Updates in Rejection Management

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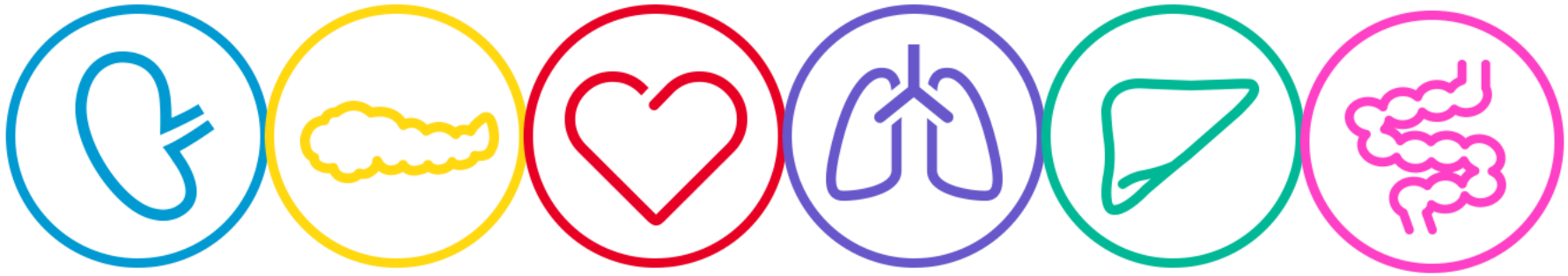

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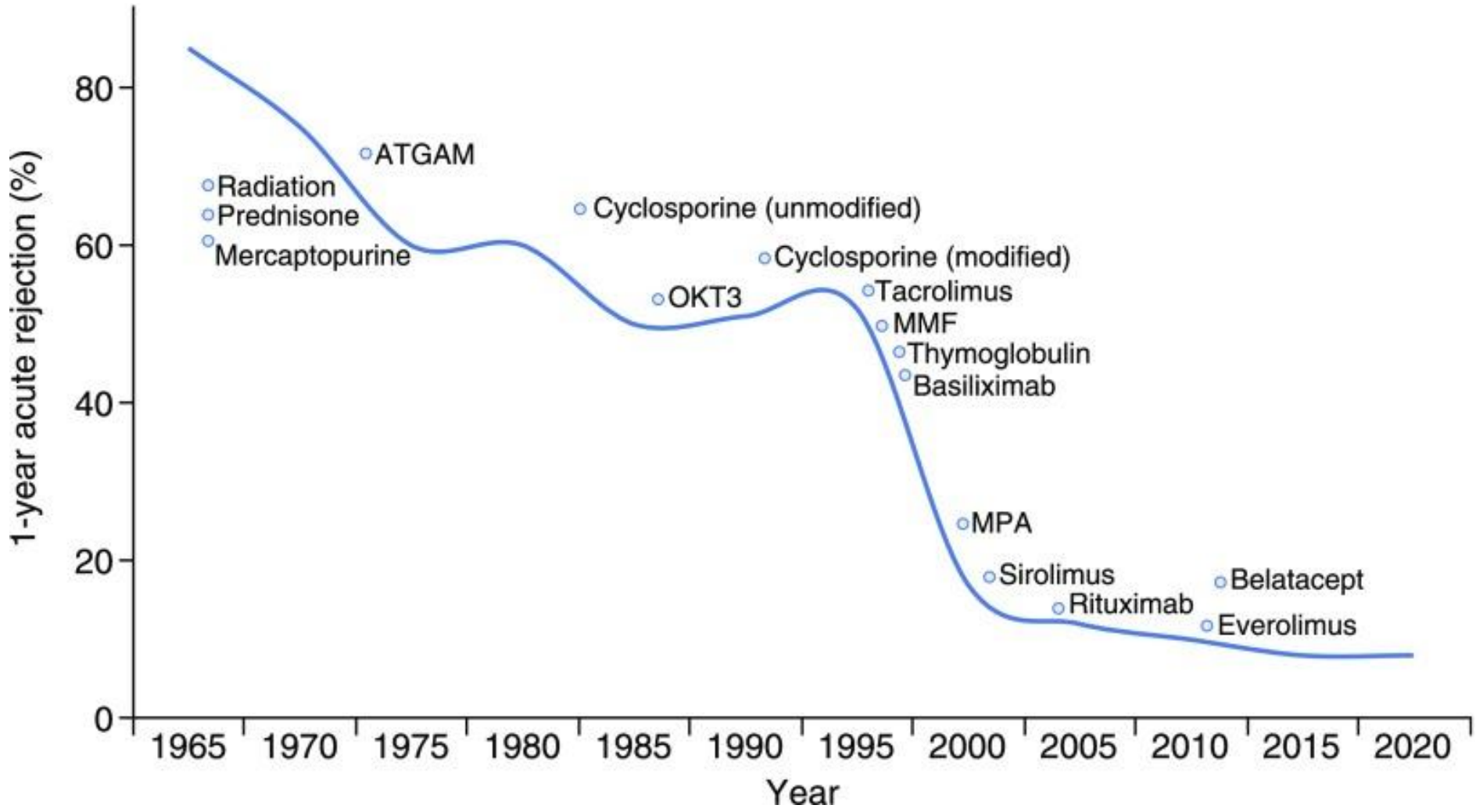
Disclosures

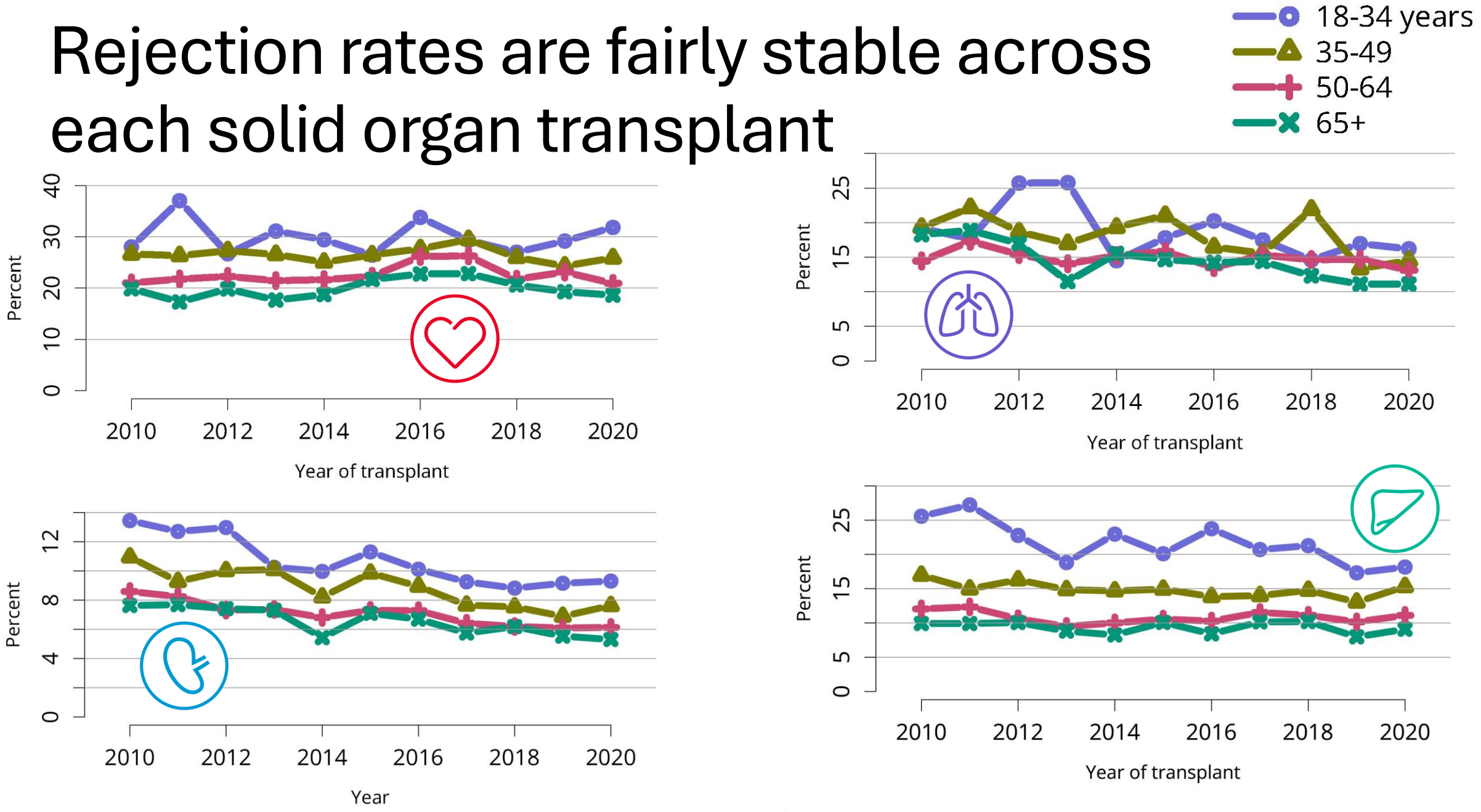
- Grant funding: NIH, Cystic Fibrosis Foundation, CareDx

Rejection remains a major problem after solid organ transplantation



Zand MS, Semin Dial, 2005; Cooper JE, CJN, 2020

Rejection rates are fairly stable across each solid organ transplant



The immunologic goals of transplantation

- Hide non-self antigens to avoid activation of injurious anti-allograft immune responses
- Respond to acute insults in a way to protect the recipient (infections) without excessive immune activation

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

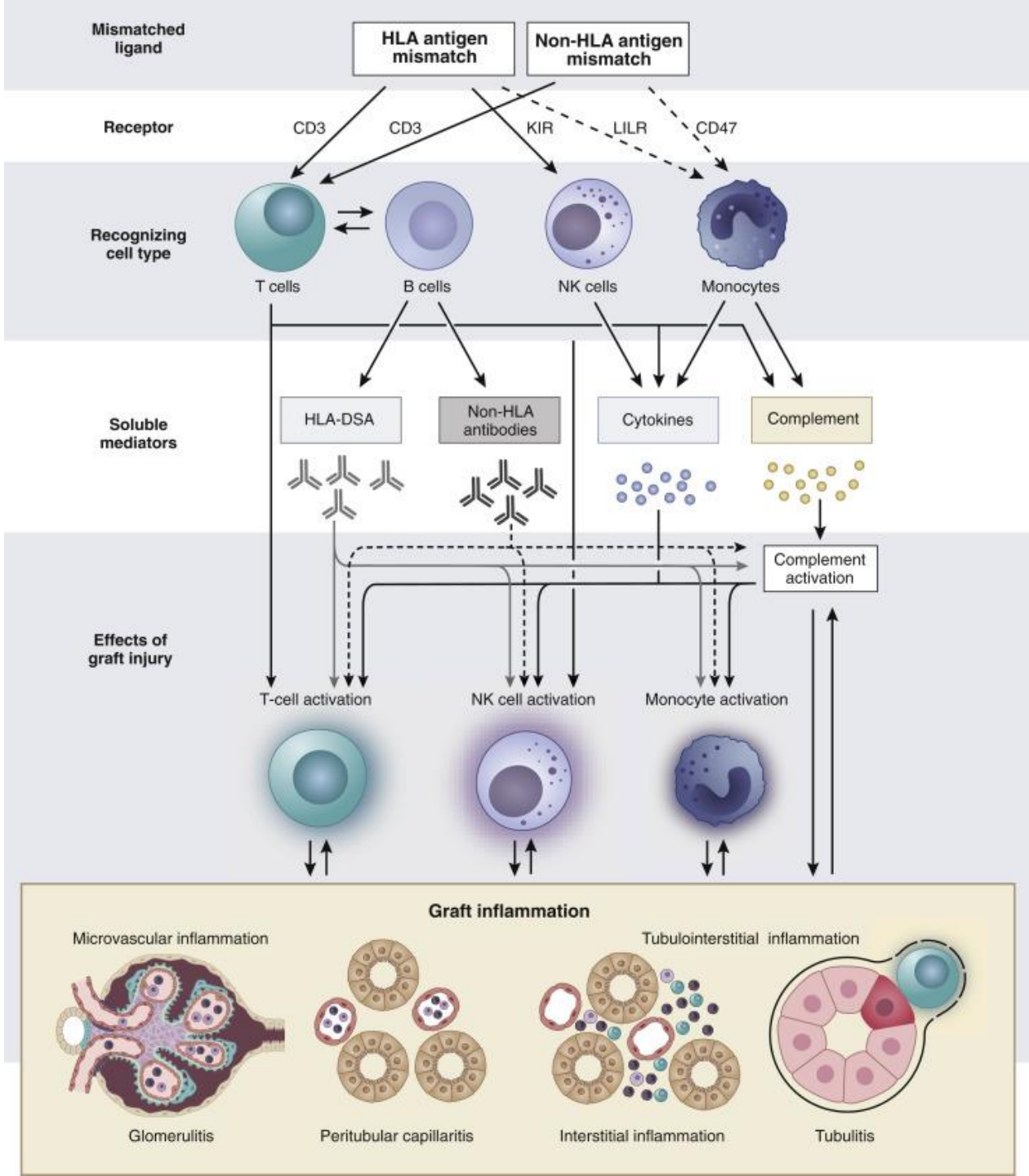
Types of acute rejection

Cellular rejection

Antibody mediated rejection

Mechanisms of rejection

- Lymphocytes
  - T cells
  - B cells
- Innate immune cells
  - NK cells
  - Monocytes / macrophages
- Soluble mediators
  - Antibodies (donor-specific, HLA or non-HLA)
  - Complement
  - Cytokines



