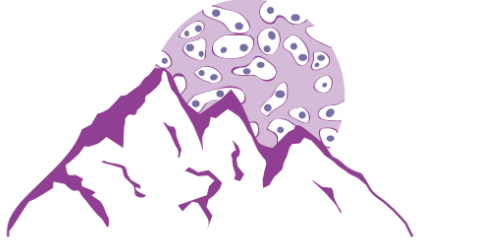


BANFF FOUNDATION
FOR ALLOGRAFT PATHOLOGY

XVIth Banff Meeting Allograft pathology, Banff Canada 19th-23rd September 2022 Joint meeting with the Canadian Society of Transplantation

Kidney Summary Session

Candice Roufosse
Maarten Naesens

Banff pre-meeting

Mission of Banff

Mission of Banff

- Banff classification is for biopsy-based DIAGNOSIS
  - For individual patient management
  - For clinical trials
  - ~~Not a prognostic tool~~

- Needs to translate to patient benefit; Needs to be usable
  - Simplify and/or algorithms
  - Where possible, related to pathophysiology: that is what we want to target with treatments

cg, Banff chronic glomerulopathy score; EM, electron microscopy; ENDAT, endothelial activation and injury transcript; g, Banff glomerulitis score; GBM, glomerular basement membrane; IF, immunofluorescence; IHC, in pillary; TCMR, T cell–mediated rejection; v, Banff arteritis score.

[1]For all ABMR diagnoses, it should be specified in the report whether the lesio frozen sections; C4d > 0 by IHC on paraffin sections) or without evident C4d deposition (C4d0 o by IF on frozen sections, C4d0 by IHC on paraffin sections).

[2]These lesions may be clinically acute, smoldering or subclinical. Biopsies showing two of the three features, except those with DSA and C4d without histologic abnormalities potentially related to ABMR or TCMR (C4d staining without evidence of rejection; see footnote 11, below) may be designated as "suspicious" for acute/active ABMR.

[3]Recurrent/*de novo* glomerulonephritis should be excluded.

[4]It should be noted that these arterial lesions may be indicative of ABMR, TCMR or mixed ABMR/TCMR. "v" lesions are only scored in arteries having a continuous media with two or more smooth muscle layers.

[5]In the presence of acute TCMR, borderline infiltrates or evidence of infection, ptc ≥2 alone is not sufficient to define moderate microvascular inflammation and g must be ≥1.

[6]At present the only validated molecular marker meeting this criterion is ENDAT expression (4), and this has only been validated in a single center (University of Alberta). The use of ENDAT expression at other centers or other test(s) of gene expression within the biopsy as evidence of ABMR must first undergo independent validation as was done for ENDAT expression by Sis et al (4).

[7]Lesions of chronic, active ABMR can range from primarily active lesions with early TG evident only by EM (cg1a) to those with advanced TG and other chronic changes in addition to active microvascular inflammation. In the absence of evidence of current/recent antibody interaction with the endothelium (those features in the Second Section), the term active should be omitted; in such cases DSA may be present at the time of biopsy or at any previous time posttransplantation.

[8]Includes GBM duplication by EM only (cg1a) or GBM double contours by light microscopy.

[9]≥7 layers in one cortical peritubular capillary and ≥5 in two additional capillaries (17), avoiding portions cut tangentially.

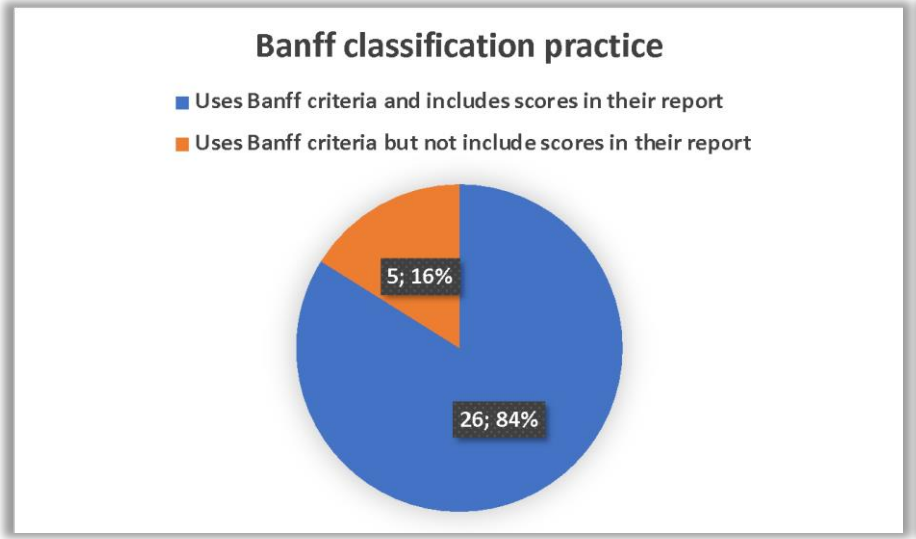
[10]While leukocytes within the fibrotic intima favor chronic rejection, these are seen with chronic TCMR as well as chronic ABMR, and are therefore helpful only if there is no history of TCMR. An elastic stain may be helpful as absence of elastic lamellae is more typical of chronic rejection and multiple elastic lamellae are most typical of arteriosclerosis, although these findings are not definitive.

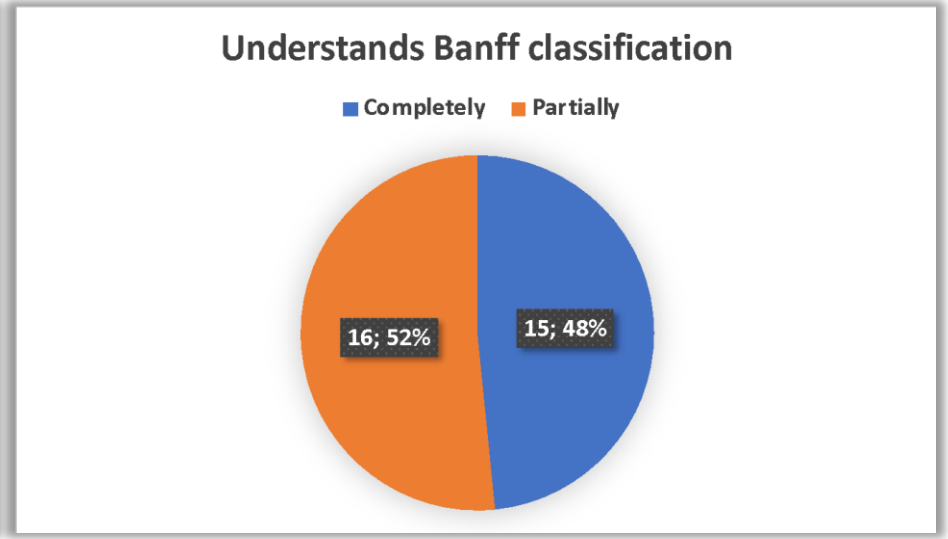
[11]The clinical significance of these findings may be quite different in grafts exposed to anti-blood-group antibodies (ABO-incompatible allografts), where they do not appear to be injurious to the graft (18,19) and may represent accommodation. However, with anti-HLA antibodies such lesions may progress to chronic ABMR (20) and more outcome data are needed.

