Cellular and Humoral Rejections: Diagnosis and Treatment

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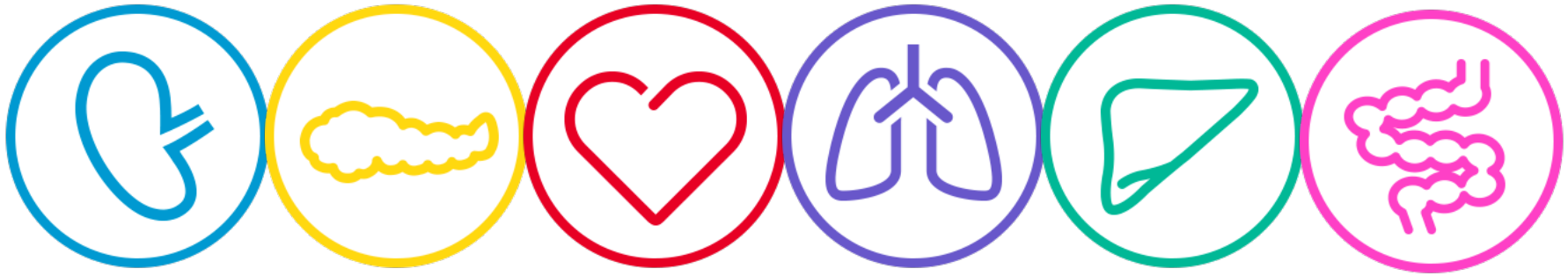
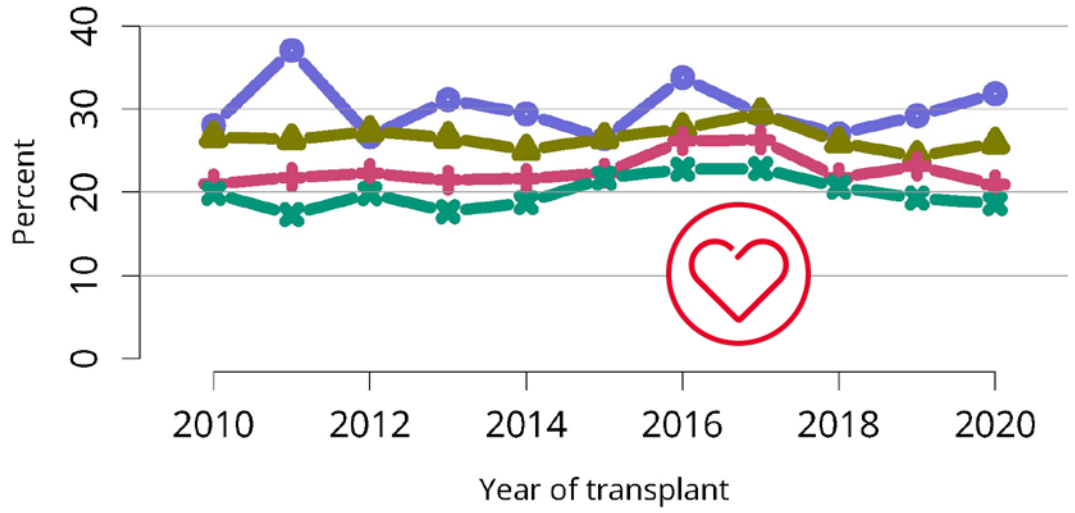
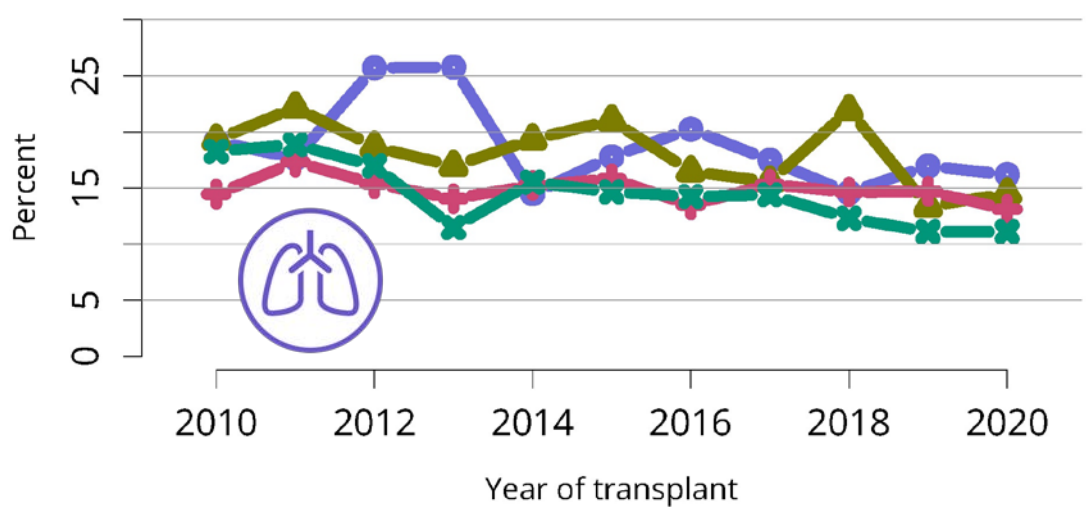


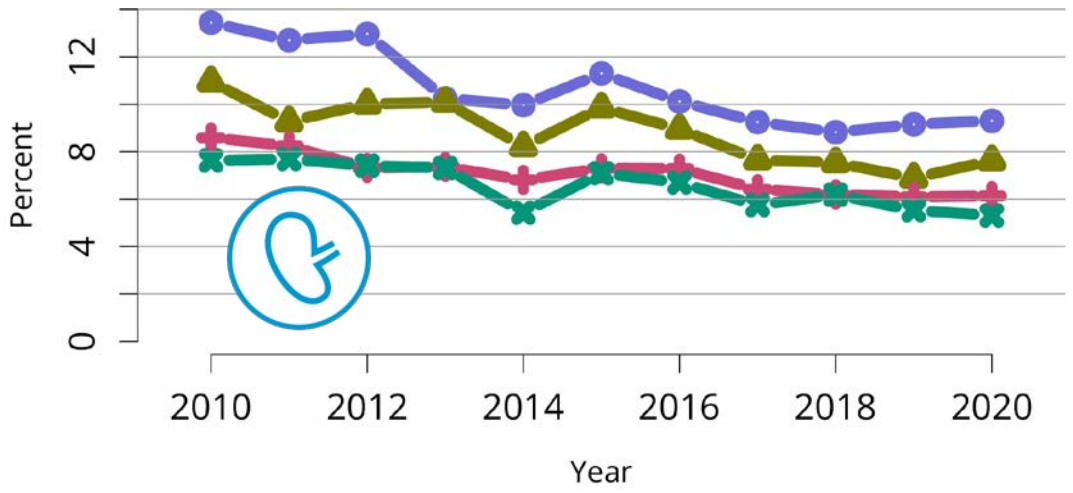
Disclosures

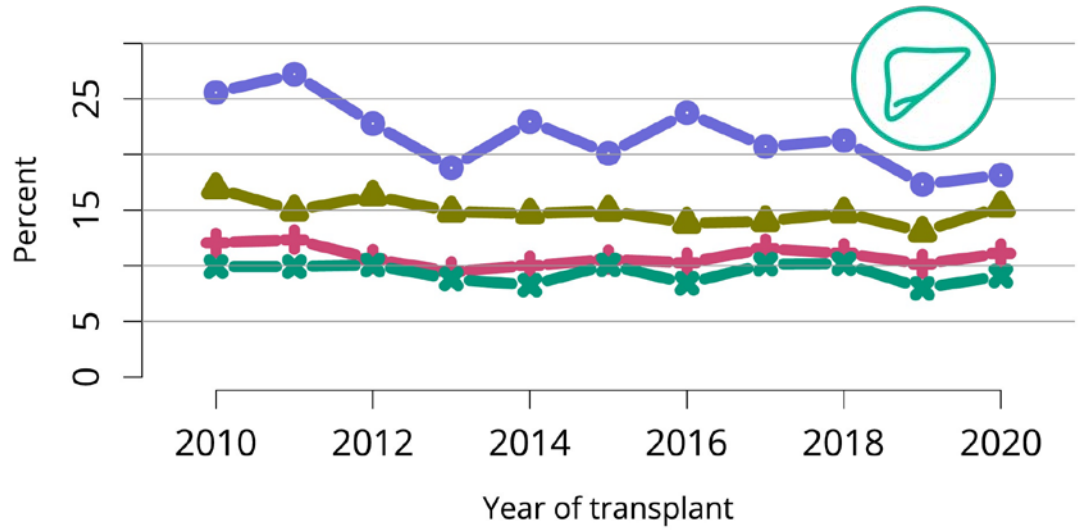
- Grant funding from NIH, Cystic Fibrosis Foundation, CareDx

Acute Rejection in Transplant – 1st year









Why do we care about acute rejection?