Callemeyn J, Kidn Intl, 2022

Biopsy is the gold standard for rejection diagnosis

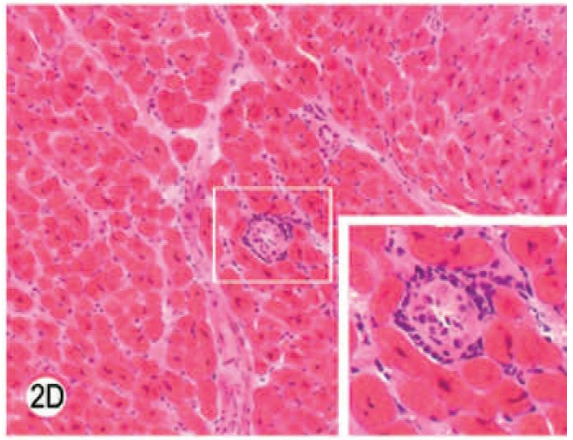
- Decades of data on interpretation
- Guidelines from professional societies for each organ

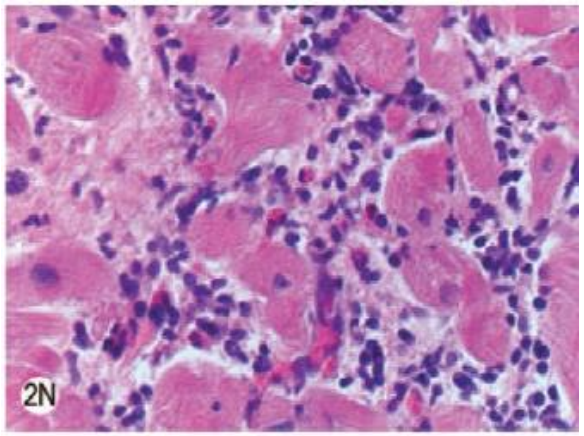
- Invasive

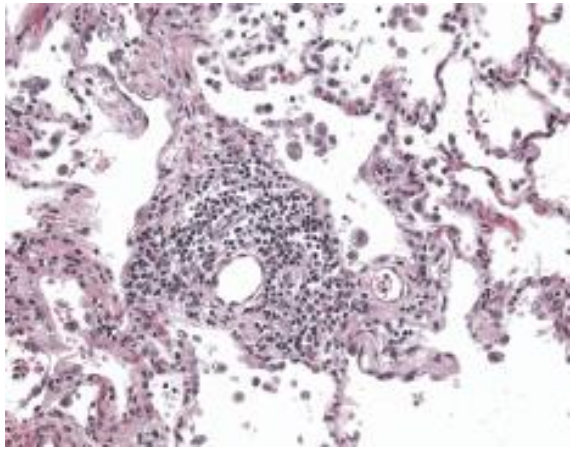
- Variability in interpretation
- Uncertain relationships with other biomarkers
  - Example: blood tests can miss important antibody deposition in the graft
  - Example: many biopsy-proven rejection episodes do not correlate with plasma biomarkers

Rejection on biopsy

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







Rejection on biopsy

Heart	Kidney	Lung	Liver
Vascular / Endothelium Myocytes	Interstitial vs intimal inflammation Tubular injury	Perivascular Airway	Portal inflammation Bile duct injury Endothelial injury

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

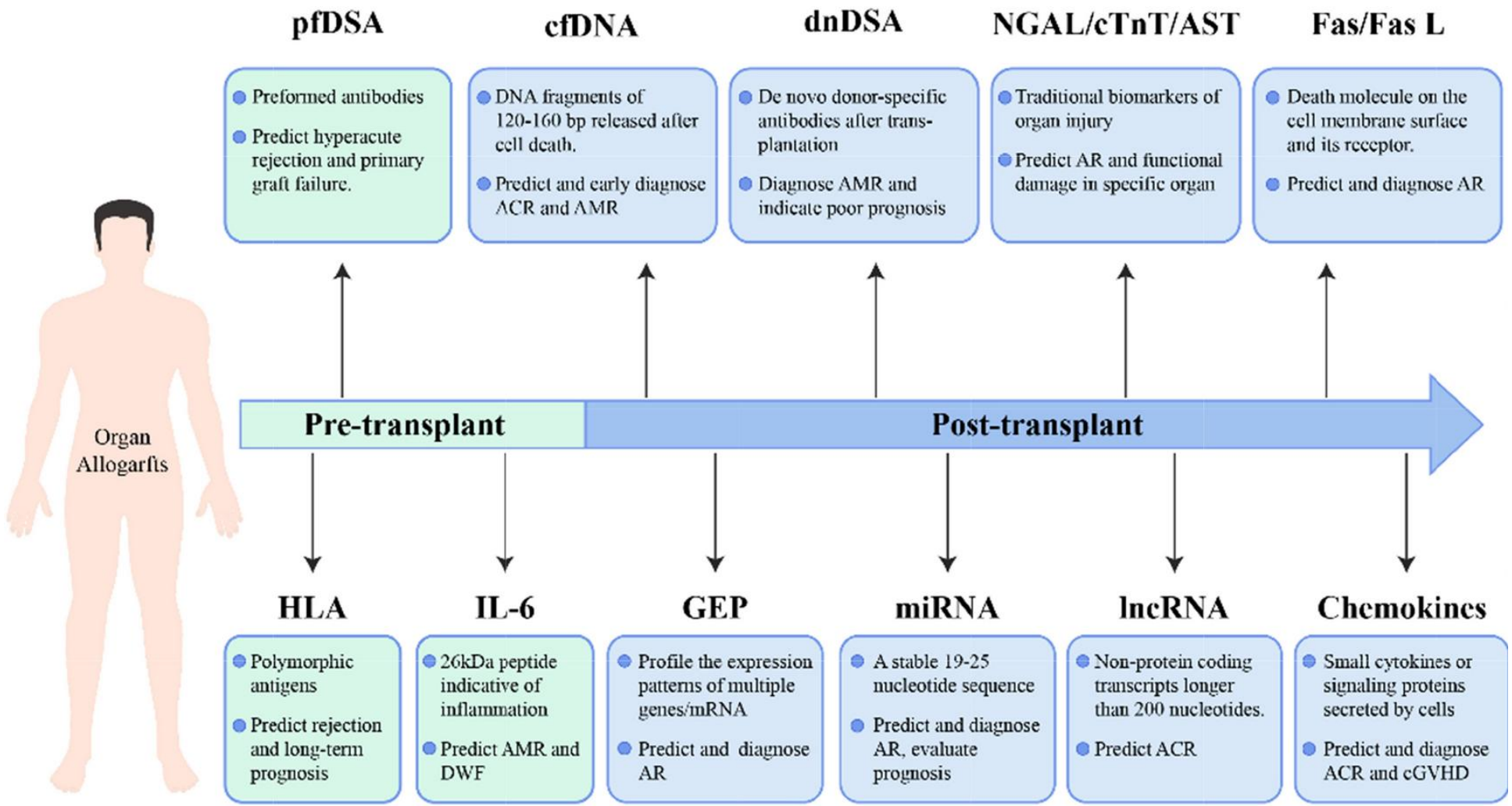
Biomarkers

DSA

Cell-free DNA

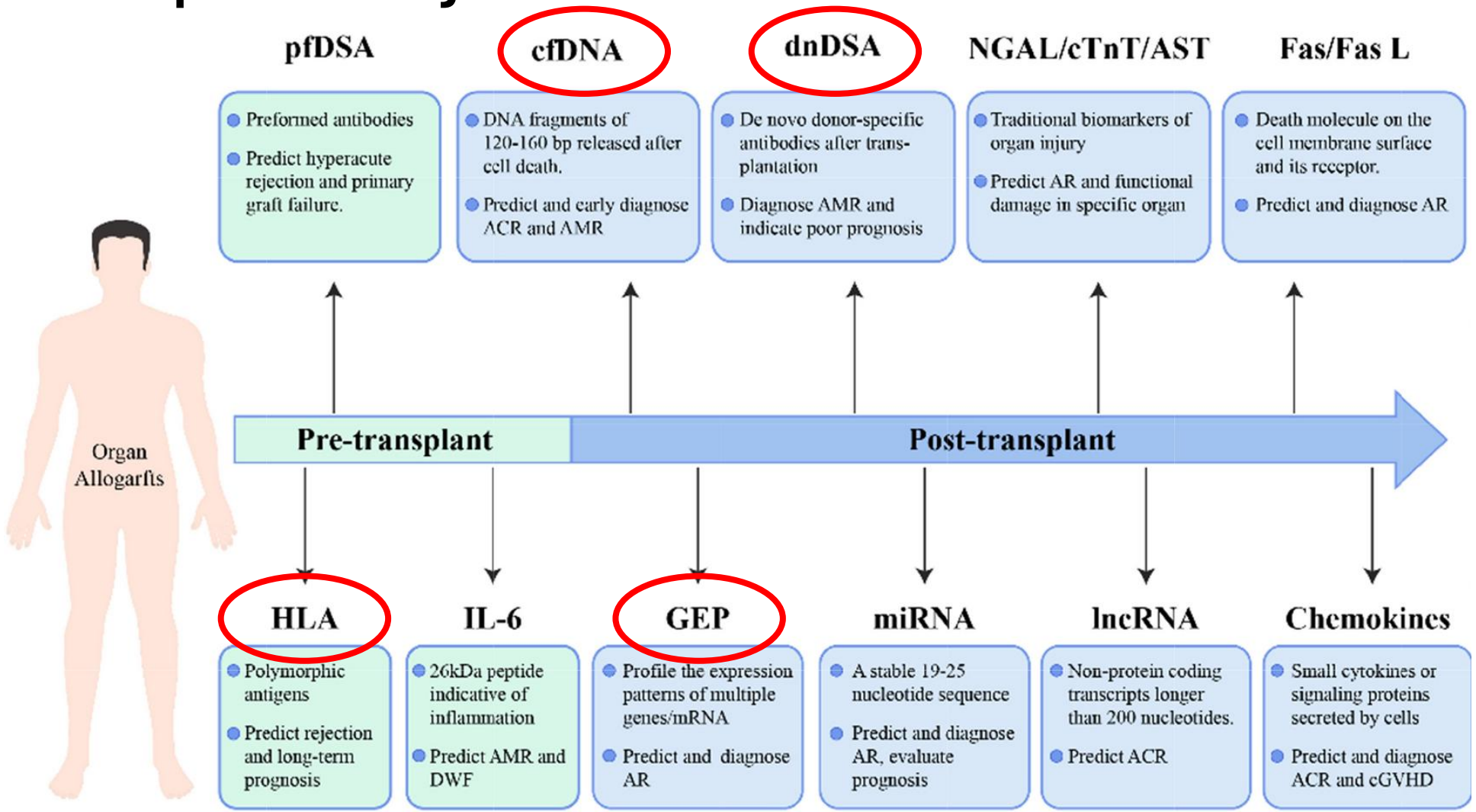
Transcriptional responses

Non-invasive biomarkers for monitoring for transplant rejection



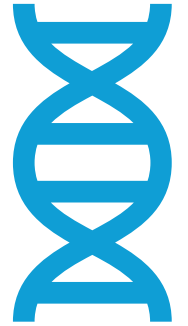
Song, *et al.* Advancements in noninvasive techniques for transplant rejection: from biomarker detection to molecular imaging. *J Transl Med* **23**, 147 (2025)

Non-invasive biomarkers for monitoring for transplant rejection



Song, *et al.* Advancements in noninvasive techniques for transplant rejection: from biomarker detection to molecular imaging. *J Transl Med* **23**, 147 (2025)

