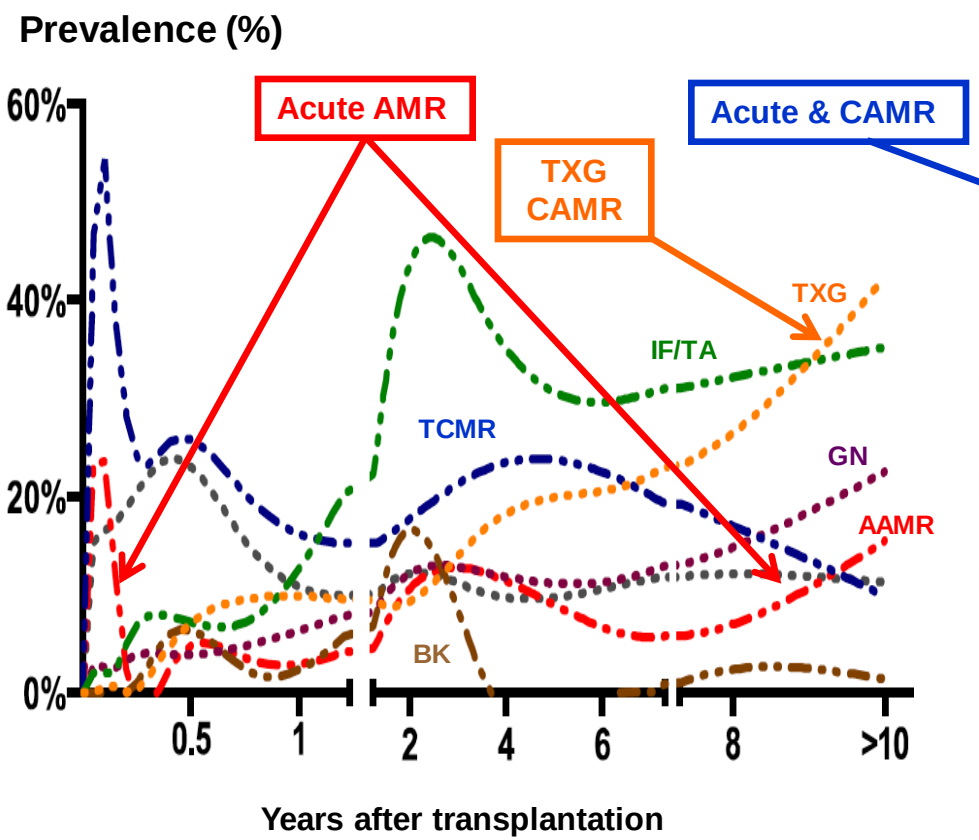
From protocol biopsies

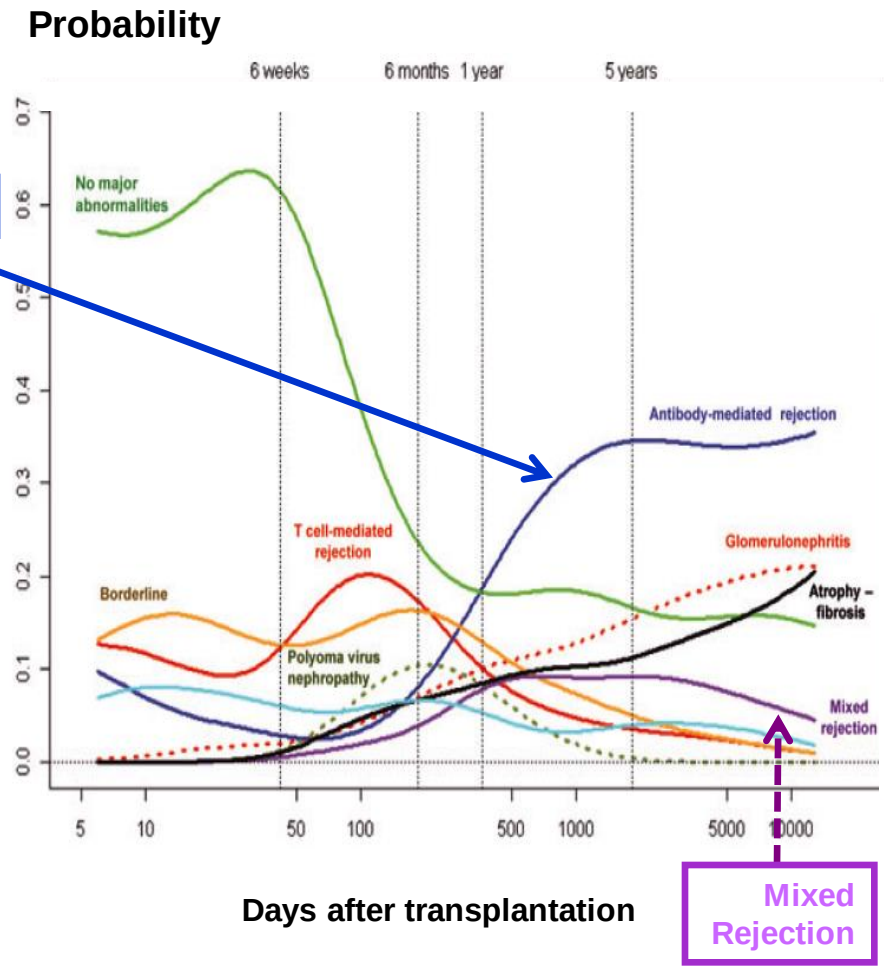
From indication biopsies

Naesens et al JASN 2013

Sellares et al AJT 2012

Antibody mediated rejection





From protocol biopsies

From indication biopsies

Naesens et al JASN 2013

Sellares et al AJT 2012

Wiebe et al AJT 2012

de novo DSA & interstitial infiltration

Table 3: Pathologic correlations with patient phenotypes at the time of dnDSA detection

DSA	Acute dysfunction dnDSA	Indolent dysfunction dnDSA	Stable function dnDSA	Dysfunction no dnDSA	Stable function no dnDSA
n	14	15	18	55	213
Clinical rejection, 0–6 months	36%*	27%*	22%	24%*	10%
Nonadherence	100%***	53%***	6%	16%*	6%
Month dnDSA positive	60 ± 35	61 ± 31	49 ± 31	–	–
Month protein, ≥0.5 g/d	63 ± 38	70 ± 33	–	51 ± 40	–
Month Cr ≥ 25% baseline	63 ± 34	73 ± 28	–	34 ± 31	–
Biopsy, n	12	13	14	35	27
Month of biopsy	63 ± 34	71 ± 26	53 ± 46	27 ± 21	24 ± 2
Creatinine at biopsy	490 ± 420***	156 ± 59***	118 ± 44	189 ± 180**	106 ± 31
g	0.92 ± 0.8***	0.92 ± 0.8***	0.14 ± 0.4	0.20 ± 0.5	0.04 ± 0.2
i	2.0 ± 1.1***	1.07 ± 0.8**	0.50 ± 0.8		0.37 ± 0.6
t	2.0 ± 1.0***	0.54 ± 0.5**	0.35 ± 0.6		0.11 ± 0.3
v	0.08 ± 0.3	0 ± 0	0.21 ± 0.8	0.03 ± 0.2	0 ± 0
ptc	2.20 ± 0.7***	1.92 ± 1.0***	0.93 ± 1.0***	0.27 ± 0.6	0.04 ± 0.2
C4d+	80%***	39%**	57%***	0%	4%
cg	0.25 ± 0.5**	0.92 ± 1.2***	0 ± 0	0.14 ± 0.4	0 ± 0
ci	1.17 ± 0.6*	1.62 ± 0.5***	0.50 ± 0.7	1.37 ± 0.7***	0.67 ± 0.6
ct	1.25 ± 0.6	1.85 ± 0.7***	0.93 ± 0.5	1.46 ± 0.6**	0.93 ± 0.6
cv	0.75 ± 0.8	0.78 ± 0.6	0.57 ± 0.7	[illegible]	0.41 ± 0.6
Months of follow-up post-dnDSA detection	29 (1–69)	45 (1–88)	19 (0–128)		–
Graft failure	57%***	40%***	0%	15%***	0%

Significance level compared to the Stable Function No dnDSA group *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Glomerulitis

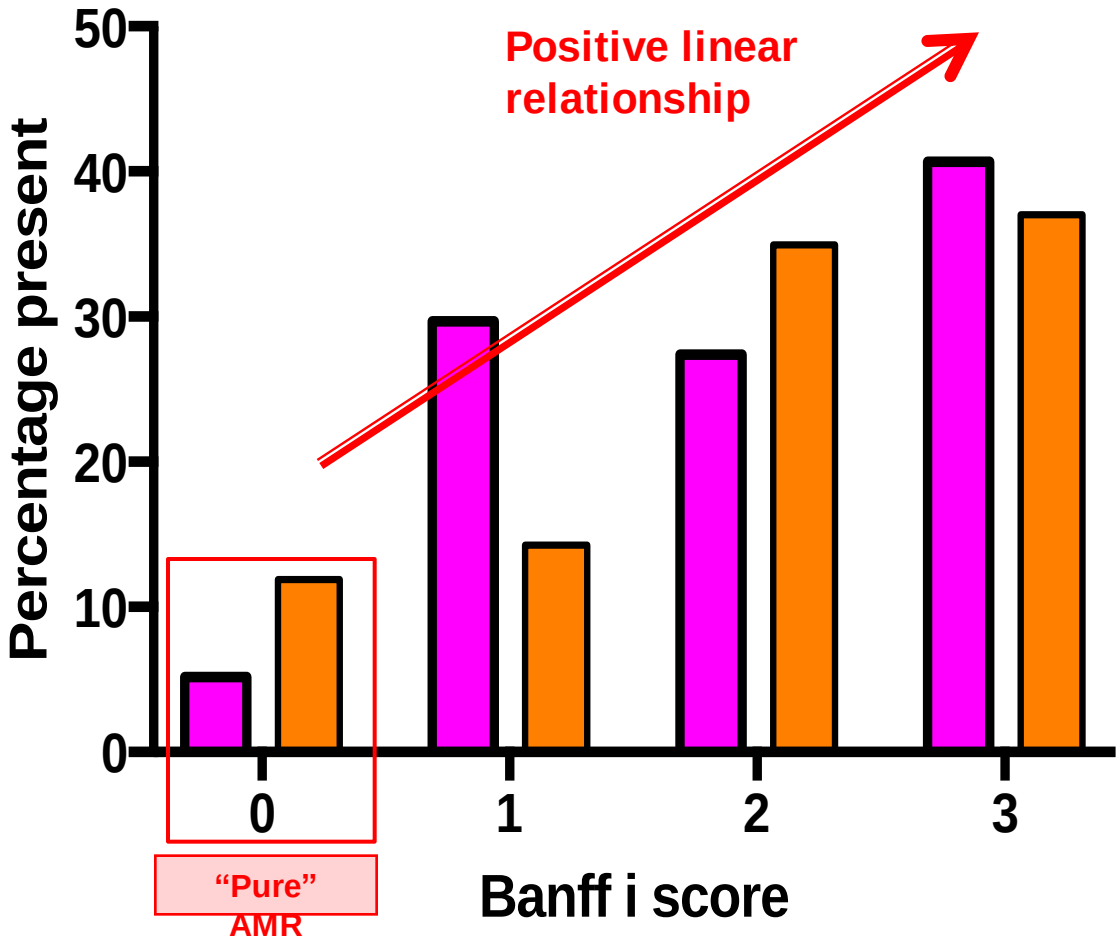
Interstitial inflammation

DSA microvascular inflammation

Nankivell unpublished

T cell & humoral marker relationships

- "continuum of mixed alloimmune response"



BFC n=483
from 1768 Bx
4 yrs conseq

MVI score≥2

C4d score≥0

MVI = ptc+g
C4D by IHC

Mixed rejection: prevalence by i score

	Banff i = 0 no TCMR	Banff i ≥ 1 TCMR
No AHR	Not rejection 52.2% (n=253 Bx)	"Pure" TCMR 24.9% 11.7% (14) DSA >500 only (n=121)
AHR marker* •C4d+ve ≥1 •MVI: ptc+g ≥2 •TXG by EM	"Pure" AHR 9.5% (n=46)	Mixed rejection 13.4% (n=65)

Other diagnoses

Normal	38
ATN	120
IFTA	75
CNI	11
BKVAN	4
Recurrent	5

*15% AHR were DSA neg with obvious AHR/TXG
38.1% of normal bx had DSA (low level) !

Nankivell unpublished

BFC n=485 (of 1768 consecutive Bx)
Adequate tissue, Westmead

Early mixed rejection: Case 1

34 y.o. female
SPK DD Tx
HLA 6/6 MM
1st graft
No pre-Tx DSA
Negative PRA

Early mixed rejection

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SPK DD Tx
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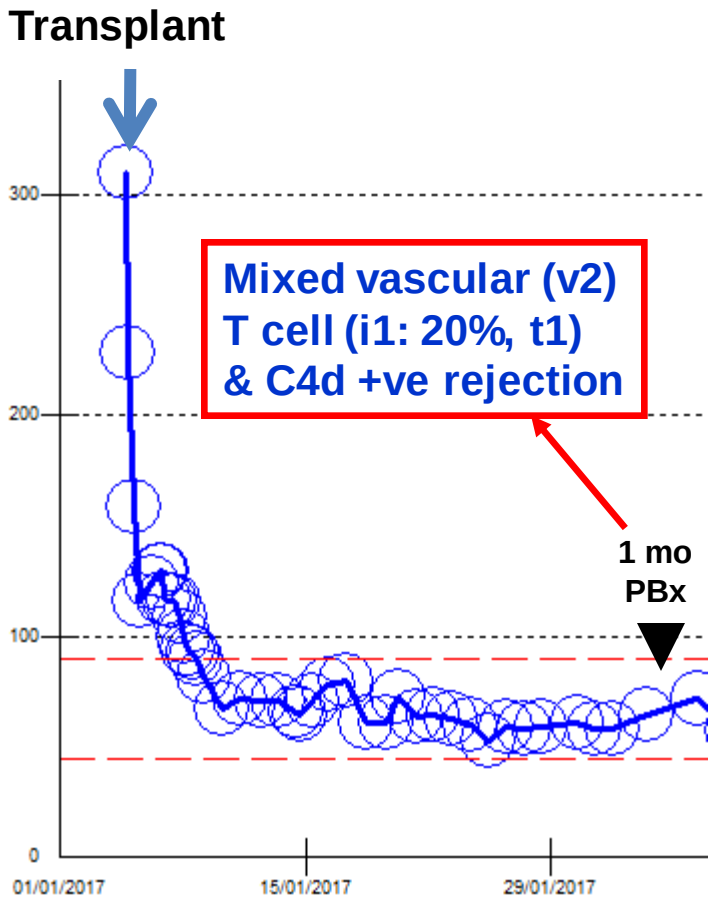
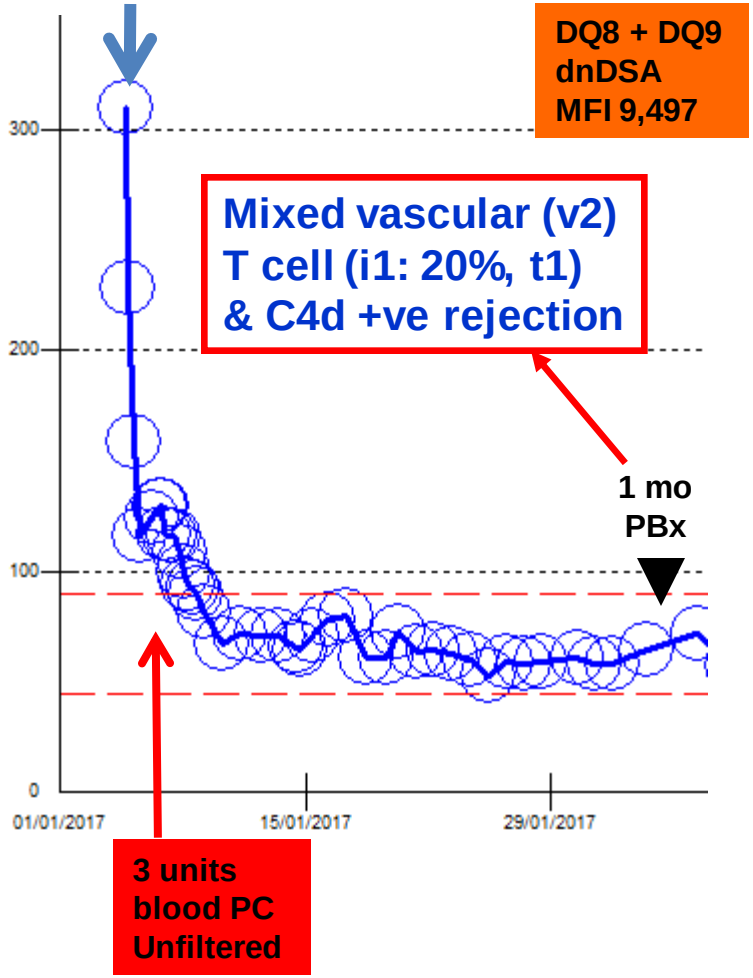
S. Creatinine (umol/L)

