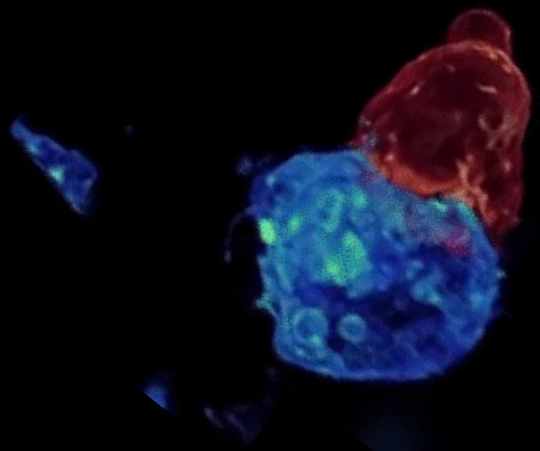
Why do we reject a transplant?



Jamil R. Azzi MD, PhD

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Endowed Chair in Transplantation Medicine
Associate Professor of Medicine, Harvard Medical School
Director, Transplantation Research Center
Brigham and Women's Hospital
Mass General Brigham

Jamil R. Azzi, MD, PhD

- Renal Fellowship @ BWH and MGH
- Transplant Fellowship @ BWH
- Post doctoral Fellowship @ BWH and HMS
- Associate Professor of Medicine @ HMS
  - Clinical focus: Transplant nephrology
  - Research focus: Basic immunology, cell and targeted therapies, and biomarker discovery