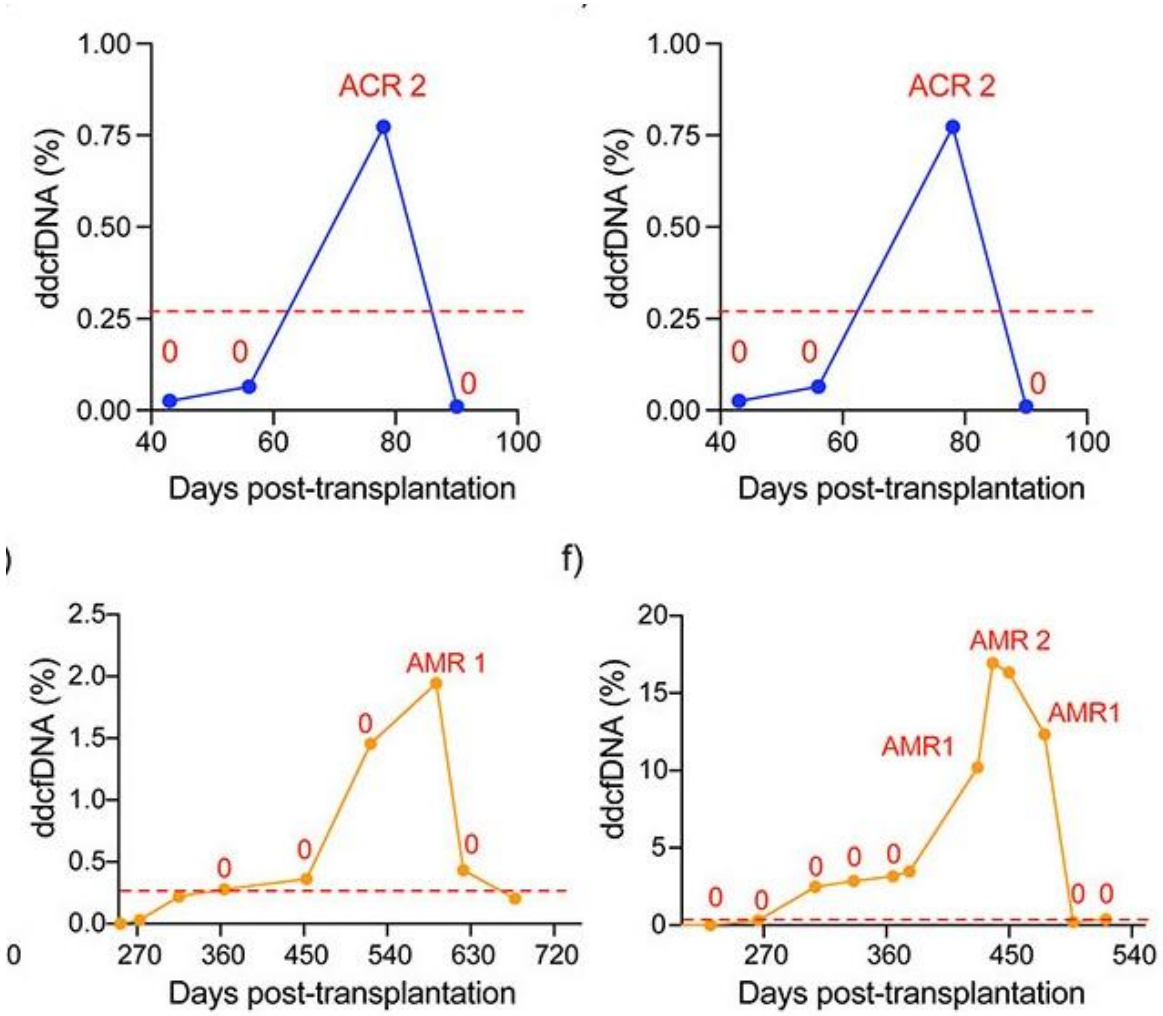
Donor-derived cell-free DNA

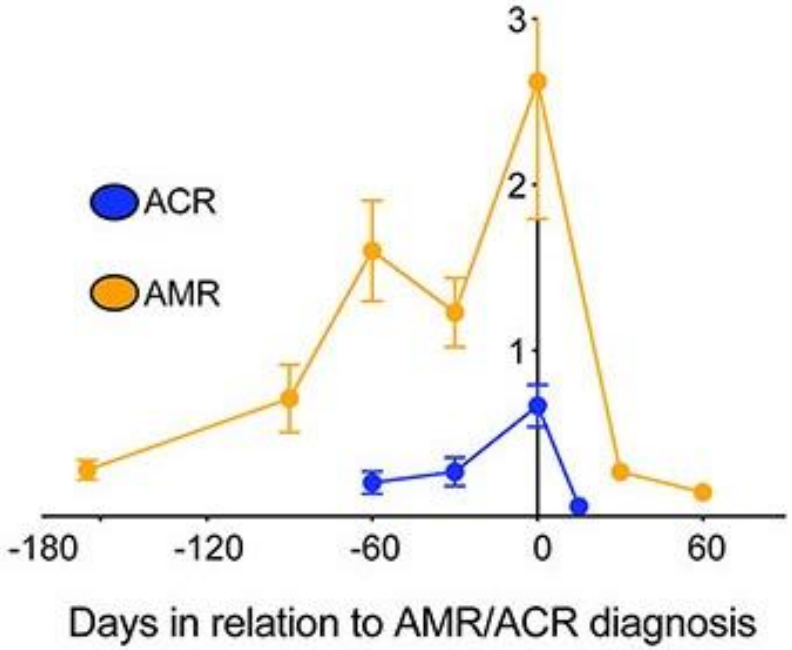


- Damage to the allograft releases DNA into the circulation
- Sequencing quantifies how much donor vs. recipient DNA is present
- Low level = graft is healthy

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

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



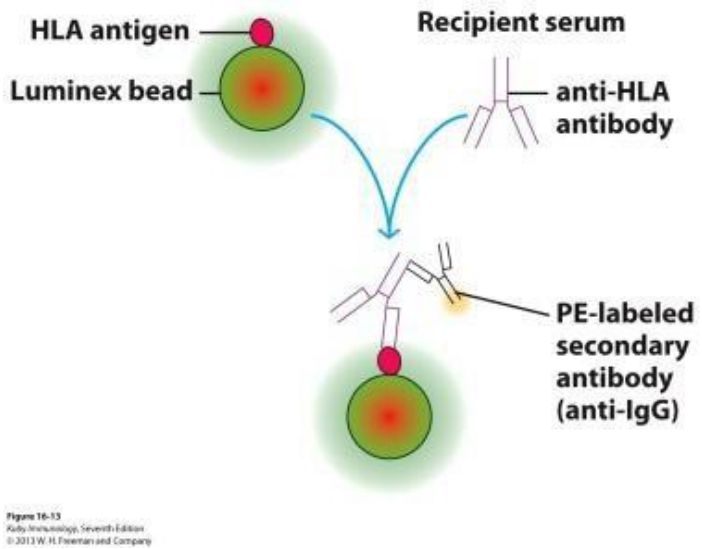


Agbor-Enoh S, *Circulation*, 2021

Testing for donor-specific antibodies (DSA)

- Test the recipient for presence of anti-HLA antibodies

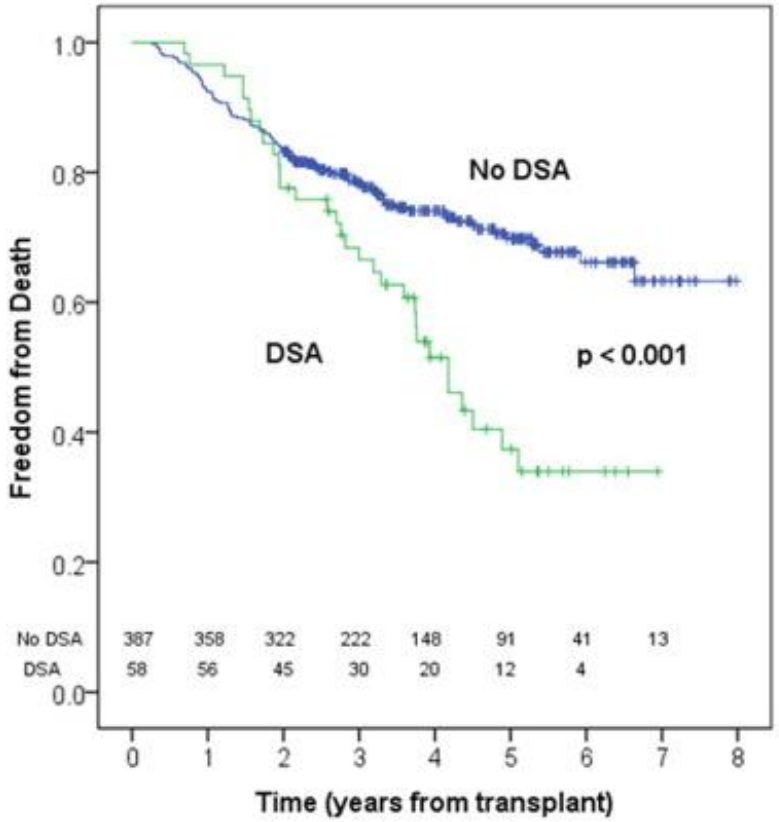
Each bead has a different HLA antigen

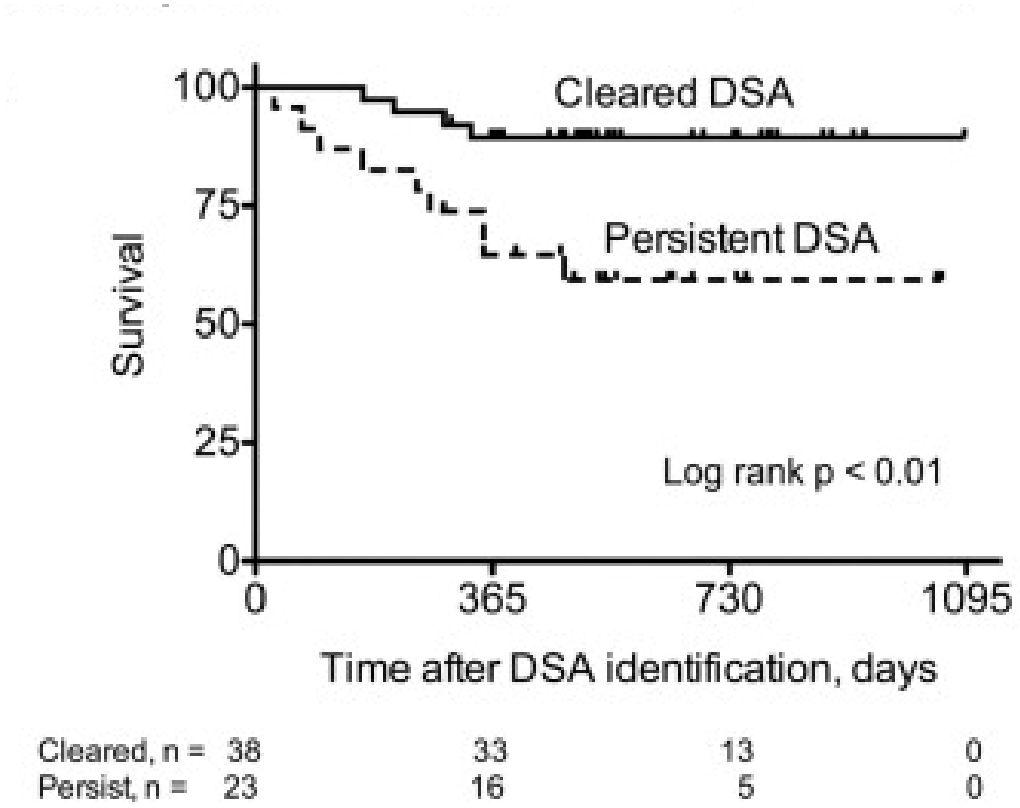


Beads can also have C1q

- 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

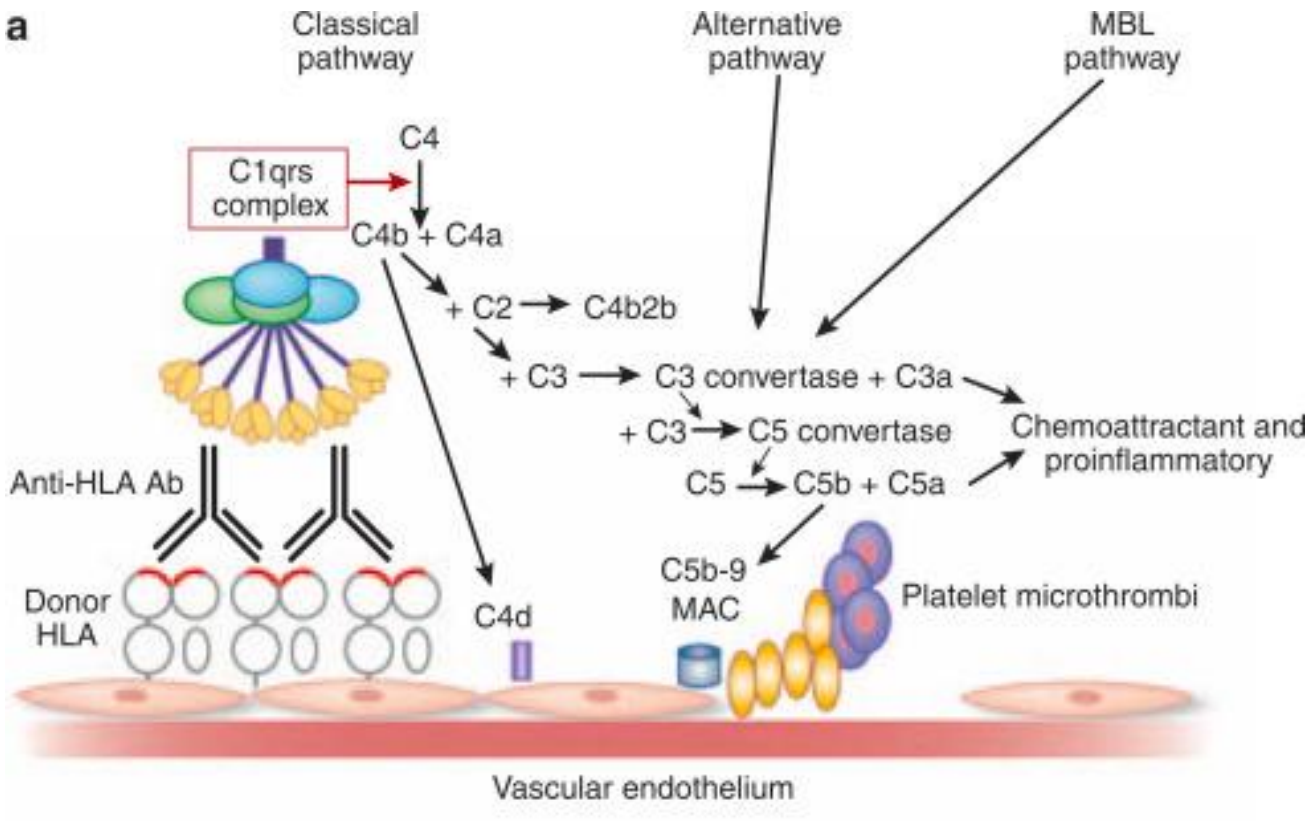




N=445
Morrell, JHLT, 2014

Hachem, JHLT, 2010

Why can anti-HLA antibodies be so bad?