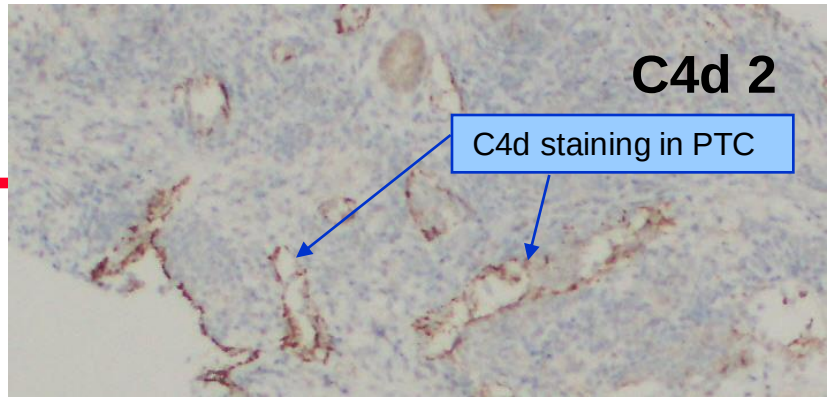


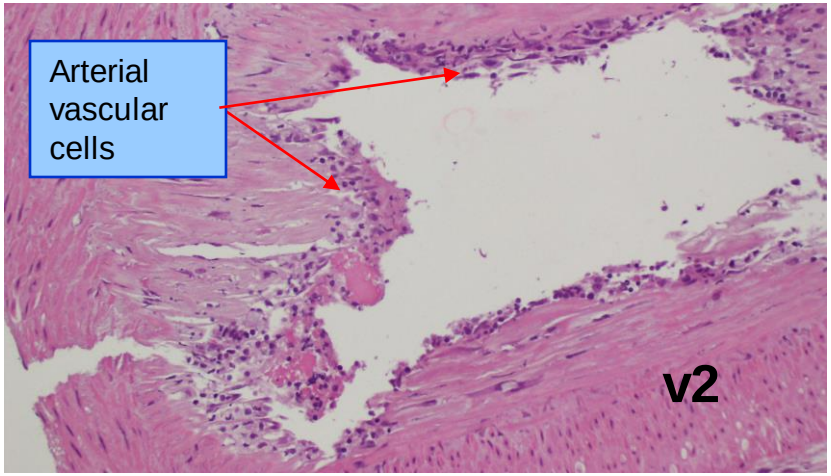
Early mixed rejection

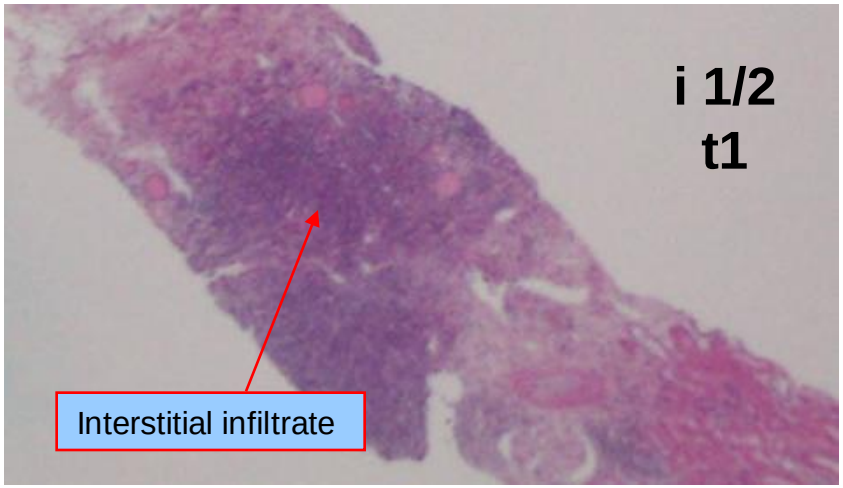
S. Creatinine (umol/L)

Transplant









Early mixed rejection

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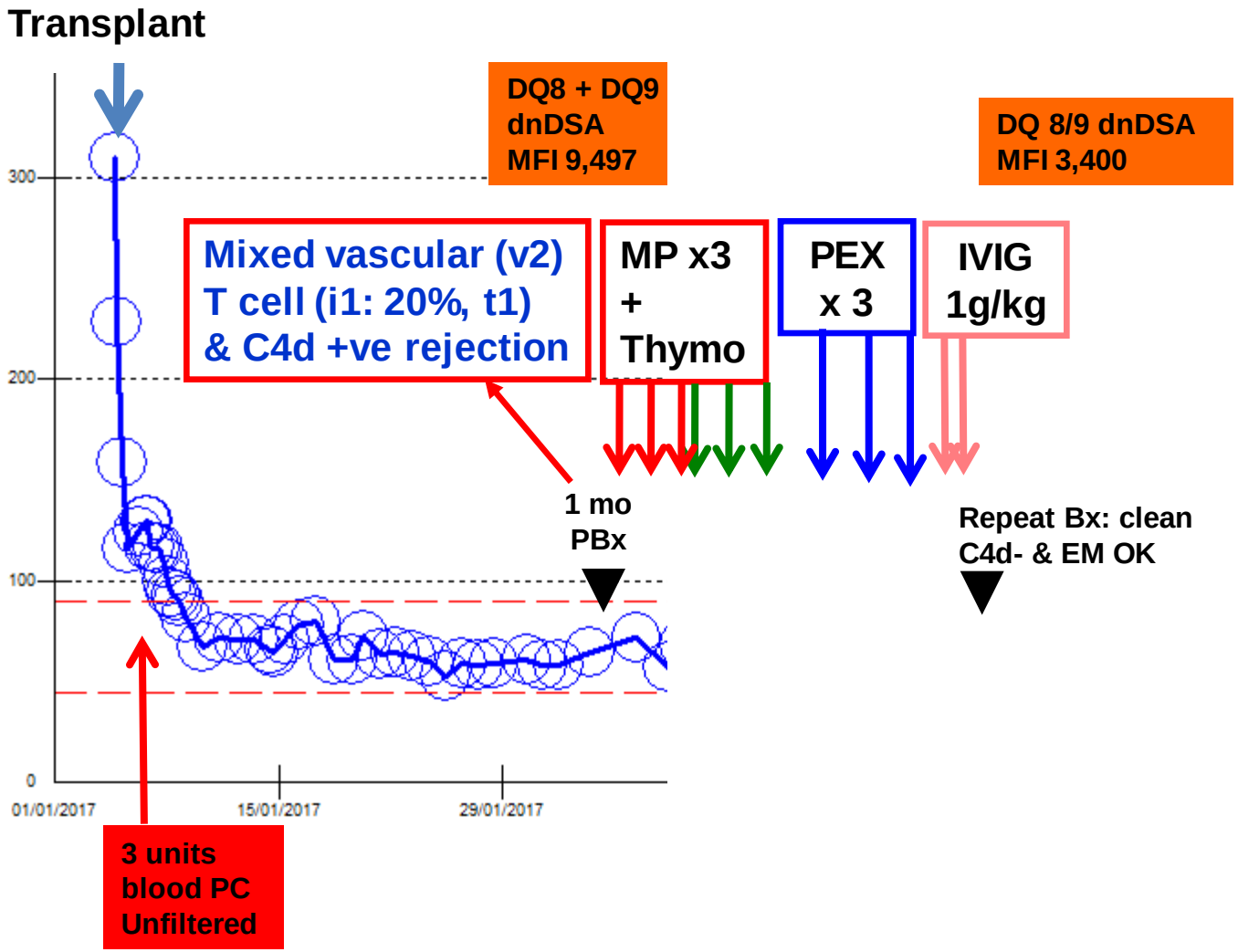
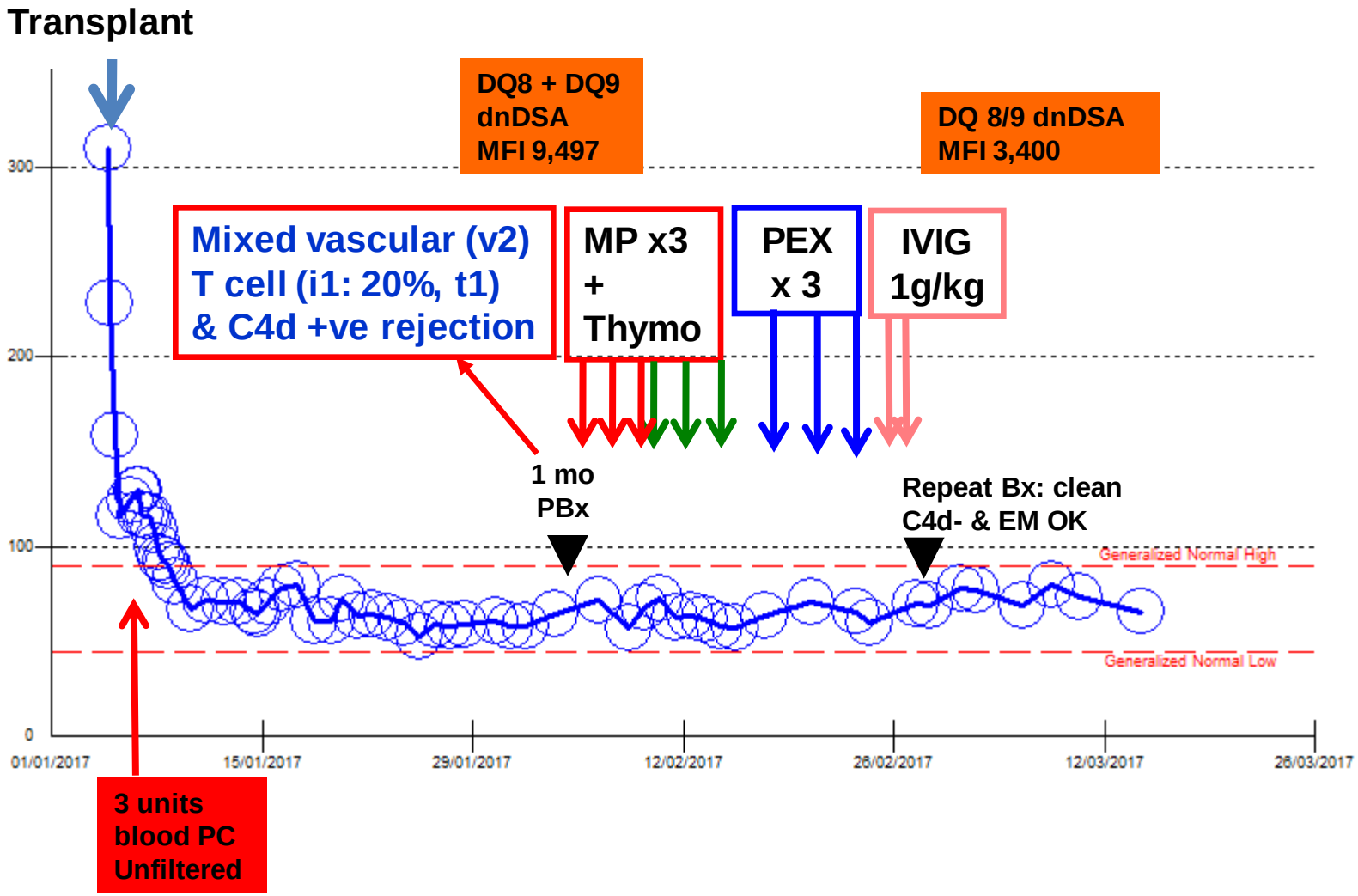


Early mixed rejection: sensitisation

34 y.o. female
SPK DD Tx
HLA 6/6 MM
1st graft
No pre-Tx DSA
Negative PRA

S. Creatinine (umol/L)



Acute rejection phenotypes & outcome

Timing: important for reversibility

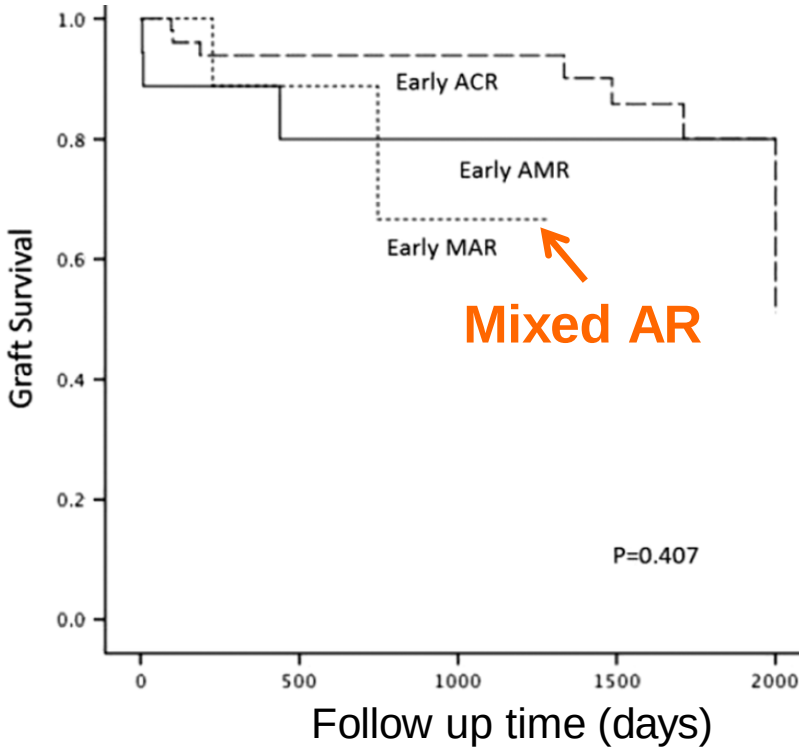
Phenotype:

- affects late AR outcomes

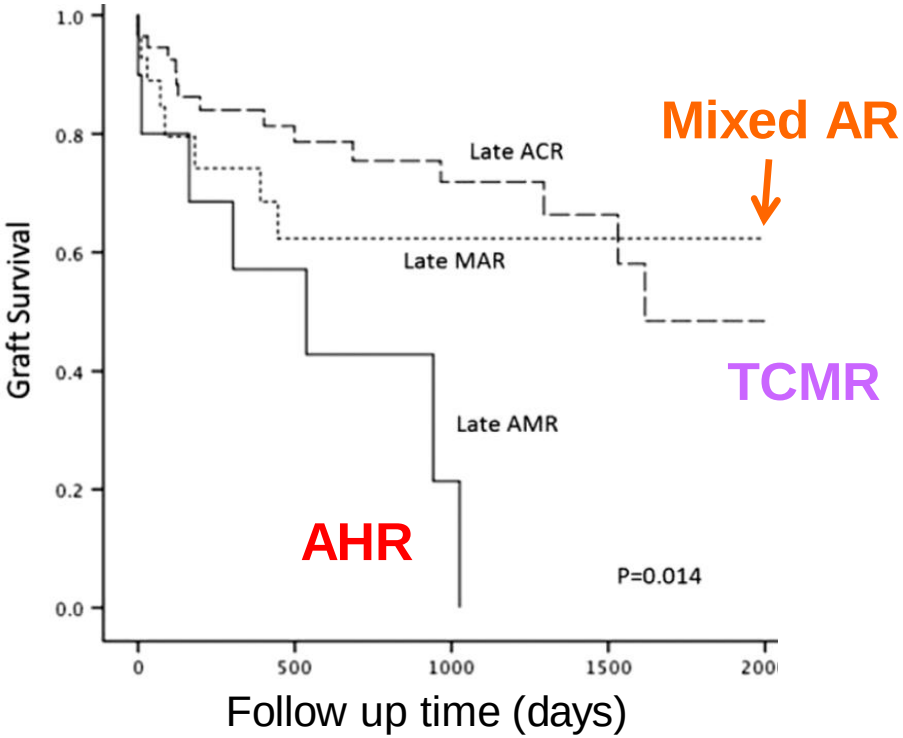
Retrospective cohort study
Single centre, Cincinnati
n=182 biopsy-proven first AR episodes
Histological AR types vs DCGS