The immune system is very sophisticated

Acute rejection is reversible

Acute rejection increases risk of chronic allograft injury

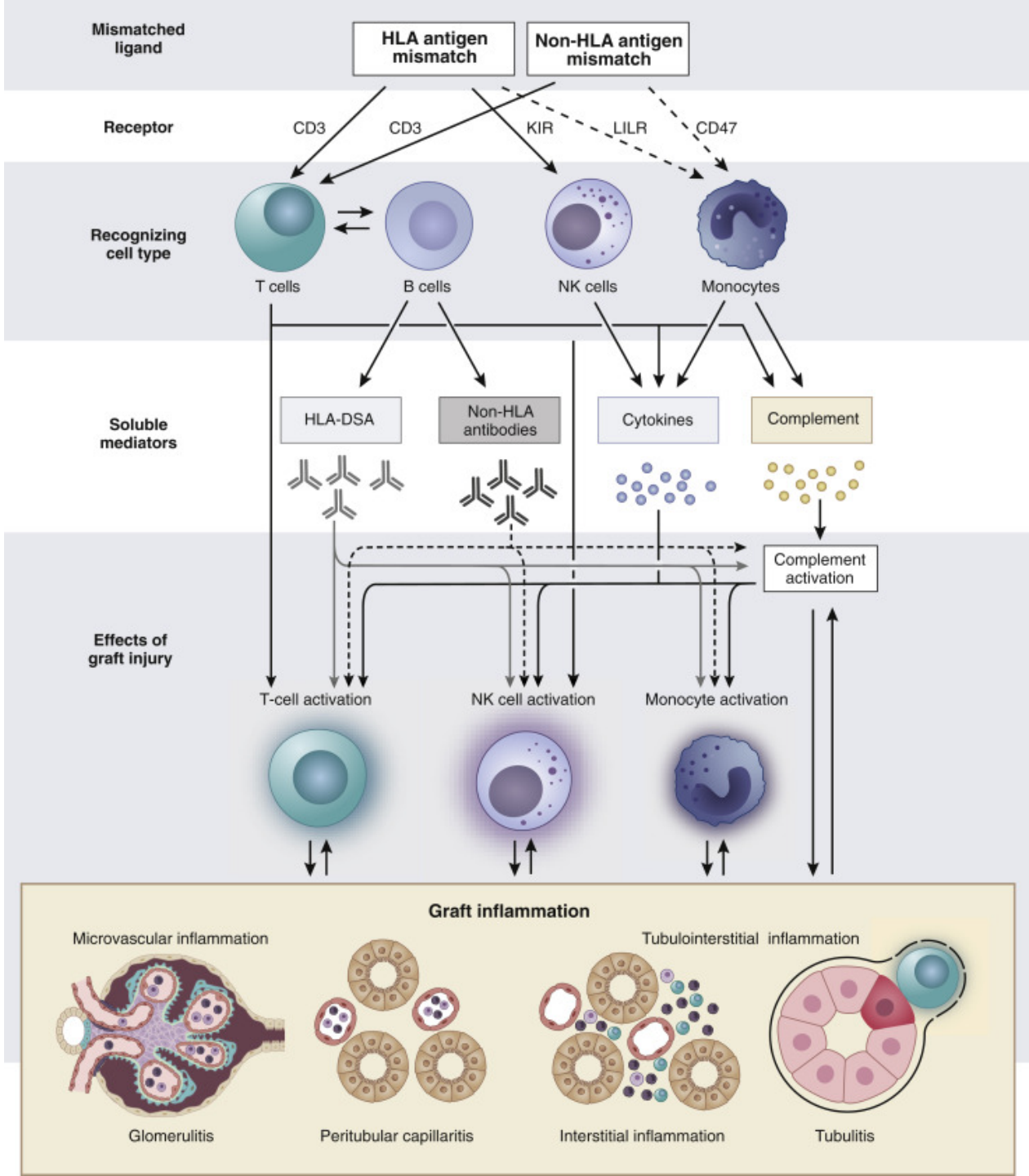
Types of acute rejection

Cellular rejection

Antibody mediated rejection

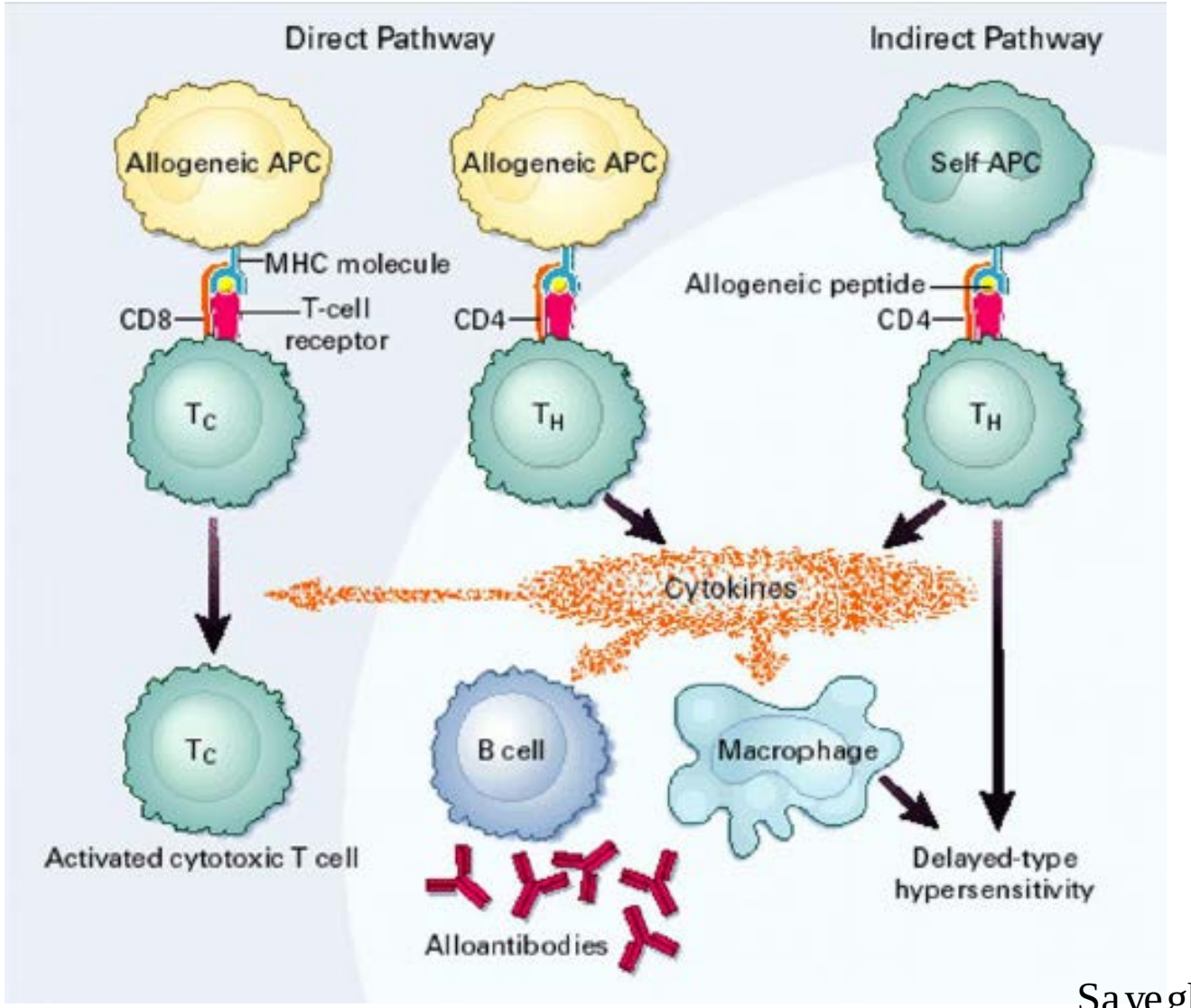
Mechanisms of rejection

- Lymphocytes
  - Tcells
  - Bcells
- Innate immune cells
  - NKcells
  - Monocytes / macrophages
- Soluble mediators
  - Antibodies
  - Complement
  - Cytokines



Callemeyn J, Kidn Intl, 2022

Mechanisms of acute rejection



Sayegh, NEJM, 1998

Diagnosis of acute rejection

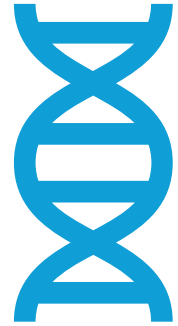
Symptoms

Shortness of breath

Fatigue

Functional testing

Biopsy



Biomarkers

DSA

Cell-free DNA

Transcriptional responses

Biopsy is the gold standard for rejection diagnosis

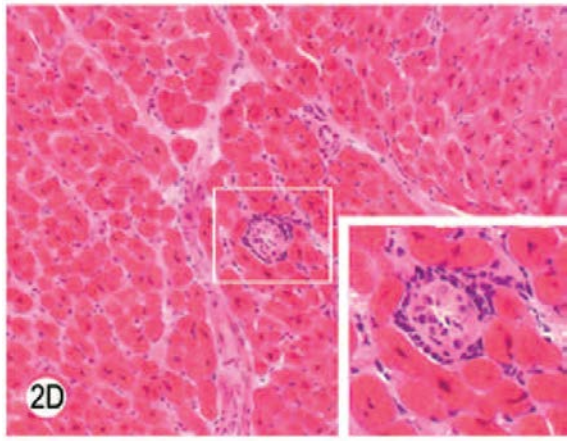
- Decades of data on interpretation
- Guidelines from professional societies for each organ

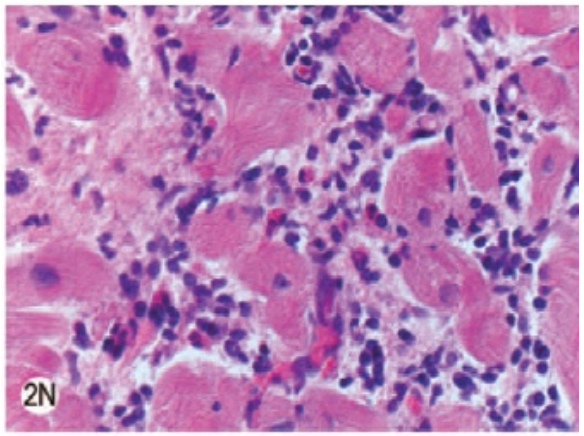
- Invasive
- Variability in interpretation

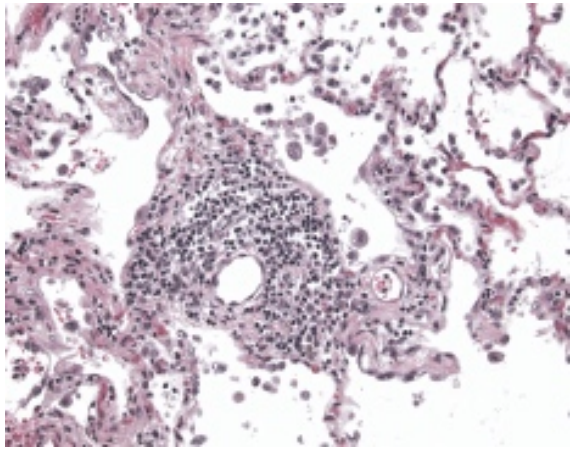
- Uncertain relationships with other biomarkers
  - Example: blood tests can miss important antibody deposition in the graft

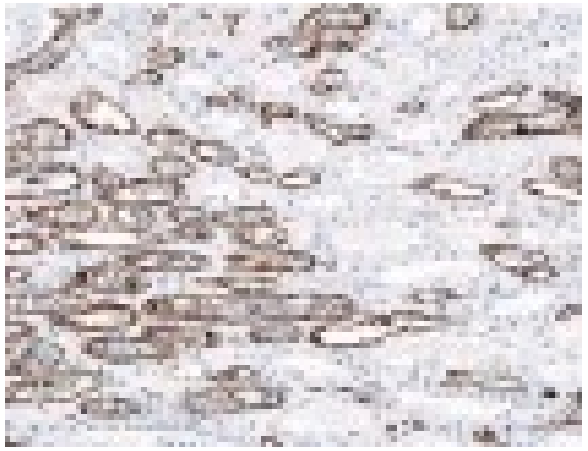
Rejection on biopsy

- Features
  - Inflammation
  - Tissue injury
  - Antibody detection or deposition
  - Complement activation (C4d)
  - Fibrosis (chronic changes)
- Location
- Magnitude









Rejection on biopsy

Heart

Vascular / Endothelium

Myocytes

Kidney

Interstitial vs intimal inflammation

Tubular injury

Lung

Perivascular

Airway

Liver

Portal inflammation

Bile duct injury

Endothelium

Could your biopsy miss the problem?

- Biopsies can show inflammation/injury without allograft dysfunction
  - Lung: ~50% is clinically silent
  - Kidney: 5% had subclinical T-cell rejection
  - Kidney: ~50% with de novo DSA+ good graft function had ABMR

- Subclinical rejection may or may not be clinically important
  - Lung: first subclinical rejection may not affect long term graft function
  - Kidney: treatment of subclinical rejection may not impact kidney function 6 months later

Rush D, AJT, 2007
Schinstock C, AJT, 2017
Orandi B, AJT, 2015

Diagnosis of acute rejection

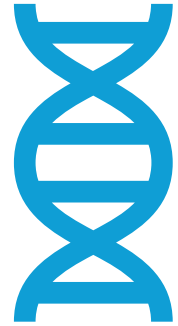
Symptoms

Shortness of breath

Fatigue

Functional testing

Biopsy



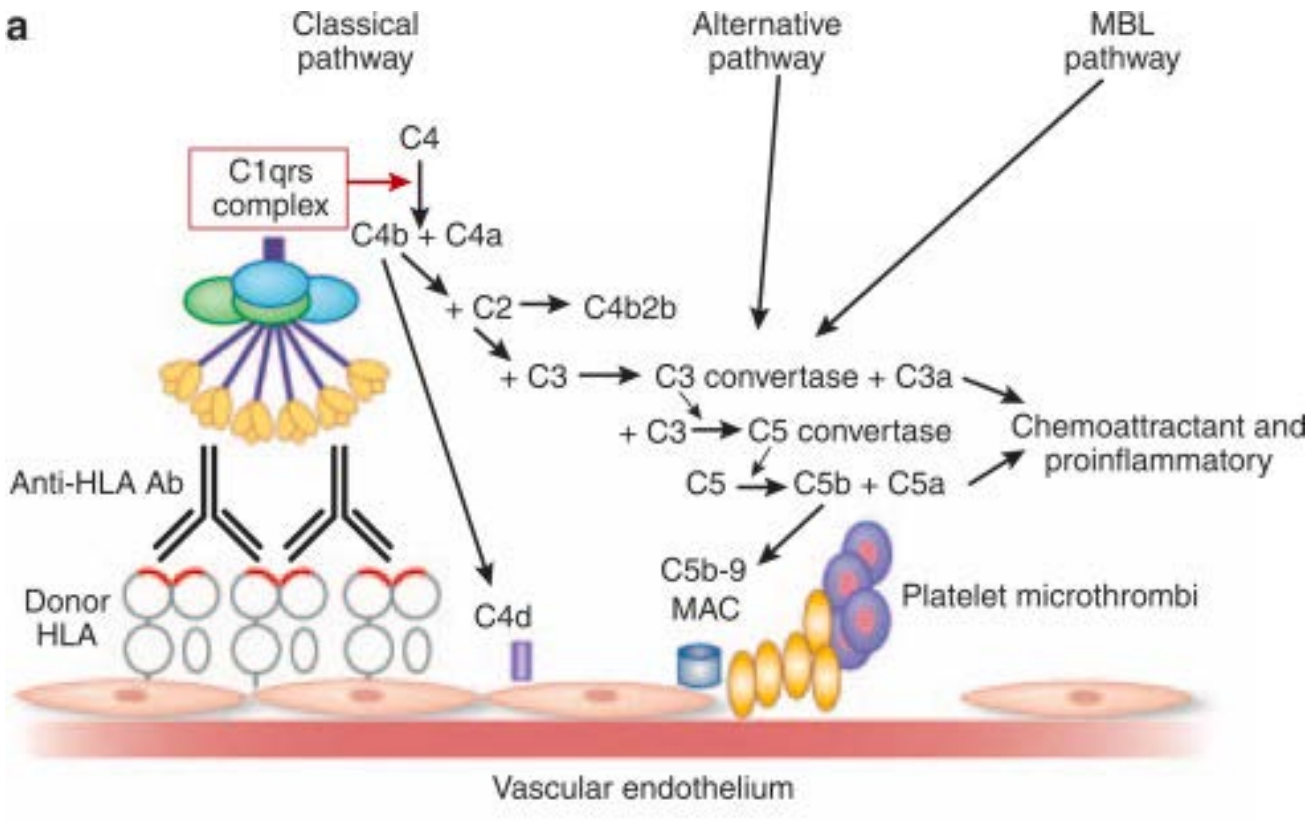
Biomarkers

DSA

Cell-free DNA

Transcriptional responses

Why can anti-HLA antibodies be so bad?



- 1. Antibody binds HLA
- 2. Complement system is activated
- 3. Membrane attack complex is formed
- 4. Endothelial cell death
- Releases more donor antigens → amplifies

Heeger, Kidney Intl, 2010

Are all DSAs bad?