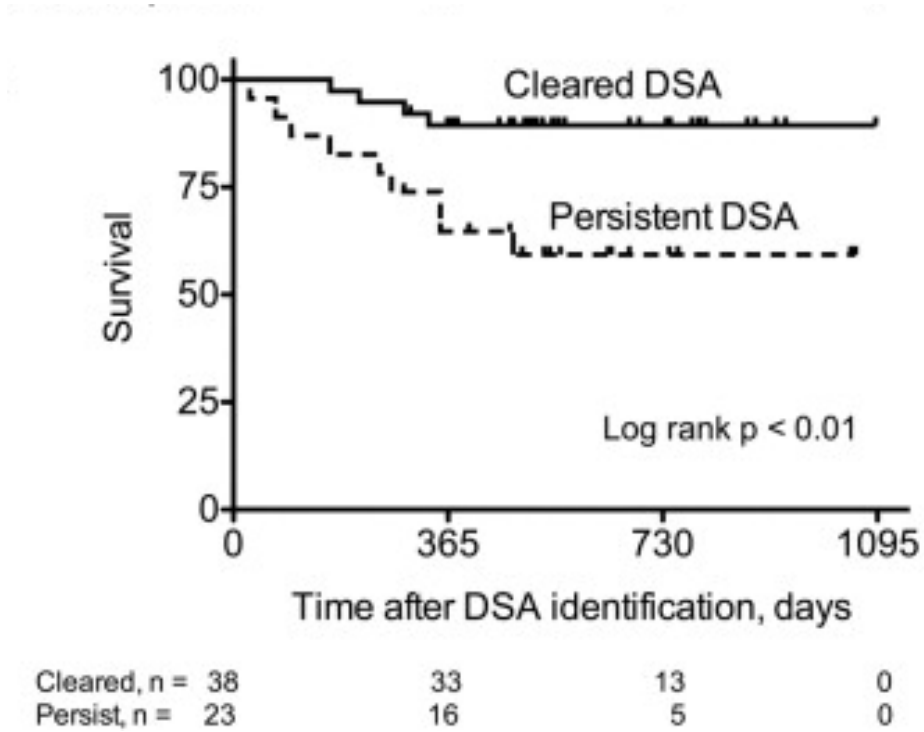


- 1. Antibody binds HLA
- 2. Complement system is activated
- 3. Membrane attack complex is formed
- 4. Endothelial cell death
- Releases more donor antigens → amplifies

Heeger, Kidney Intl, 2010

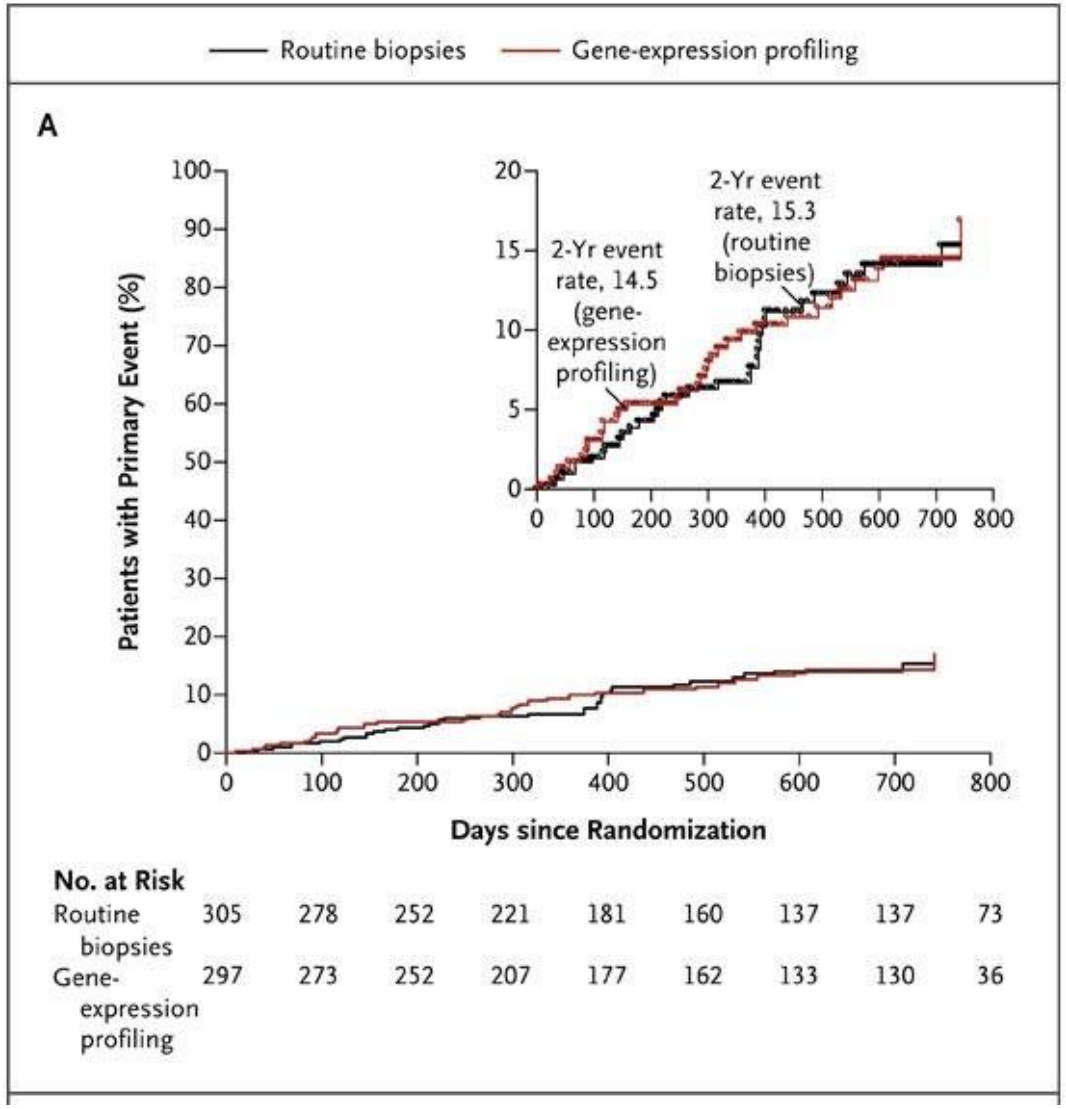
Are all DSAs bad?

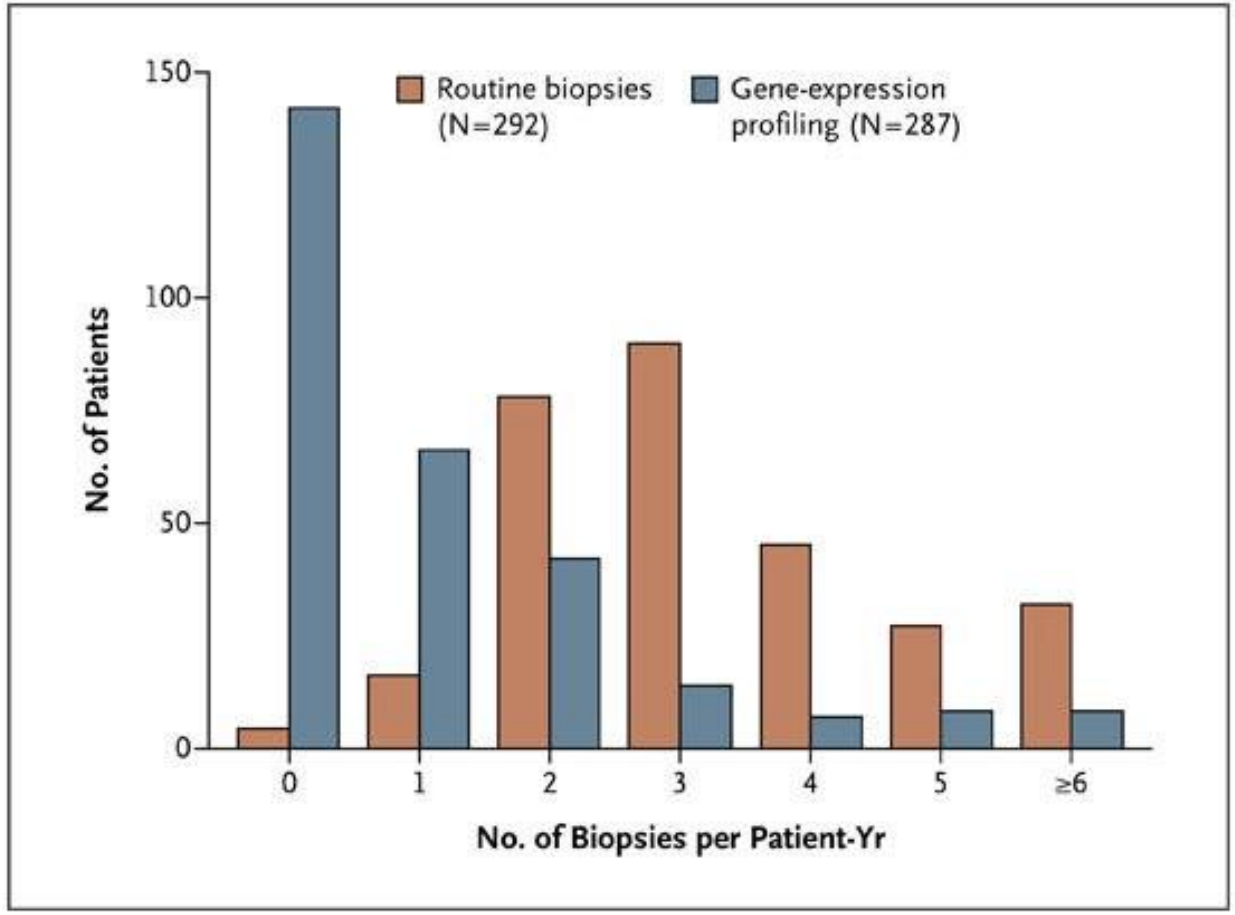
- Some antibodies are worse than others
  - Fix complement → more likely to cause cell death
  - High MFI or high titer → more likely to cause injury
  - Class II are usually worse than Class I
- Non-HLA antibodies are increasingly appreciated as important contributors to graft failure
  - Some are donor-specific, but others are not