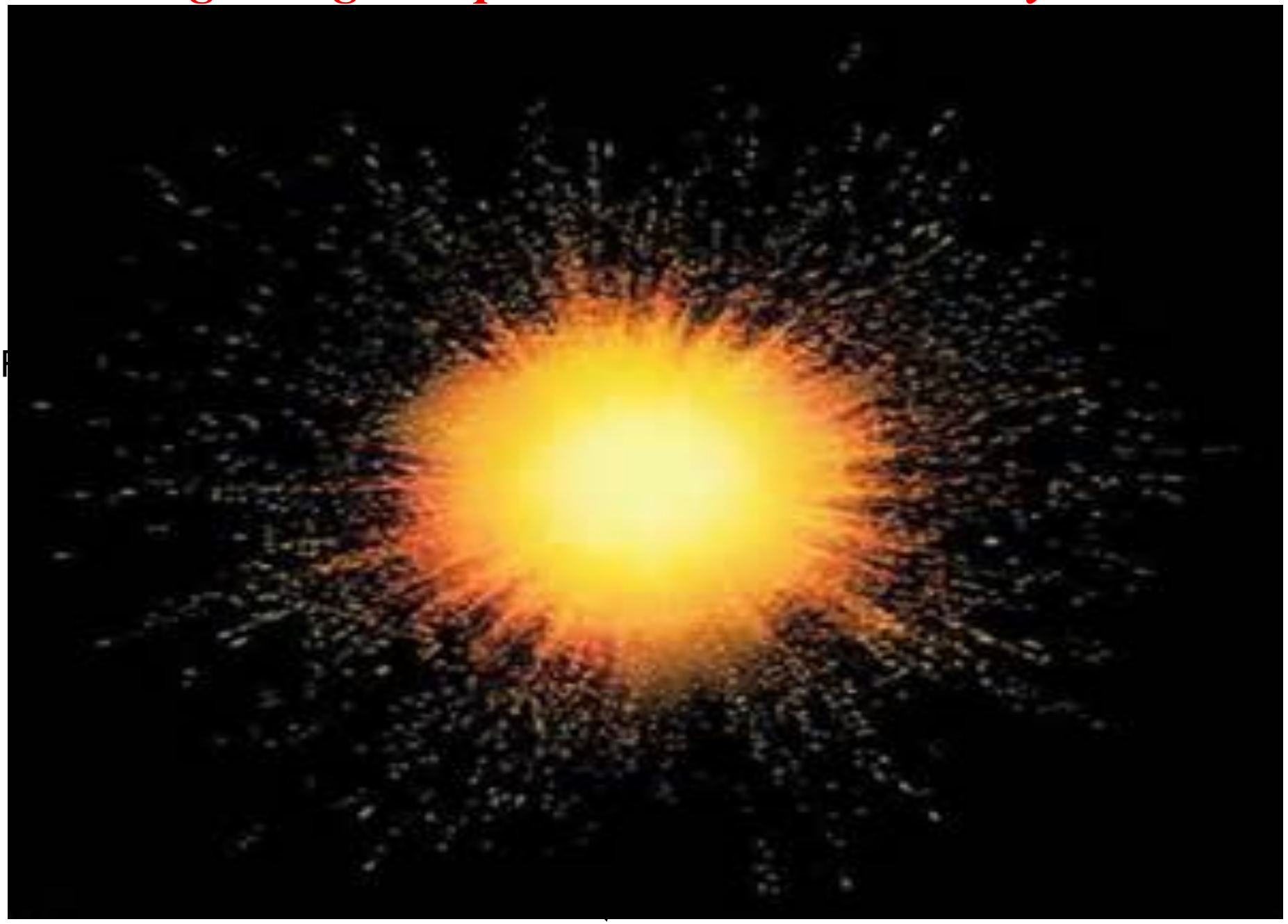
BRIGHAM AND WOMEN'S HOSPITAL

HARVARD MEDICAL SCHOOL

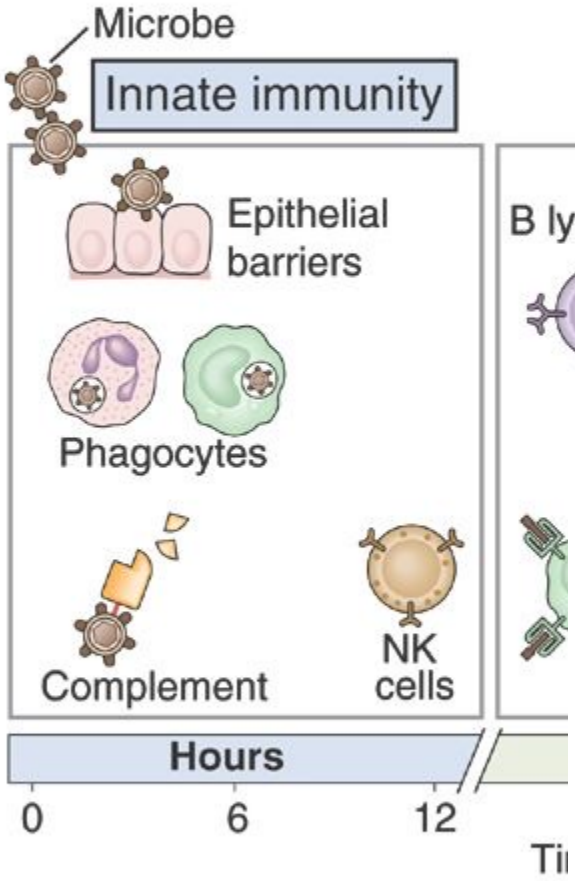
Disclosures:

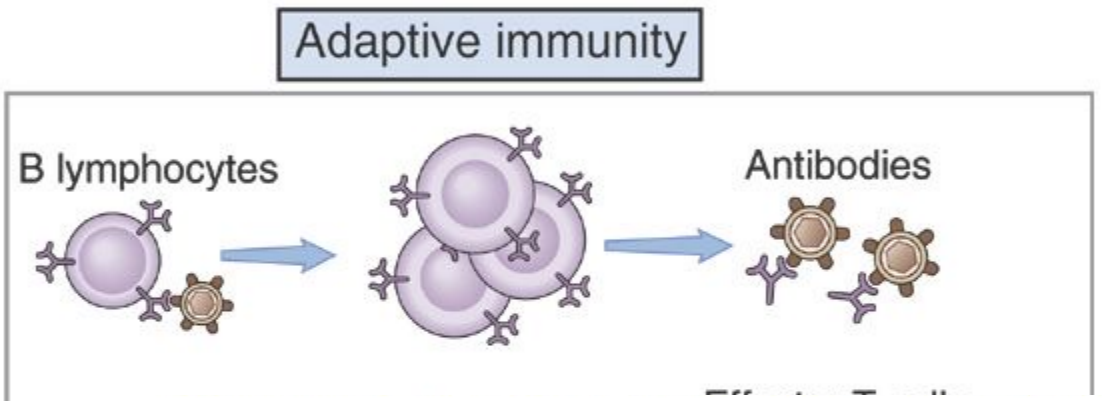
- ***Intellectual properties:*** ExosomeDx (Biotechne-Brand), Thermo Fisher and Accrue inc.
- ***Founder:*** *Lybra Bio., ImmunNanoDx*
- ***Royalties:*** Accrue inc.
- ***Grants:*** ExosomeDx (Biotechne-Brand), CareDx, Moderna Inc., Alexion.
- ***Consultancy:*** Moderna Inc., CareDx, Trustech, Kezar Life Sciences, CSL Behring, Sanofi,
- ***Advisory Boards:*** Trustech, CareDx, Moderna Inc., Vertex.

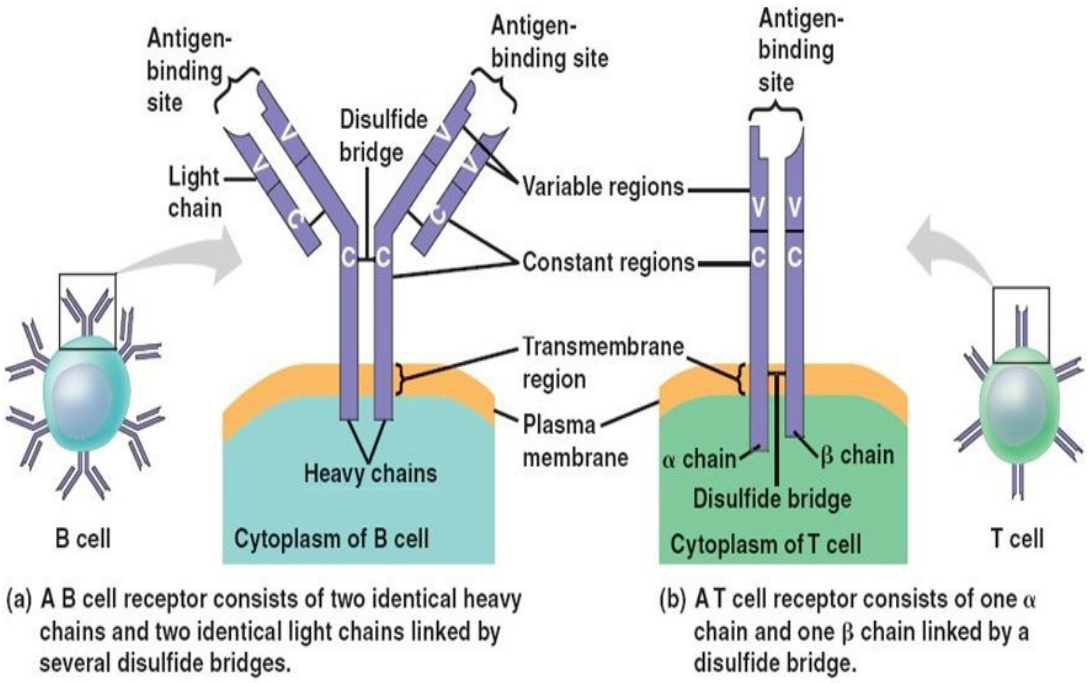
The beginning: Adaptive and Innate immunity



The beginning: Adaptive and Innate immunity







(a) A B cell receptor consists of two identical heavy chains and two identical light chains linked by several disulfide bridges.