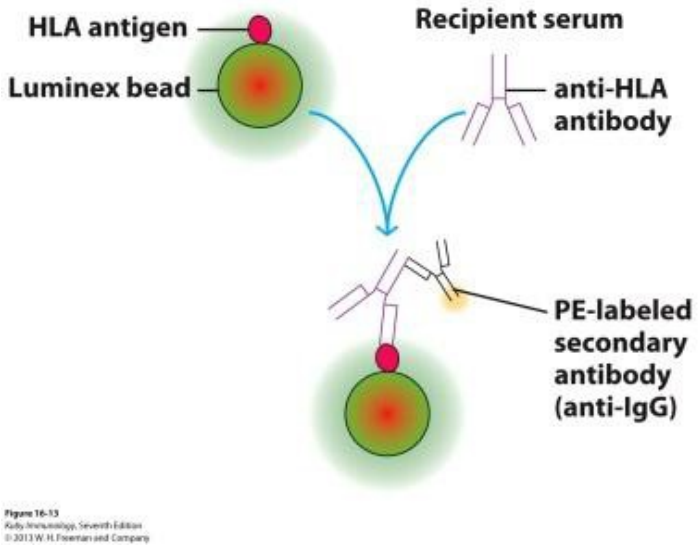
- Some antibodies are worse than others
  - Fix complement → more likely to cause cell death
  - High MFI or high titer → more likely to cause injury
  - Class II are usually worse than Class I

- Non-HLA antibodies are increasingly appreciated as important contributors to graft failure
  - Some are donor-specific, but others are not

Testing for anti-HLA antibodies

- Test the recipient for presence of anti-HLA antibodies

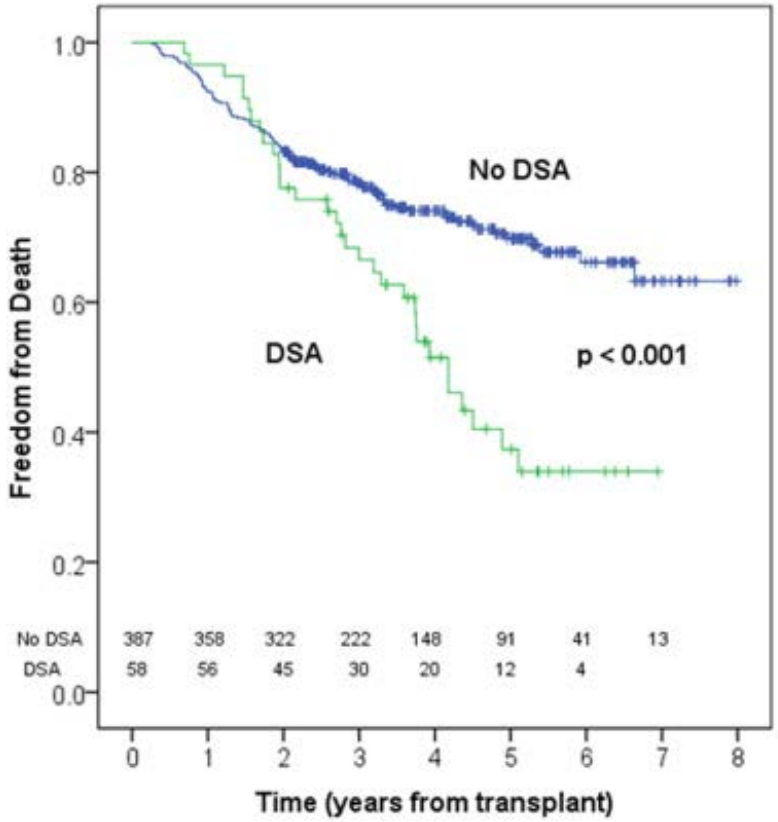
Each bead has a different HLA antigen

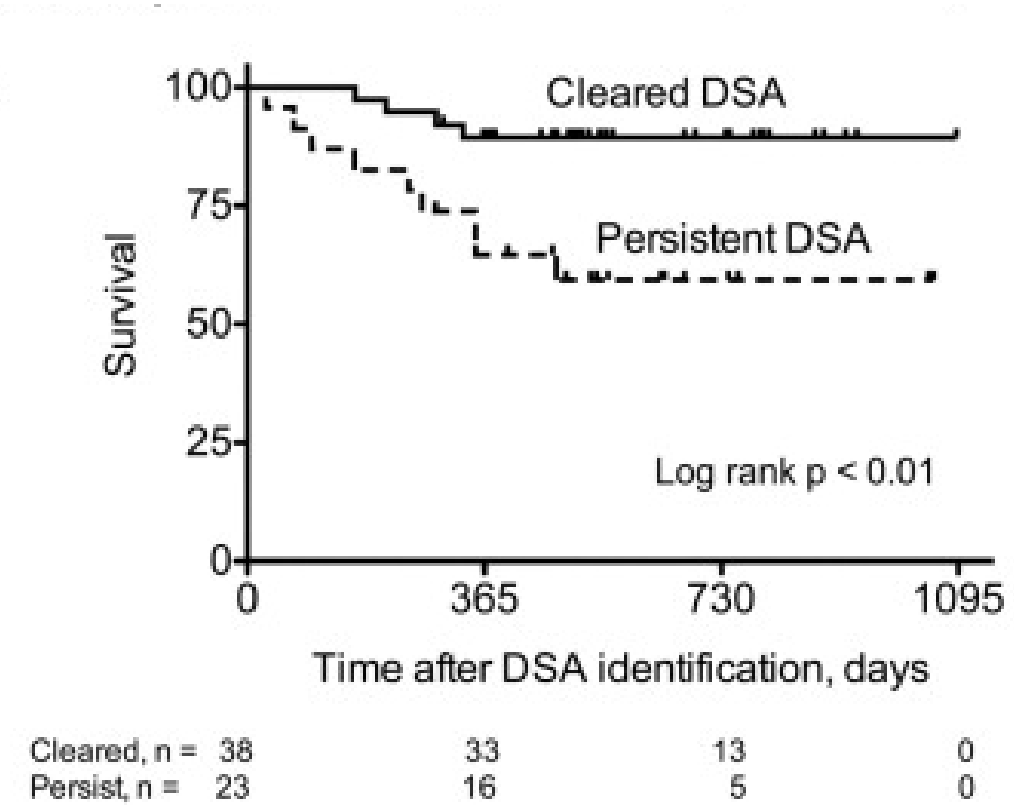


Beads can also have C1q

- If HLA-antibodies are found, compare to the donor HLA type to determine if donor-specific
- Then repeat assay with C1q added to see if complement fixing

Lung transplant patients with DSA have reduced survival





N=445
Morrell, JHLT, 2014

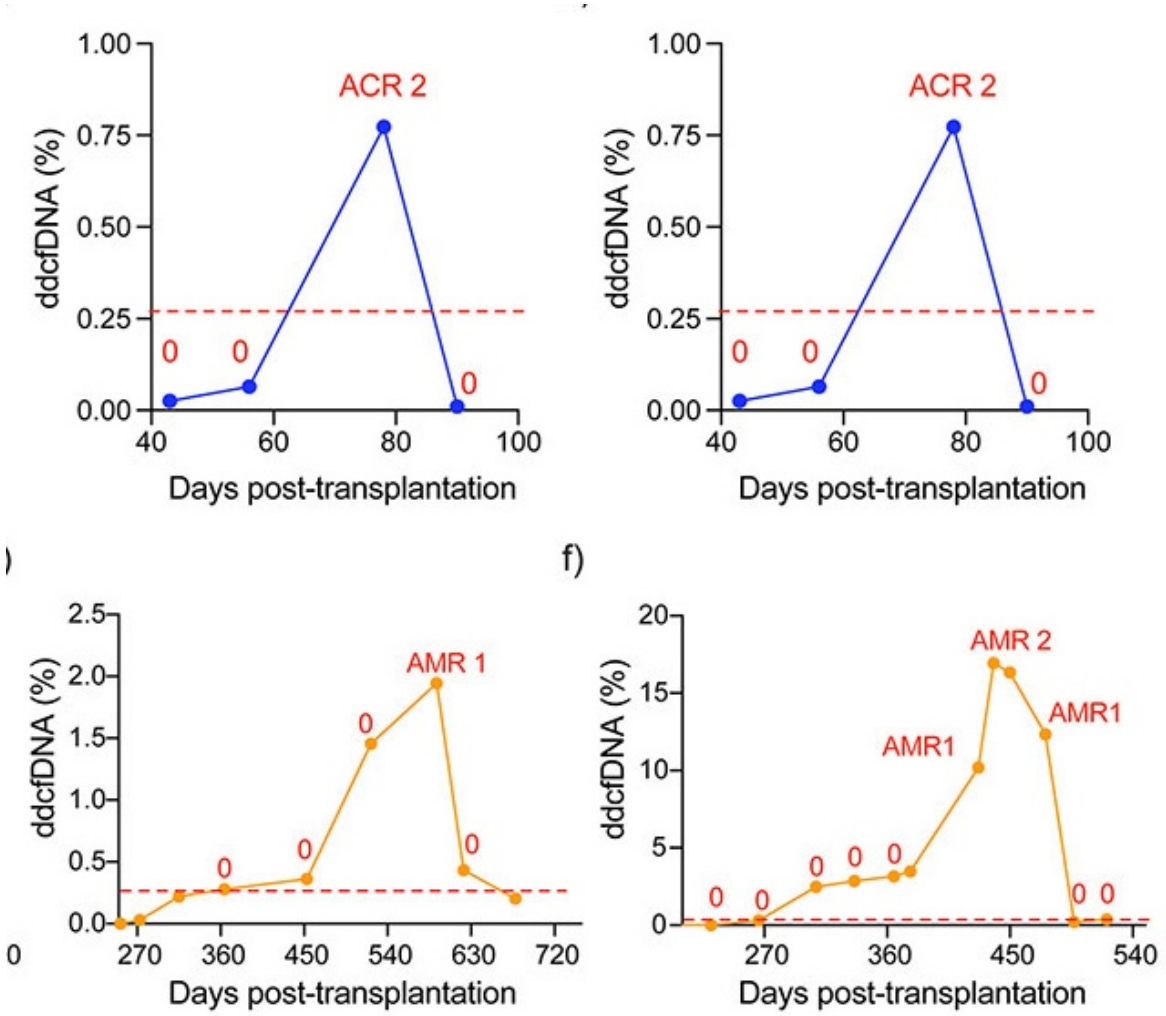
Hachem, JHLT, 2010

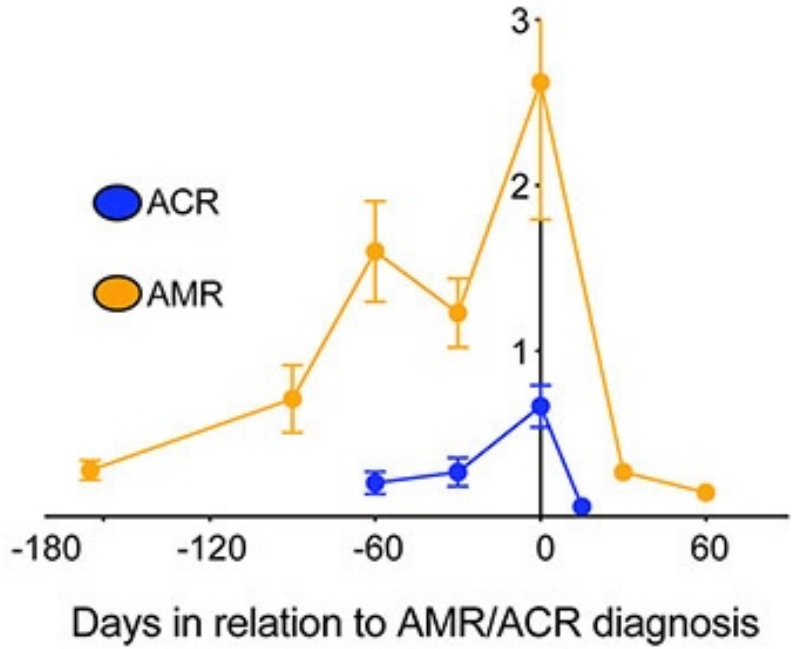
Donor-derived cell-free DNA

- Damage to the allograft releases DNA into the circulation
- Sequencing quantifies how much donor vs. recipient DNA is present
- Low level = graft is healthy

- Example from kidney:
  - Cutoff of 1%: negative predictive value for antibody-mediated rejection of 96%
  - Cutoff of 0.74%: negative predictive value 100%, positive predictive value 69%
    - Did not discriminate between those with and without Tcell–mediated rejection