Hachem, JHLT, 2010

Gene expression profiling - Allomap

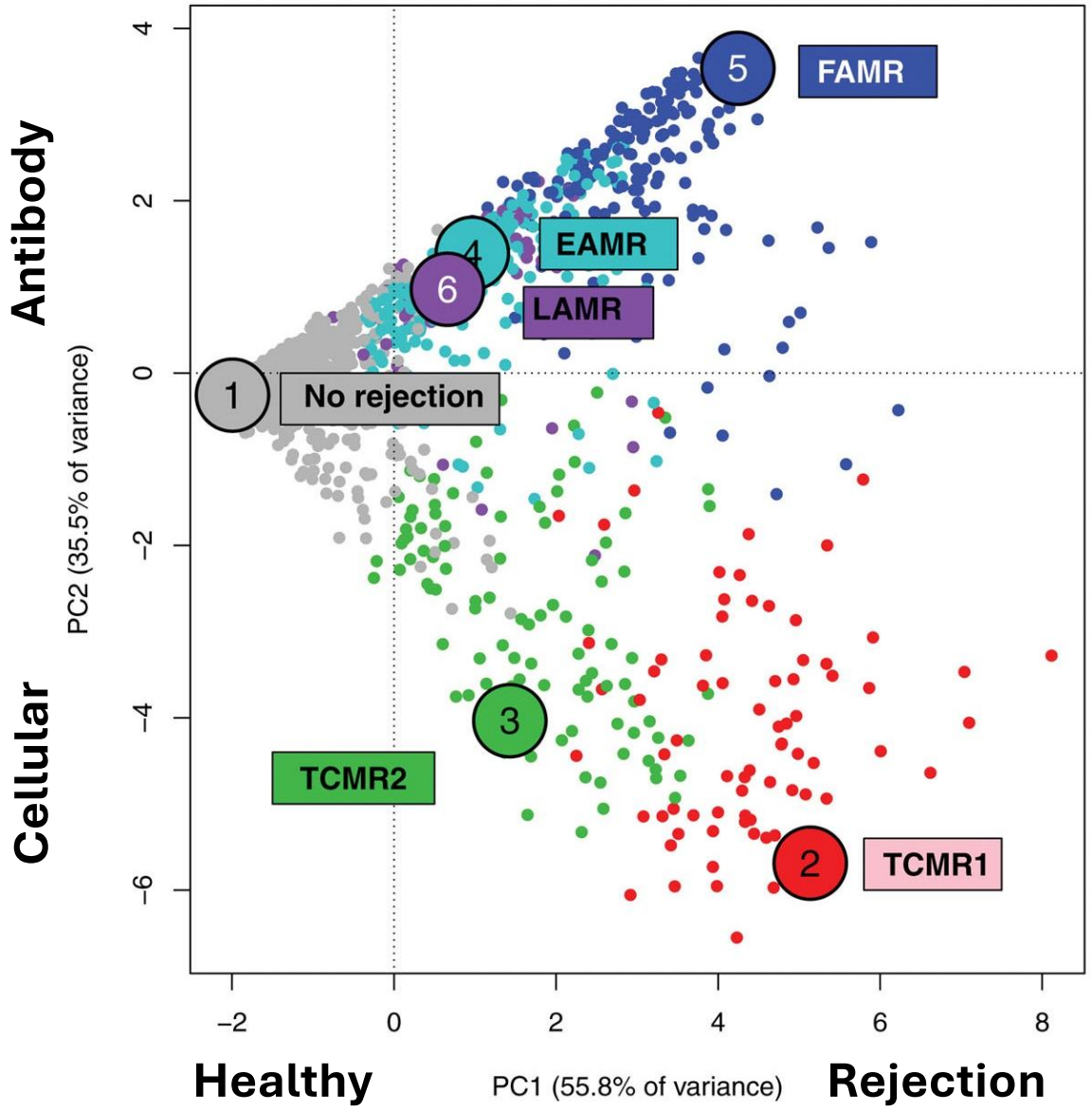




Pham, NEJM, 2010

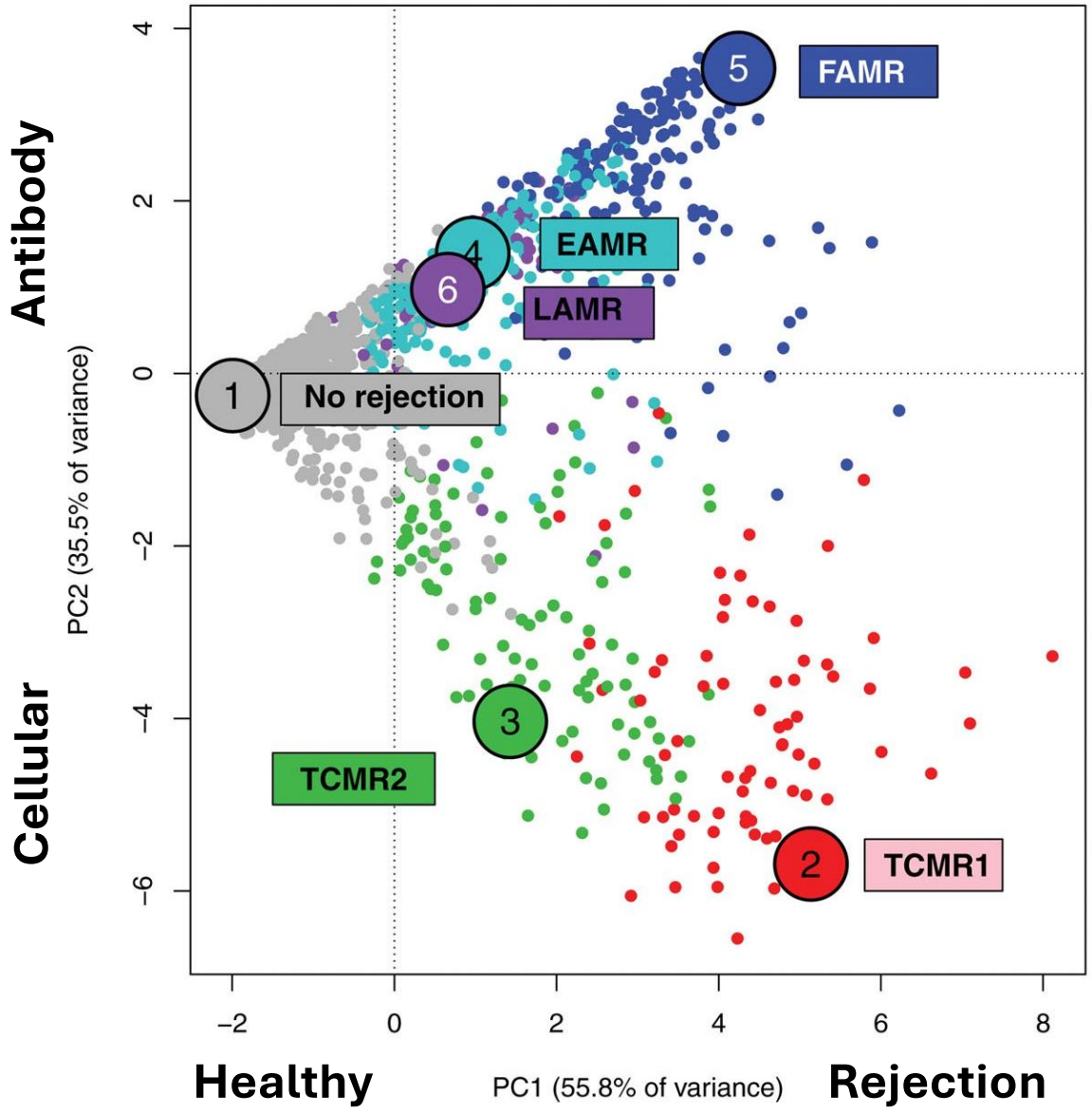
Gene expression profiling - Molecular Microscope

- Analyzes patterns of gene expression to give likelihood of different types of organ injury
- References to >1,800 biopsies during testing development
- Similar platform being developed for lung and heart



Gene expression profiling - Molecular Microscope

- Analyzes patterns of gene expression to give likelihood of different types of organ injury
- References to >1,800 biopsies during testing development
  - The current biopsy result can be depicted on this type of map
- Similar platform being developed for lung and heart



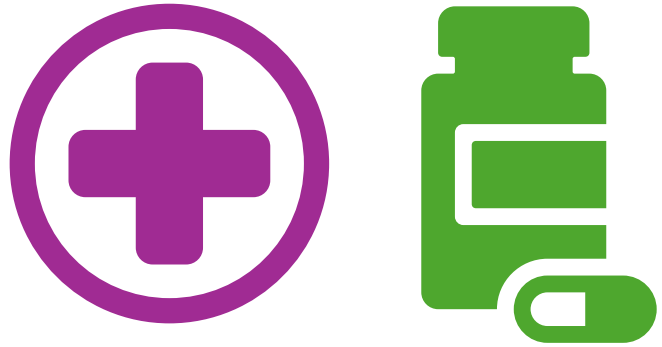
You diagnosed rejection – what do you do?

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)
    - Sequential treatments vs. Multifaceted treatment
  - Potential for side effects

- There are very few clinical trials that directly compare treatment options
  - Many have <50 patients per group
  - Those that do compare are often negative or have only transient effects

Treatment strategies

CELLULAR	Augment	Immunosuppression with calcineurin inhibitors and steroids
	Suppress	Suppress T cell activation
ANTIBODY MEDIATED	Remove	Remove existing pre-formed antibodies
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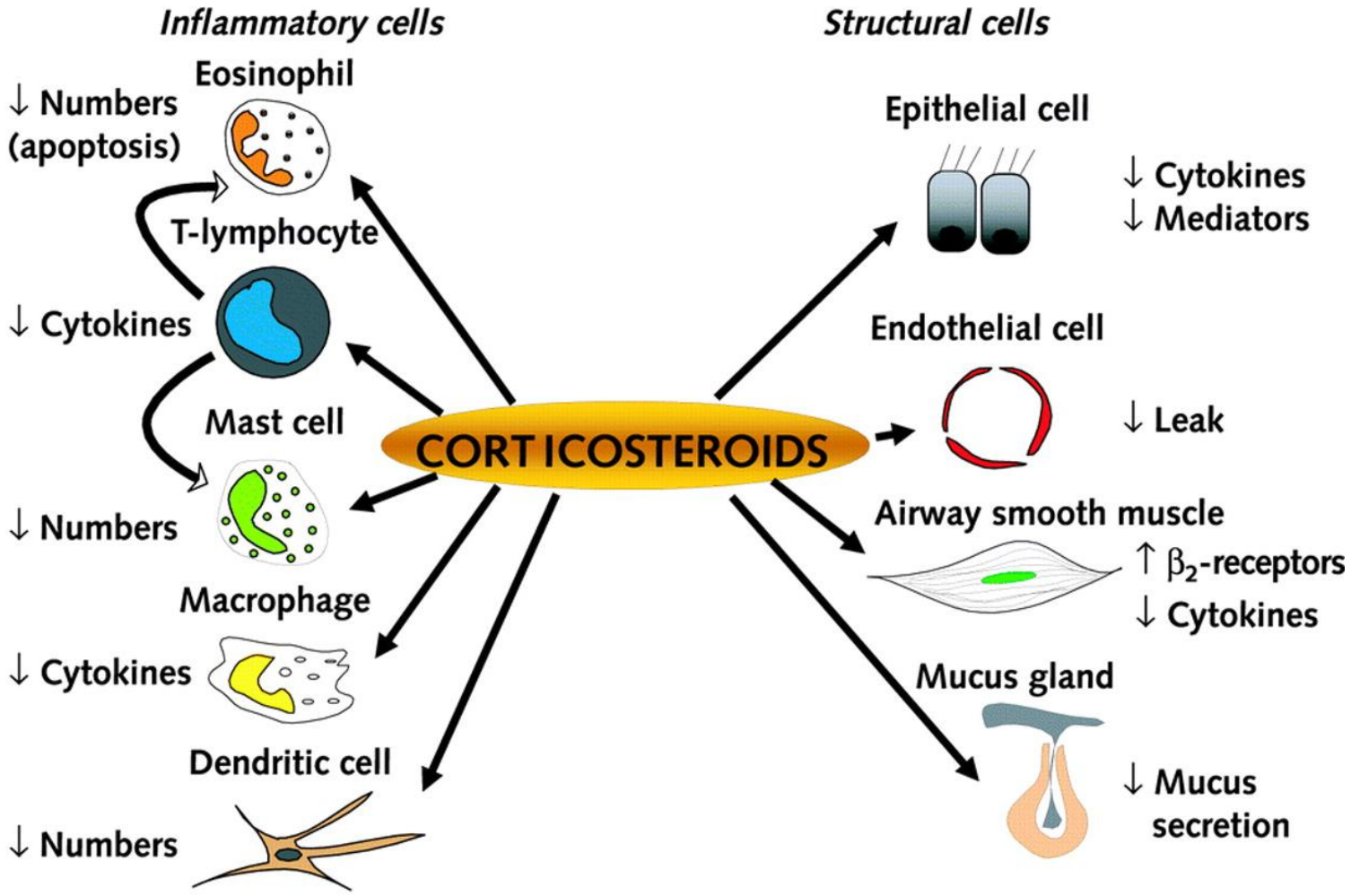
Glucocorticoids

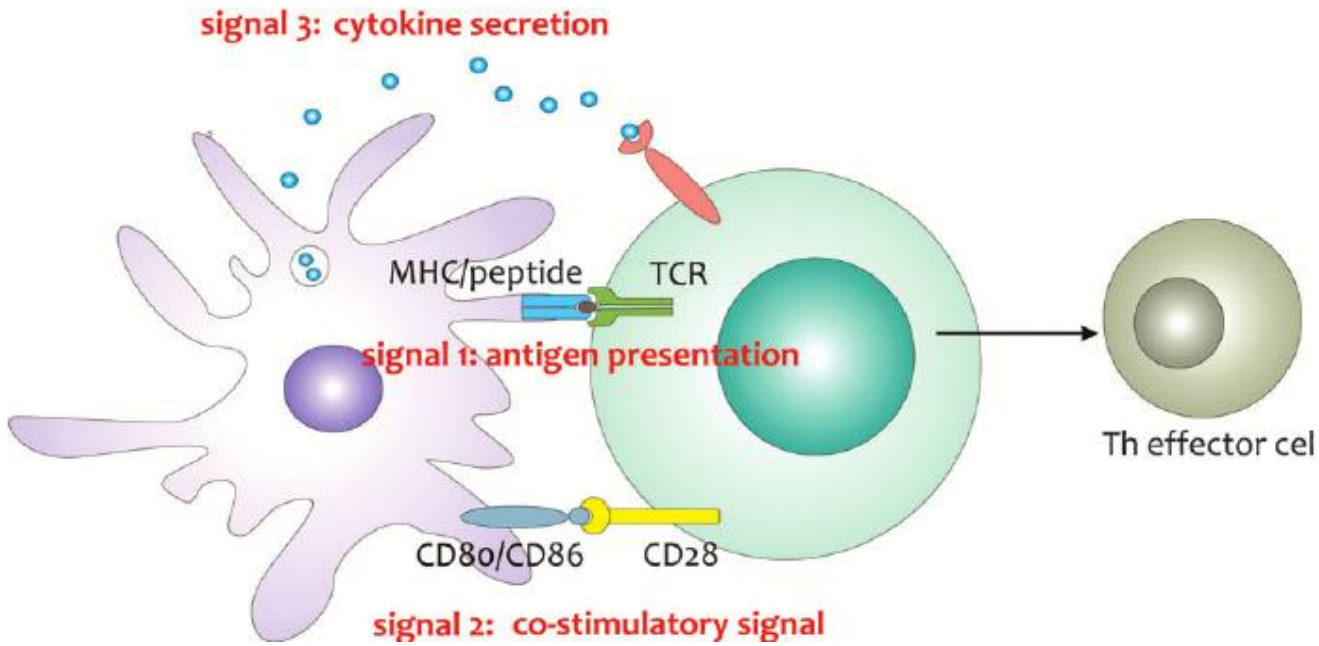
Steroids ***broadly*** reduce cytokine production, communication between immune cells, and innate immune cell function