Krisl et al Transplant 2014

Early acute rejection



Late rejection: Bad



1. T cell mediated rejection: treatments

Methyprednisolone 500mg IV x 3

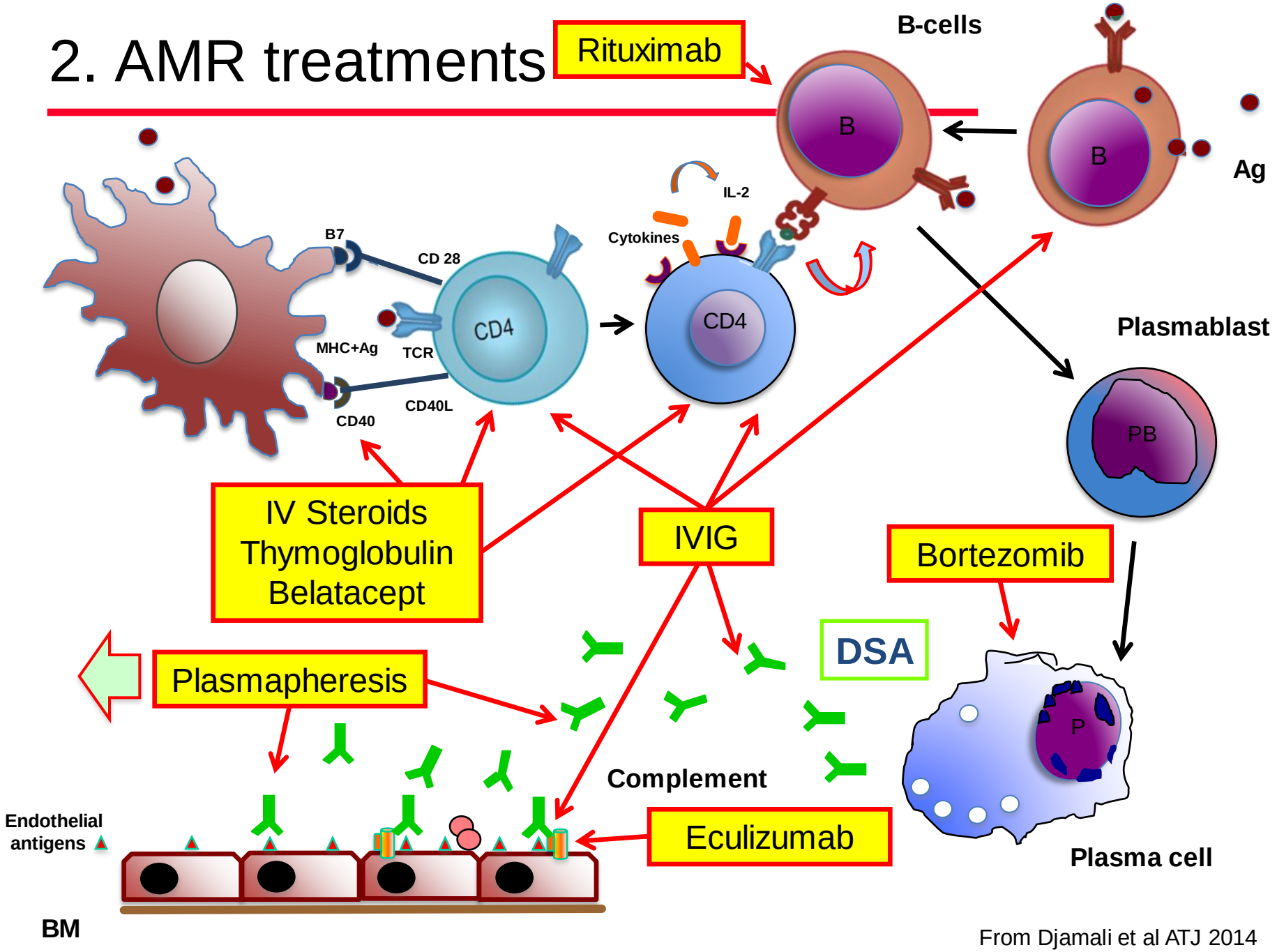
- monitor serum creatinine

Lymphocyte depleting agent

- anti-thymocyte globulin “ATG” or OKT3
- anti-infective prophylaxis (CMV, fungal, PJP)

- Follow up biopsy for clearance

2. AMR treatments



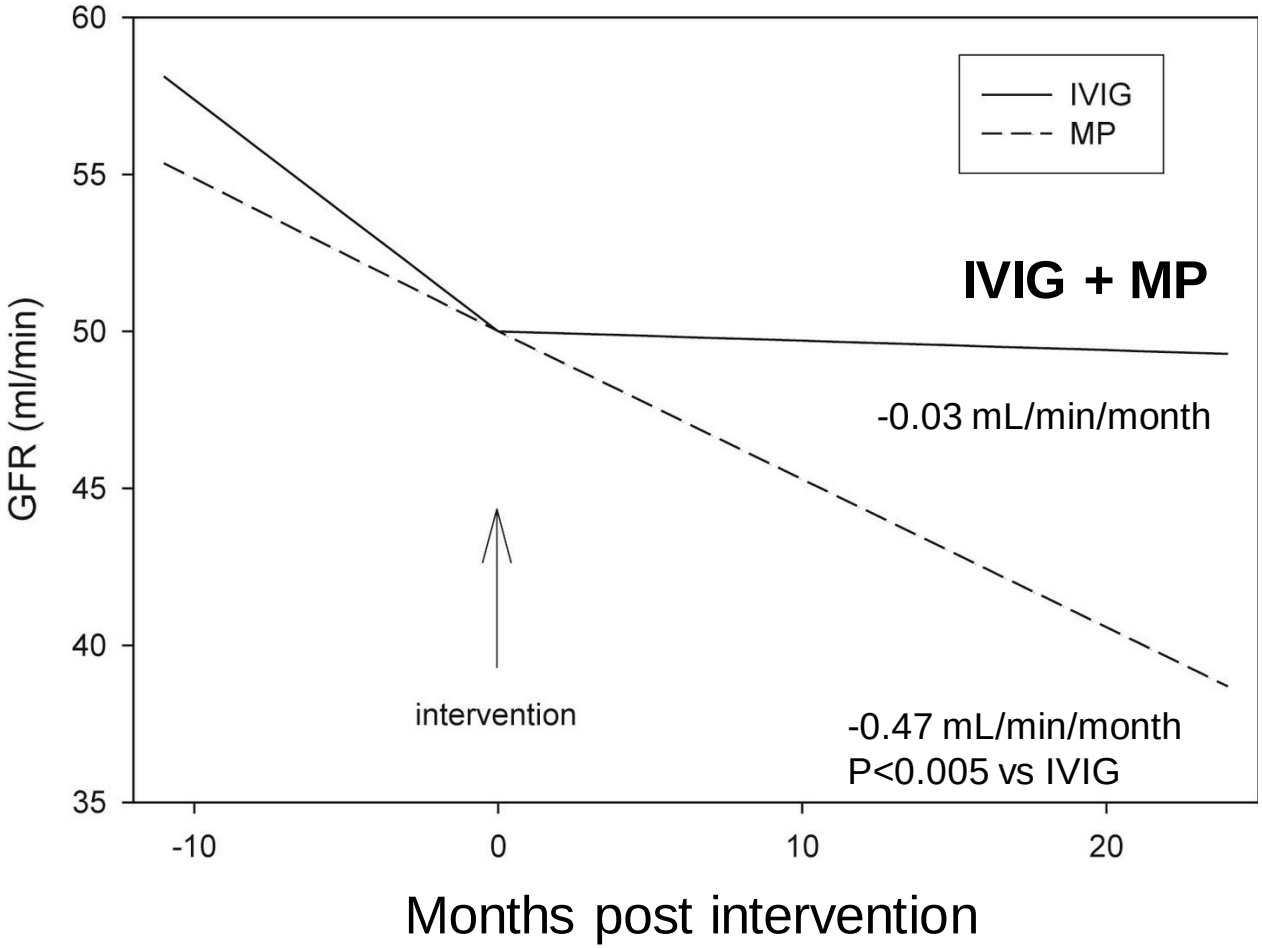
From Djamali et al ATJ 2014

Treatment of AMR: quality of evidence

Therapy	Action	Evidence base / Benefit	
			GRADE system
IV Steroids	↓ DSA / T cells	V low	unknown
Plasmapheresis	↓ DSA titre	low	inconsistent
Immunoadsorption	↓ DSA titre	low	benefit
IVIG	DSA modulation	V. low	¼ with benefit
Rituximab	RCT ac. AHR	low	no benefit as add on
	small cohort studies with some benefit		
Bortezomib	plasma cell	low	benefit
Eculizumab	Complement	low	no clear benefit
Anti-thymocyte agents	helper T	V. low	not proven
MMF	↓ T & B cells	V. low	v low, may help
DSG/ cyclophosphamide / splenectomy		V. low	v low, may help

Roberts et al Transplant 2012

IVIG for late onset AMR



Change in MDRD eGFR

Less in IVIG group

IV pulse Methyprednisolone

Biopsy-proven AMR, n=39
Retrospective analysis, single centre
non-randomised & different entry non-comliiance pts
MP vs MP+IVIG: 1-3 g/kg 2 days

Furmańczyk-Zawiska et al T Proc 2016

Antibody-mediated vascular rejection

Treatment:

- pulse IV corticosteroids
- plasma exchange
- IV immunoglobulin (+ rituximab)

	Number of patients	Number of events	Hazard ratio (95% CI)	p value
Treatment strategy				
Steroids and intravenous immune globulin	13	7	1	..
Steroids plus muromonab-CD3 or rabbit antithymocyte globulin	29	11	0·4 (0·2–1·3)	0·1
Steroids, plasmapheresis, intravenous immune globulin, and rituximab	22	3	0·16 (0·04–0·66)	0·01

Lefaucheur et al, Lancet 2012

Rituximab for AMR: RITUX ERAH study

Treatment early AMR

- 500mg MP IV x3 then 1mg/kg/d PO tapered
- 3x PE first 5 days + IVIG (100mg/kg post PP)
- 1x Rituximab 375mg/m^2 IV vs. placebo

Results

- No difference in functional recovery & graft loss

Multi-centre, Double-blind RCT, France
Prospective Phase III, Tx. 38 Kidney Tx
Inclusion: Bx proven early AMR
Intervention: Day 5 Ritux 375m2 vs. Placebo (1:1)
All with PE, IVIG, steroid pulse as SOC
Composite endpoint:
graft loss & failed functional recovery day 12

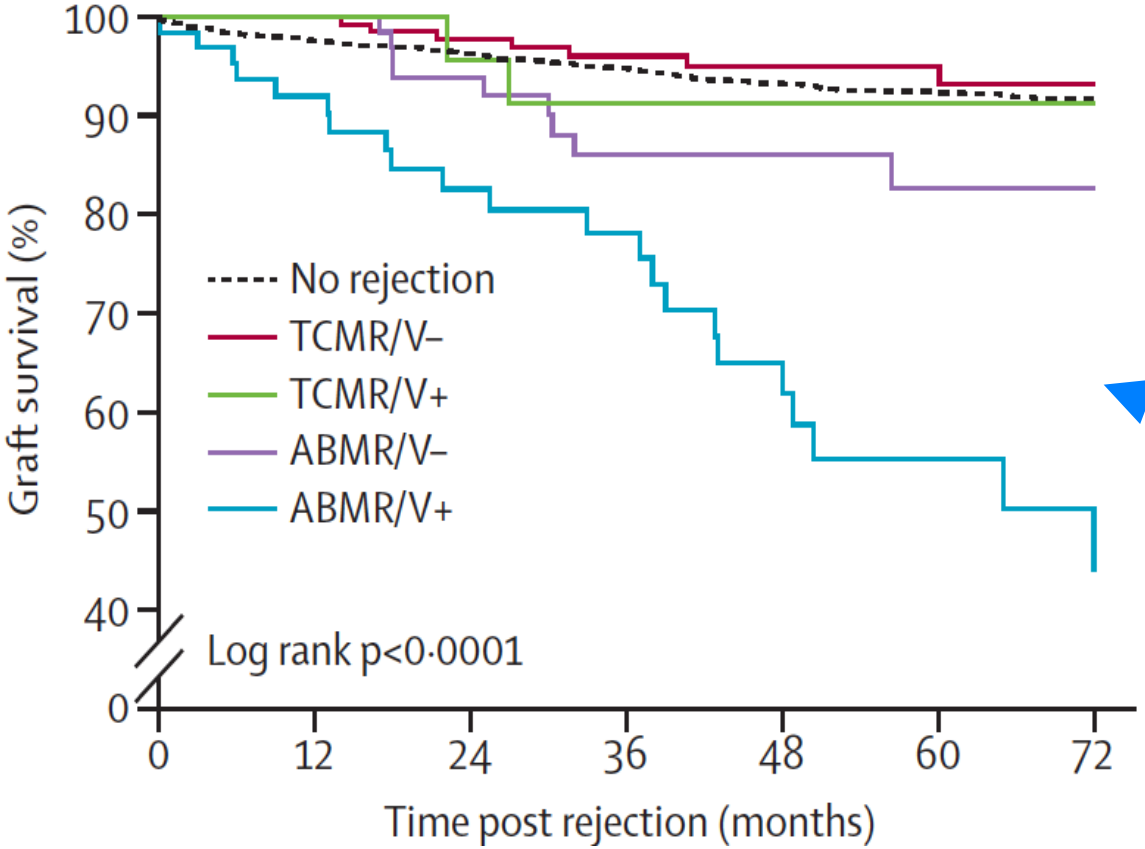
Endpoint
52.6% vs 57.9%

Sautenet et al Transplant 2016

Antibody-mediated vascular rejection

Vascular rejection with DSA

- 21% of all acute rejections: steroid resistant
- Onset early at 1.1 months



Outcome poor:

6 year survival: 50.3%

Predicted by:

- arteritis grade
- DSA MFI level
- T cell infiltrate

Lefaucheur et al Lancet 2013

Late mixed rejection: Case 2

39 y.o male
LRD Mother
HLA 3/6 mismatch
1st graft
No pre-Tx DSA
Low dose CSA + EVR + Pred: RCT