"visual assault"

"impossible not to make errors"

A short survey on the Banff classification practice from Turkey





n=31 respondents
Nephropathology WG

Foam Cell Arteriopathy:

No

Percent Luminal Narrowing:

20

Number (or estimate) of Arterioles

12

Arteriolitis:

No

Fibrinoid Necrosis:

No

Smooth Muscle Necrosis:

No

HUS Intimal Lesion:

No

Errors and Warnings

Error: Type and extent of PTC inflammation must be specified when severity is positive

None

COPY

Diagnosis Highlights:

KIDNEY ALLOGRAFT, LEFT, NEEDLE BIOPSY (INDICATION):
Biopsy Adequacy: Adequate

Category 3: Suspicious (Borderline) for Acute TCMR:
TCMR Borderline: Yes, Suspicious

Category 4: TCMR:

Chronic Active TCMR: IA

Chronic Changes:

COPY

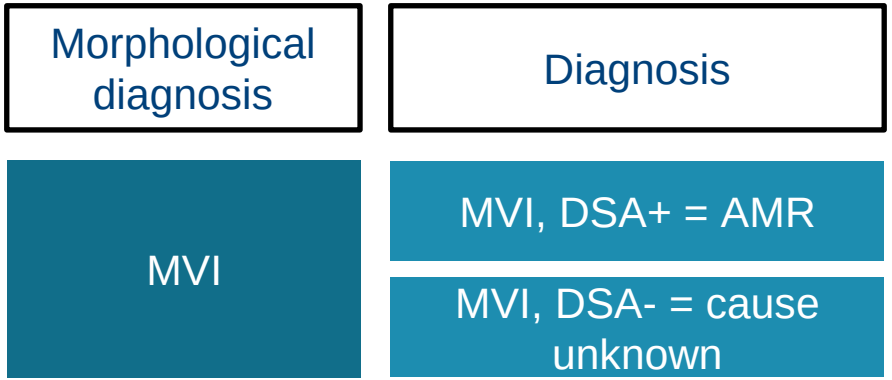
Possible Evidence of Calcineurin Inhibitor Toxicity:

Yes; Evidence of Isometric Tubular Vacuolization

Specified Diagnosis / Consult Comments:

Rather than algorithms: a mandate for simplification!

- It should be universally applicable
  - Hierarchy in time and/or in availability in tests



The main clinical difficulty with BANFF CLASSIFICATION is in AB AMR category

ABMR = **MVI**

antibody mediated rejection

microvascular inflammation

Help clinicians to guide therapy?

?

We prefer to use

the term“microvascular inflammation”

with or without DSA,

instead of AMR

Consensus and changes to the classification

Banff Classification for Allograft Pathology

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
	1991	1993	1995	1997	1999	2001	2003	2005	2007	2009	2011	2013	2015	2017	2019
Change	n/a		x	x		x		x	x			x	x	x	x

☐ minor change

“The banff classification is changing too often”

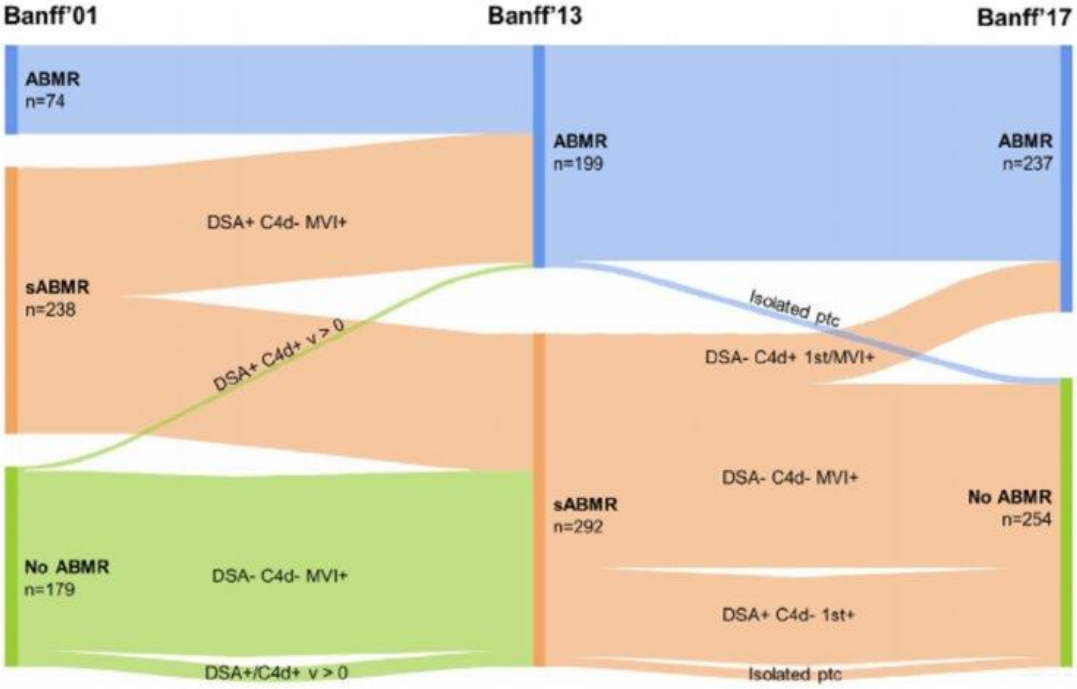
Rethinking the consensus process

- Evidence level: “ at least 2 independent groups provide evidence”
- Banff consensus using KDIGO, DELPHI,…

Stress-testing the changes?

Simulating changes to the Banff classification before final implementation

Multicenter!



Innovation and the future

Banff Classification for Allograft Pathology is forward-looking

- Via Multi-disciplinary Working Groups
  - Pathologists, nephrologists, immunologists, computer/data scientists, tissue typers,…

- Conflict between innovation and regulation/implementation

The iBox roadmap

2012
- **iBox** project design

2013
- **Cohort** securisation
- Registration
- **Protocol** finalization

2014
- **Data curation** and internal validation
- Studies on biopsies, DSA and longitudinal data acquisition

2015 - 2016
Statistical modeling Generation of scoring system
- Development cohort

2017
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2017 - 2018
- **External validation cohort & RCTs** data acquisition
- Cohorts enhancement

2019
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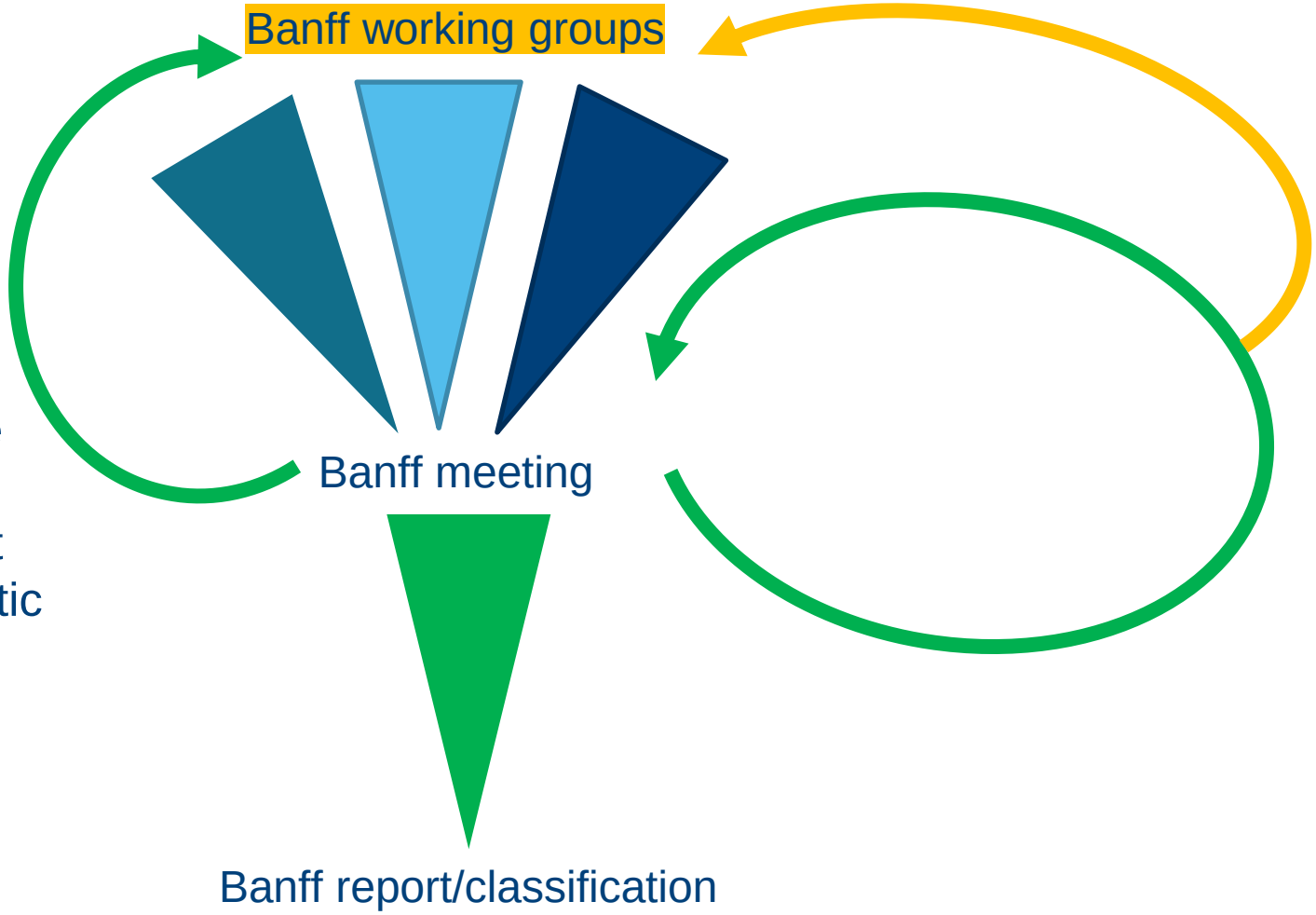
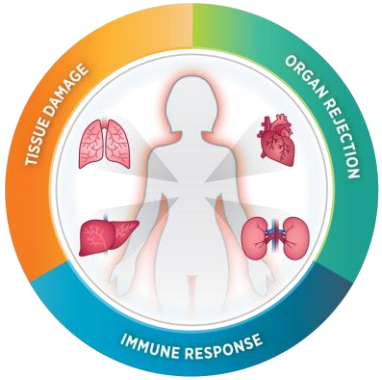
2020
- FDA **letter of intent**
- Further **refinements** on cohorts and studies