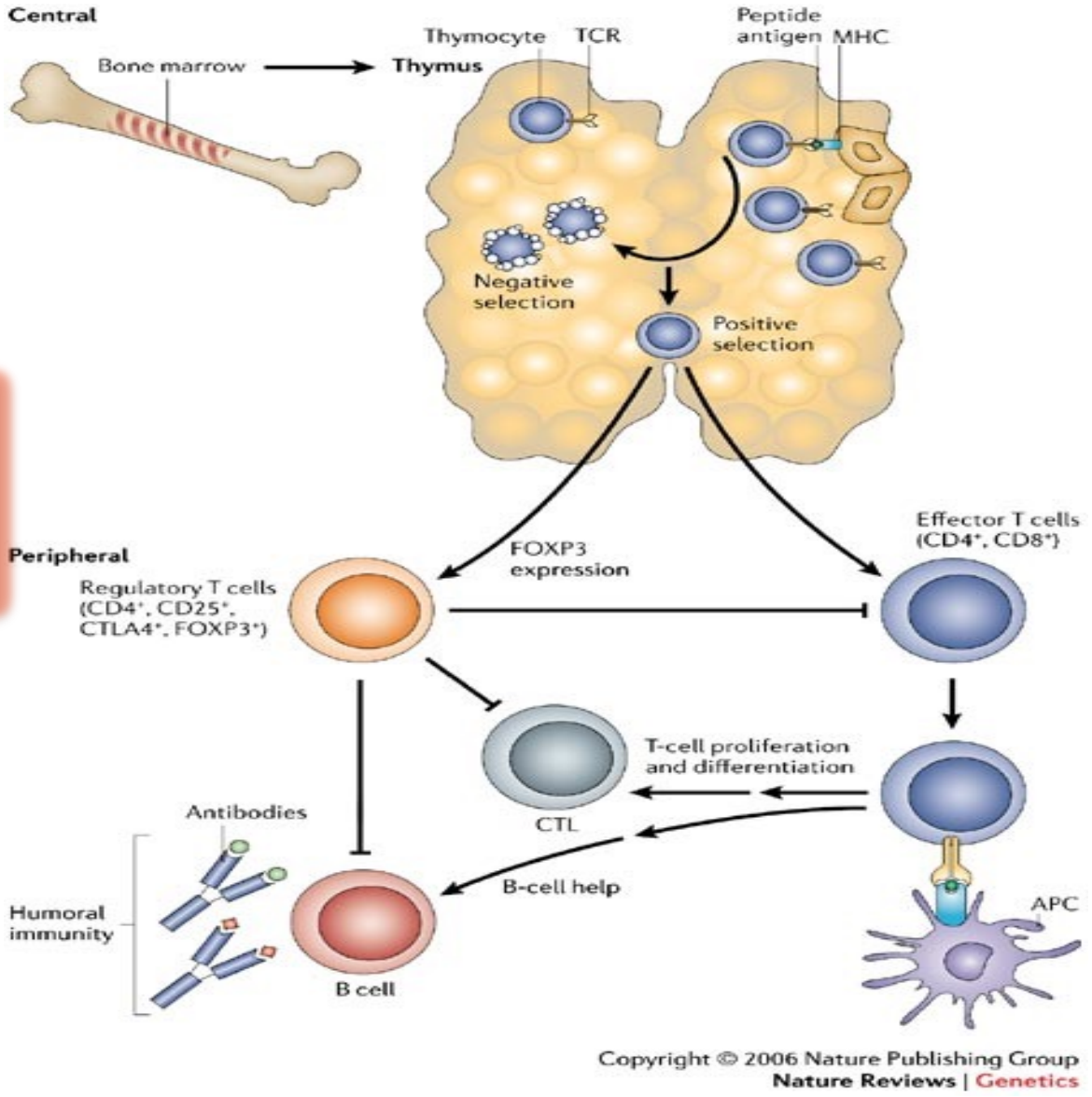
(b) A T cell receptor consists of one α chain and one β chain linked by a disulfide bridge.

T cell development in the Thymus

- T cells acquire their TCR in the thymus

- **T cell clone:** T cells with similar TCR for one specific antigen.

- T cell clones recognizing self antigens get eliminated in the thymus (Negative selection)



Gregersen et al. Nature Review. 2006.

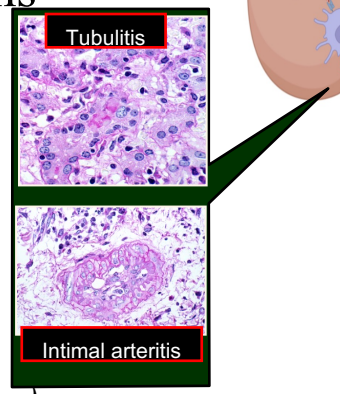
Allograft rejection

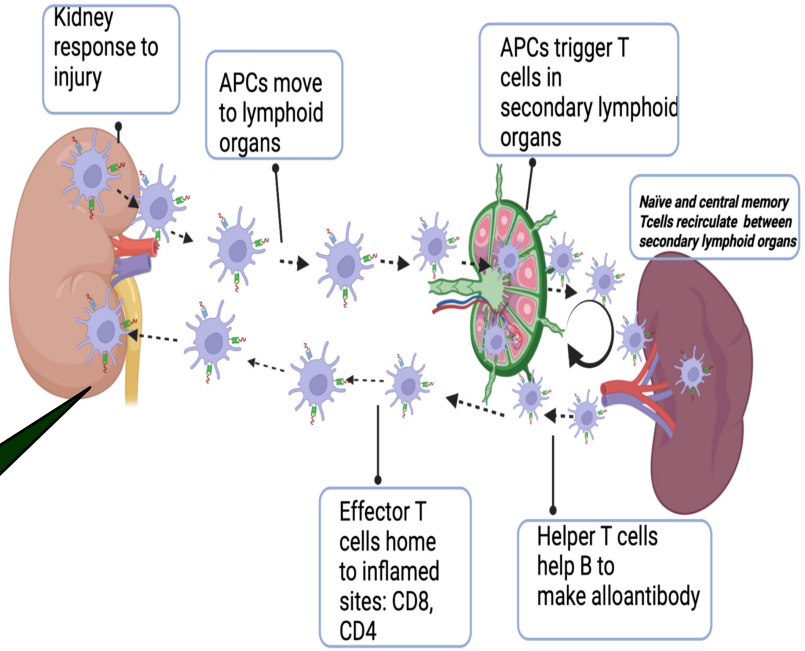
1- Recognition of the allo-antigens
- *Ischemia reperfusion*
- *Major Histocomptability complex (MHC)*
- *T cell receptor (TCR)*