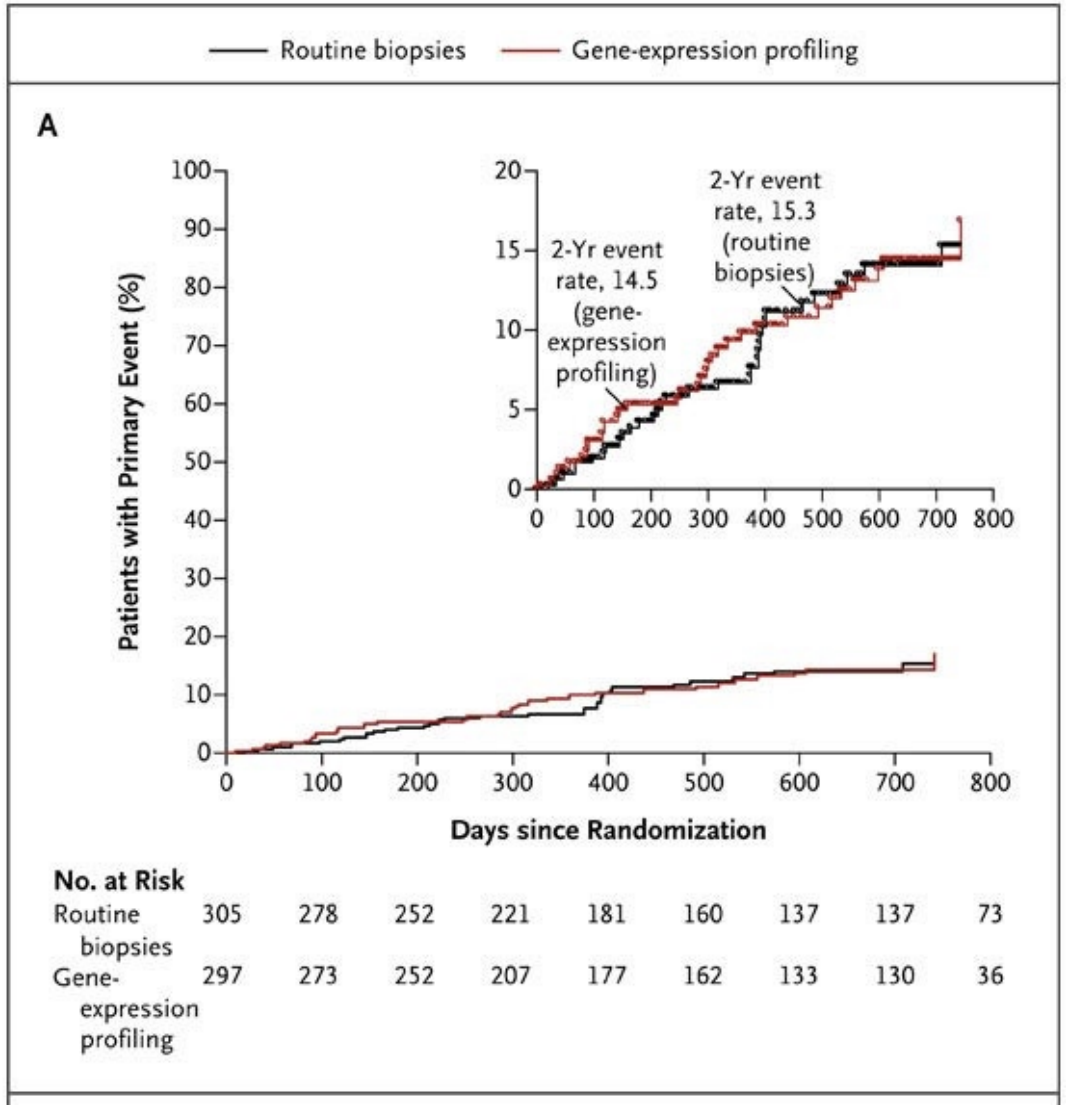
Donor-derived cell-free DNA release associates with and precedes rejection

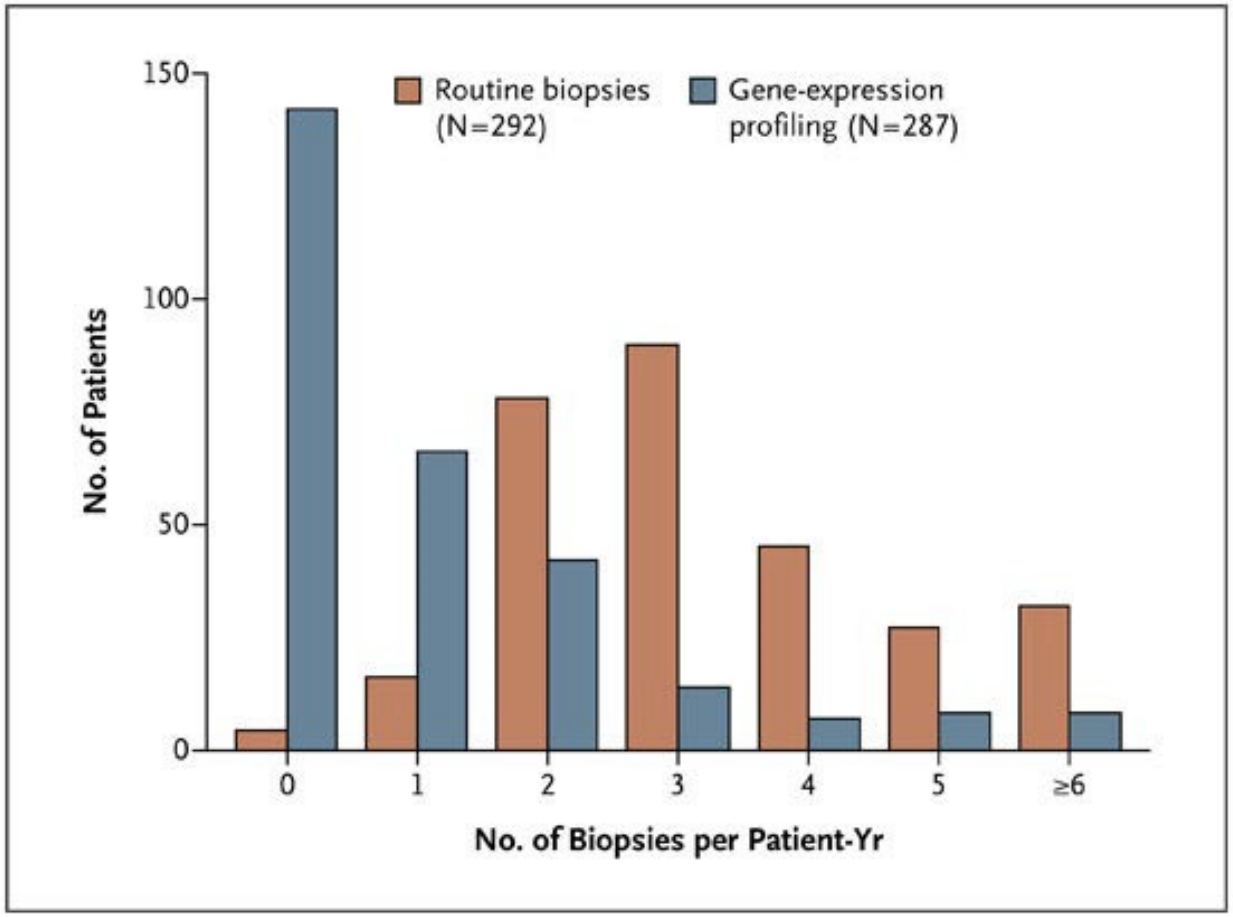




Agbor-Enoh S, Circulation, 2021

Gene profiling for heart - Allomap





Pham, NEJM, 2010

Combination of plasma Allomap and cell-free DNA help assess utility of heart biopsy

HIGH ALLOMAP / LOW ALLOSURE

A biopsy is unlikely to reveal ACR (ACR positivity in SHORE[4] = 1.9%)

Consider biopsy or repeat HeartCare testing earlier if:

- AlloSure level is close to threshold and increased from prior measurement

Consider other pathological causes of an increased AlloMap:

- CMV infection

Medication adherence review recommended

DUAL POSITIVE HEARTCARE (High AlloSure / High AlloMap)

A biopsy is more likely to reveal ACR
(ACR positivity in SHORE[4] = 9.2%)

- Biopsy should be considered

Medication adherence review recommended

DUAL NEGATIVE HEARTCARE (Low AlloSure / Low AlloMap)

A biopsy is unlikely to reveal ACR
(ACR positivity in SHORE[4] = 1.5%)

- Continue protocol immuno-optimization

LOW ALLOMAP / HIGH ALLOSURE

A biopsy is unlikely to reveal ACR (ACR positivity in SHORE[4] = 4.3%)

Consider a biopsy or repeat HeartCare testing earlier if:

- AlloMap is close to threshold and AlloSure has increased by ≥0.2% from prior measurement
- Recent treatment for rejection (<21 days) or current prednisone >20 mg
- At risk of Antibody Mediated Rejection/markedly elevated AlloSure

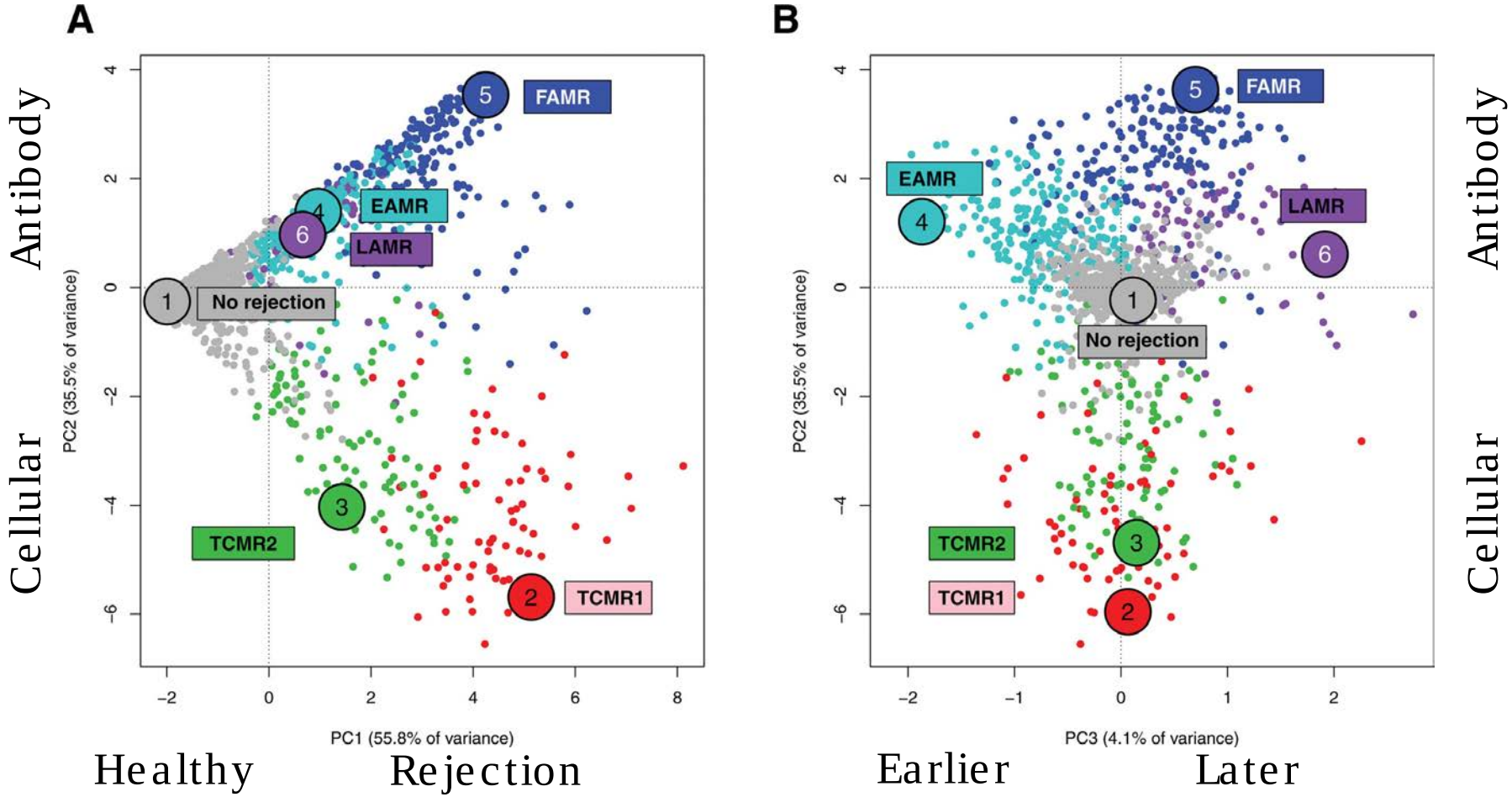
Consider other possible pathological causes of an increased AlloSure:

- Cardiac allograft vasculopathy
- Severe infection
- Antibody Mediated Rejection (AMR) / Donor specific antibodies

Medication adherence review recommended

Molecular microscope for kidney

- Transcriptomics of 1679 biopsy samples → analyzes patterns of gene expression to give likelihood of different types of organ injury



THE MMDx-KIDNEY REPORT

Clinical information
Time post-transplant; biopsy indication, DSA

6. Summary of additional molecular scores
Rejection, injury-related binary classifiers (and AKI transcript set)

Comparison to normal
Scores of this biopsy interpreted vs. relatively normal biopsies

Patient information
Date of transplant, date of biopsy, etc.