2021
- Publication on dynamic iBox (**Lancet Digital Health**)
- First application of iBox (**BMJ open**): Transform Study
- eGFR trajectories (**Kidney Int**)

2022 - 2023
- iBox as a **surrogate endpoint**
- Qualification process iBox Clinical use : recruiting RCT

How can Banff both encourage innovation and ensure a robust clinical diagnostic classification?





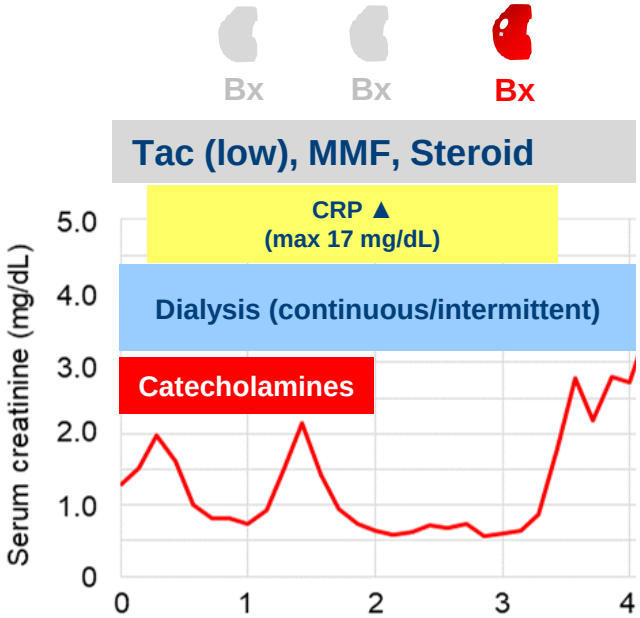
Transcriptomic analysis of kidney transplant biopsies

Banff pre-meeting: Molecular diagnosis - Invasive

- Molecular tests likely become companion tools for diagnosis soon
- Several groups presented equivocal cases “solved” with molecular diagnosis

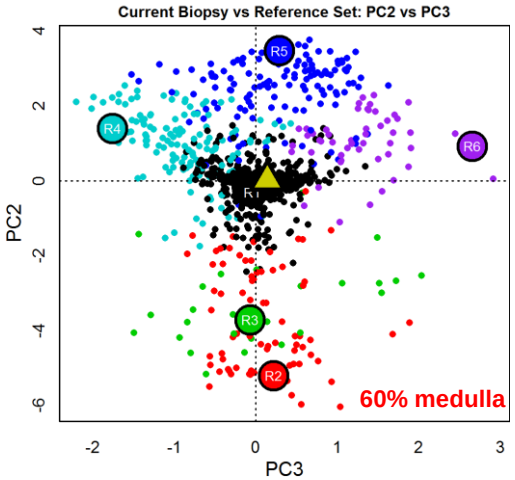
Vienna Case #1

Post-Tx DSA negative



Third kidney biopsy

Morphology
v0, i0, t0
g0, ptc1, C4d0



Extensive AKI score

► **no rejection treatment**

MVI in Early Post-Tx Allografts with DGF C4d Deposition (case 2)

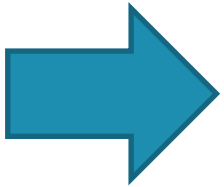
- 42 year male; ESRD secondary to GN NYD
- DGF; allograft Bx at 9 days post-Tx
- Moderate ATN with regenerative changes
- Focal cortical ptc3 with mononuclear cells
- Focally prominent medullary vasa recta inflammation
- PTCs diffuse C4d + (C4d3) by IF; focal C4d+ by IHC
- Banff 2019 scores: g0, i0, ti0, t0, v0, ptc3
  cg0, mm0, ci0, ct0, i-IFTA 0, t-IFTA 0, cv1, ah0
- **"highly suspicious for active ABMR"**

MVI in Early Post-Tx Allografts with DGF C4d Deposition (case 2)

- Pre-Tx PRA 0%
- Negative for HLA class I and class II DSA pre-Tx and at time of biopsy
- AT_1R Ab <10 both pre-Tx and at time of Bx
- Non-HLA Ab screen negative
- ABMR gene expression of 190; below threshold (<236) for “molecular ABMR” (NanoString 34-gene set, University of Alberta, Ben Adam & Michael Mengel)

Kidney biopsy

- ptc 2 g1 cg0 c4d0
- Aah2
- FIAT III
- No BKV nephropathy



DSA negative!!

AT1R Ab: 38 U/ml

Gene expression based probabilities

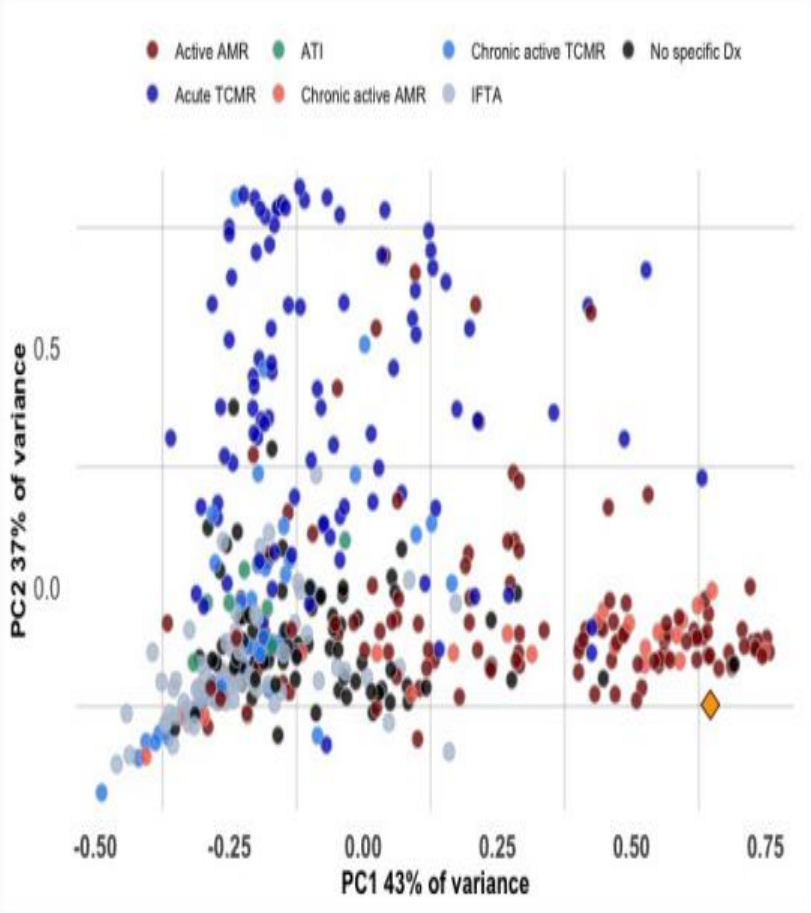
Diagnosis based probabilities (%):