Late mixed rejection

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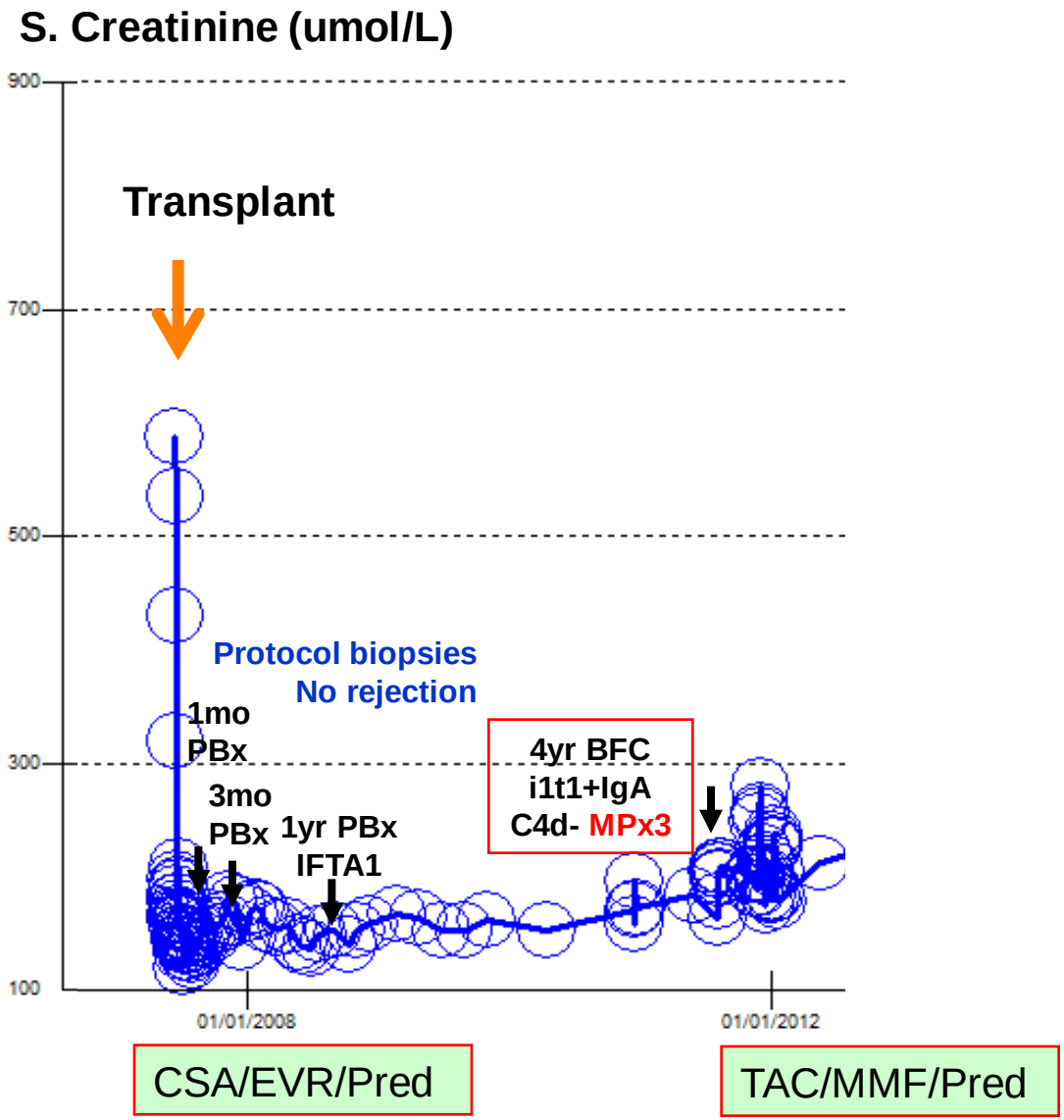
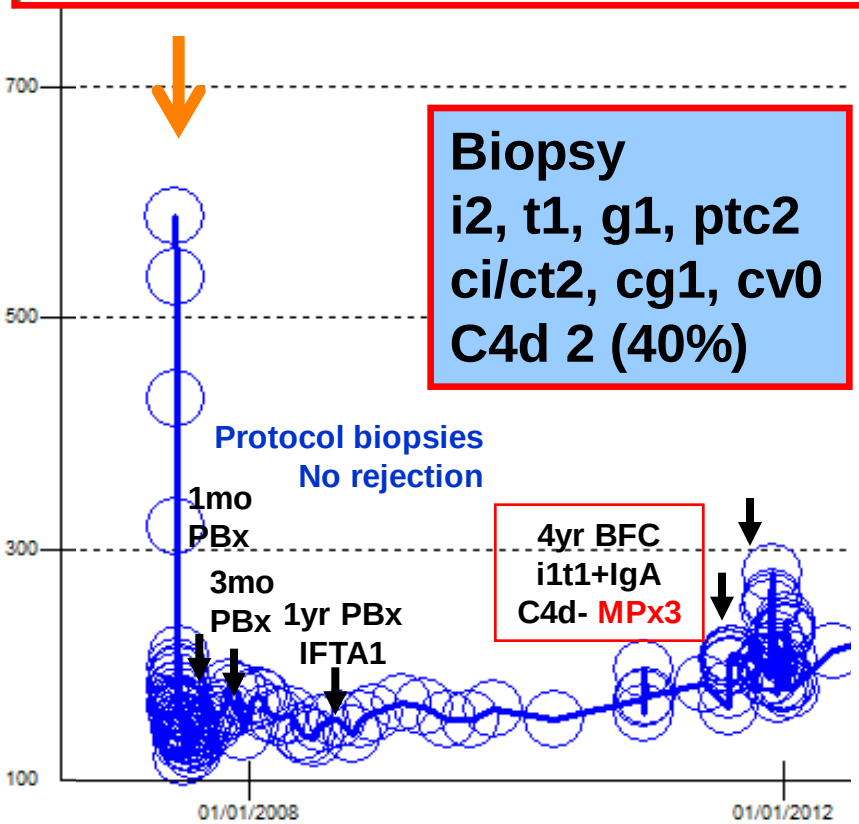


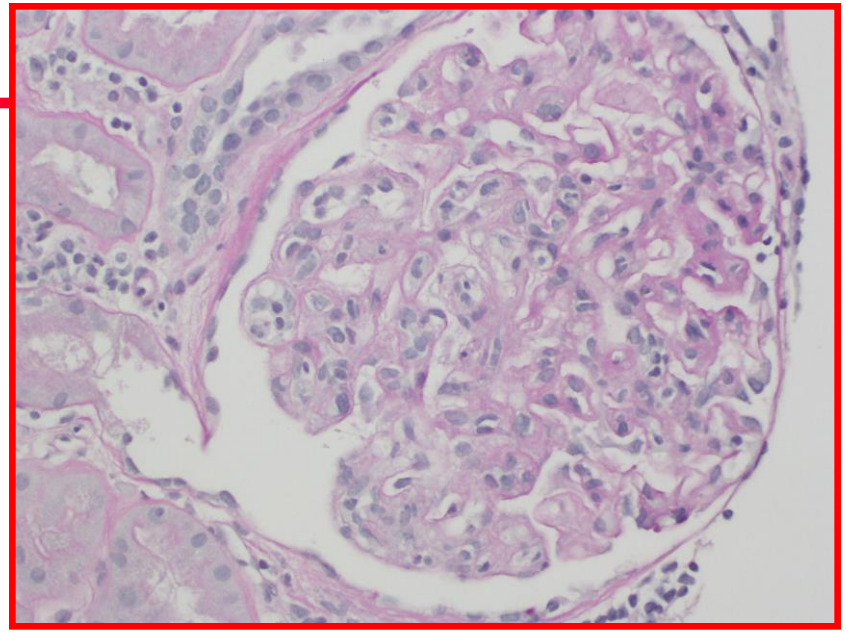
Late mixed rejection

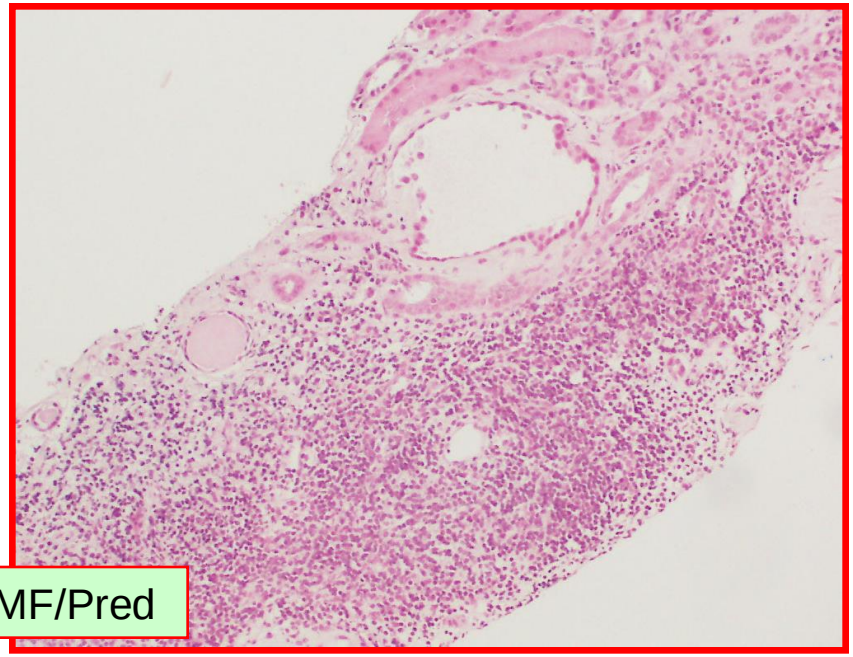
Addendum Diagnosis

RENAL TRANSPLANT BIOPSY(>4 YEARS):
EQUIVOCAL POSITIVITY FOR C4D STAIN
NEGATIVE BK VIRUS STAIN



Biopsy
i2, t1, g1, ptc2
ci/ct2, cg1, cv0
C4d 2 (40%)





CSA/EVR/Pred

TAC/MMF/Pred

Late mixed rejection: iatrogenic

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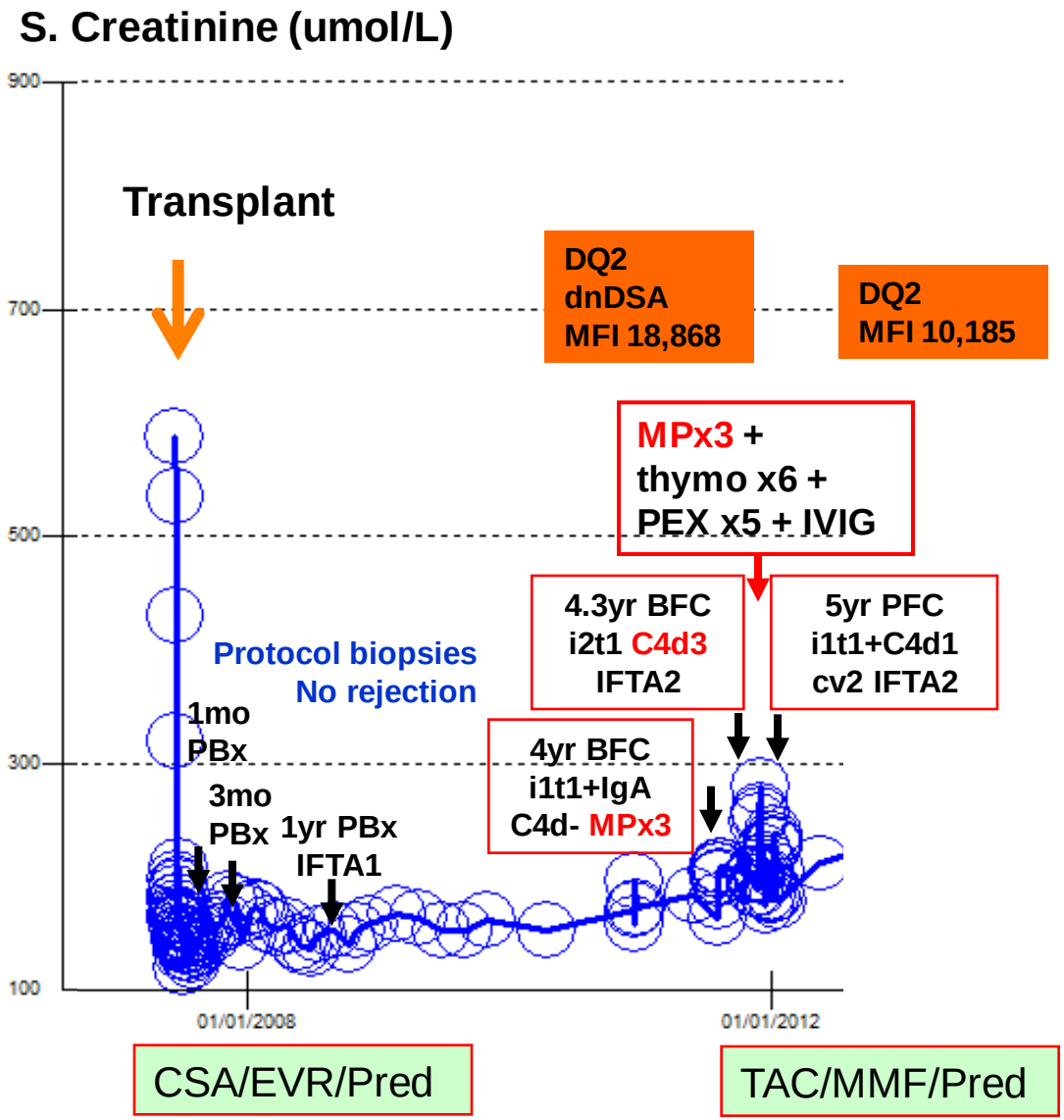
LRD Mother
HLA 3/6 mismatch
1st graft
No pre-Tx DSA
Low dose CSA + EVR + Pred: RCT



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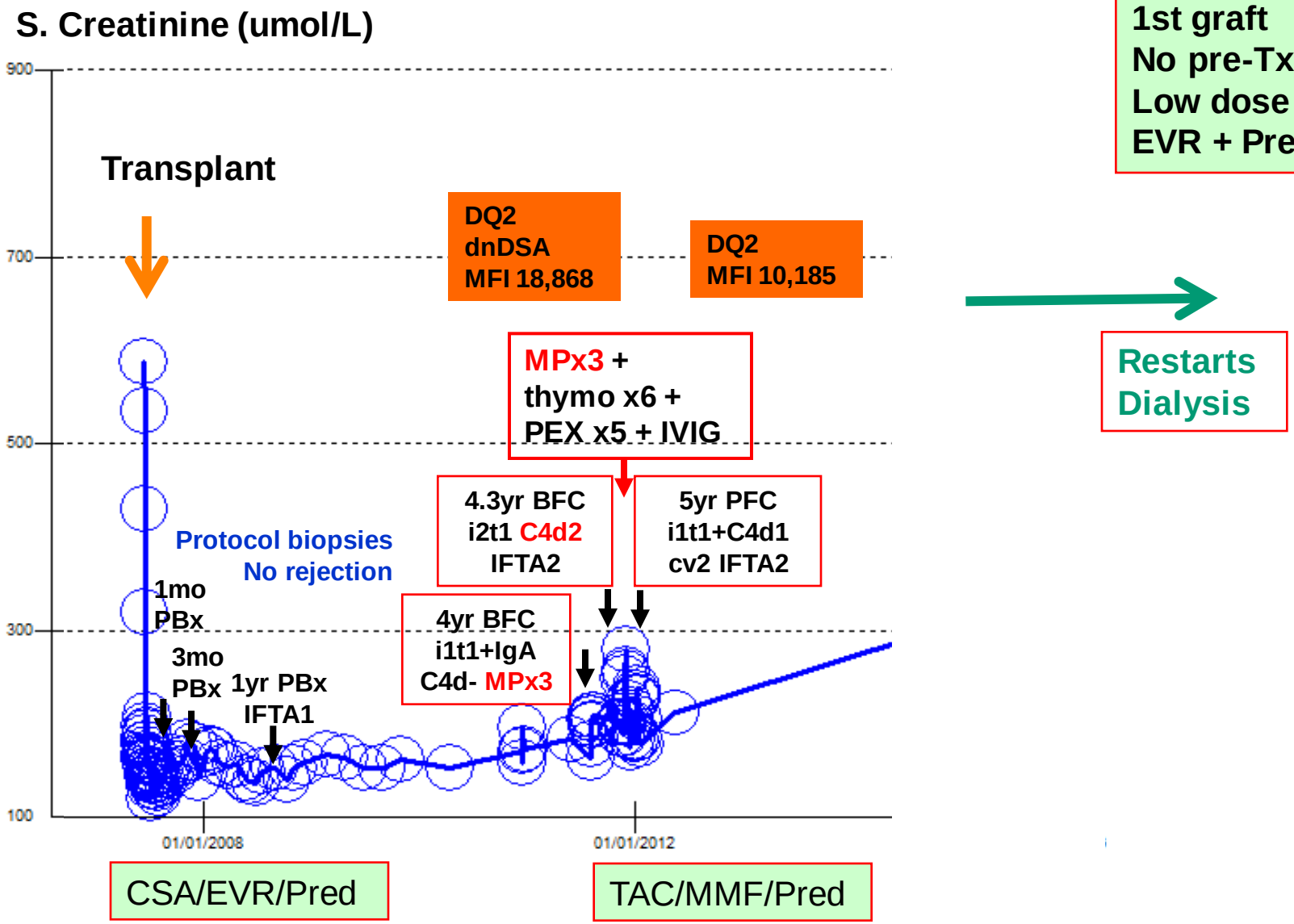