Barnes PJ, Annals of Internal Medicine, 2003

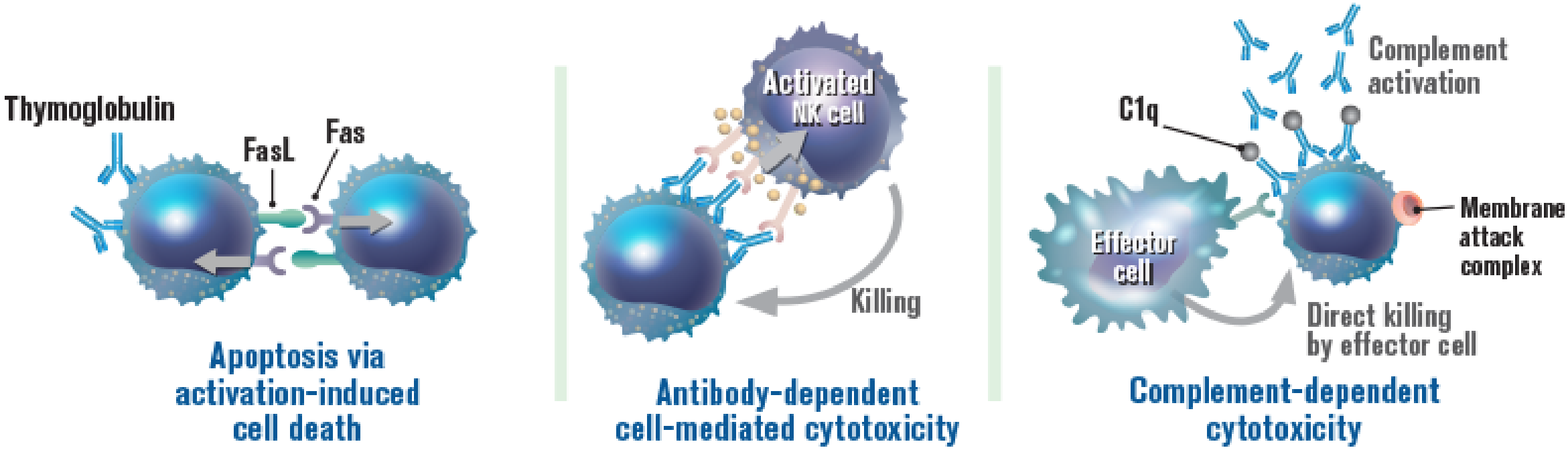
Suppress T cell activation

- 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 T and B lymphocytes
- Profound immunosuppression lasting >6 months

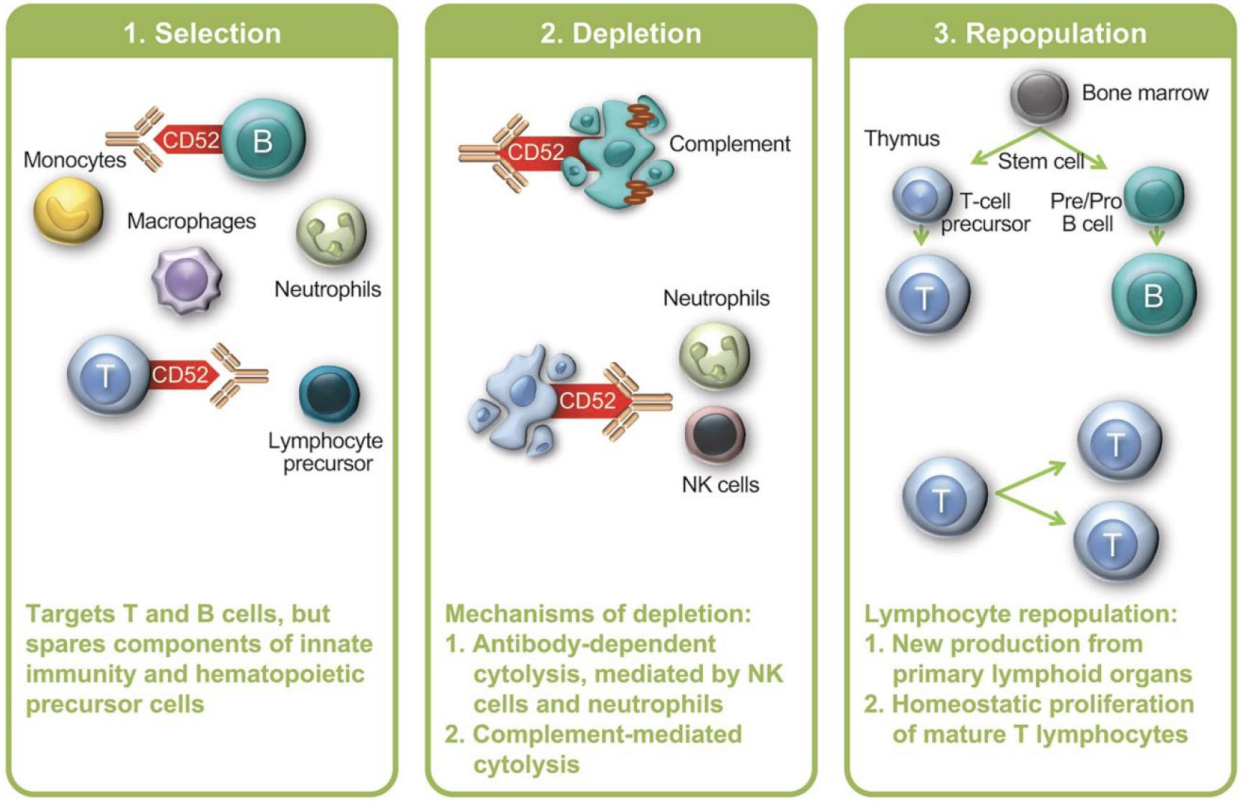
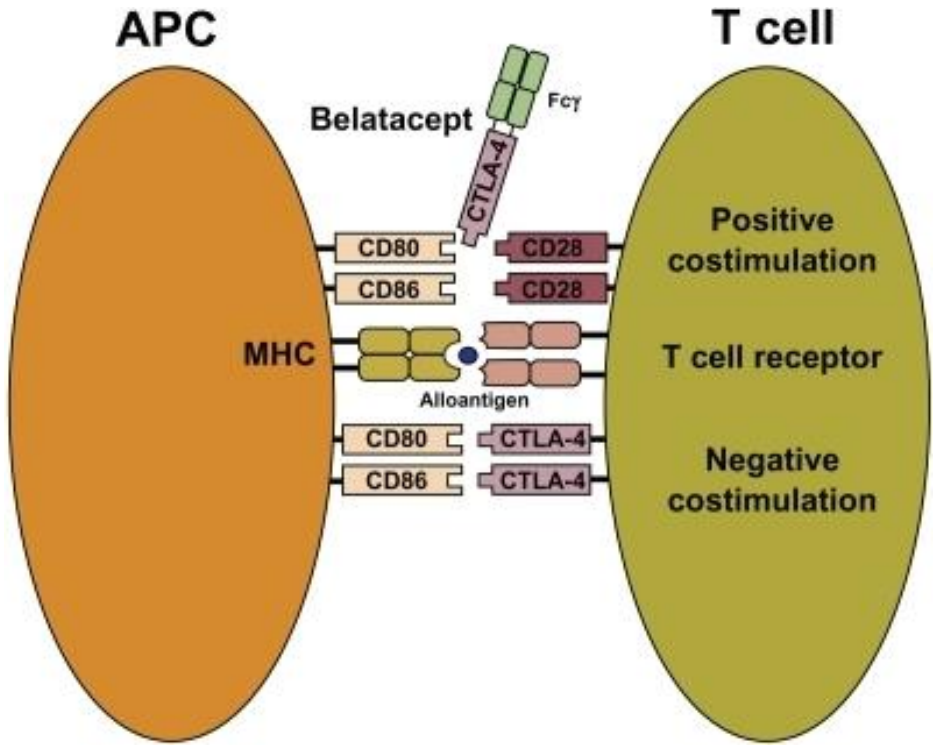


Figure 1. Alemtuzumab proposed mechanism of action.
NK, natural killer.

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
  - T cells get negative signal
  - Apoptosis



Gupta G, Drug Des Devel Ther, 2010

Treatment strategies

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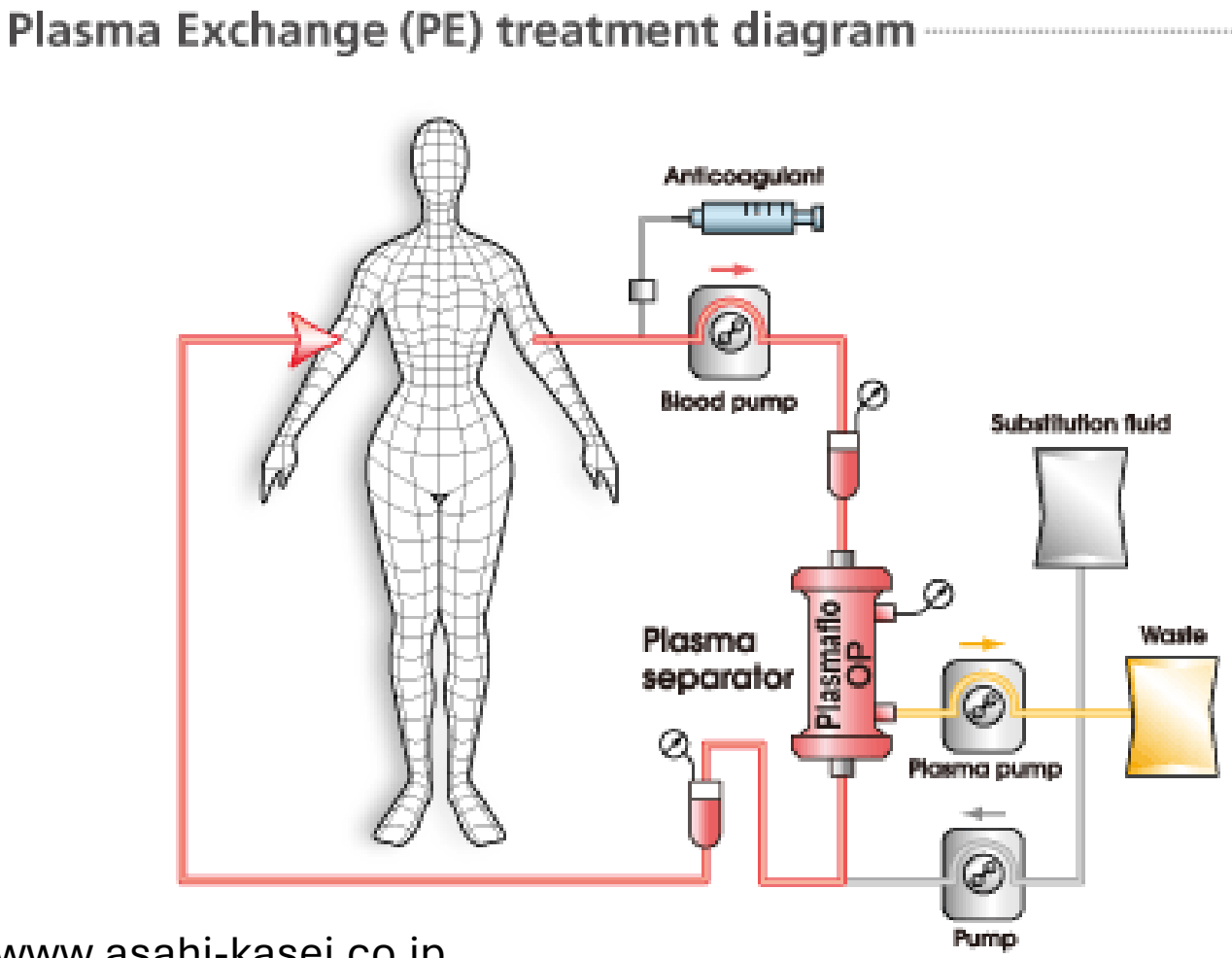
Removing pre-formed antibodies

- Plasmapheresis
  - Removes (all) antibodies from the circulation

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

Plasmapheresis

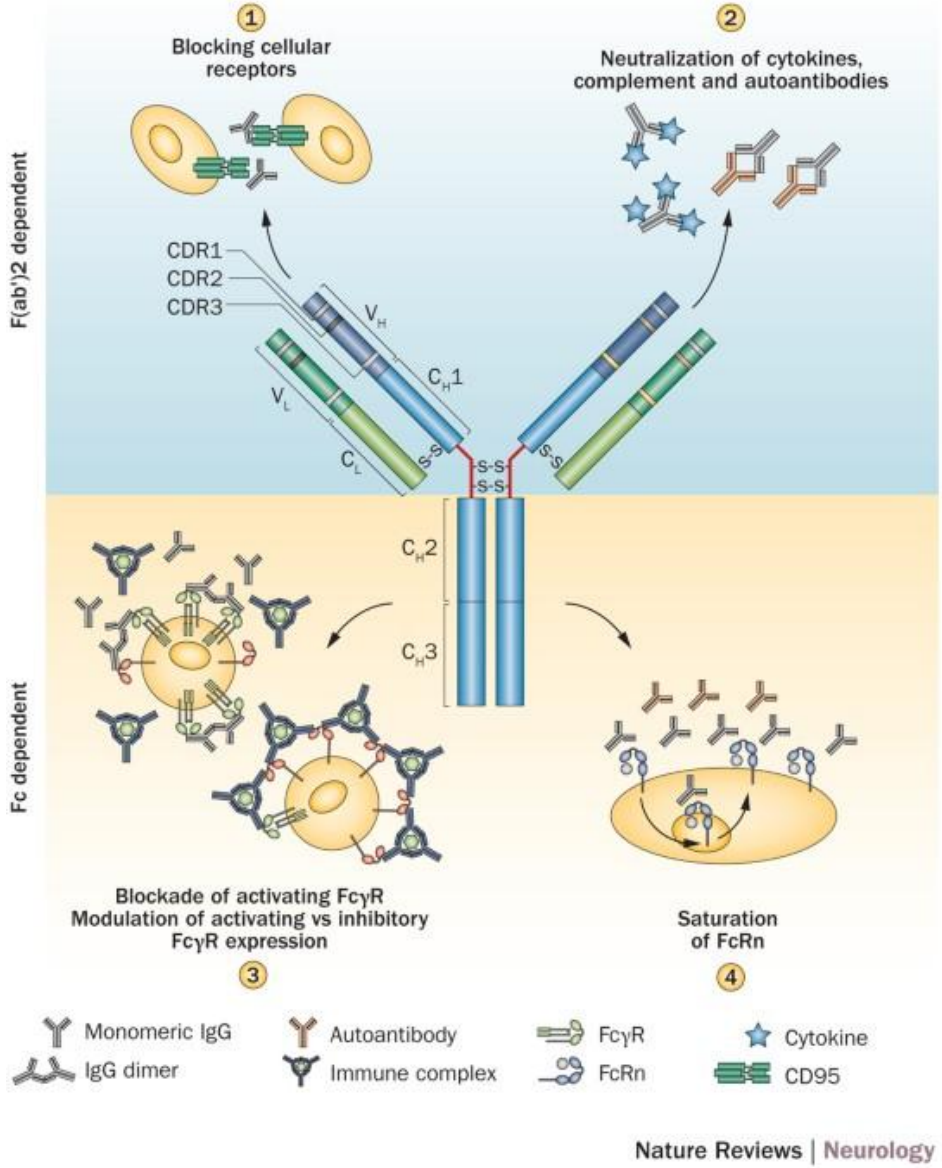


www.asahi-kasei.co.jp

- 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

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Stop production of additional antibodies