Diagnosis	SL.203	Normal range	Interpretation
AMR	99.0	3.7 - 24.6	very high probability
TCMR	25.1	8.2 - 31	moderate probability
ATI	34.7	0.1 - 0.7	moderate probability
IFTA	18.7	7.9 - 19.7	low probability

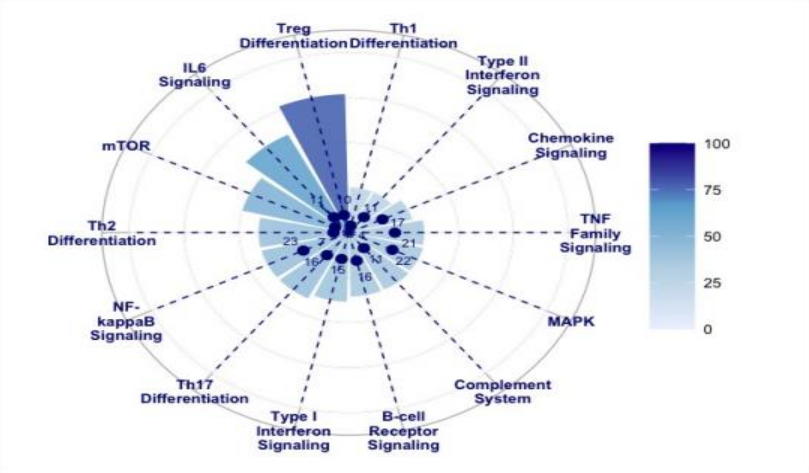
Banff lesion based probabilities (%):

Banff lesion	SL.203	Normal range	Interpretation
g > 0	25.2	12.5 - 32.5	moderate probability
ptc > 0	94.4	14.2 - 33.9	very high probability
cg > 0	0.2	1 - 3.9	unlikely
i > 1	34.7	8.6 - 28.3	moderate probability
t > 1	60.0	14.7 - 40.1	moderate/high probability
iifta > 0	77.2	15.3 - 48.9	high probability
v > 0	36.2	1 - 6.6	moderate probability
cv > 1	63.6	27.5 - 57.3	high probability
ci > 1	95.3	15.5 - 44.7	very high probability
ct > 1	95.8	13.5 - 43.3	very high probability

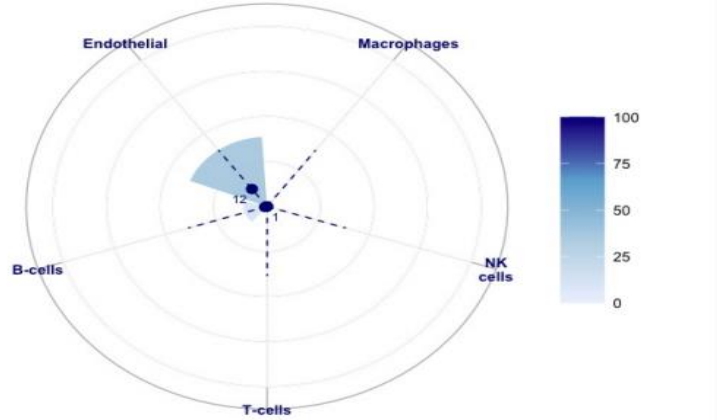
Principal Components Analysis (PCA) of molecular scores:



Pathway analysis



Cell type analysis



Banff pre-meeting: Molecular diagnosis - Invasive

- Defined classifiers with thresholds being tested for added value to diagnosis in a variety of situations
  - AMR
  - BL/TCMR

- Banff Molecular Working Group seeking how to integrate into the classification
  - Define COU
  - Regulatory approval!

Chronic active TCMR

Chronicity in rejection and the i-IFTA challenge

- Universal agreement that i-IFTA is a feature of poor prognosis
- Threshold of prognostic value of i-IFTA may be too high
  - i-IFTA 1 and ti1 may have prognostic value

- Evidence for i-IFTA as a feature of chronic TCMR remains conflicting
  - Some groups showing evidence to support causality of TCMR in iIFTA, though not in all cases
  - Other groups showing more mixed picture
    - Molecular profile showing inactive inflammatory infiltrates and/or evidence of AMR (=non-specificity of i-IFTA)
    - Limited response to anti-TCMR therapies

caTCMR: still a d entity

- Unresolved issues
  - i-IFTA belongs on R category? Stress-test
  - Could be a score of main diagnostic group (like v, cv)?
  - How/if to
  - Borderlin ? Molecular?

- TC include
  clin tions

OPTION 1

Keep it as it is since 2019

Tubulitis is scored **in all but severely atrophic tubules**

cortex/tubules	Normal	Mildly atrophic	Moderately atrophic	Severely atrophic
No IF	t	t	t	-
IF	t-IFTA	t-IFTA	t-IFTA	-

- **t in areas of preserved cortex**
- **t-IFTA in areas of IF** (which in practice is mostly in mildly-moderately atrophic tubules).

PROs:

No new change

No need to evaluate the size of tubules

Will allow to compare the impact of « t » in IF and « t » in preserved areas in future studies

CONs:

Big change compared to 2017 and before, so no consistency between studies before and after 2017

Incorrect registry data before 2017 and any prognostic scores generated with the version of t prior to 2017

Banff and clinical trial design

Banff and clinical trial design

- Banff classification is not only used clinically, but also for clinical trials:
  - As endpoint (BPAR)
  - For inclusion

- Problem: most clinical trials (e.g. in AMR) use derivatives (keeping certain aspects but not all) and (much) older versions of the Banff classification
- Consensus to not have separate Banff classifications for clinical use and for use in trials

-> Mission to make Banff great again (for clinical trials)

Paths towards reinstalling the consensus Banff classification as standard for clinical trials

1. Simplify the classification for MVI/AMR
   - Reduce the footnotes
   - Clear definition of DSA+AMR vs. DSA-MVI (see next)

1. Define in the classification what is substantiated by evidence and clinically available, and what is not (yet)

3. Use algorithms, based on lesion scores, that automate and standardize the final diagnosis
   - The Paris system for automated Banff scoring
   - The Pittsburgh system for automated Banff scoring
   - Integration of both systems?