2- T cell activation

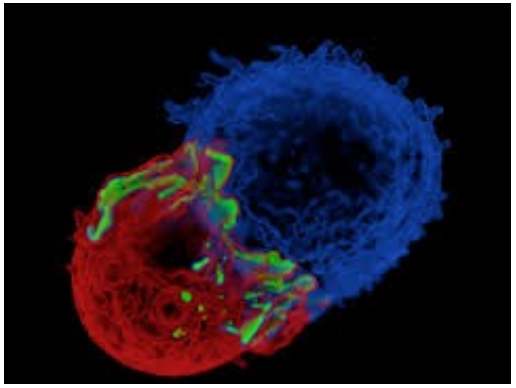
3- Activation of the effector mechanisms
- *Cytotoxic T cells (CD8)*
- *B cells*
- *Innate immunity*

4- Resolution of the immune system:
- *Memory formation and sensitization*
- *Regulatory mechanisms (Tregs).*





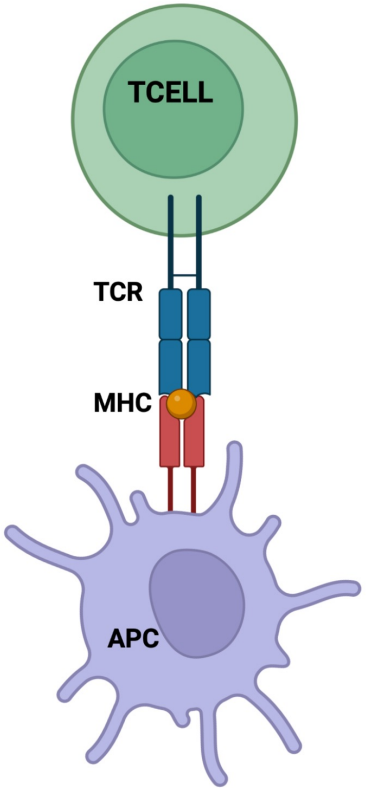
Step One: Recognition of an antigen



Recognition of an antigen requires:

- A receptor on T cells (TCR) that recognizes a specific antigen presented on the surface of cells (APC or endothelial cells…)

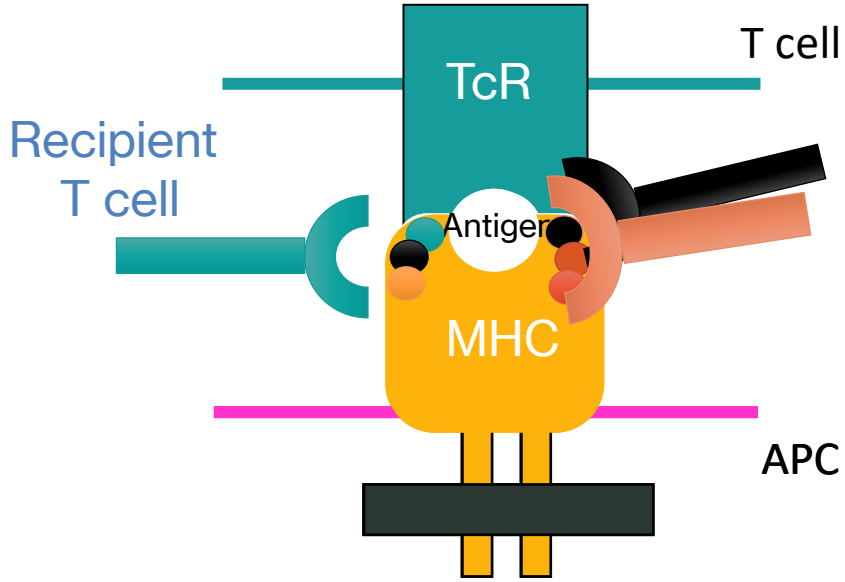
- Cells present antigens through MHC molecules



Step One: Recognition of the allo-antigens

Recognition of an antigen requires:

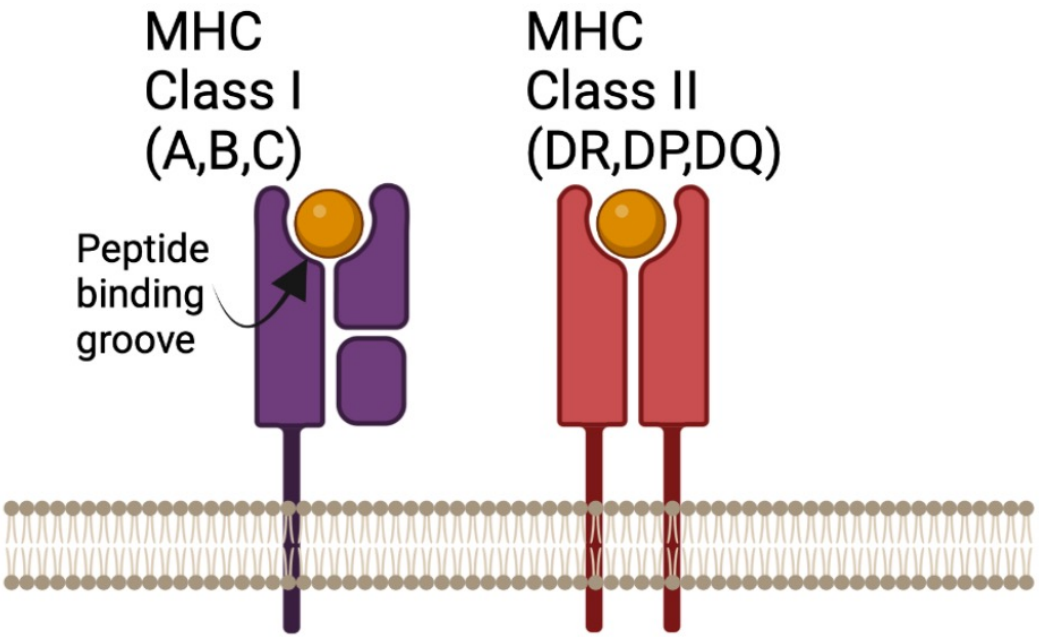
- T cell receptors can directly recognize peptides on HLA molecule.