Iatrogenic under immunosuppression

Severe acute illness / ICU / overwhelming sepsis

- immunosuppression stopped except steroids

Life threatening sub-acute chronic infection

e.g. abdominal sepsis, bronchiectasis, fungal etc

BKVAN – late rejection with reduced IS

Cancer - localized or metastatic

Scheduled minimization or withdrawal of ISD meds

Medication side effects

Medication side effects

Things that patients experience from meds

Tacrolimus

- tremor
- NODAT / IGT

MMF

- diarrhoea
- nausea bloating

Prednisolone

- weight gain / thin skin

Image: indiamart.com

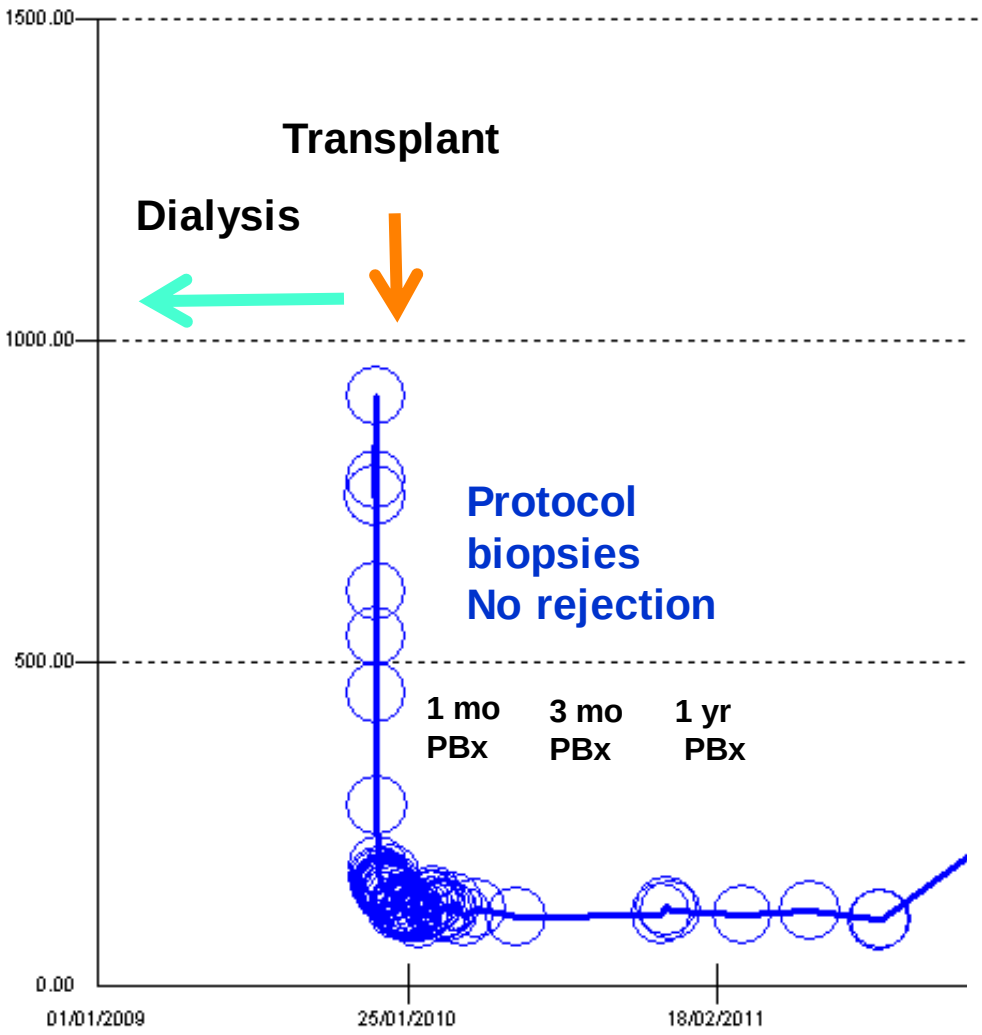
Late graft failure: Case 3

25 y.o. male
LRD brother
HLA 3/6 mismatch
1st graft
No pre-Tx DSA
Negative PRA

Late graft failure: non-compliance

25 y.o. male
LRD brother
HLA 3/6 mismatch
1st graft
No pre-Tx DSA
Negative PRA

S. Creatinine (umol/L)



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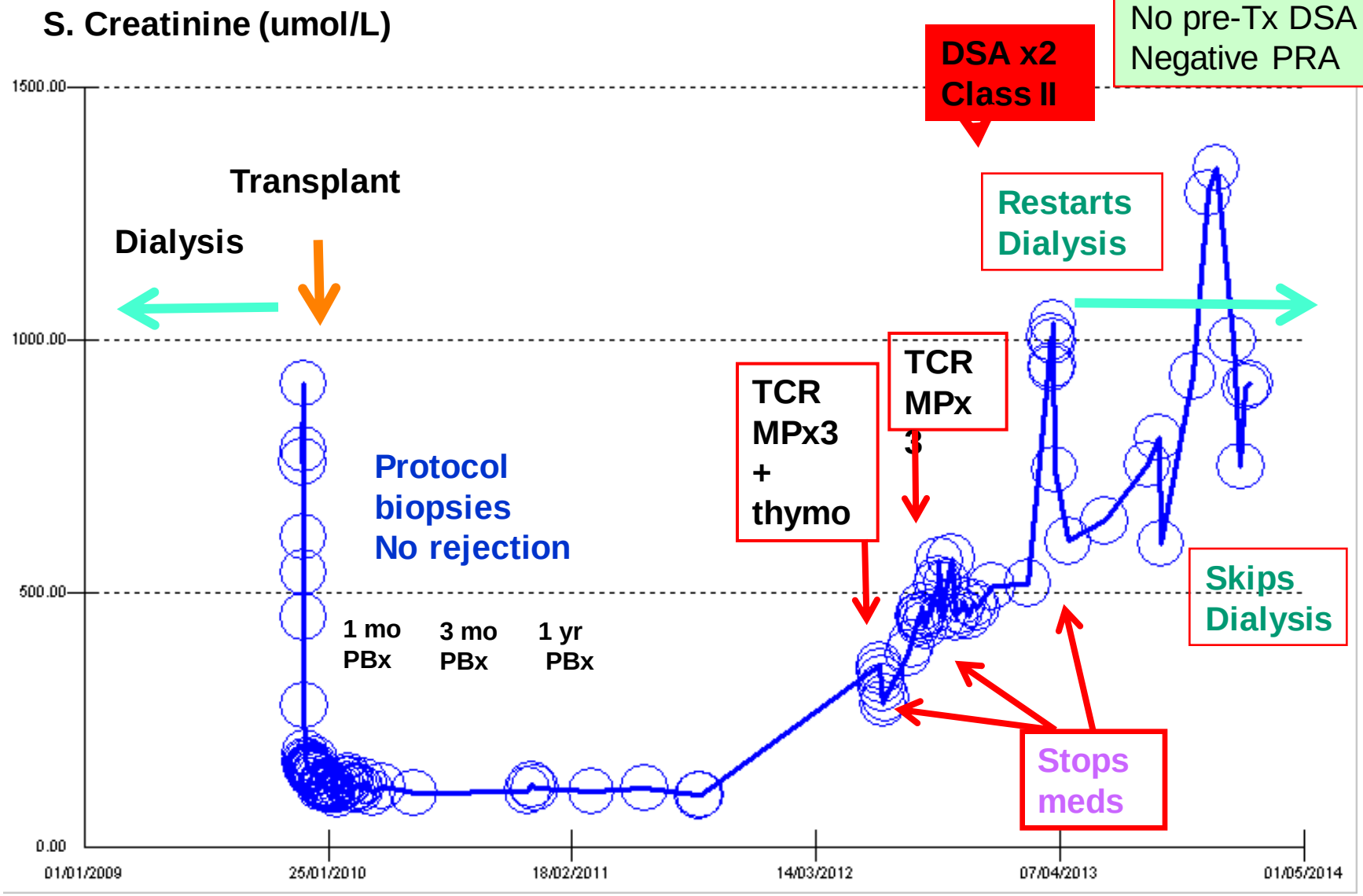
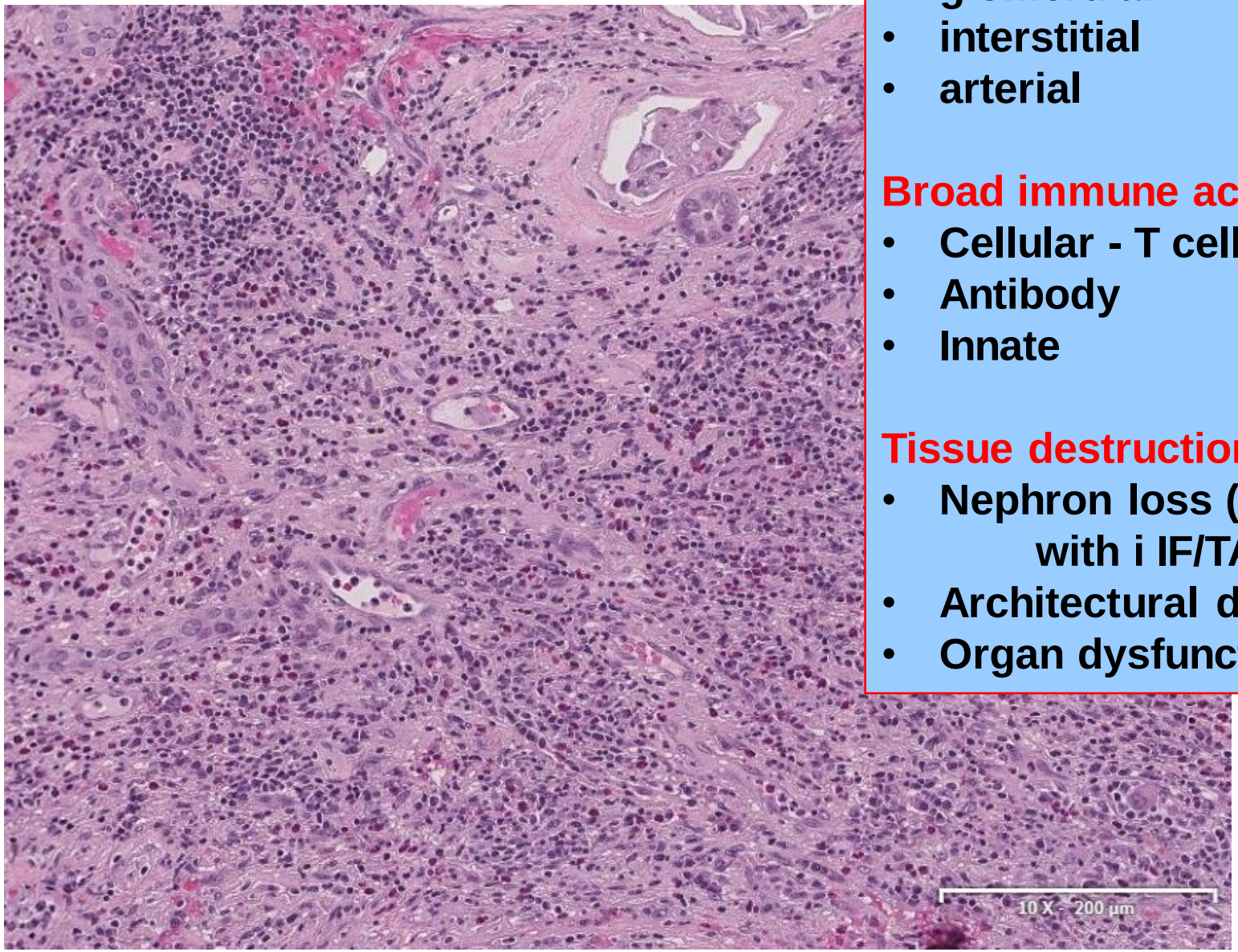
No pre-Tx DSA
Negative PRA



Late graft failure: Features



Widespread inflammation

- **microcirculation**
- **glomerular**
- **interstitial**
- **arterial**

Broad immune activation

- **Cellular - T cells etc.**
- **Antibody**
- **Innate**

Tissue destruction

- **Nephron loss (IF/TA)**
  with i IF/TA
- **Architectural distortion**
- **Organ dysfunction**

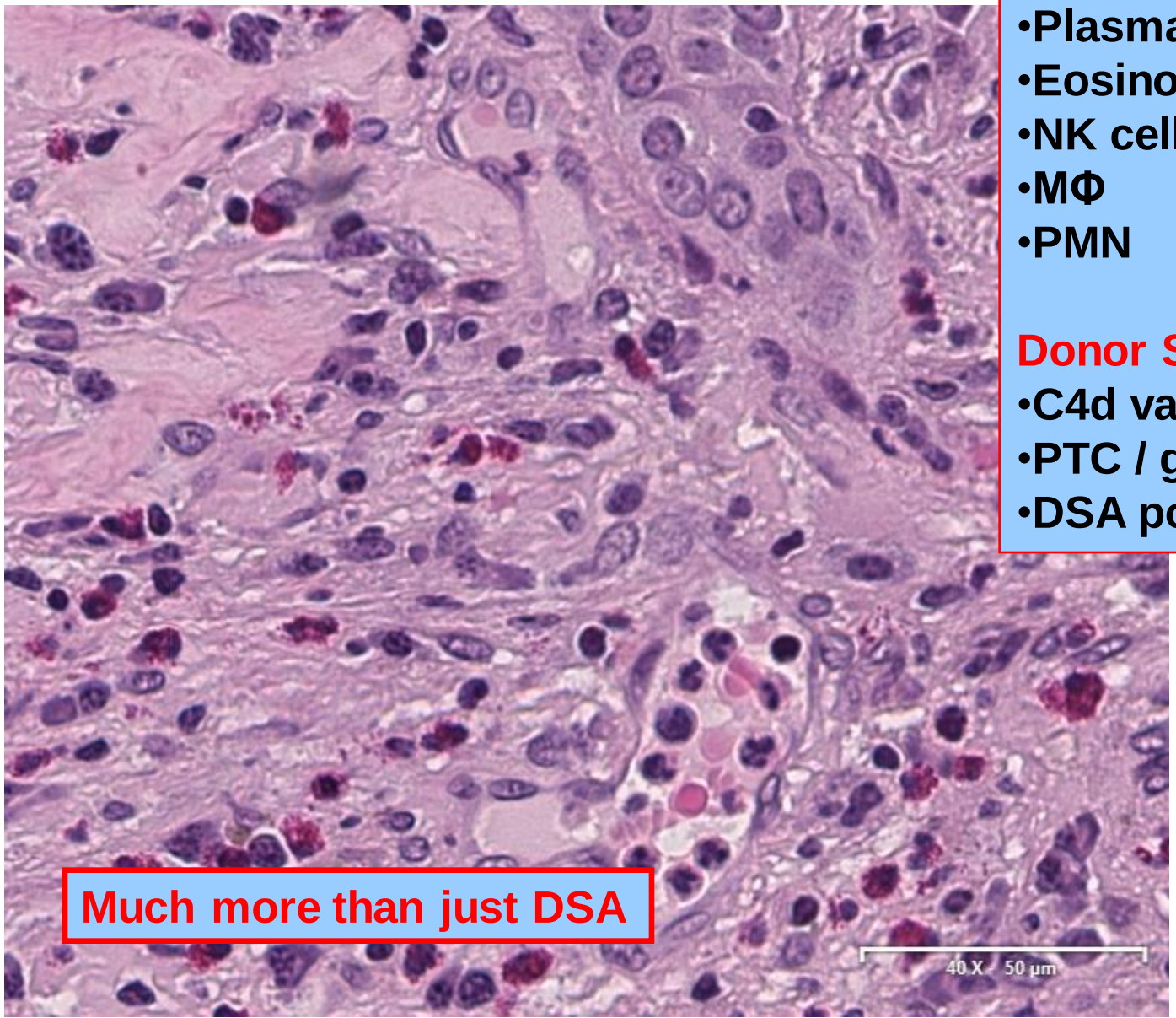
Late graft failure: Features

Widespread inflammation

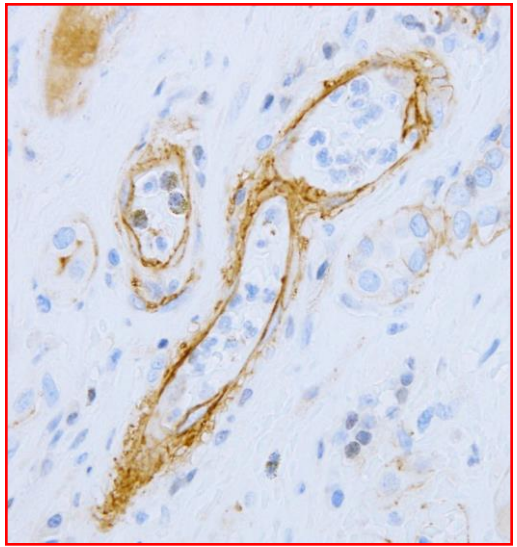
- **T cells**
- **B cells**
- **Plasma cells**
- **Eosinophils**
- **NK cells**
- **MΦ**
- **PMN**

Donor Specific Antibody

- **C4d variable positive**
- **PTC / glomeruli**
- **DSA positive in 30%**



Much more than just DSA



Why do patients stop immunosuppression ?

Why do patients stop immunosuppression ?