Paths towards reinstalling the consensus Banff classification as standard for clinical trials

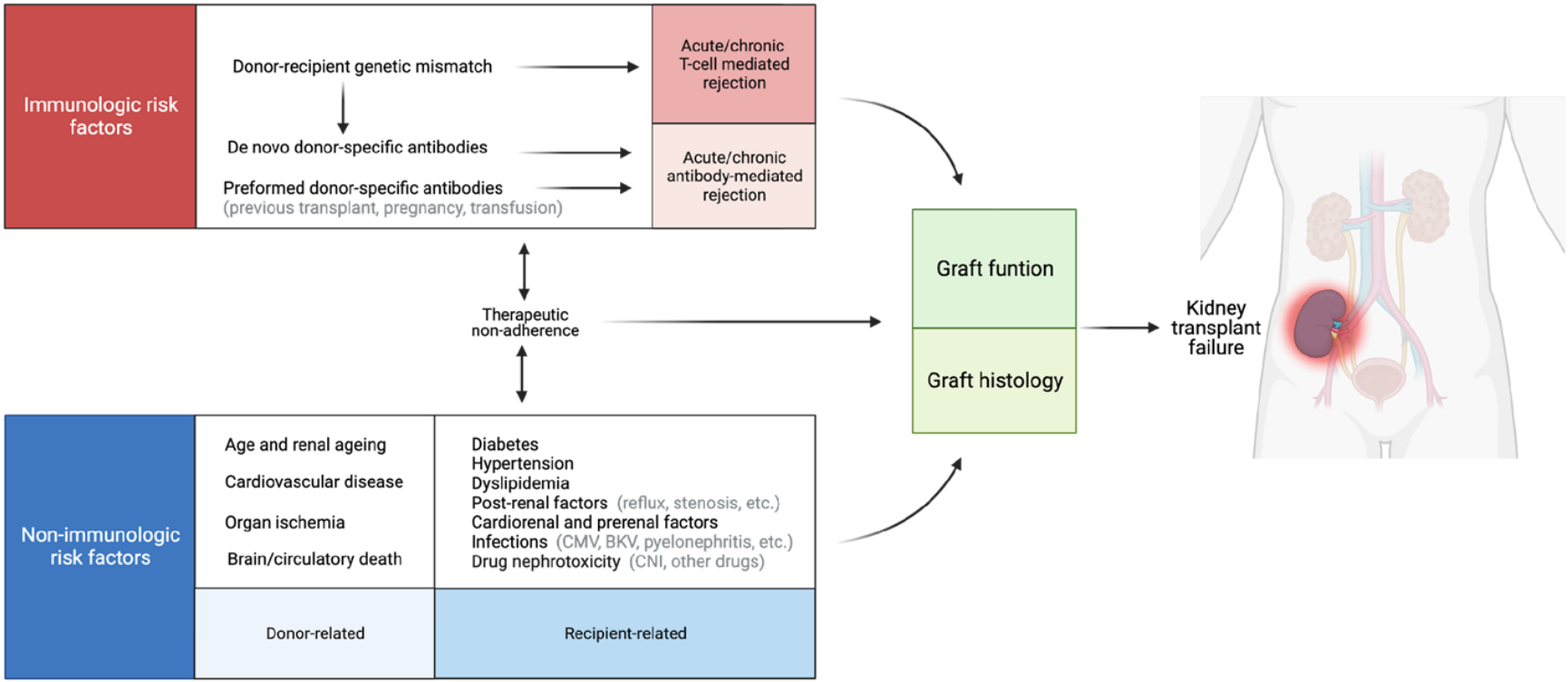
4. Clinical trial design is hampered by current lowly reproducible discontinous scoring
    - Morphometry might help, algorithms are being developed

5. Central pathology with clinical information provided to pathologists improves the accuracy

6. Move away from using BPAR as endpoint, but differentiate between TCMR and AMR, and take the difference into account in clinical trial design

7. Involve Banff community and pathologists in the international consensus on clinical trial design and endpoints, example of the TTS-ESOT-ATS-Banff TCMR Working Group

Surrogate endpoints for late graft failure may to take into account the multiple factors acting together



Let's celebrate the success story of the iBox

2012
- **iBox** project design

2013
- **Cohort** securisation
- Registration
- **Protocol** finalization

2014
- **Data curation** and internal validation
- Studies on biopsies, DSA and longitudinal data acquisition

2015 - 2016
Statistical modeling
Generation of scoring system
- Development cohort

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- **Project discussion** with Societies and agencies (FDA, EMA)

2017 - 2018
- **External validation cohort & RCTs** data acquisition
- Cohorts enhancement

2019
- Publication in **BMJ**
- Transplant societies endorsement (**TTS, ESOT, AST**)

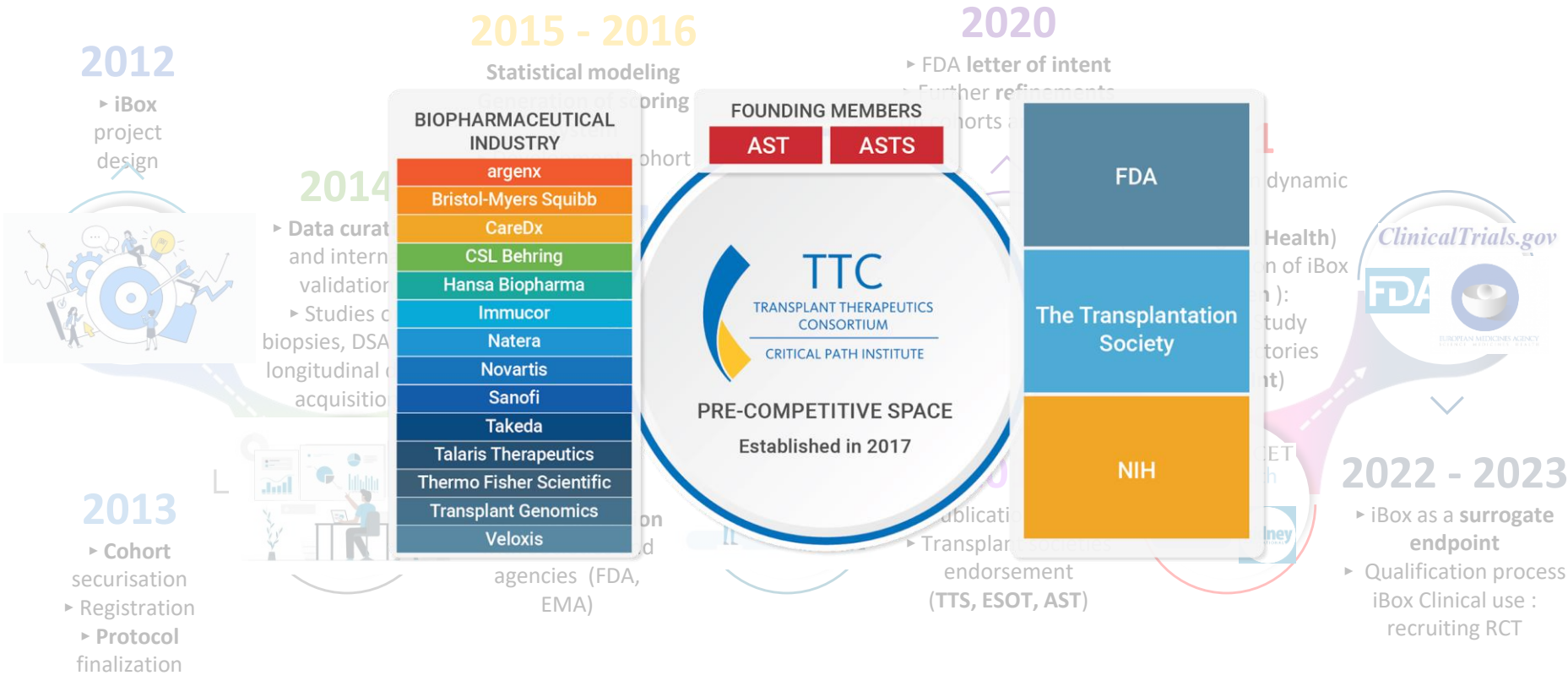
2020
- FDA **letter of intent**
- Further **refinements** on cohorts and studies

2021
- Publication on dynamic iBox (**Lancet Digital Health**)
- First application of iBox (**BMJ open**): Transform Study
- eGFR trajectories (**Kidney Int**)