MHC molecules are major targets for alloreactive T cells causing rejection } Donor APC

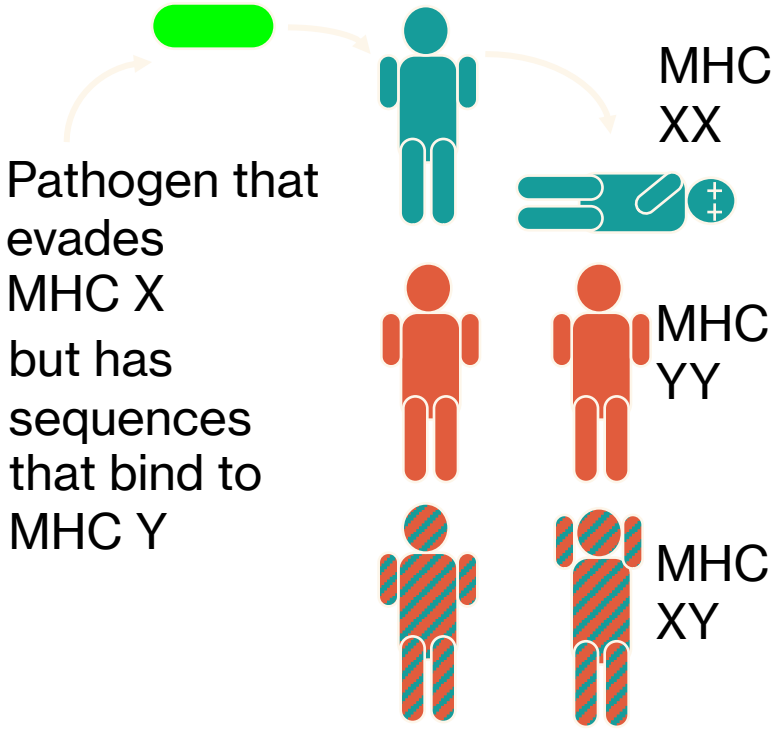
Major Histocompatibility Complex

- Major Histocompatibility Complex
  - *Cluster of genes found in all mammals*
  - *Its products (MHC Moleucles) pla role in discriminating self/non-self*
- MHC molecules Act As Antigen Presenting Structures
- In Human MHC Is Found On Chromosome 6
  - *Referred to as HLA complex*
- *Extensive Polymorphism*



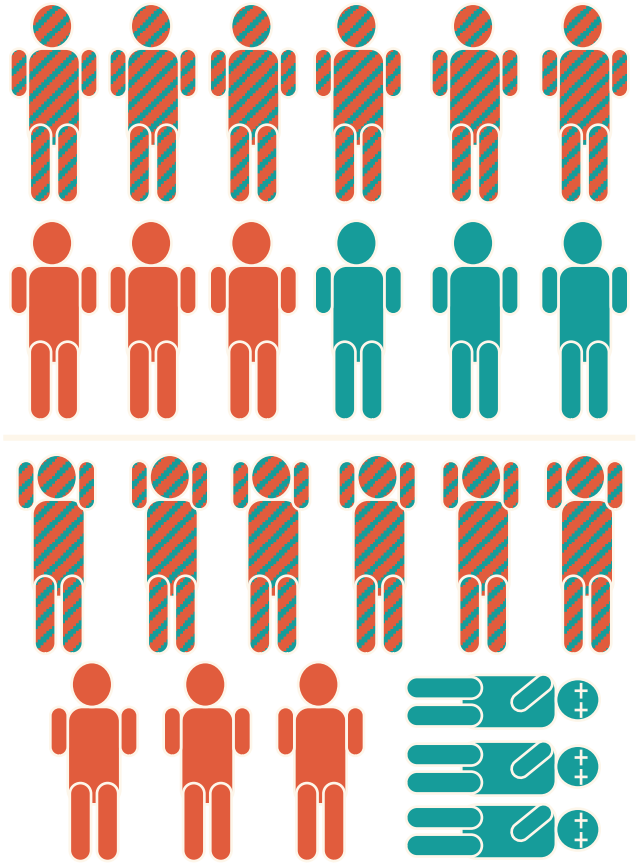
MHC class I expressed on all nucleated cells and present antigens to Cytotoxic CD8 T cells
MHC class II expressed on antigen presenting cells and present antigens to helper CD4 T cells

MHC molecules are targets for immune evasion by pathogens



Pathogen that evades MHC X but has sequences that bind to MHC Y

Impact on the individual depends upon genotype



Population survives

Polymorphism protects against pathogen immune evasion.

Not so good for matching kidneys

Diversity of MHC molecules in the individual

MHC molecules are CODOMINANTLY expressed
Two of each of the six types of MHC molecule are expressed

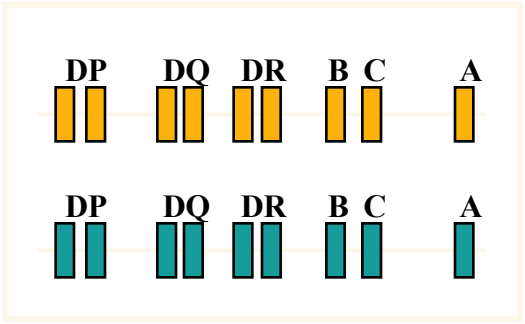
Genes in the MHC are tightly LINKED and usually inherited in a unit called an MHC HAPLOTYPE

{ 0 antigen Mismatch (matched on A, B and DR only) deceased kidney transplant is not a perfect match }