All happy families are alike:
each unhappy family is unhappy in its own way

Leo Tolstoy 1873
Anna Karenina

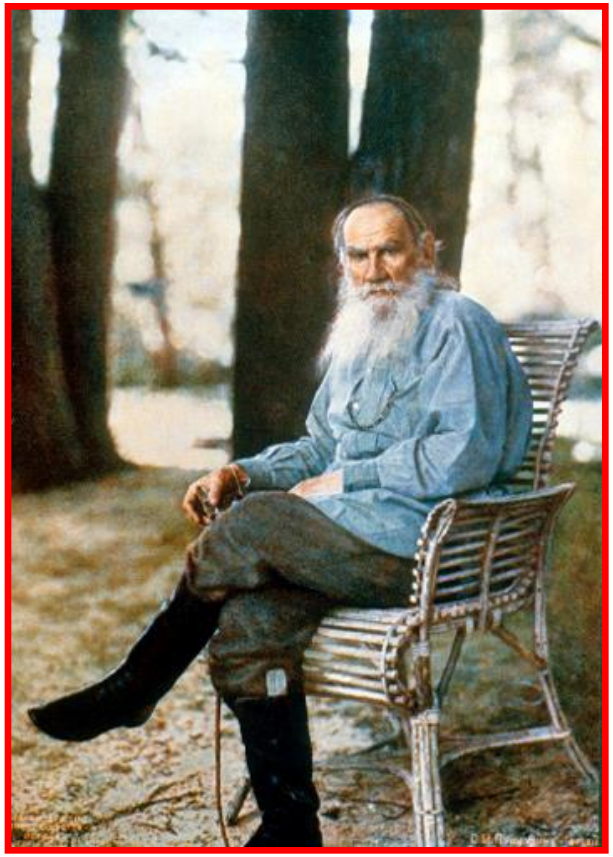
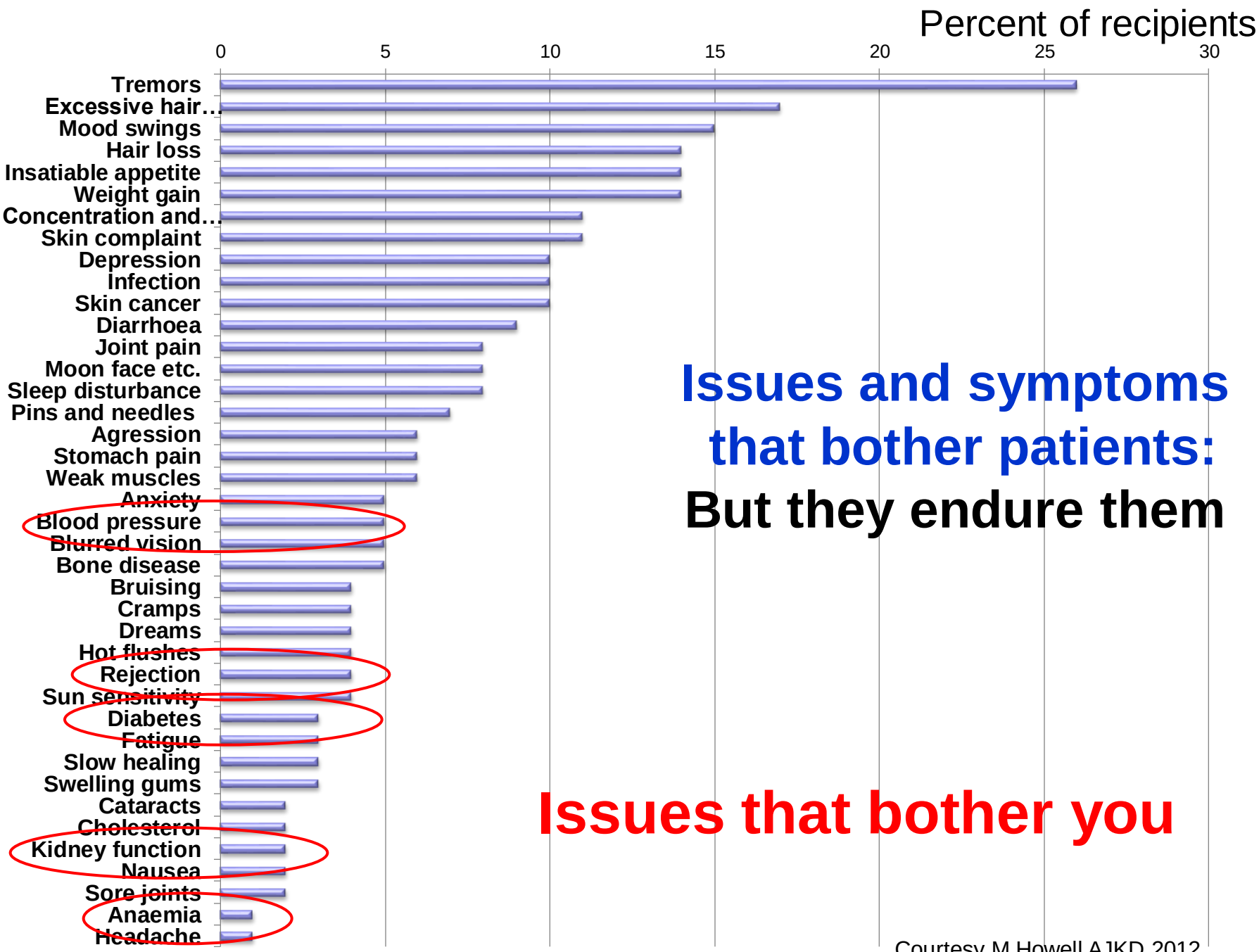


Photo: Sergey Prokudin-Gorsky (from wiki)

Under immunosuppression: Non-compliance

- Situational stress: Divorce, relationship breakup etc.
- Adolescent / young adults: feel normal & stop, want to "be normal" and not have an illness
- Shift workers - forget evening dose
- Take "Drug holiday"
- Living recipients: manipulation of donor
- Financial – cannot afford meds
- Medication confusion, elderly, dementia, forgetfulness
- Consult naturopath, friends & stop
- Don't know why they stop

Medication side effects: Steroids, MMF & CNI



Courtesy M Howell AJKD 2012

Non compliance

Prevalence 23-50%

Late rejection losses

Clues:

- missed follow-up
- differences - prescribed vs dispensed meds
- fluctuating or undetectable CNI levels
- unexpected acute rejection episode

Questions

- non-threatening: listen carefully to answers
- side effects & situational questions

Preventive strategies for adherence

1. Education:

- individualized
- repeated
- by a specialized transplant pharmacist

2. Minimisation of:

- drug side effects
- medication complexity
- costs

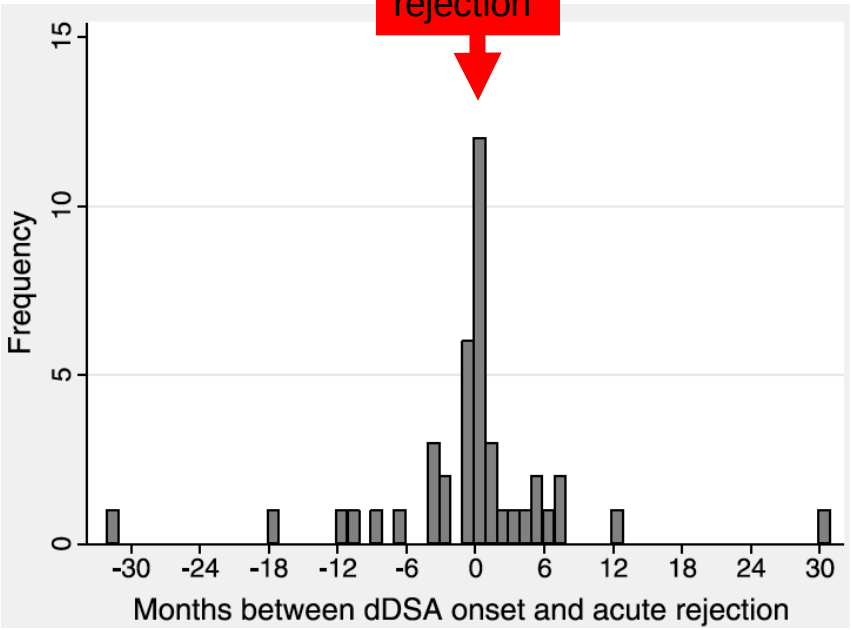
3. Regular clinical support

Patients need to take their meds regularly

Many dnDSA detected with acute rejection

Onset of DSA (median mo.)

Acute rejection



DeVos et al Trans 2014
Cooper et al Transplant 2011
Everly et al Transplant 2013
Wiebe et al AJT 2012

Risk of *de novo* DSA

Deceased-donor recipients

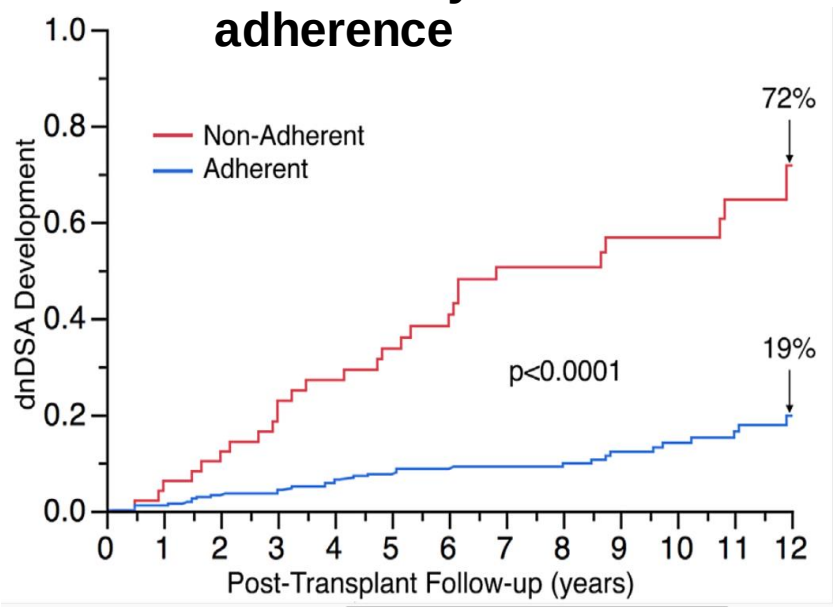
Pre-transplantation HLA (non-DSA) antibodies
Prior cellular rejection
African American
SPK
Any class II mismatch / HLA mismatch
DQ mismatch
HLA-DRβ1 MM > 0

Young / pediatric recipients
Non-adherence

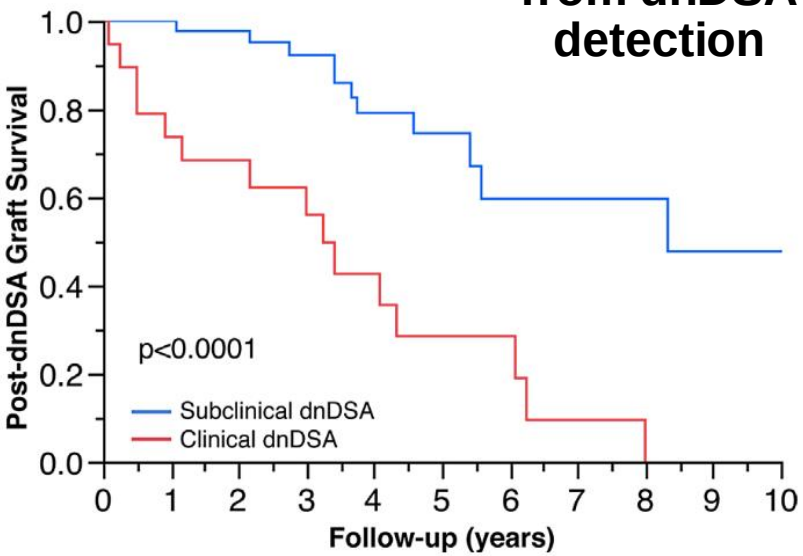
Data from prospective studies

dnDSA follows non-adherence

1. dnDSA by adherence



2. Graft failure from dnDSA detection



Follows subclinical TCMR & non-adherence

Constitutes a continuum of mixed alloimmune-mediated injury

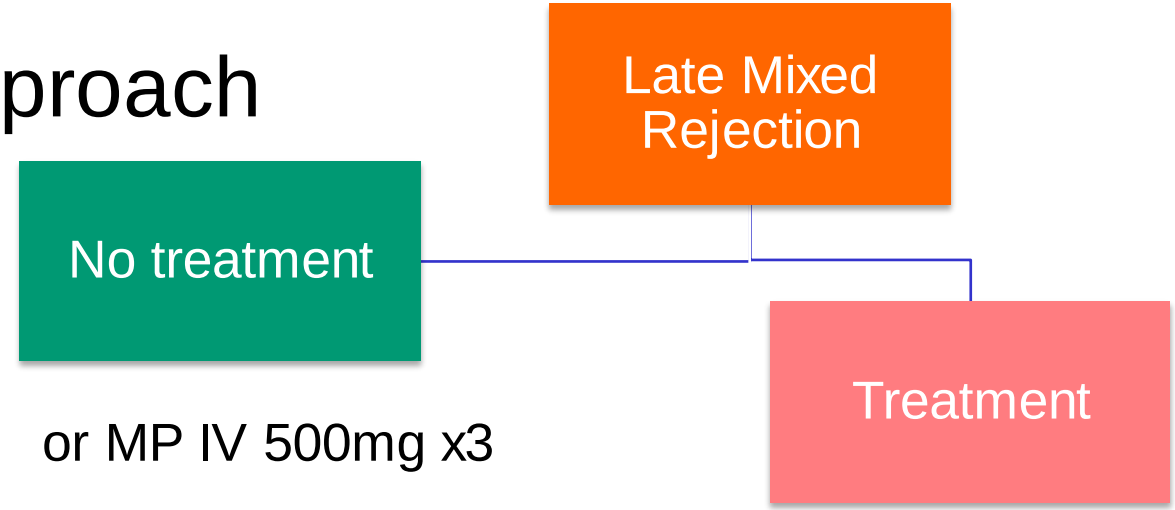
Wiebe et al AJT 2015

Single centre prospective observational cohort study
Tertiary referral centre, Winnipeg
Adult & pediatric
N=508 Kid Tx

Approach

Late Mixed Rejection

Approach



or MP IV 500mg x3

Assessment of late acute rejection

TRANSPLANTED KIDNEY

1. Acute rejection by dominant immunophenotype

- TCMR vs. AMR vs. Mixed: by Banff histology + i-IFTA

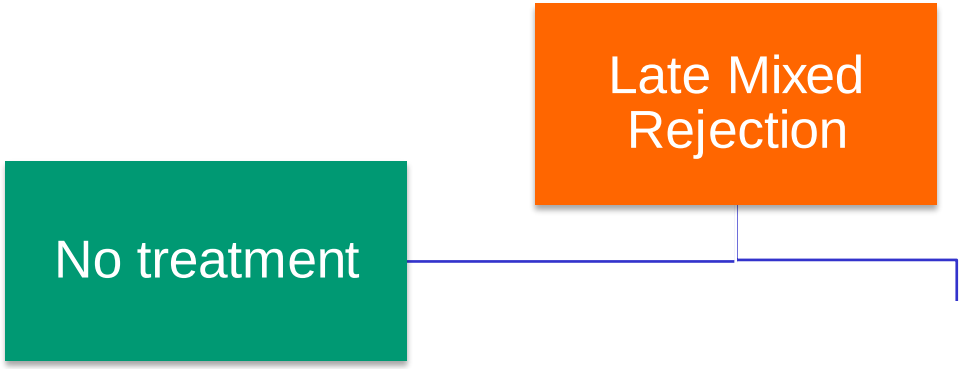
2. Humoral markers: C4d (ptc & glomerular IHC), DSA, EM

- prognosis & treatment priority

3. Scarring functional graft recovery: trichrome stain

PATIENT

- age, fitness, frailty, comorbidity
- intercurrent illness: cancer, infection, BKVAN etc
- compliance, access, social situation
- dialysis options