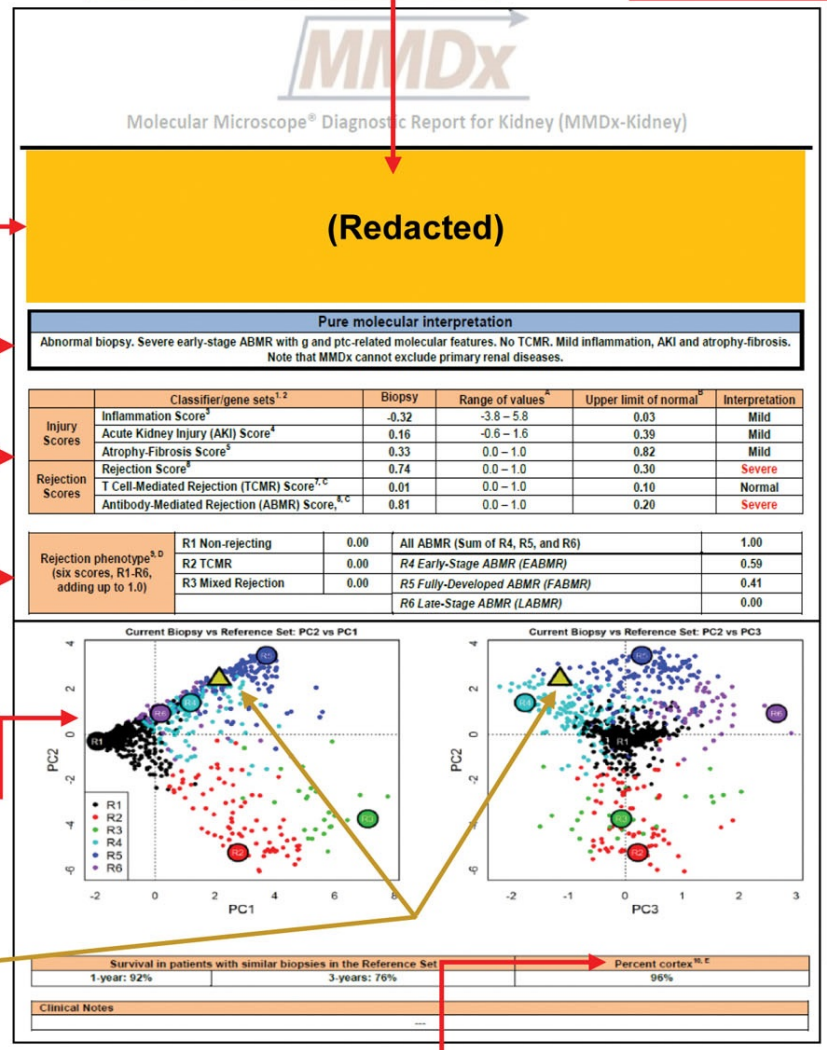
1. Sign-out
Interpretation of the molecular phenotype

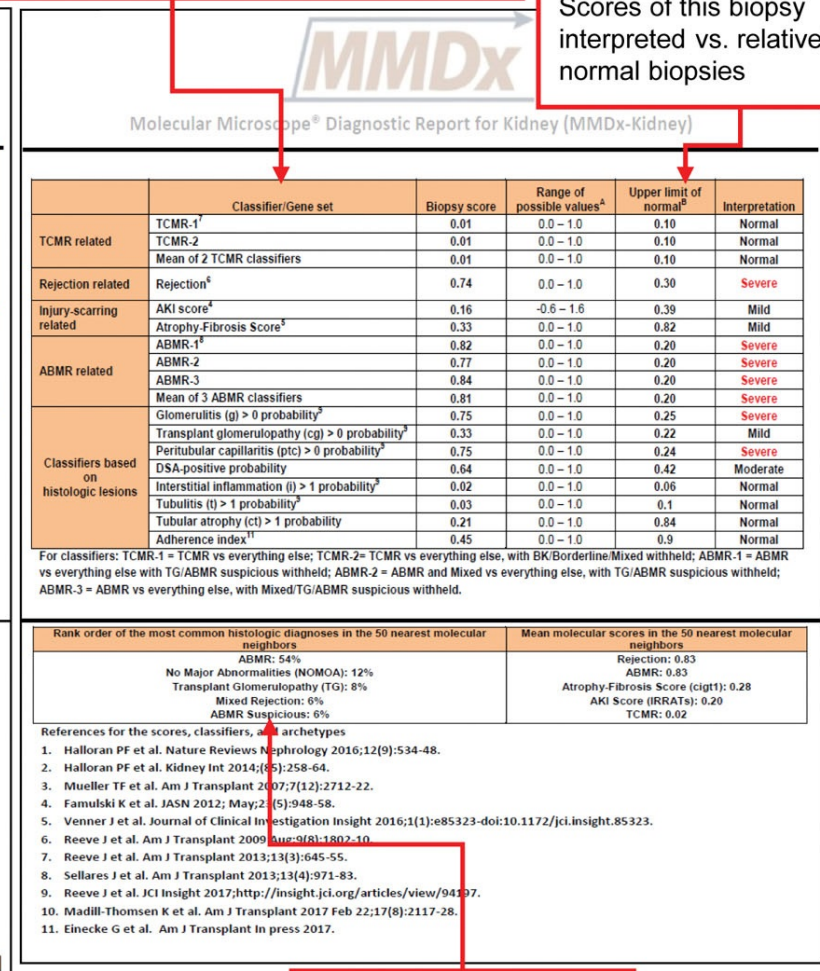
2. Summary of molecular scores
(Injury, rejection)

3. Archetypal analysis
Rejection-related archetype scores (Normal, TCMR, ABMR)

4. Visualization
Principal component analysis (PCA) plots. Relationship of this biopsy to others in the reference set:
- PC2 vs. PC1
- PC2 vs. PC3

This biopsy
Yellow triangle





5. Percent cortex
Molecular estimate of the % of this biopsy core that is cortex

7. Characteristics of nearby biopsies
Based on the nearest neighbors in 3-dimensional space from the PCA plots

THE MMDx-KIDNEY REPORT

Clinical information

6. Summary of additional molecular scores

Patient inf
Date of trans
biopsy, etc.

1. Sign-ou
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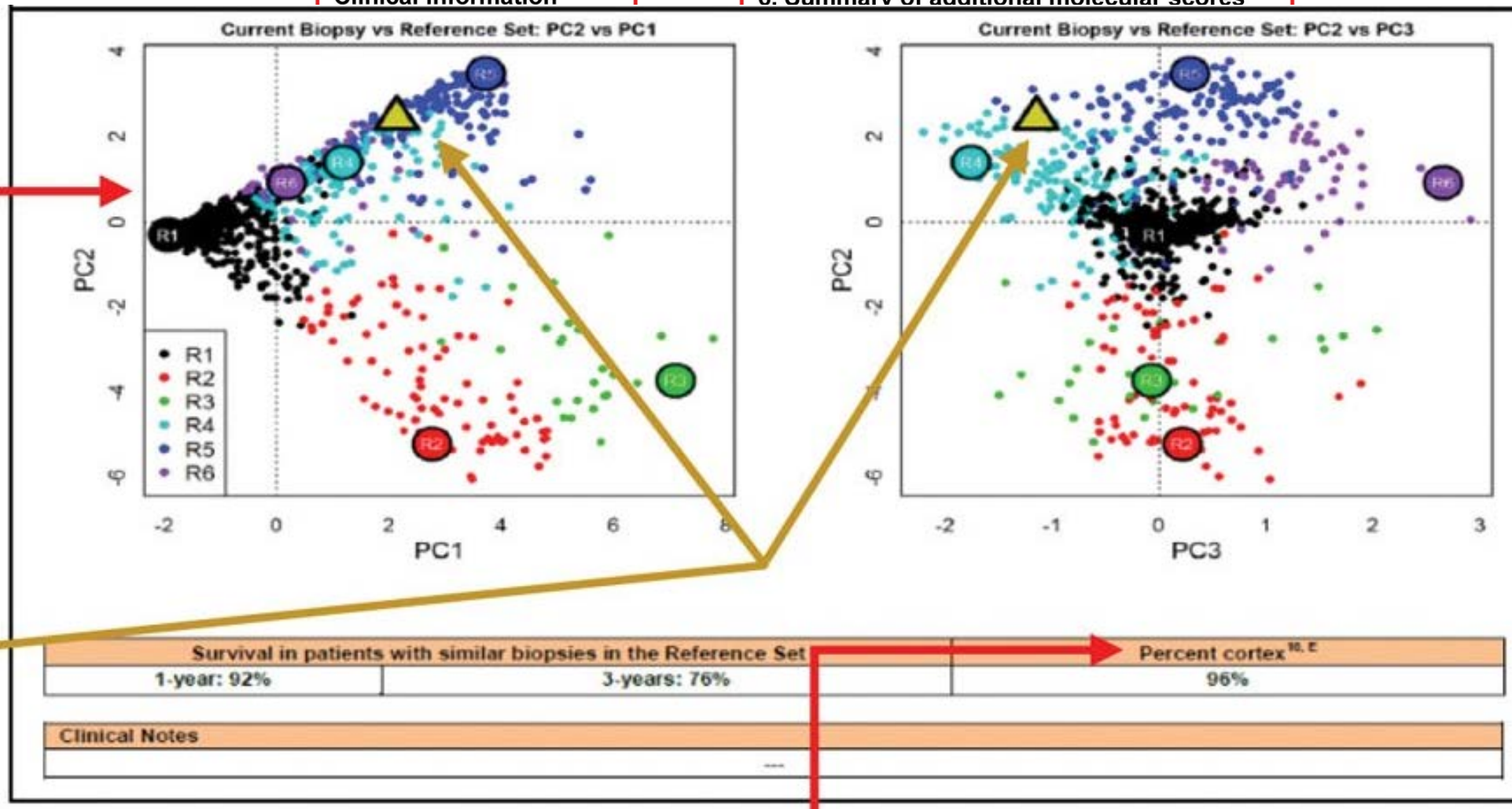
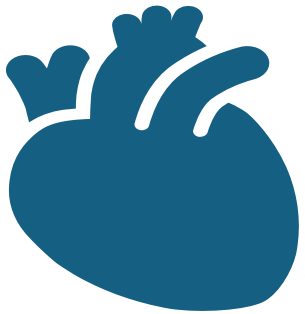


This biopsy
Yellow triangle

5. Percent cortex
Molecular estimate of the % of this biopsy core that is cortex

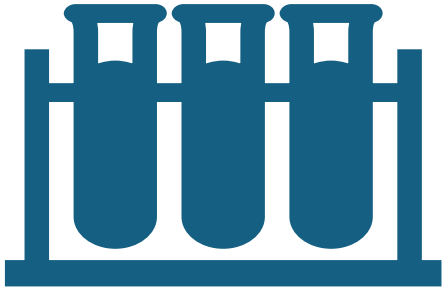
7. Characteristics of nearby biopsies
Based on the nearest neighbors in 3-dimensional space from

You diagnosed rejection – what do you do?



Is the organ failing?

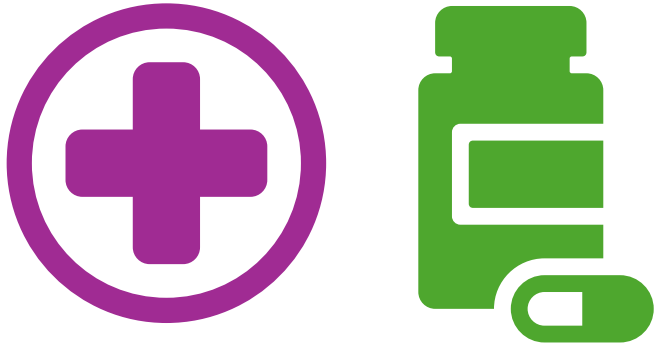
Are the abnormalities mild or severe?



Cellular or antibody related injury? Both?