Inheritance of MHC haplotypes

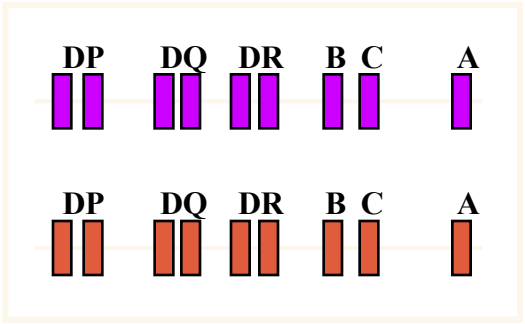
Parents

DP-1,2
DQ-3,4
DR-5,6
B-7,8
C-9,10
A-11,12



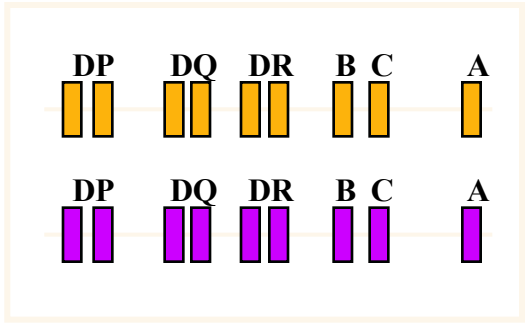
X

DP-9,8
DQ-7,6
DR-5,4
B-3,2
C-1,8
A-9,10

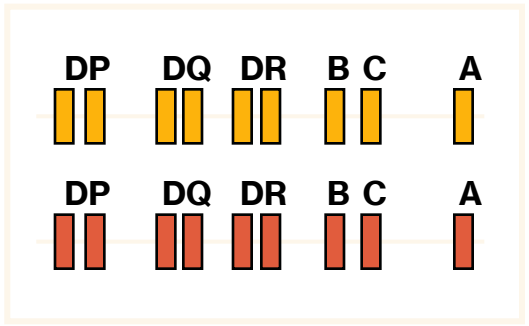


Children

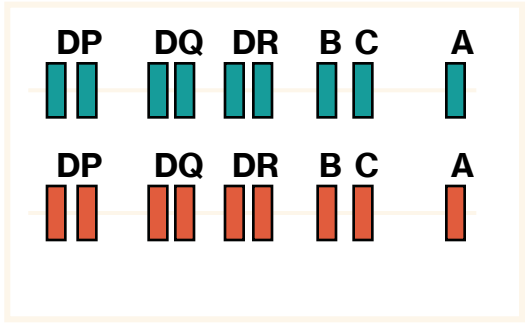
DP-1,9
DQ-3,7
DR-5,5
B-7,3
C-9,1
A-11,9



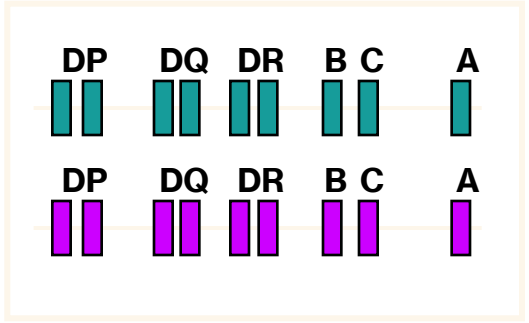
DP-1,8
DQ-3,6
DR-5,4
B-7,2
C-9,8
A-11,10



DP-2,8
DQ-4,6
DR-6,4
B-8,2
C-10,8
A-12,10



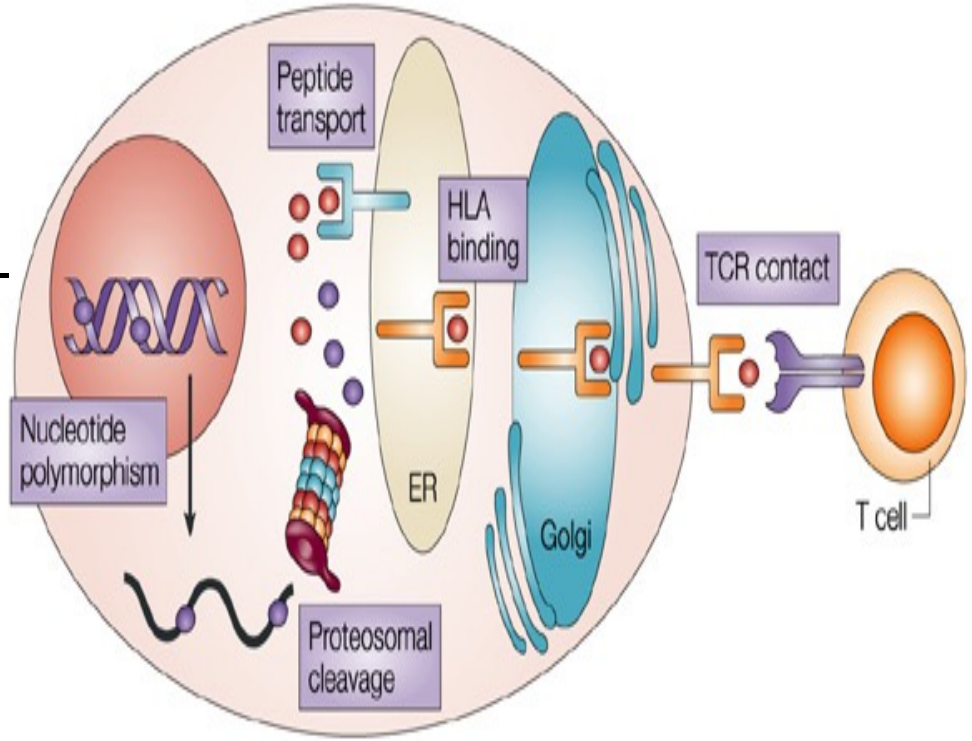
DP-2,9
DQ-4,7
DR-6,5
B-8,3
C-10,10
A-12,9



2 haplotype match LRT from a sibling is a perfect match

Minor transplantation antigens

- 'Minor histocompatibility antigens' (MiHA):
  - Due to normal proteins that can be polymorphic.
  - Processed and presented on self-MHC
  - Potentially elicit an anti-graft response.
- A recipient can reject a graft matched at all MHC loci
- Proteins encoded on the Y chromosome (H-Y), mitochondrial proteins (MTF), myosin related protein (HA-2)…