2022 - 2023
- iBox as a **surrogate endpoint**
- Qualification process iBox Clinical use : recruiting RCT

Let's celebrate the success story of the iBox

and the closing of the Banff surrogate endpoints WG



AMR and MVI

The Banff schema overly simplifies the full spectrum of anti-HLA DSA associated AMR

ABMR continuum

	Early acute ABMR (+XM)	Acute ABMR	Active (smoldering) ABMR	Chronic active ABMR
Clinical setting	Clinically apparent: AKI, <1 month post-transplant	Usually clinically apparent: AKI	Subclinical	Subclinical or clinically apparent: Progressive renal insufficiency, proteinuria, hypertension
Histology	ATN, thrombi, mild capillaritis, v lesions	ATN, thrombi, capillaritis, v lesions	Capillaritis only (g, ptc)	Capillaritis and TG, TA, or PTCBMML
C4d	Diffuse +	+	Negative, focal +, occasionally diffuse +	Negative, focal +, occasionally diffuse +
Serum DSA	High	High	Low, mid	Low, mid

"Need to recognize exceptions"

The Banff schema overly simplifies the full spectrum of anti-HLA DSA associated AMR

ABMR continuum

	Early acute ABMR (+XM)	Acute ABMR	Active (smoldering) ABMR	Chronic active ABMR
Clinical setting	Clinically apparent: AKI, <1 month post-transplant	Usually clinically apparent: AKI	Subclinical	Subclinical or clinically apparent: Progressive renal insufficiency, proteinuria, hypertension
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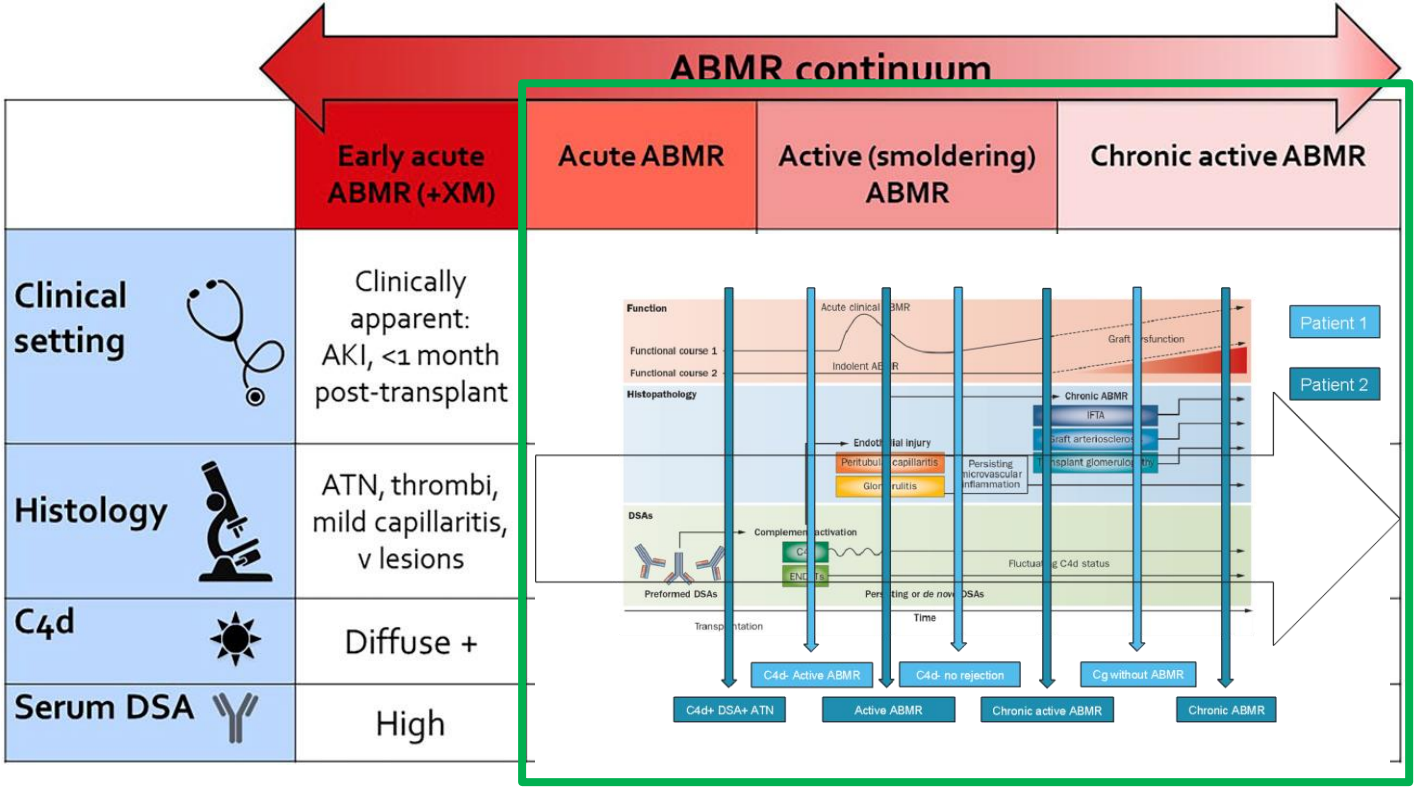
Serum DSA	High	High	Low, mid	Low, mid

The Banff schema overly simplifies the full spectrum of HLA DSA positive MVI = AMR



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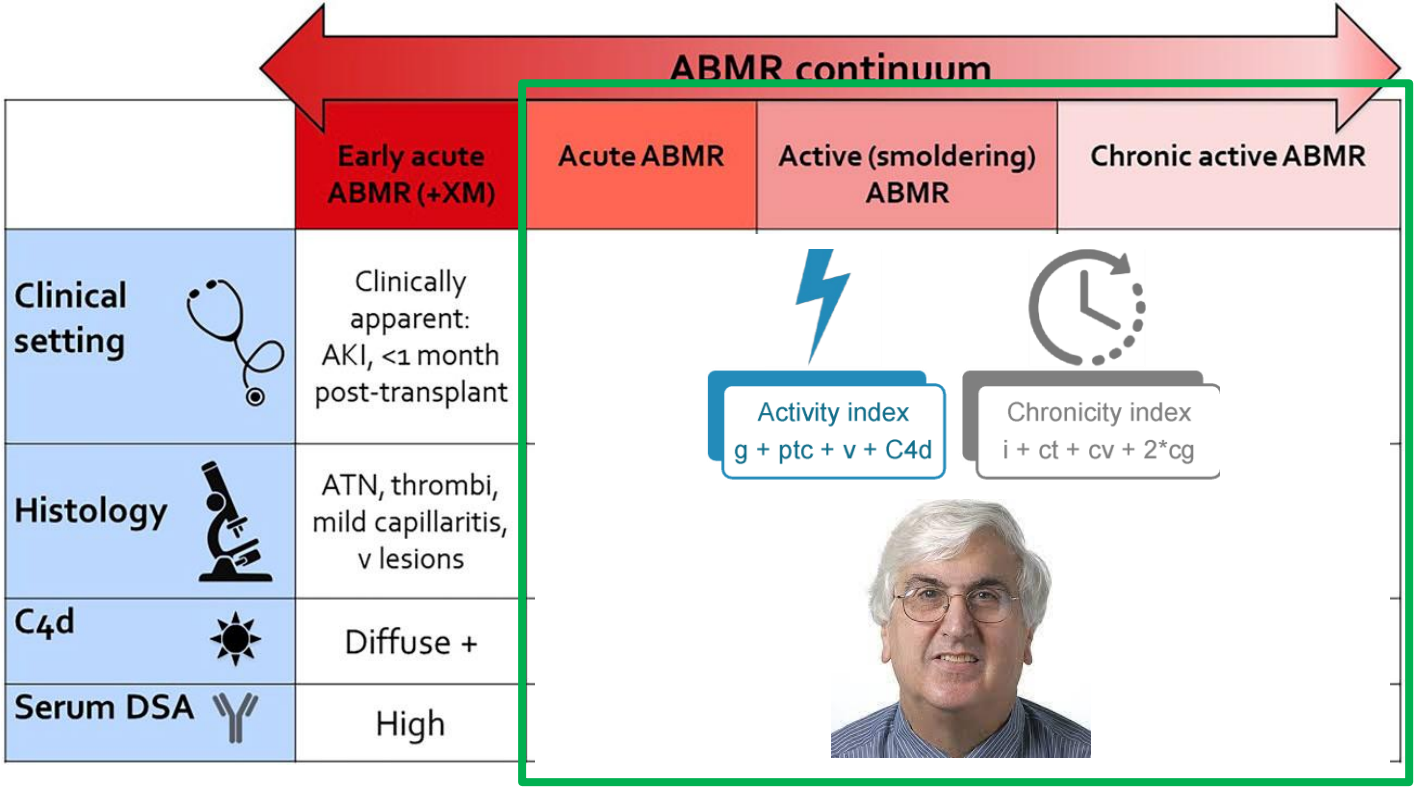