Treatment of acute rejection

- Considerations
  - Cellular vs. antibody-mediated vs. both
  - Degree of allograft dysfunction (or lack thereof)
  - Potential for side effects



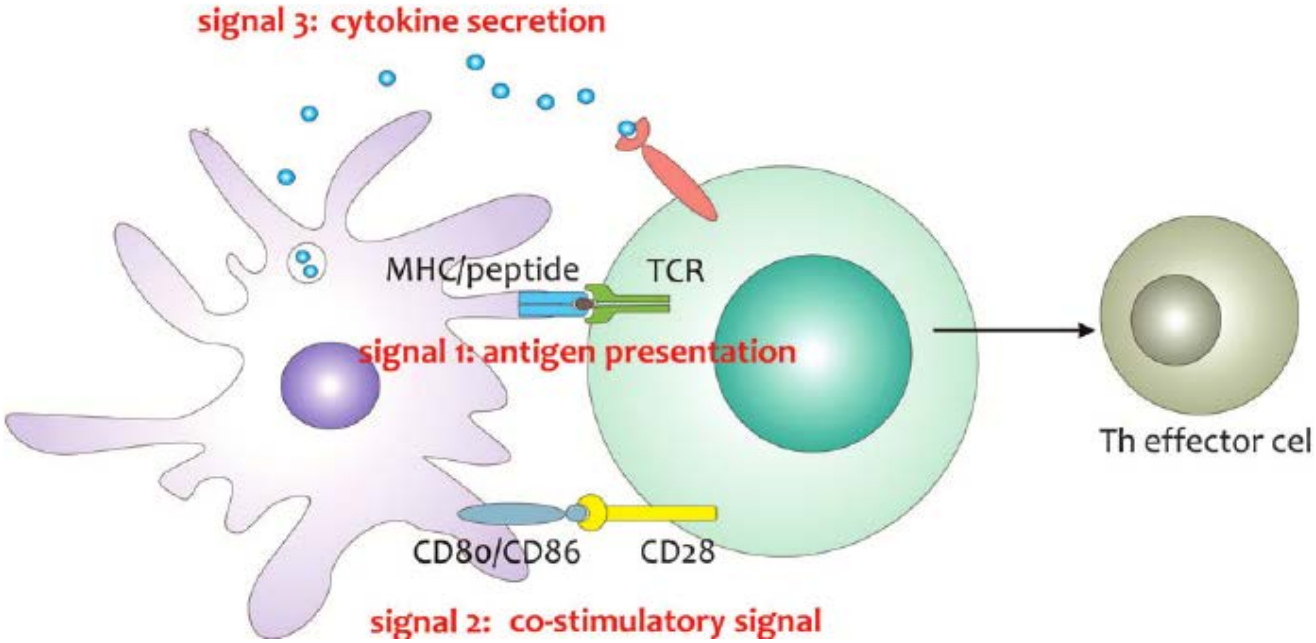
- There are very few clinical trials that directly compare treatment options
  - Many have <50 patients per group

Treatment strategies

Suppress	Suppress Tcell activation
Remove	Remove existing pre-formed antibodies
Stop	Stop production of additional antibody
Suppress	Suppress signals driving antibody production
Stop	Stop complement activation

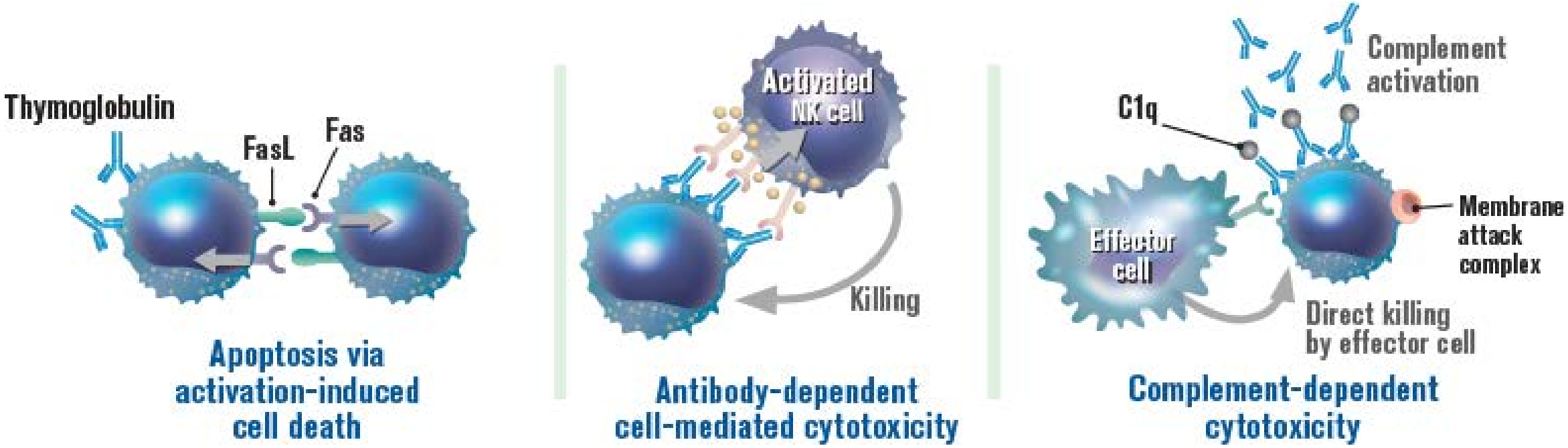
Suppress Tcell activation

- Anti-thymocyte globulin
  - Polyclonal antibody preparation
- Alemtuzumab
  - Anti-CD52
- Co-stimulation blockade
  - Belatacept



Thymoglobulin / ATG / R-ATG

- Rabbit polyclonal antibodies against human thymocytes
- Depletes T cells for several months



http://www.thymoglobulin.com

Alemtuzumab

- Targets CD52, an antigen of unknown function expressed on Tand B lymphocytes

- Profound immunosuppression lasting >6 months

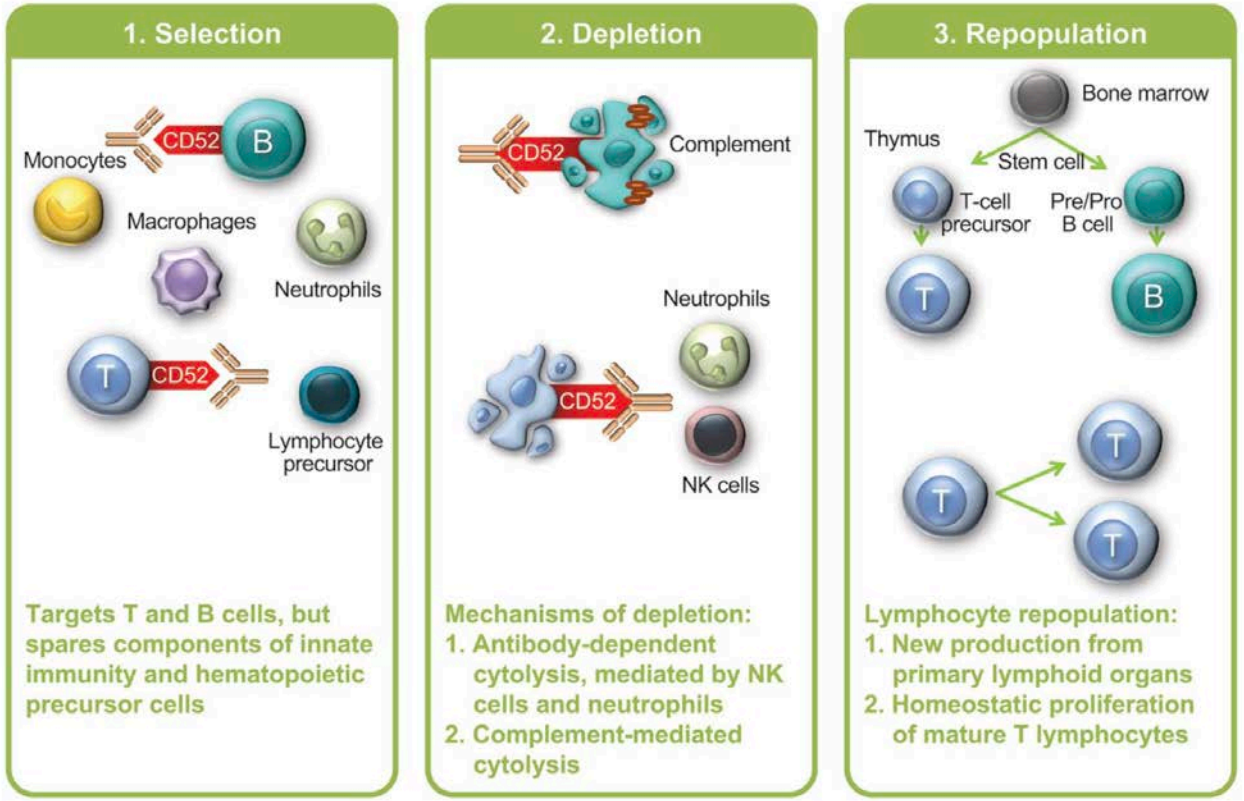
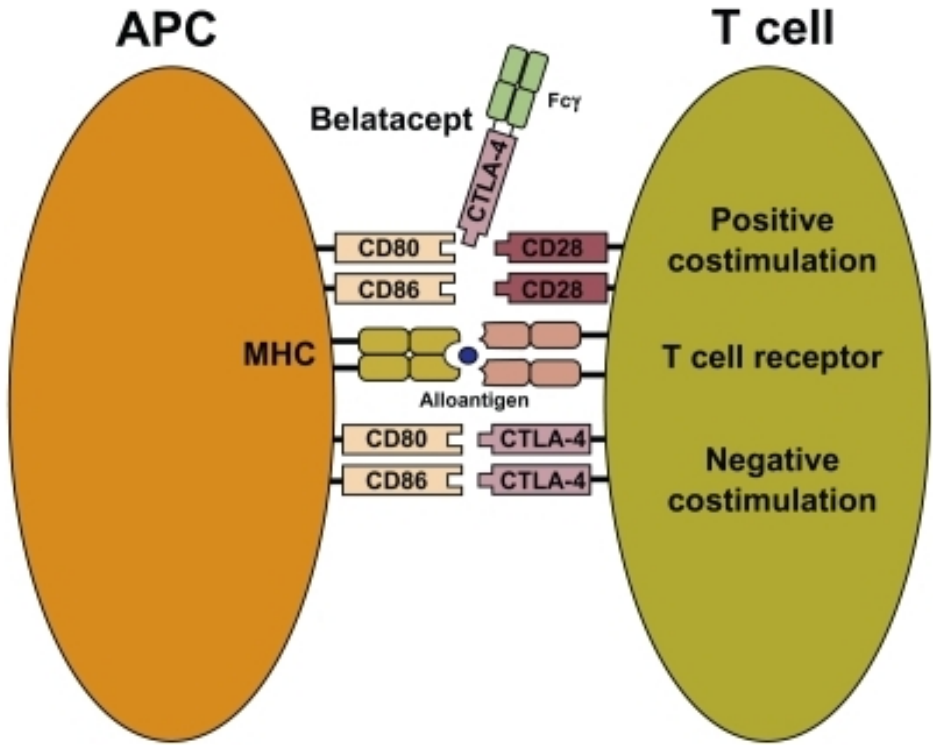


Figure 1. Alemtuzumab proposed mechanism of action.
NK, natural killer.

https://www.researchgate.net/figure/Alemtuzumab-proposed-mechanism-of-action-NK-natural-killer_fig1_270909982

Belatacept

- Fusion protein of Immunoglobulin with CTLA4
- Higher affinity for CD80/CD86 than CD28
  - CD28 not activated
  - Tcells get negative signal
  - Apoptosis



Gupta G, Drug Des Devel Ther, 2010

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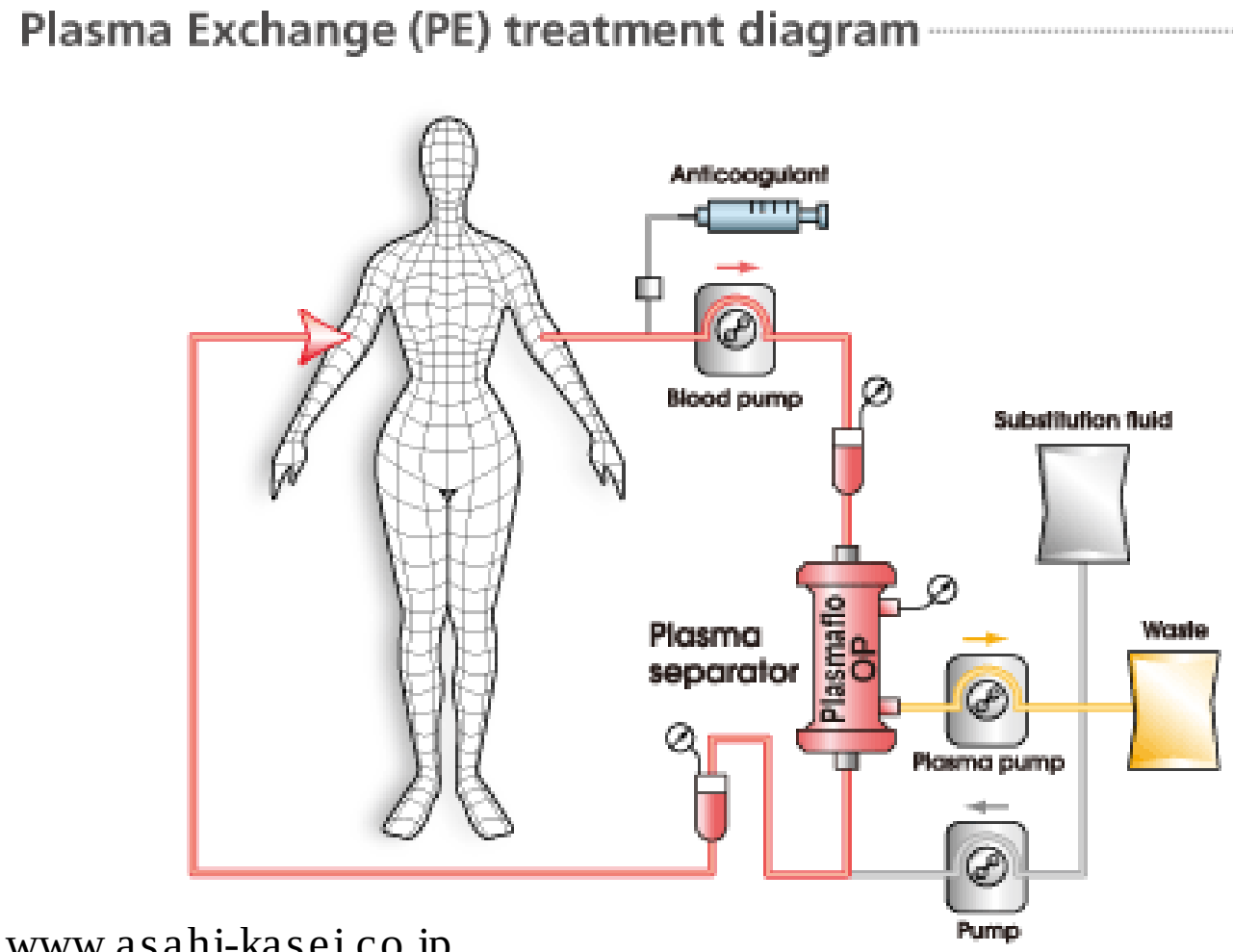
Removing pre-formed antibodies