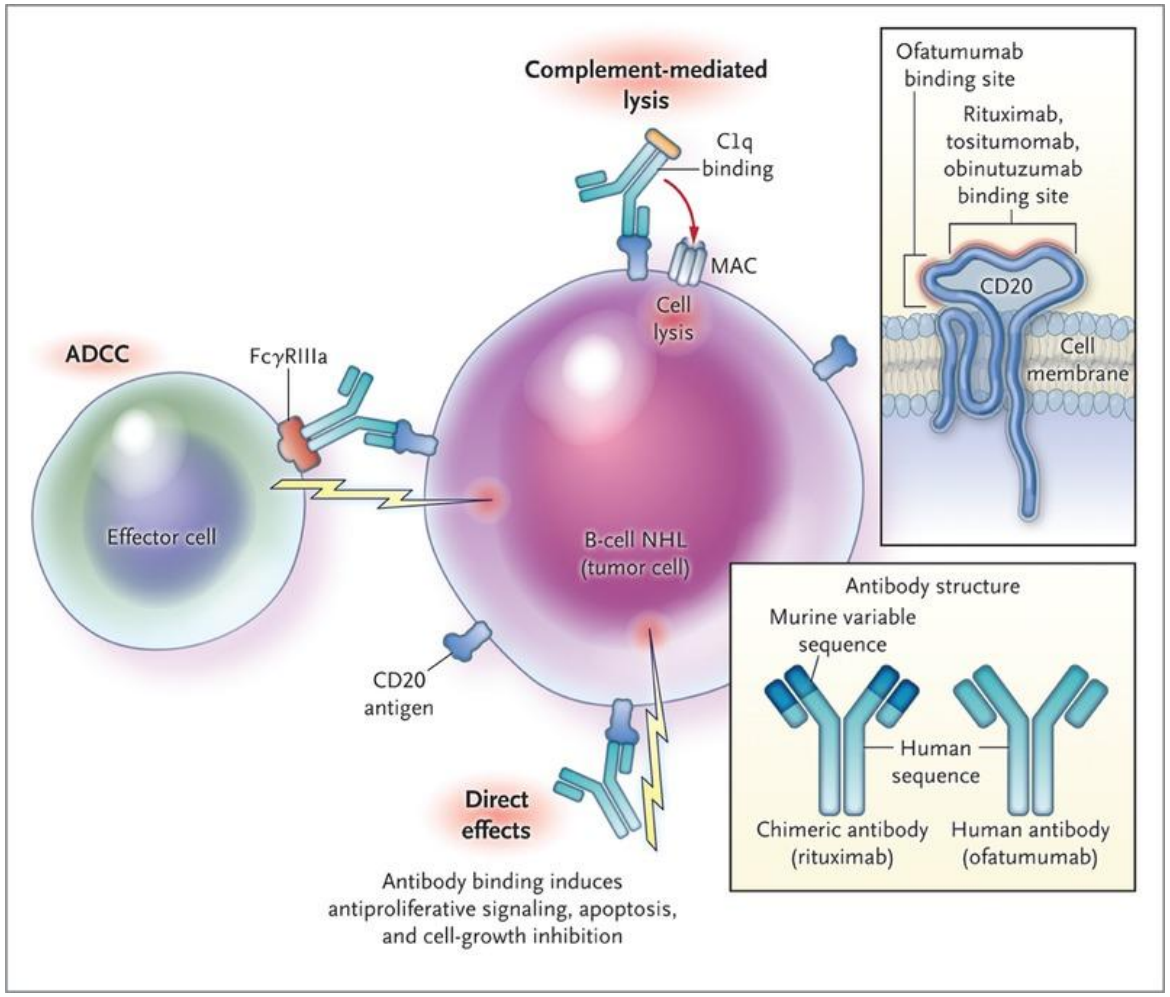
- Anti-CD20 – rituximab
  - Targeted removal of CD20+ B cells
- Proteosome inhibitors – bortezomib, carfilzomib
  - Apoptosis of plasma cells
- Anti-CD38 – daratumumab
  - Targeted removal of CD38+ plasma cells and NK cells

Rituximab

- Anti-CD20 antibody (B cells)

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

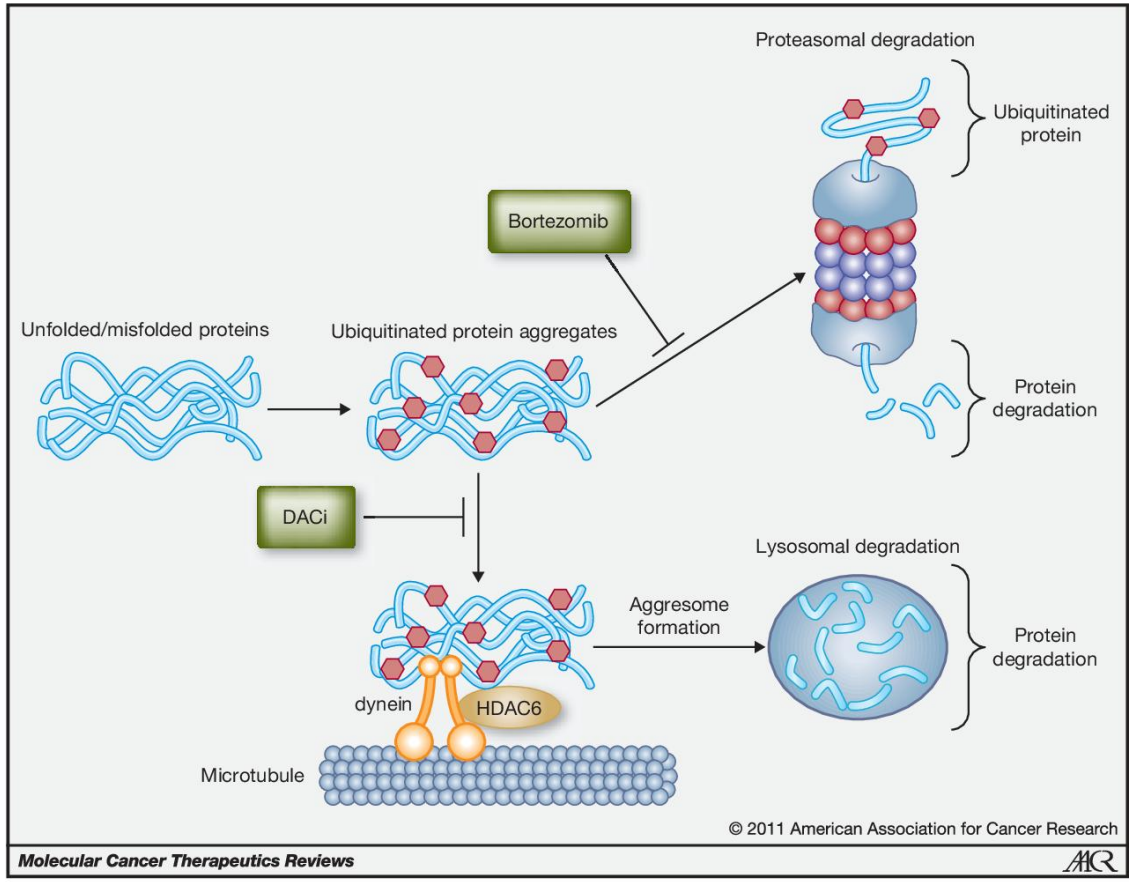
- Successfully reduces Ab levels and cPRA
  - Many grafts have Ab resurgence within 1 month (Vo, Transplantation, 2014)



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, 2011

Daratumumab

- Anti-CD38 monoclonal antibody (plasma cells, NK cells)
- Mechanism 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

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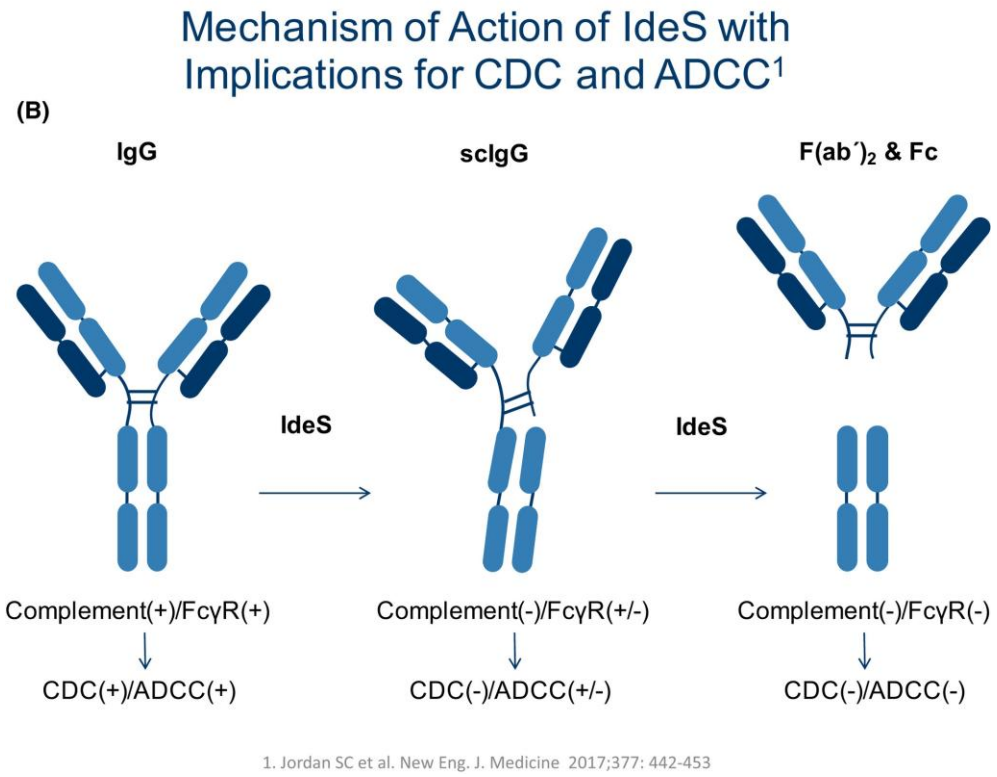
Suppress signals driving antibody production

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

Inflimidase

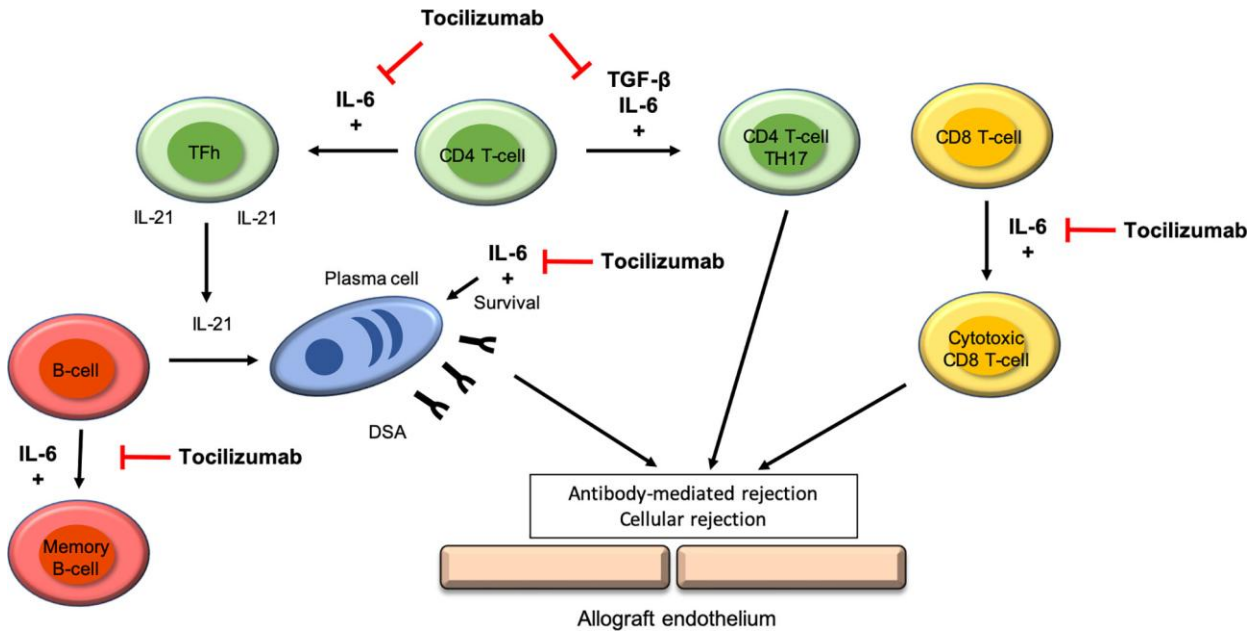
- Streptococcal protein
- Cleaves circulating IgG into F(ab) and Fc
  - Inhibits Ab-dependent and complement-dependent cytotoxicity
- Circulating antibody is depleted within 6 hours
  - Also cleaves B cell receptors → inhibits Ag binding, may reduce plasma cell differentiation
- Rebound IgG levels within 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 T helper, Th17, and CD8
  - Inhibits regulatory T cells
  - Promotes plasma cell survival
- Growing data in kidney transplant