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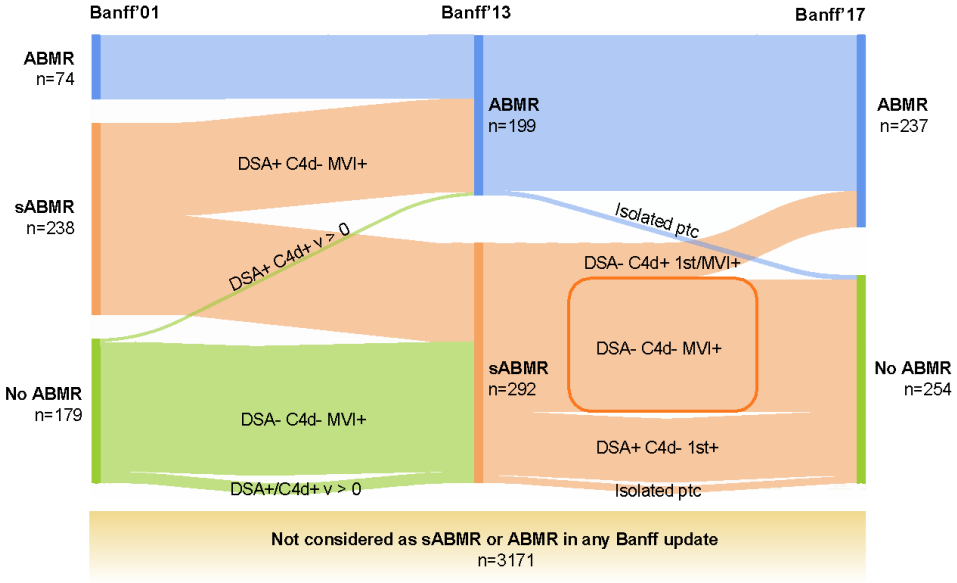
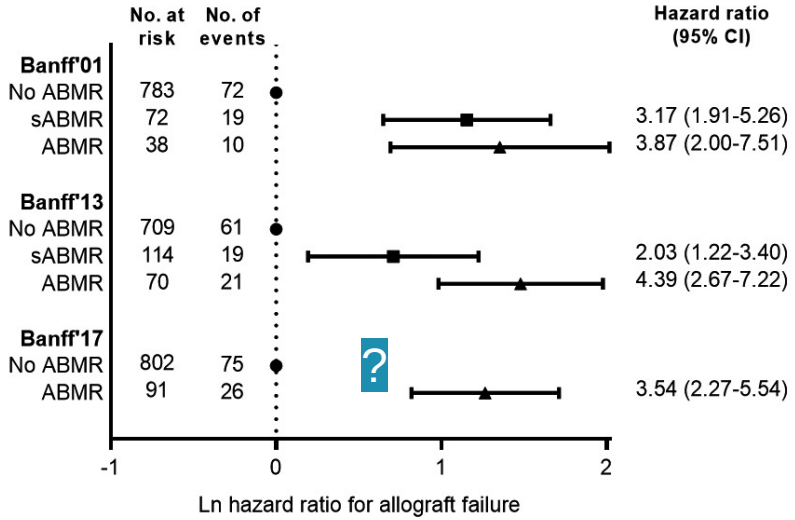




Definition of HLA-DSA
- Technical hurdles
- STAR guidance
- AMR Working Group

MVI triggers interaction with HLA lab

Non-HLA antibodies
- allo-immune?
- auto-immune?

STAR review

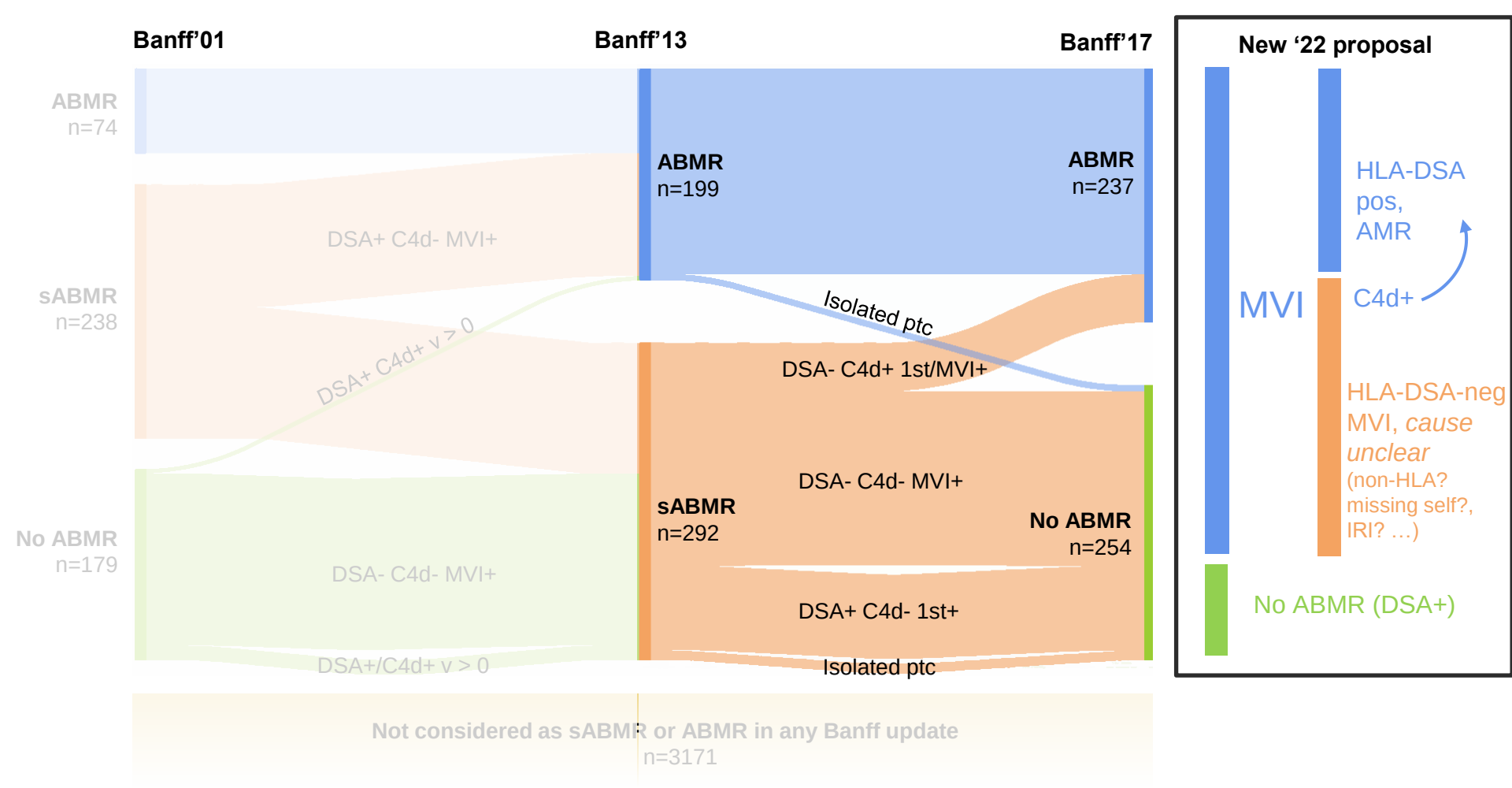
Tools needed

NK cells
- Missing self
- Other activation mechanisms

Validation needed

Ischemia reperfusion injury
Different types of infiltrates?