- Plasmapheresis
  - Removes (all) antibodies from the circulation

- IVIg (immunoglobulin)
  - Binds and facilitates removal of existing antibodies

Plasmapheresis

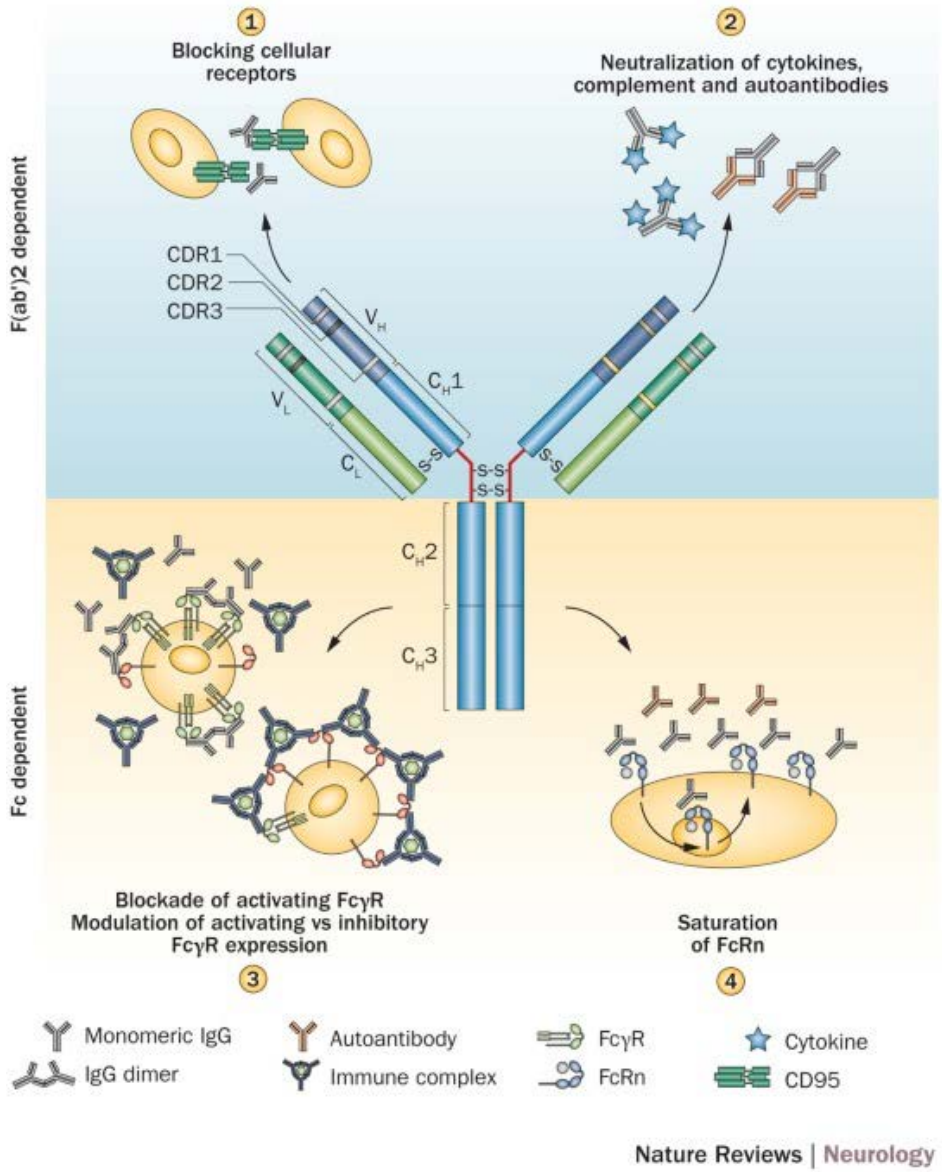


www.asahi-kasei.co.jp

- All antibodies (pathogenic and protective) are affected
- Replace volume with FFP or albumin
- Can adjust the number of exchanges

IVIG

- Binds to circulating antibodies → neutralizes, facilitates immune complex removal
- Saturates FcRn → prevents recycling of Ab and facilitates degradation in lysosomes
- Blocks complement and other cellular receptors



Lunemann, Nat Rev Neurol, 2015

Treatment strategies

Suppress	Suppress Tcell activation
Remove	Remove existing pre-formed antibodies
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Stop	Stop complement activation

Stop production of additional antibodies

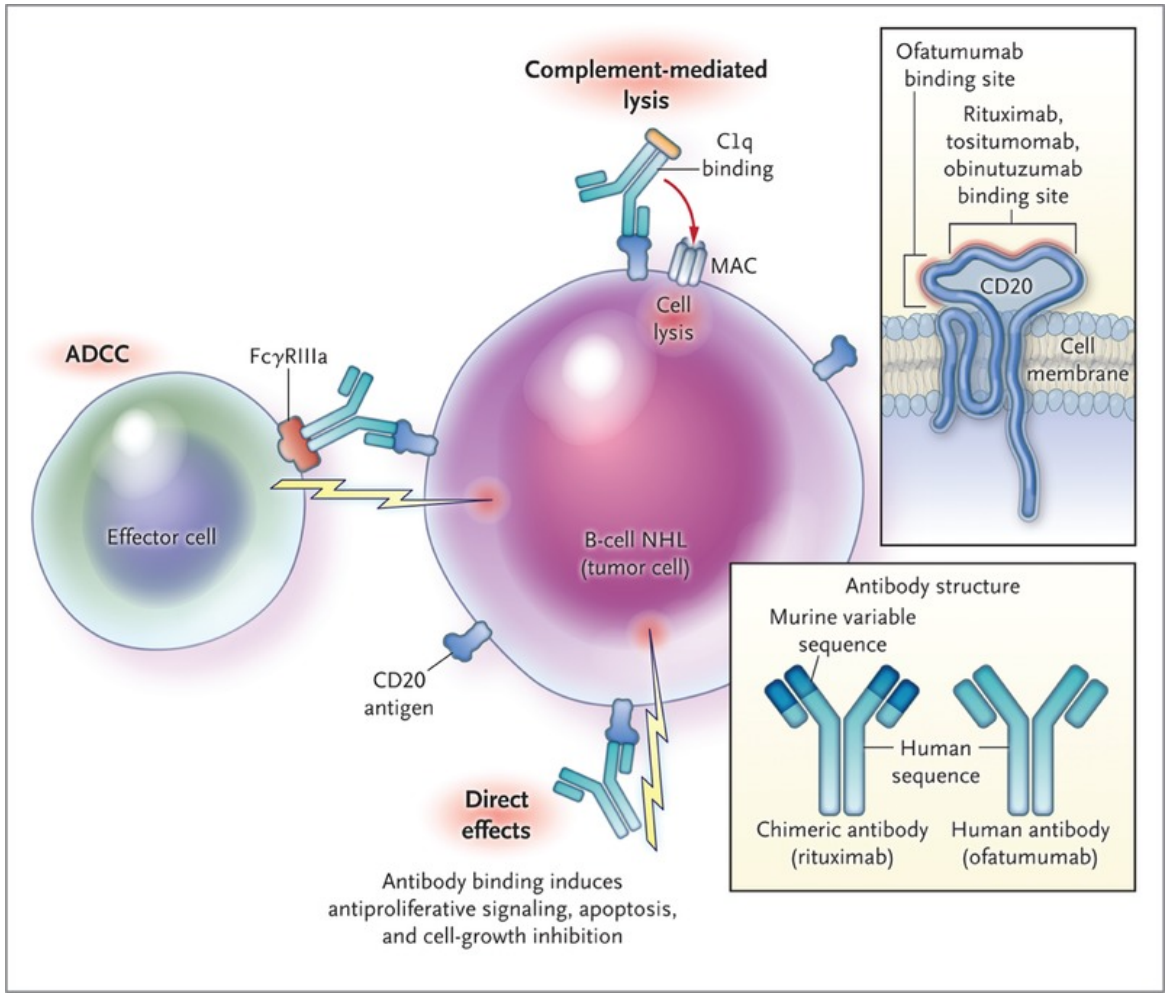
- Anti-CD20 – rituximab
  - Targeted removal of CD20+ B cells
- Proteosome inhibitors – bortezomib, carfilzomib
  - Apoptosis of plasma cells
- Anti-CD38 – daratumumab
  - Targeted removal of CD38+ plasma cells and NK cells

Rituximab

- Anti-CD20 antibody (B cells)

- Antibody-dependent cytotoxicity
- Complement-dependent cell lysis
- Antibody-dependent phagocytosis
- Apoptosis due to signal interruption

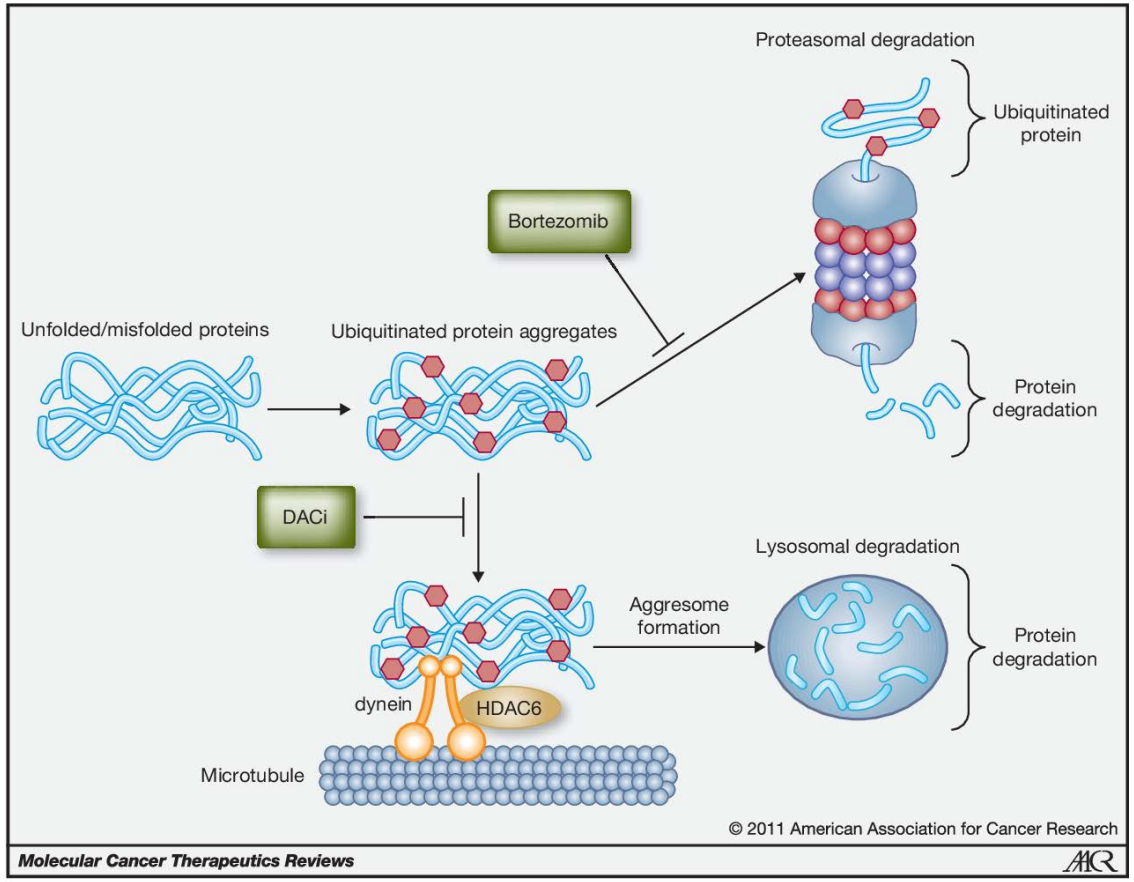
- Successfully reduces Ab levels and cPRA
  - Many grafts have Ab resurgence within 1 month (Vo, Transplantation, 2014)



Maloney, NEJM, 2012

Proteosome inhibitors

- Bortezomib (reversible), carfilzomib (irreversible)
- Misfolded proteins accumulate
  - Apoptosis
- Targets plasma cells
  - Make enormous amounts of protein
- Numerous side effects
- Therapeutic effect lasts up to 6m and then rebounds



Hideshima, Mol Cancer Ther Rev, 20

Daratumumab

- Anti-CD38 monoclonal antibody (plasma cells, NK cells)
- Mechanism of effect is similar to rituximab
  - Antibody-dependent cytotoxicity
  - Complement-dependent cell lysis
  - Antibody-dependent phagocytosis
  - Apoptosis
- Reduced Ab levels and improved graft survival, but had worse rebound in non-human primates (Kwun, Am J Soc Neph, 2019)
  - CD38 is also on regulatory B cells and some suppressor cells and therefore suppresses some “good” immune responses