1. Unsuitable kidney
- unsalvageable kidney: futility
- IF/TA score 2/3 + inflammation

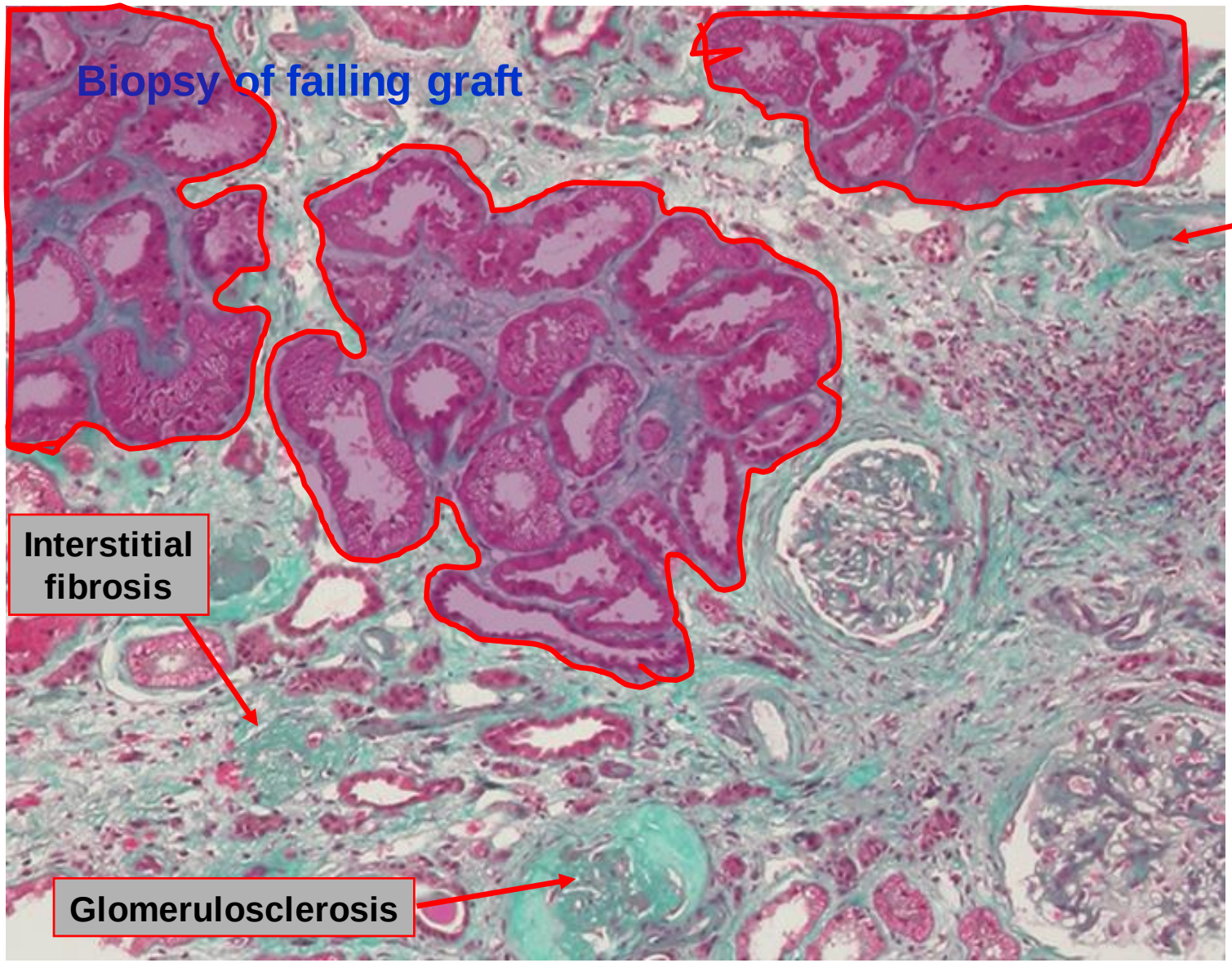
2. Unsuitable patient
- non-compliance / refusal
- frail, elderly, unwell, cardiovascular

3. Circumstances of reduced IS
- severe infection / ongoing sepsis
- metastatic cancer / PTLD

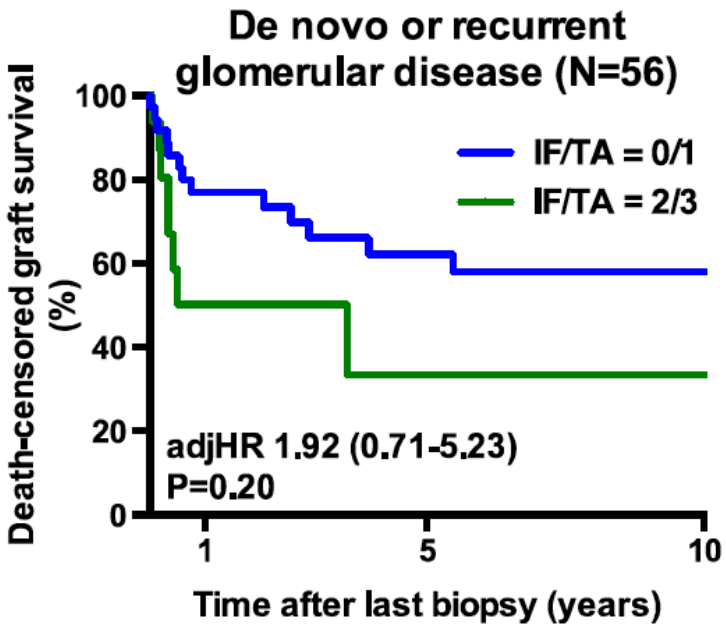
Overall:

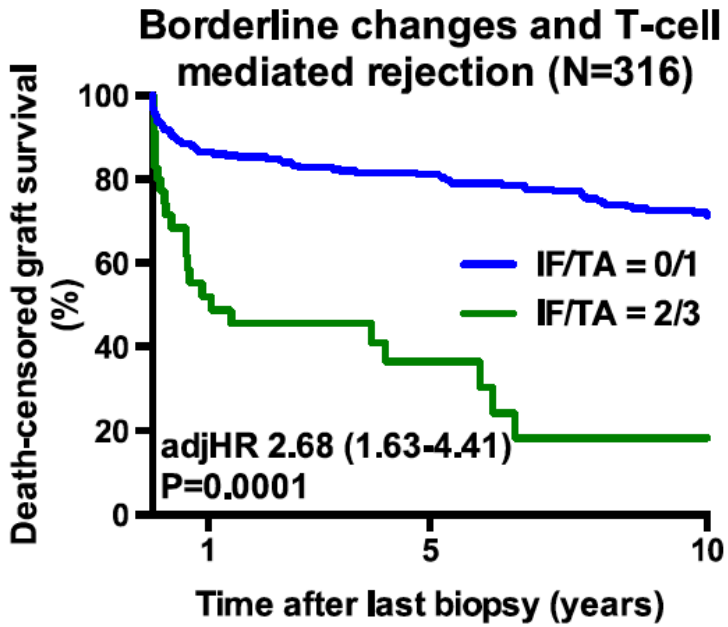
- Little benefit
- High risk of complications

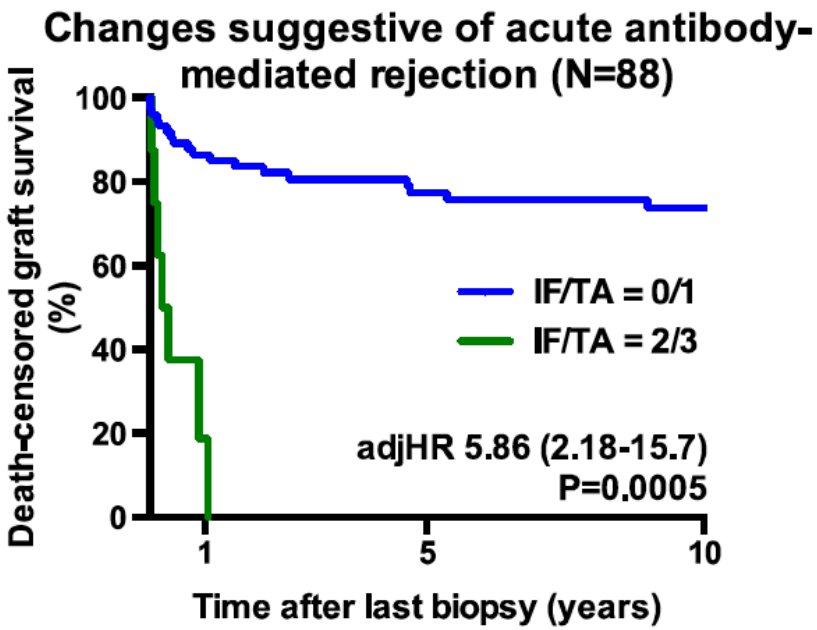
Severe IFTA limits treatment options

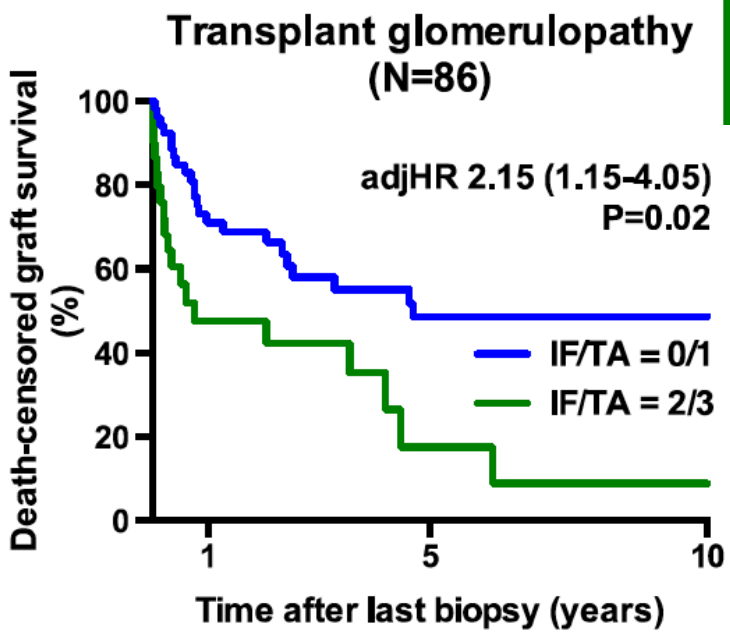


ASSESS
Functional nephrons remaining









IF/TA "CAN" still matters

N=1197 Kid Tx
PBx & BFC
N=1365
351 graft losses
FU 14.5+/-2.8 years

Naesens et al JASN 2013

Treatment

Late Mixed Rejection

No treatment

Approach: sequential therapy

Treat: TCMR dominant

500mg IV MPx3

+ Thymoglobulin 1.5mg/kg x 4-6

Treat: AMR dominant

500mg IV MPx3
+ PEX 2 weeks+
+ IVIG low dose 0.1mg/kg post PP
+/- rituximab 375m^2

Bortezomib
Eculizumab
Tocilizumab

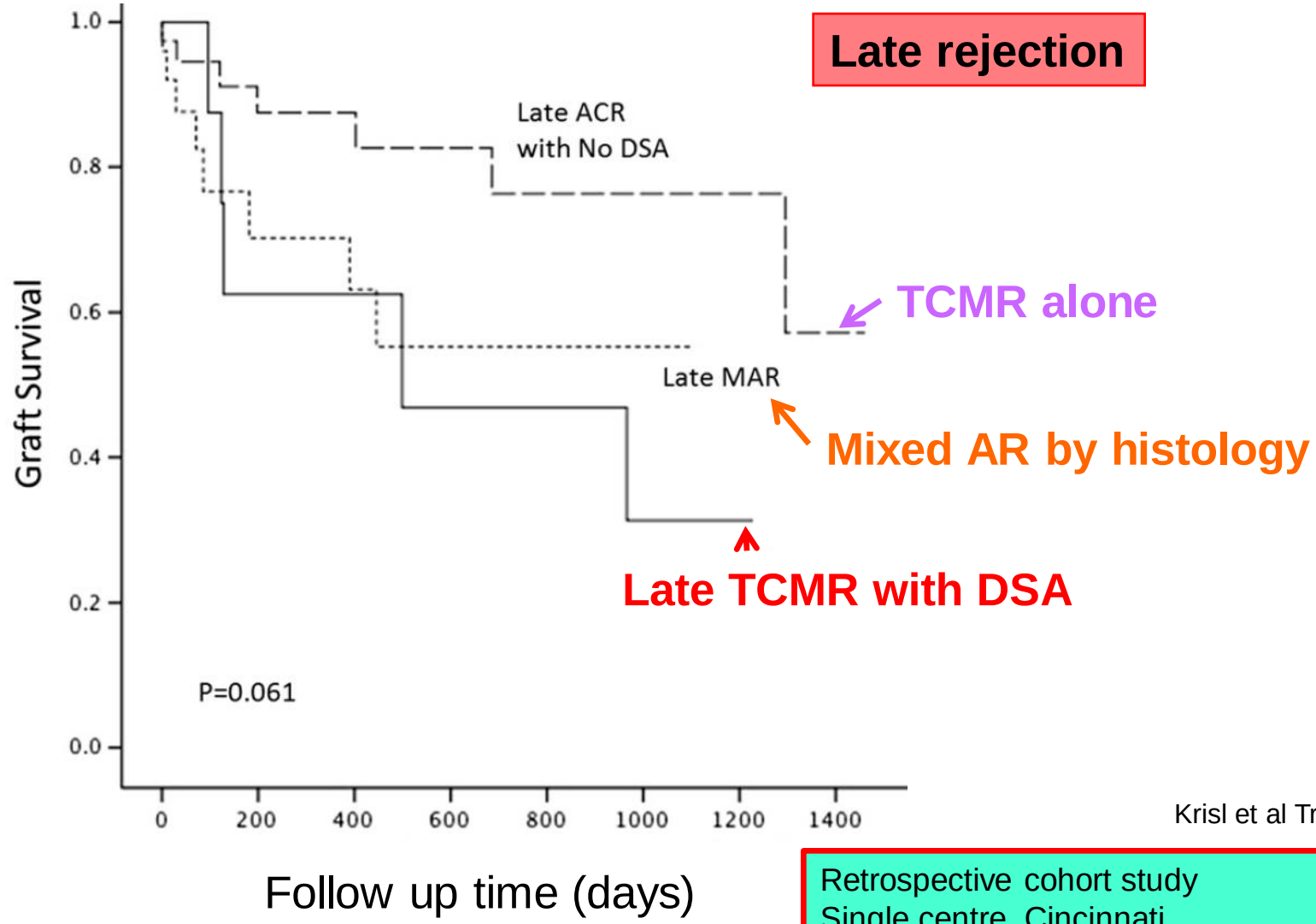
Mixed Treat: Sequential

500mg IV MPx3
+ Thymoglobulin 1.5mg/kg x 3-4

+ PEX 1 to 2 weeks

+ IVIG high-dose
Total 1-2g/kg divided

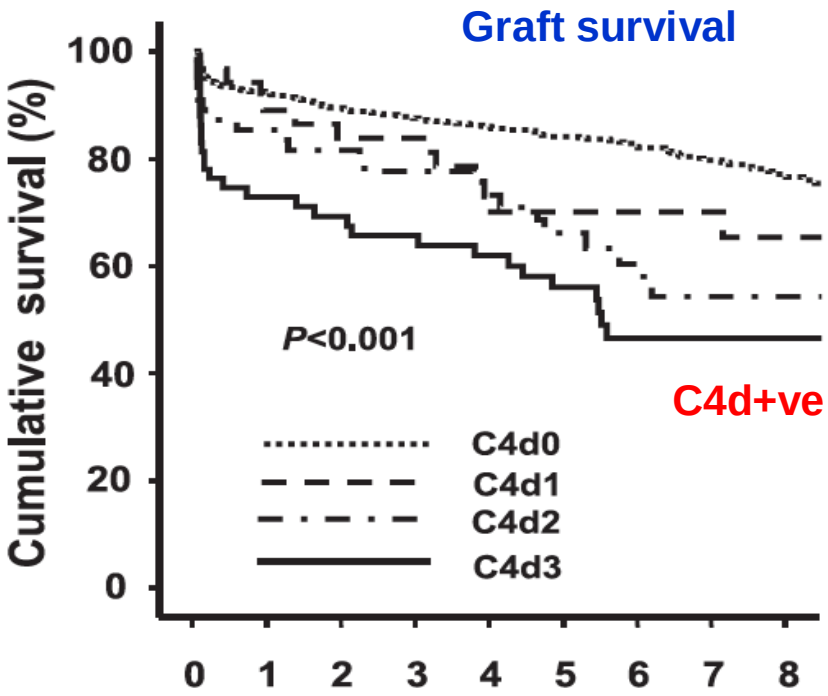
TCMR with any humoral marker is worse

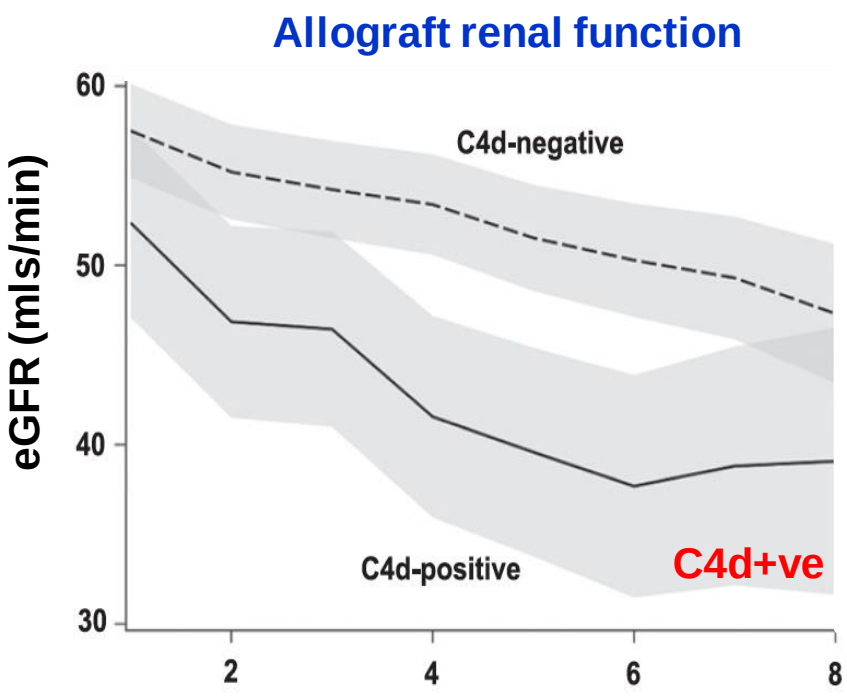


Krisl et al Transplant 2014

Retrospective cohort study
Single centre, Cincinnati
n=182 biopsy-proven first AR episodes
Histological classification of AR types vs DCGS