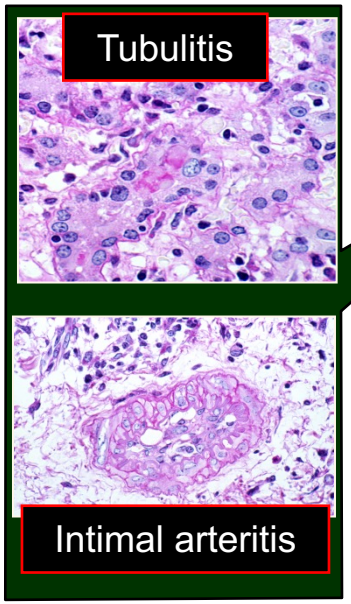


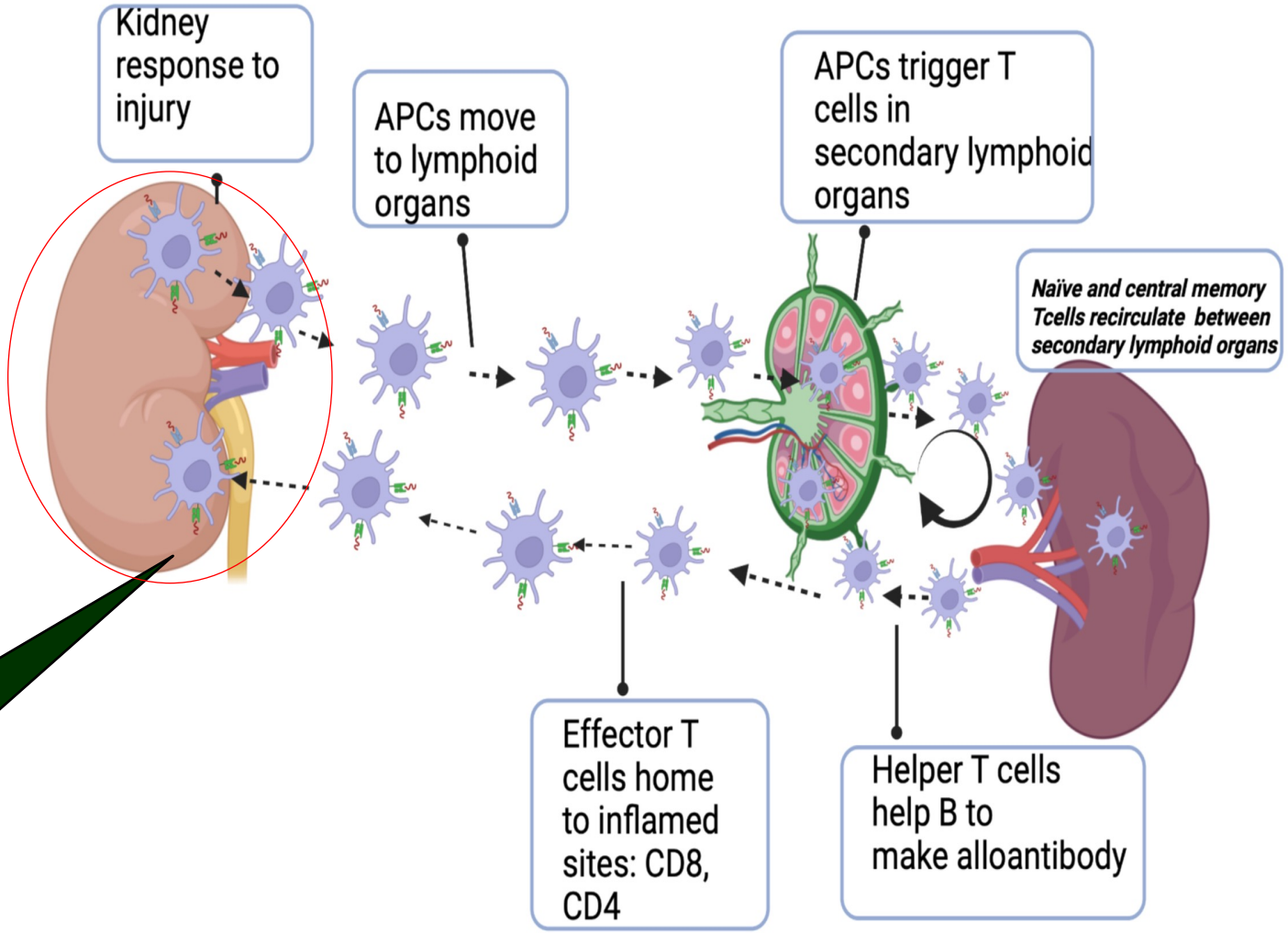
Bleakley et al. Nature Reviews Cancer 4, 371-380 (May 20

Nature Reviews | Cancer

- Perfect match siblings still differ in their Minor histocomptability (MiHA) antigens unless identical twins.
- Only identical twins do not need immunosuppressive drugs.
- Our current Immunosuppressive therapies are not antigen specific