Treatment strategies

Suppress	Suppress Tcell activation
Remove	Remove existing pre-formed antibodies
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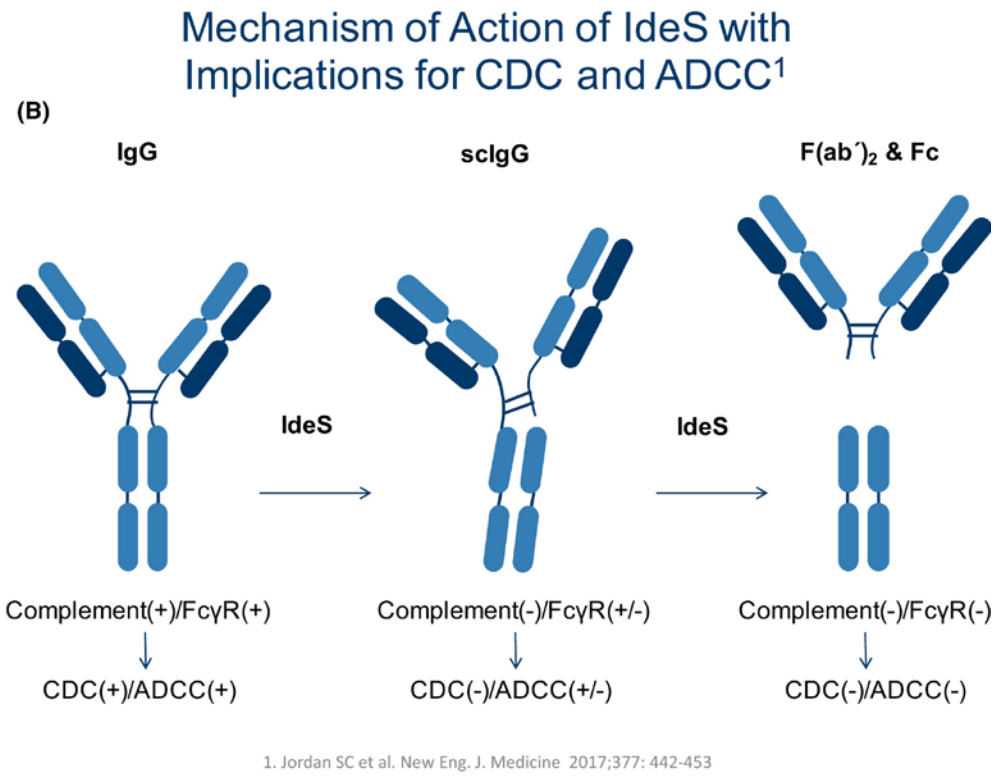
Suppress signals driving antibody production

- Lymphocyte depletion
  - Anti-thymocyte globulin
  - Alemtuzumab
- IgG cleavage proteins
  - Inflimidase
- IL-6 pathway therapies
  - Tocilizumab
  - Clazakizumab

Inflimidase

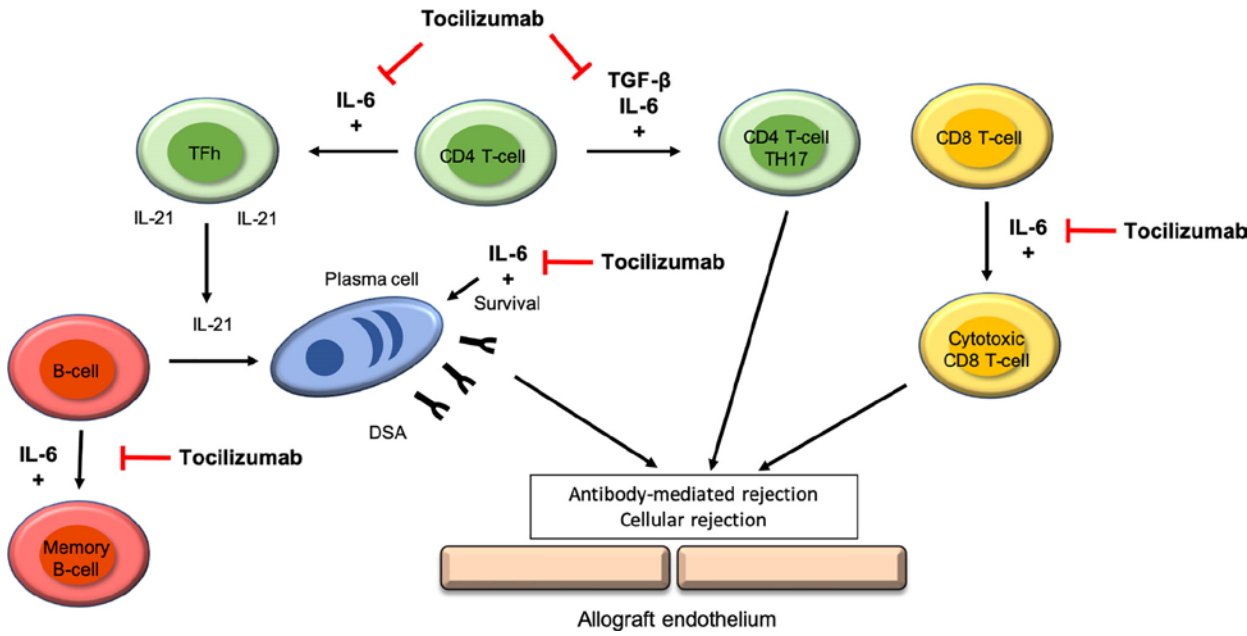
- Streptococcal protein
- Cleaves circulating IgG into F(ab) and Fc
  - Inhibits Ab-dependent and complement-dependent cytotoxicity
- Circulating antibody is depleted within 6 hours
  - Also cleaves B cell receptors → inhibits Ag binding, may reduce plasma cell differentiation
- Rebound IgG levels within 1-2 weeks
  - Used successfully in kidney, usually in combination with other agents



Huang, AJT, 2021

IL-6 pathway inhibition

- Tocilizumab (IL-6R antagonist)
- Clazakizumab (direct IL-6 inhibitor)
- IL-6 functions
  - Stimulates Thelper, Th17, and CD8
  - Inhibits regulatory T cells
  - Promotes plasma cell survival
- Growing data in kidney transplant

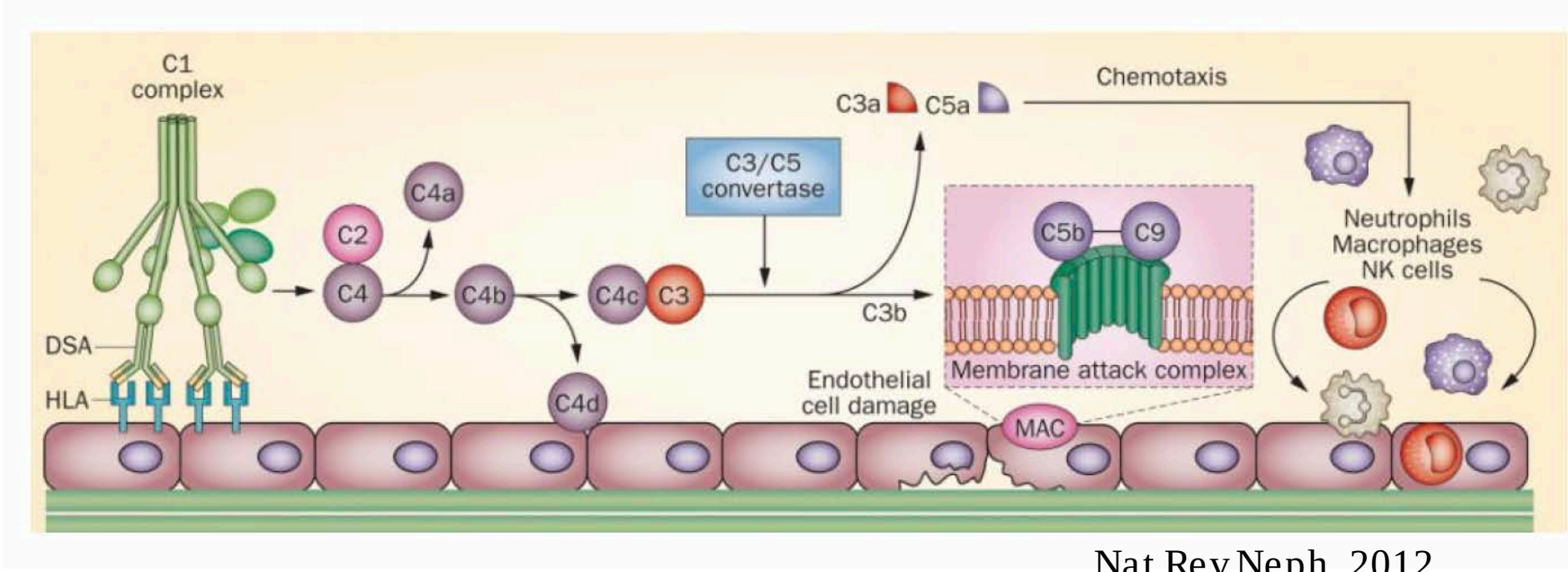


Treatment strategies

Suppress	Suppress Tcell activation
Remove	Remove existing pre-formed antibodies
Stop	Stop production of additional antibody
Suppress	Suppress signals driving antibody production
Stop	Stop complement activation

Stop complement activation

- Eculizumab
  - Anti-C5 antibody
  - No effect on antibody levels or binding
  - Prevents formation of MAC complex



Nat Rev Neph, 2012

Treatment strategies

Suppress	Suppress Tcell activation
Remove	Remove existing pre-formed antibodies
Stop	Stop production of additional antibody
Suppress	Suppress signals driving antibody production
Stop	Stop complement activation

Treatment options

Acute cellular rejection

- Thymoglobulin
- Alemtuzumab
- Belatacept

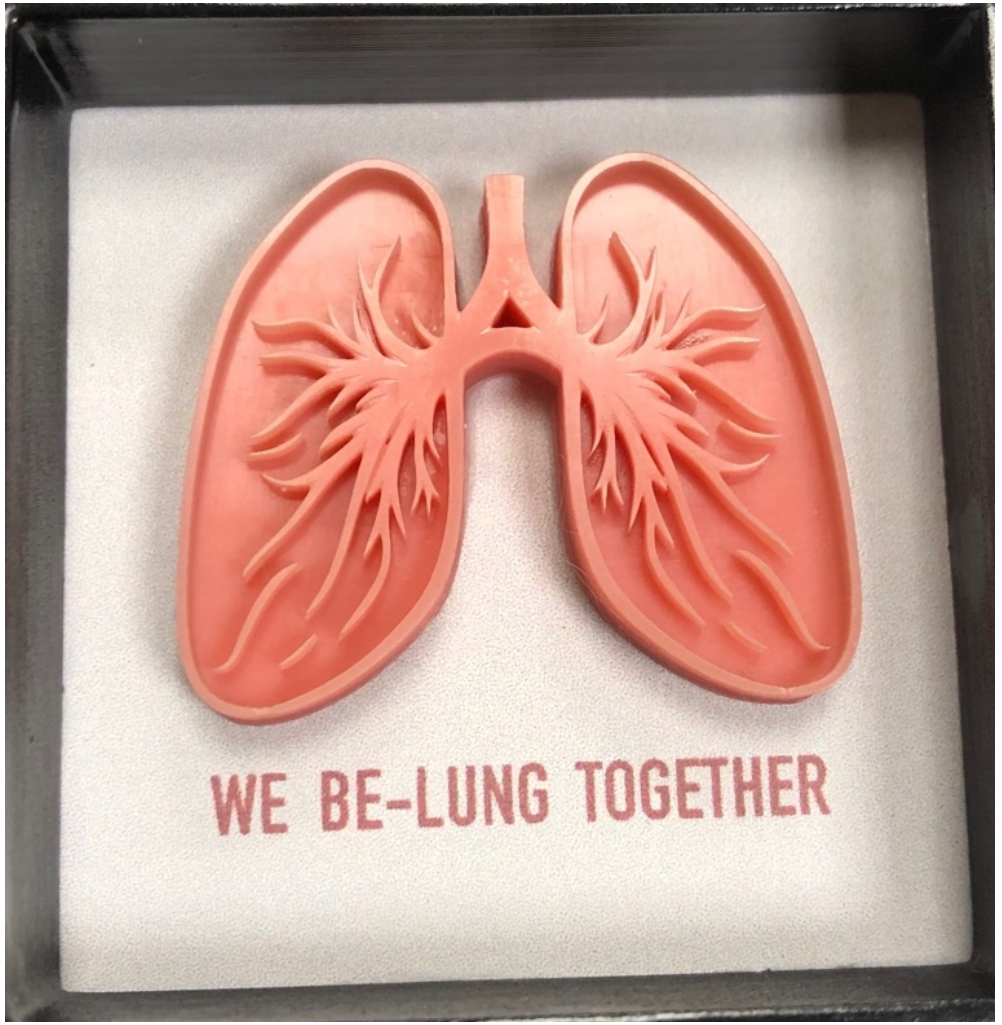
Antibody mediated rejection

- Plasmapheresis
- IVIG
- Rituximab
- Bortezomib / Carfilzomib
- Daratumumab
- Inflimidase
- Eculizumab

Combination

- Corticosteroids
- Tocilizumab / Clazakizumab

Questions?



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