Capillary C4d+: Worse outcomes





Independent risk of graft failure

C4d+	HR=1.85 (1.34-2.57)	P<0.001
AMR*	HR=1.91 (1.38-2.66)	P<0.001

Retrospective clinico-pathologic study, n=885 kidney Tx biopsy (of 1248 transplanted)
Single centre, Vienna,
C4d by IHC vs g, ptc, cg

*AMR on biopsy

Kikic et al CJASN 2015

Late Mixed Rejection

No treatment

Approach: sequential therapy

Treat: TCMR dominant

500mg IV MPx3

+ Thymoglobulin 1.5mg/kg x 4-6

Treat: AMR dominant

500mg IV MP
+ PEX 2 weeks+
+ IVIG low dose 0.1mg/kg post PP
+/- rituximab 375m^2

Bortezomib
Eculizumab
Tocilizumab

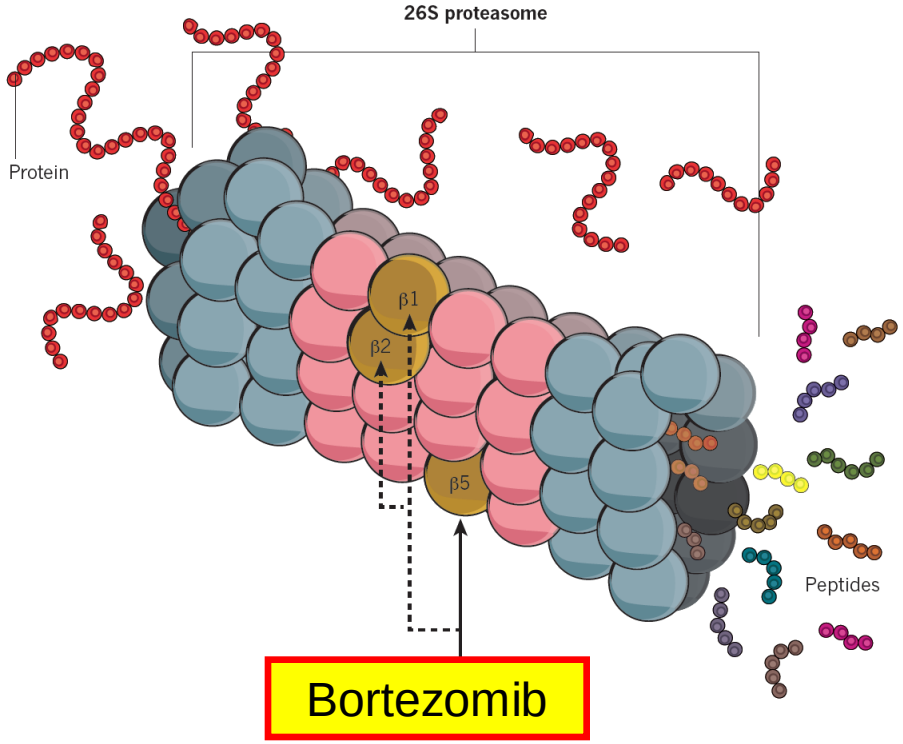
Mixed Treat: Sequential

500mg IV MPx3
+ Thymoglobulin 1.5mg/kg x 3-4

+ PEX 1 to 2 weeks

+ IVIG high-dose Total 1-2g/kg divided

Bortezomib inhibits 26S proteasome



Function of proteasome:
•degrades unneeded, damaged or misfolded proteins

RC Walsh et al Transplant 2011
SM Flechner et al Transplant 2010
Woodle et al AJT 2011 (abs 428)
Gupta at al transplant 2014

Mechanisms

- Failed clearance of misfolded proteins
- ER accumulation & stress
- plasma cell apoptosis

From Appel Nature 2011

Use in late AHR

1.3mg/m^2 2nd wk IV x 4

with PE±IVIG± Rit± rATG

Results

Lower iDSA (50%)	35-40%
Better FU biopsy	54-58%
eGFR stabilized	53.8%

The long lived plasma cell

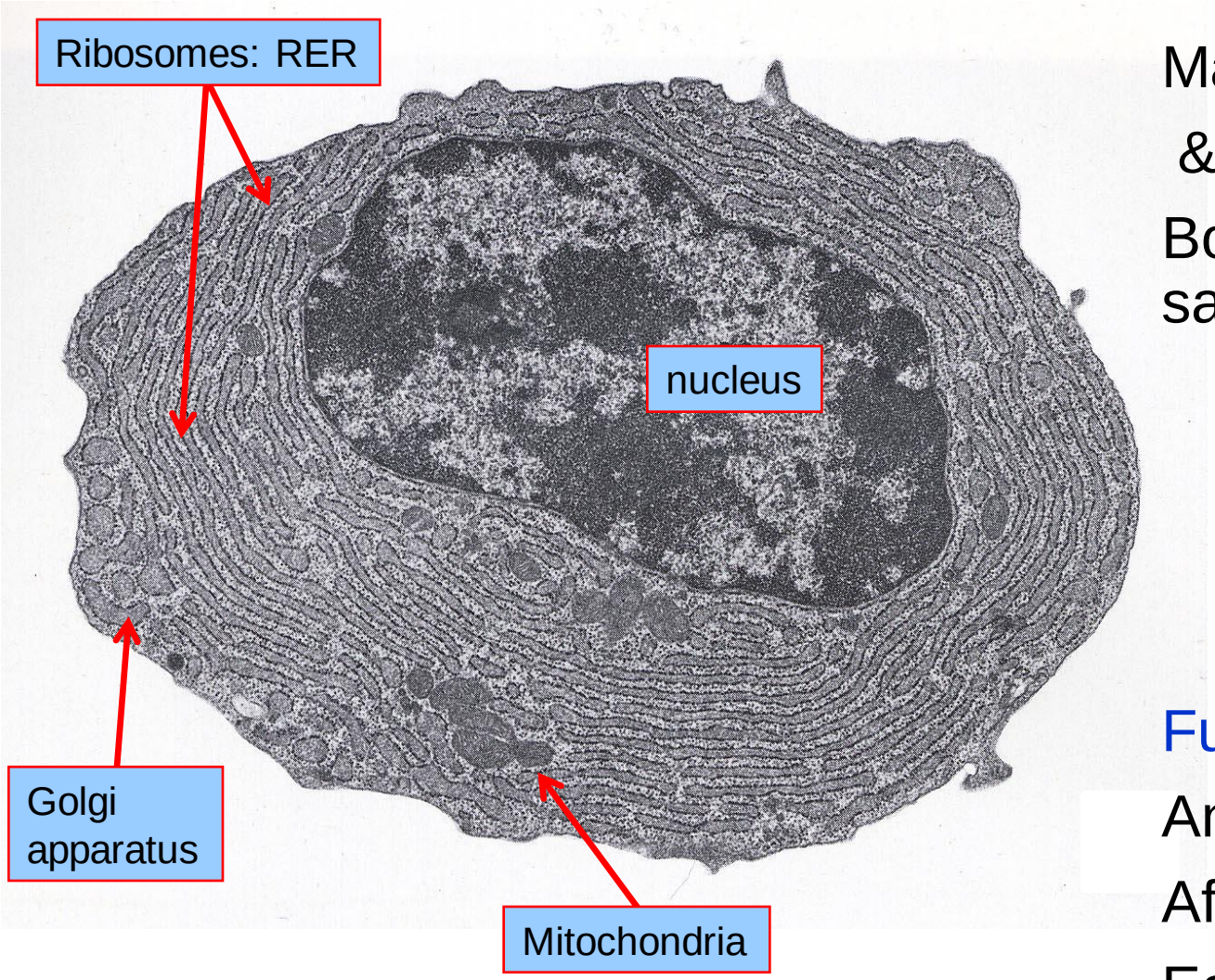


Image form Dr J Swierkoszje
www.slu.edu/~swierkoszje

Massive RER & Golgi

Bone marrow sanctuary sites

Function:

Antibody production

Affinity matured

Each cell makes 100-1000 DSA / sec

Pre-emptive eculizumab sensitized recipients

Table 2: Posttransplant outcomes in the eculizumab-treated and control groups

Category	Eculizumab group (n = 26)	Control group (n = 51)	p-Value
Follow-up	11.8 ± 6.3	48.8 ± 14.1	
(mean months ± SD, range)	(3.0–27.5)	(7.8–69.8)	
Graft survival at 1 year (n, %)	16/16 (100%)	49/51 (96%)	1.00
Antibody-mediated rejection ≤ 3months (n, %)	2 (7.7%)	21 (41%)	0.0031
Patients developing high DSA levels ≤ 3 months[1]	13 (50%)	22 (43%)	0.63
High DSA biopsies C4d+ (n, %)	13 (100%)	20 (91%)	0.52
High DSA and C4d+ biopsies showing AMR (n, %)	2 (15%)	20 (100%)	<0.0001
Cellular rejection ≤3 months (n, %)	1 (6.2%)	1 (2%)	0.42
Plasma exchange posttransplant			
Patients receiving PE (n, %)	3 (12%)	39 (76%)	<0.0001
Number of PE treatments (mean ± SD)	0.35 ± 1.1	7.9 ± 7.5	<0.0001
Splenectomy (n, %)	0 (0%)	9 (18%)	0.025
Graft dysfunction in first month (mg/dL) (maximum serum creatinine – nadir serum creatinine)	0.45 ± 0.37	0.93 ± 1.15	0.05
Histology at 1 year			
Transplant glomerulopathy incidence (n, %)	1/15 (6.7%)	15/42 (36%)	0.044
Cg score (mean ± SD)	0.20 ± 0.78	0.74 ± 1.13	0.17
Ci score (mean ± SD)	1.00 ± 0.76	0.79 ± 0.80	0.31
Ct score (mean ± SD)	1.13 ± 0.74	0.91 ± 0.80	0.33
Cv score (mean ± SD)	0.80 ± 0.68	0.59 ± 0.74	0.23

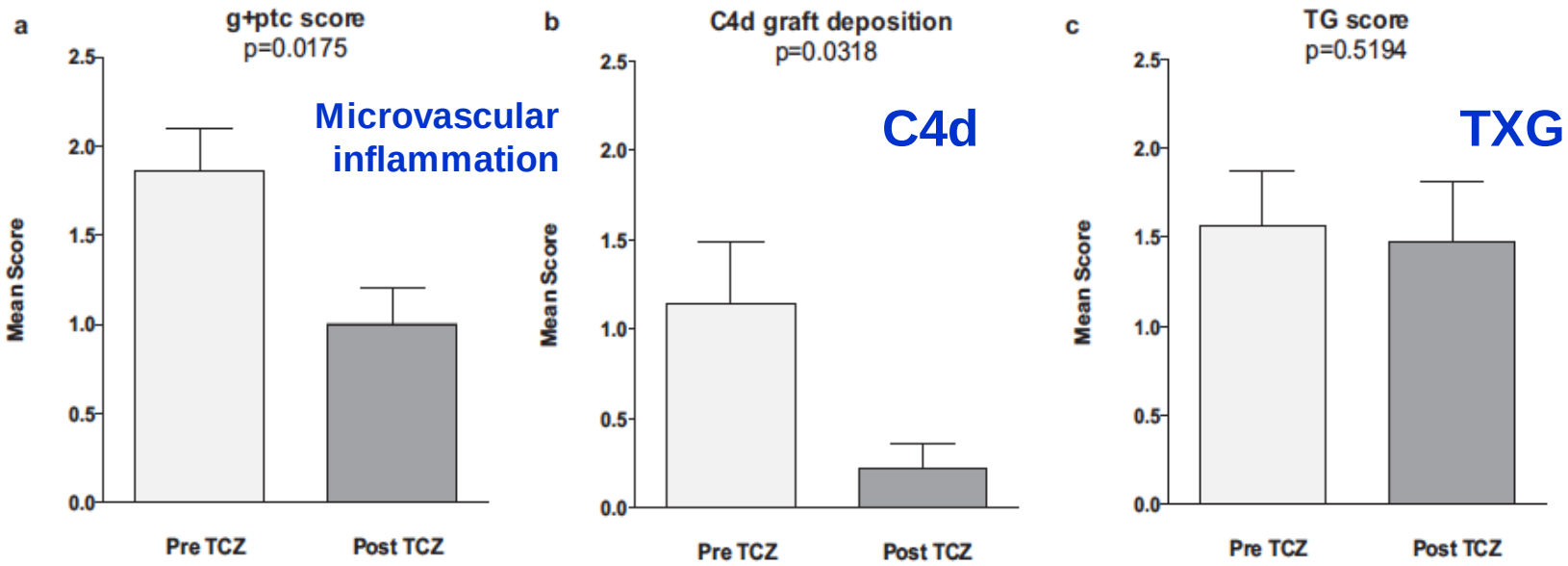
Single centre open-label cohort study, Mayo
N=26 kidney Tx ECUL vs 52 historical controls
Pre-emptive therapy for LRD recipients of XM flow+ve
Desensitization: PEX, Thymo, TAC, MMF, Pred +/- ECUL
Outcome: prevention of AMR
Follow-up 3.26 (IQR 1.82–3.81) years

Reduced early AMR

Stegall et al AJT 2011

Tocilizumab for CAMR: IL-6 blockade

- N=36 CAMR rescue therapy after failed SOC
- 6-year graft survival 80%, stable eGFR, DSA reduced



Repeat Bx n=9

Single centre open label cohort study (Cedars-Sinai)
n=36 kidney Tx biopsy failed SOC (Ritux + IVIG +/- PEX)
Rescue therapy, uncontrolled, investigator driven
Entry: CAMR by histology with DSA +/-TXG
TX: TAC, MMF, Pred + IV TOC (8 mg/kg mo x 6–25 mo to 800mg)
Follow-up 3.26 (IQR 1.82–3.81) years

Choi et al AJT 2017

C1 INH for acute AHR

- human plasma-derived C1 esterase inhibitor
- approved hereditary angioedema

Study:

- 2b multicentre double-blind RCT “pilot” POC
- Entry: early (<12 mo) AHR by Bx (inc. ABOi)
- High dose C1 INH 2 weeks + SOC for AMR

Results:

- Failed primary endpoint day 20 pathology
- Subset 6mo PBX: TXG 0/7 C1 INH vs 3/7 placebo

Biopsy-proven AMR, n=39
Single centre RCT n=9 (+C1 INH) vs n=9 control
Second daily C1 INH for 2 weeks to total 20 000 units

Montgomery RA et al AJT 2016

Late Mixed Rejection

No treatment

Approach: sequential therapy

Treat: TCMR dominant

500mg IV MPx3

+ Thymoglobulin 1.5mg/kg x 4-6

Treat: AMR dominant

500mg IV MP
+ PEX 2 weeks+
+ IVIG low dose 0.1mg/kg post PP
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Mixed Treat: Sequential

500mg IV MPx3
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+ PEX 1 to 2 weeks

+ IVIG high-dose Total 1-2g/kg divided

Summary

1. Establish clinico-pathological diagnosis

- plus DSA, C4d, EM, trichrome

2. Strategy guided by:

- allograft damage & reversibility
- patient factors

    Iatrogenic causes versus non-compliance

    Comorbidity

3. Combination pharmacological therapy targeted to immunodominant pathology

4. Patient treatment: compliance & dialysis planning

Mixed rejection: treatment goals