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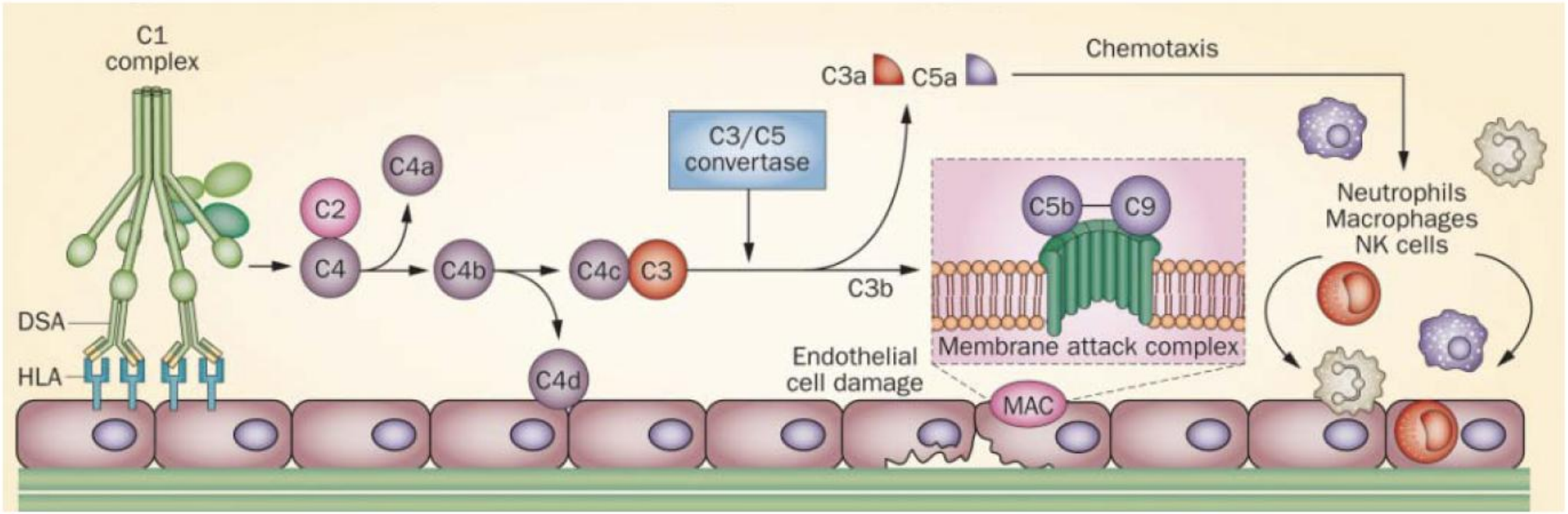
Stop complement activation

- Eculizumab
  - Anti-C5 antibody → Prevents formation of MAC complex
  - No effect on antibody levels or binding
- C1 esterase inhibitor



Nat Rev Neph, 2012

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Treatment options

Acute cellular rejection

- Thymoglobulin
- Alemtuzumab
- Belatacept

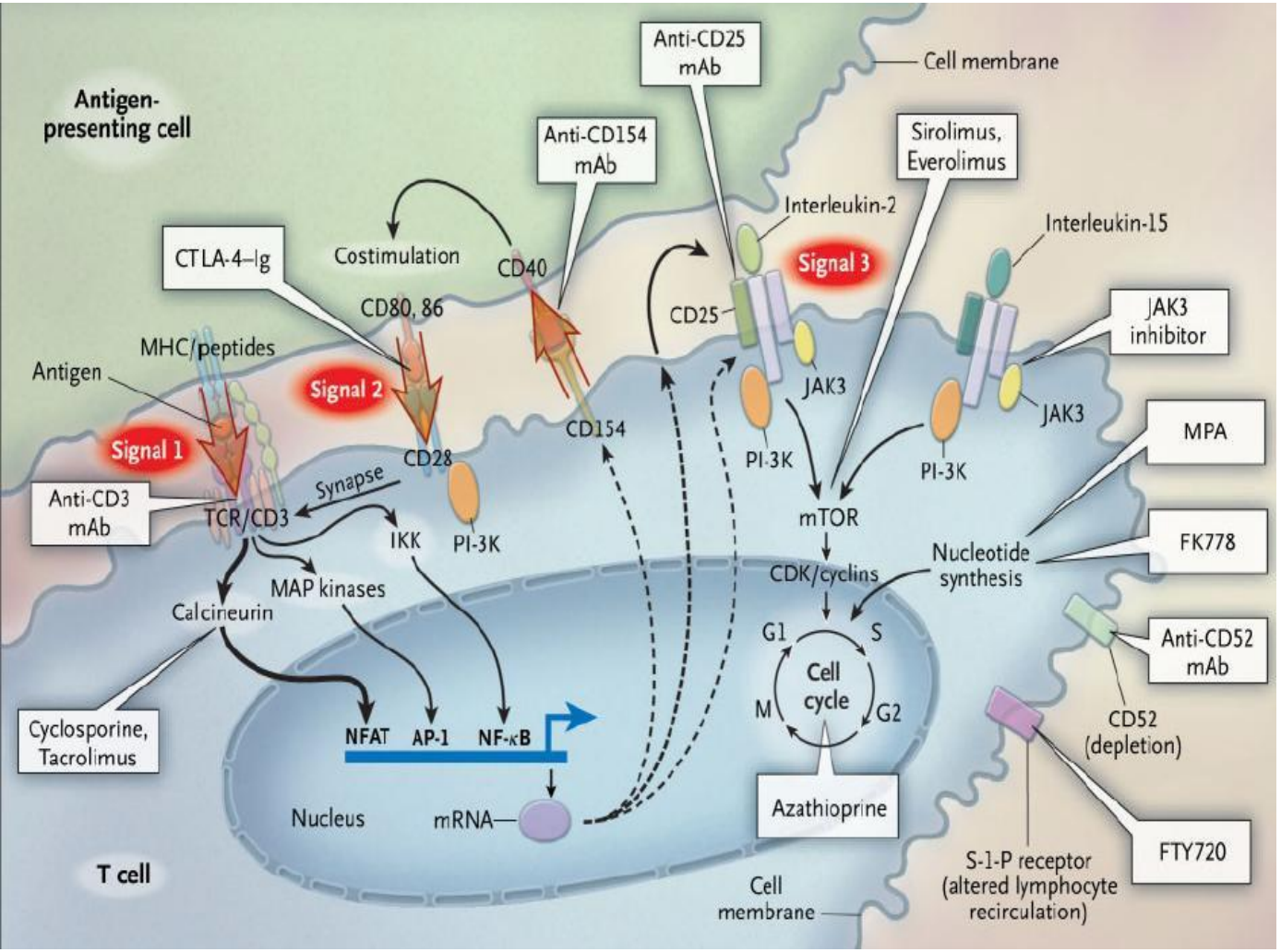
Antibody mediated rejection

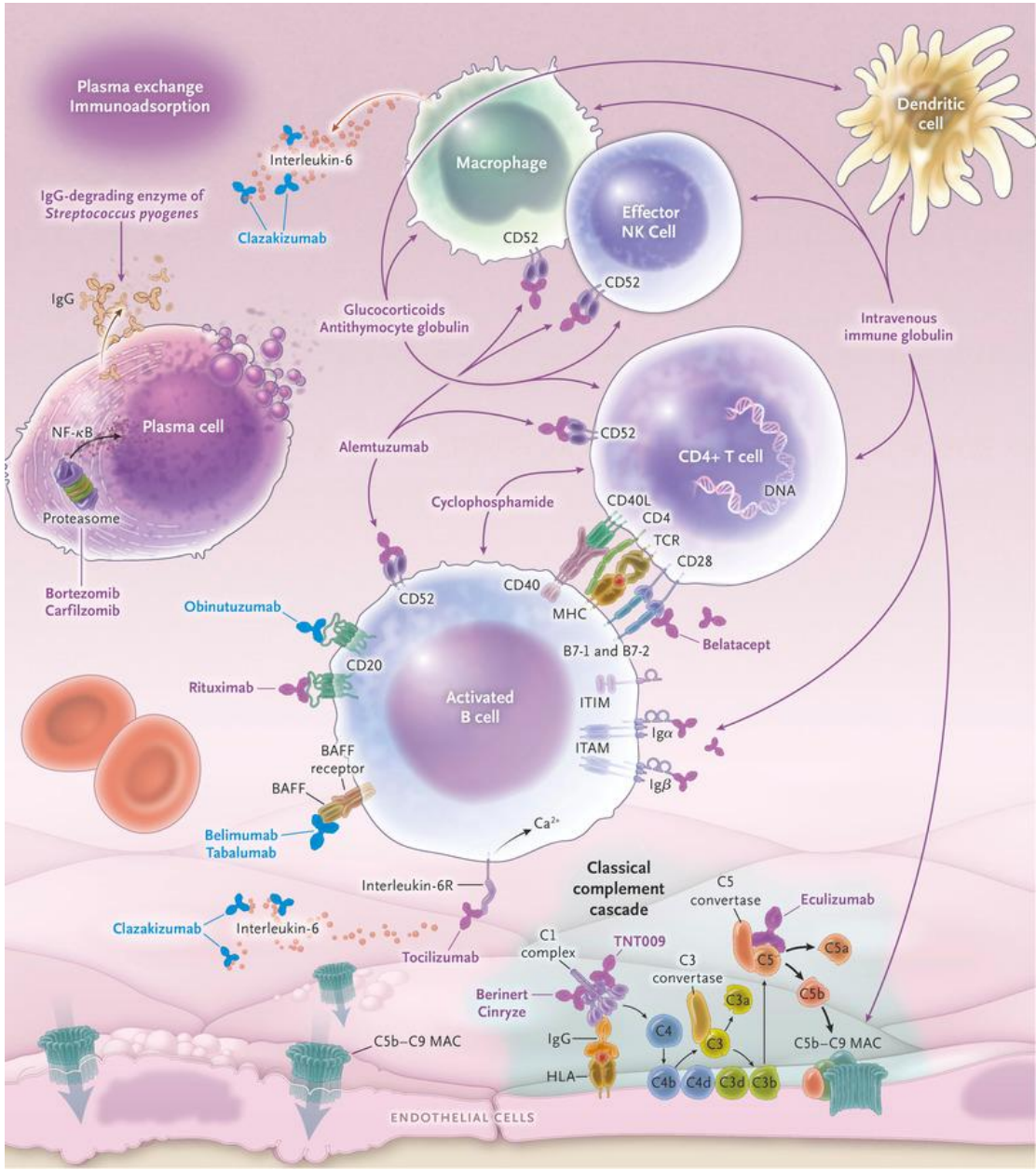
- Plasmapheresis
- IVIG
- Rituximab
- Bortezomib / Carfilzomib
- Daratumumab
- Inflimidase
- Eculizumab

Combination

- Corticosteroids
- Tocilizumab / Clazakizumab

Summaries


Antigen-presenting cell
Anti-CD25 mAb
Cell membrane
Anti-CD154 mAb
Sirolimus, Everolimus
Interleukin-2
Interleukin-15
CTLA-4–Ig
Costimulation
CD40
Signal 3
CD80, 86
CD25
JAK3 inhibitor
MHC/peptides
Antigen
Signal 2
JAK3
CD154
MPA
Signal 1
CD28
PI-3K
PI-3K
Anti-CD3 mAb
Synapse
TCR/CD3
IKK
PI-3K
mTOR
FK778
MAP kinases
Calcineurin
CDK/cyclins
Nucleotide synthesis
G1
S
Cell cycle
M
G2
Anti-CD52 mAb
Cyclosporine, Tacrolimus
NFAT
AP-1
NF-κB
CD52 (depletion)
Nucleus
mRNA
Azathioprine
T cell
S-1-P receptor (altered lymphocyte recirculation)
FTY720
Cell membrane


Plasma exchange Immunoadsorption
Interleukin-6
Macrophage
Dendritic cell
IgG-degrading enzyme of Streptococcus pyogenes
Clazakizumab
Effector NK Cell
CD52
CD52
IgG
Glucocorticoids Antithymocyte globulin
Intravenous immune globulin
NF-κB
Plasma cell
Alemtuzumab
CD52
CD4+ T cell
DNA
Cyclophosphamide
CD40L
CD4
TCR
CD28
Proteasome
Bortezomib Carfilzomib
CD52
CD40
MHC
Obinutuzumab
B7-1 and B7-2
Belatacept
CD20
Rituximab
Activated B cell
ITIM
Igα
ITAM
Igβ
BAFF receptor
BAFF
Belimumab Tabalumab
Ca2+
Interleukin-6R
Classical complement cascade
C5 convertase
Eculizumab
Clazakizumab
Interleukin-6
C1 complex
TNT009
C5
C5a
Tocilizumab
C3 convertase
Berinert Cinryze
C3a
C5b
C4
C3
IgG
C5b–C9 MAC
C5b–C9 MAC
HLA
C4b
C4d
C3d
C3b
ENDOTHELIAL CELLS