Validation needed


Banff'01
Banff'13
Banff'17
ABMR
n=74
sABMR
n=238
No ABMR
n=179
DSA+ C4d- MVI+
DSA+ C4d+ v > 0
DSA- C4d- MVI+
DSA+/C4d+ v > 0
ABMR
n=199
sABMR
n=292
Isolated ptc
DSA- C4d+ 1st/MVI+
DSA- C4d- MVI+
DSA+ C4d- 1st+
Isolated ptc
ABMR
n=237
No ABMR
n=254
Not considered as sABMR or ABMR in any Banff update
n=3171
New '22 proposal
MVI
HLA-DSA pos, AMR
C4d+
HLA-DSA-neg MVI, cause unclear (non-HLA? missing self?, IRI? …)
No ABMR (DSA+)

Options for a definition of “DSA-negative MVI”

Microvascular inflammation needs to be defined

- ❑ g + ptc ≥ 2[#]
- ❑ Aligned with Banff for AMR: 1st + 2nd Banff criterion:

  1. Histologic evidence of acute tissue injury, including 1 or more of the following: • Microvascular inflammation (g > 0 and/or ptc > 0), in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 1 alone is not sufficient and g must be ≥ 1 • Intimal or transmural arteritis (v > 0) • Acute thrombotic microangiopathy, in the absence of any other cause • Acute tubular injury, in the absence of any other apparent cause
  2. Evidence of current/recent antibody interaction with vascular endothelium, including 1 or more of the following: • Linear C4d staining in peritubular capillaries or medullary vasa recta (C4d2 or C4d3 by IF on frozen sections, or C4d > 0 by IHC on paraffin sections) • At least moderate microvascular inflammation ([g + ptc] ≥2) in the absence of recurrent or de novo glomerulonephritis, although in the presence of acute TCMR, borderline infiltrate, or infection, ptc ≥ 2 alone is not sufficient and g must be ≥1

- ❑ Restricted definition of MVI*: g+ptc ≥ 2, in the absence of glomerulonephritis, with g > 0 in the presence of TCMR or borderline changes
- ❑ Other option?

Senev et al *AJT* 2018: 97% overlap

[#]Irrespective of concomitant TCMR
*Roufosse et al Transplant Int 2022

Ptc working group

- Banff ptc score: first discussion 2003 and 2005, included in Banff 2007
- Current ptc score rel… …st affected ptc
- Single-center data su… …nt in addition to the current ptc score

- Primary aim of the … …whether the ptc score + ptc extent are mo…
- Question of the r… …er questions to be answered

- Currently setting up the DTA, distribution of samples, etc.

Digital pathology

Banff Digital Pathology Working Group

IMAGE BANK(S) / COLLECTION(S)

PLATFORM TO SHARE ALGORITHM(S)

COMPETITION(S)/ TRIAL(S)

Pilot Image Bank: DPLab

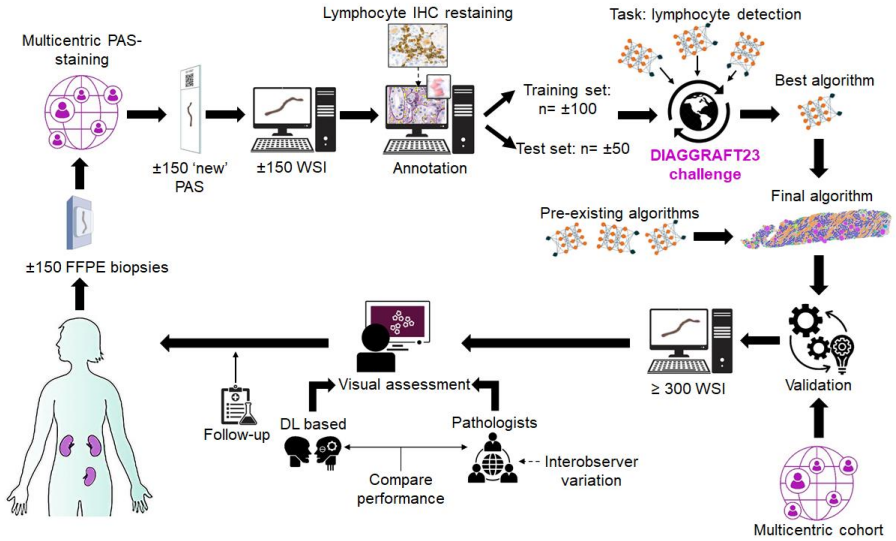
https://dplab.gsu.edu/

@banffdpwg YouTube

Am J Transplant. 2020.
PMID: 32185875.

- **Dr. Renate Kain** on the "Big Picture"
- **Dr. Richard Levenson** on novel microscopy
- **Dr. Peter Boor** on their server
- **Dr. Laura Barisoni** and their group's peritubular capillary, glomerular, & other algorithms
- Dr. Ul Balis on his novel image analysis tool
- Dr. Lynn Cornell on immunofluorescence scanners

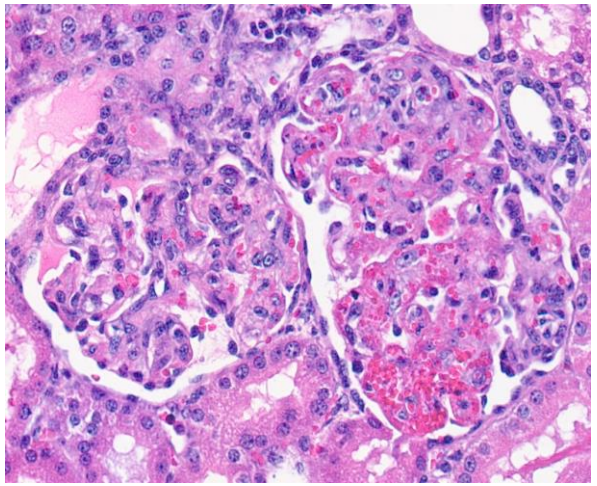
Radboudumc Diagnostic Image Analysis Group/ **DIAGGRAFT Study**



https://www.computationalpathologygroup.eu/projects/diaggraft/

Xenotransplantation

- Pre-clinical models
  - pig to primate
  - GTKO pig to primate
  - TKO pig to primate



- Early (time post-transplant) observations in humans (pig to human decedent) mirror primate observations
- Molecular diagnosis: “B-POT” – first cross-species gene expression panel

Proposal for Banff scoring for xenografts

	Thrombotic microangiopathy scoring				
		0	1	2	3
glomeruli	gTMA	no thrombi	≤25%	26–50%	≥50%
arteriolar	aTMA	no thrombi	≤25%	26–50%	≥50%

	C4d scoring				
		0	1	2	3
glomeruli	gC4d	no staining	≤25%	26–50%	≥50%
vascular	vC4d*	no staining	≤25%	26–50%	≥50%
	*arteries and arterioles				

Working groups

- PTC, sensitised, TCMR, digital, non-invasive diagnostics, molecular, Rules and dissemination presented
- EM WG met
- HIV, TMA, recurrent glomerular disease sent in reports

- Implantation biopsy WG may be revived
- Activity and chronicity indices WG
- Xenotransplantation WG

Thank you very much for all your hard work