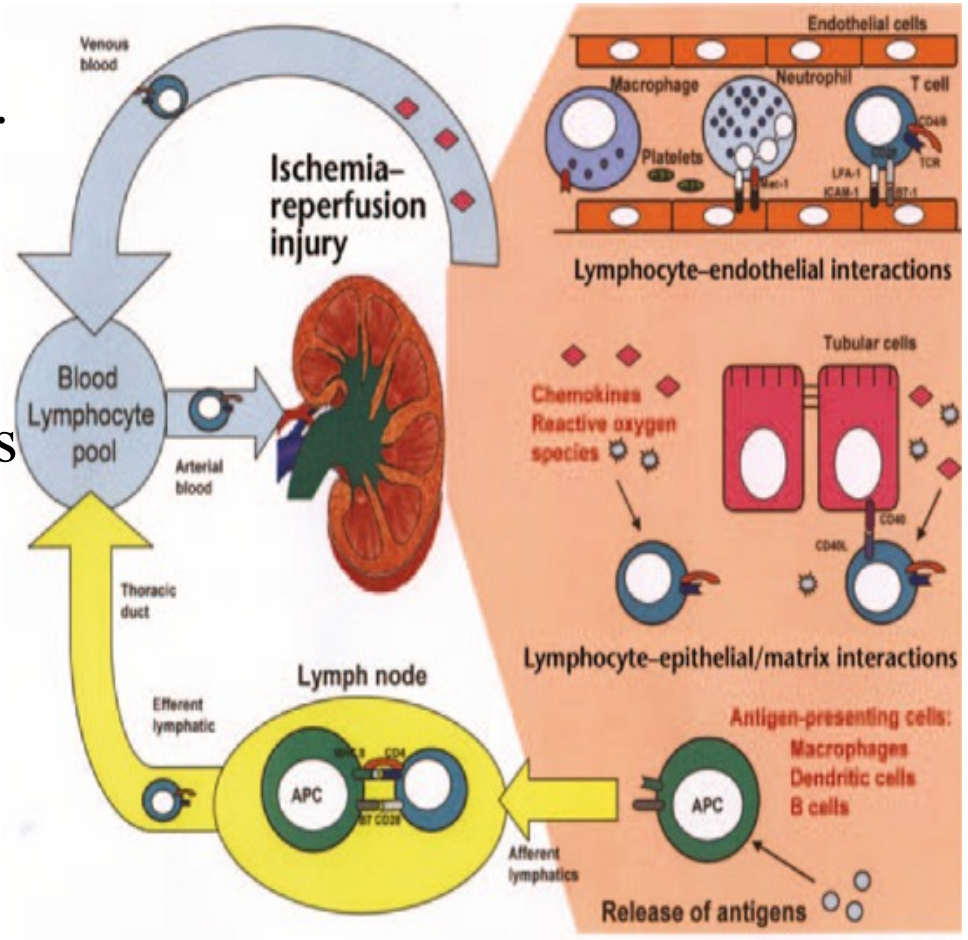
Allograft rejection



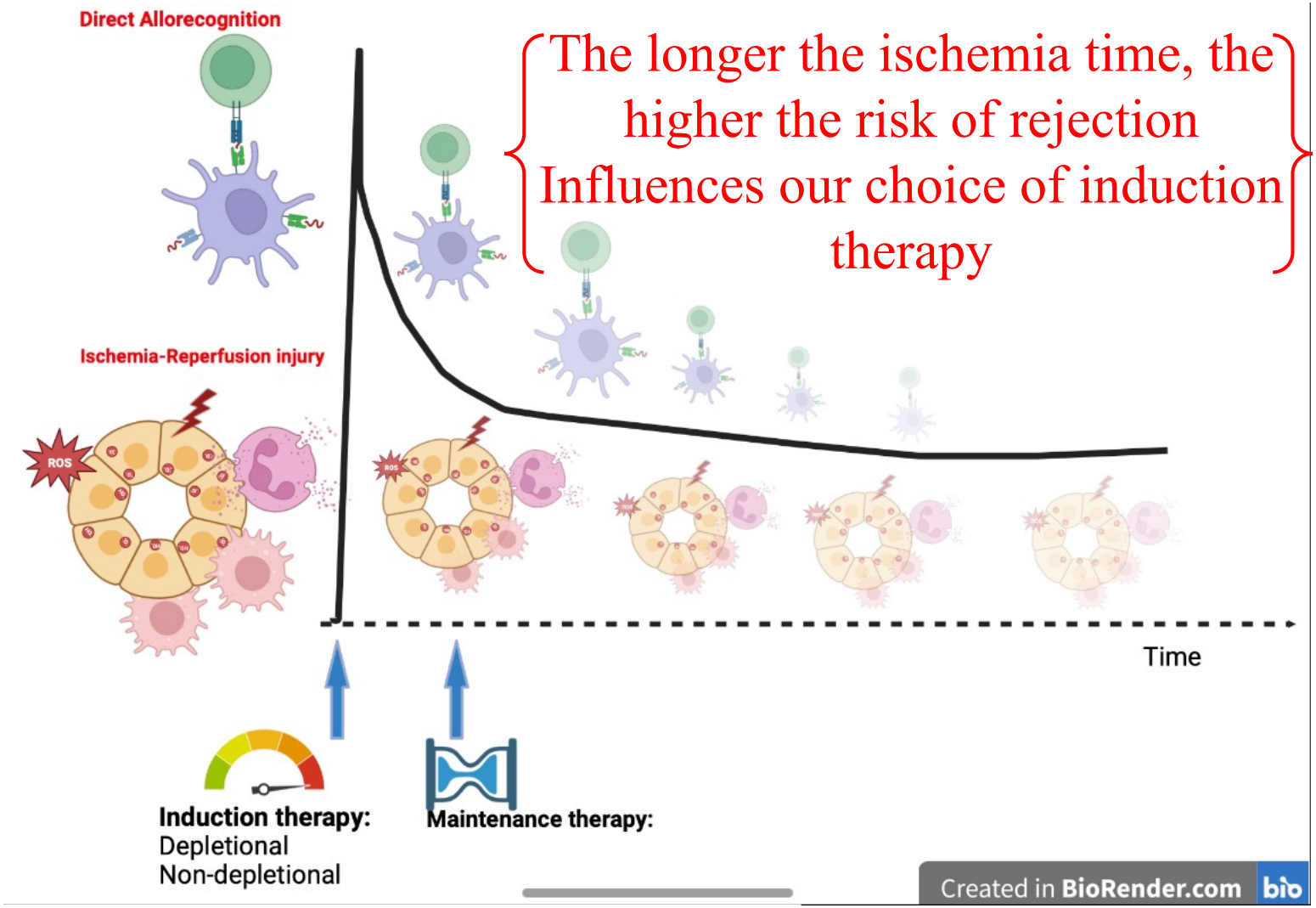


Ischemia and Antigen presentation

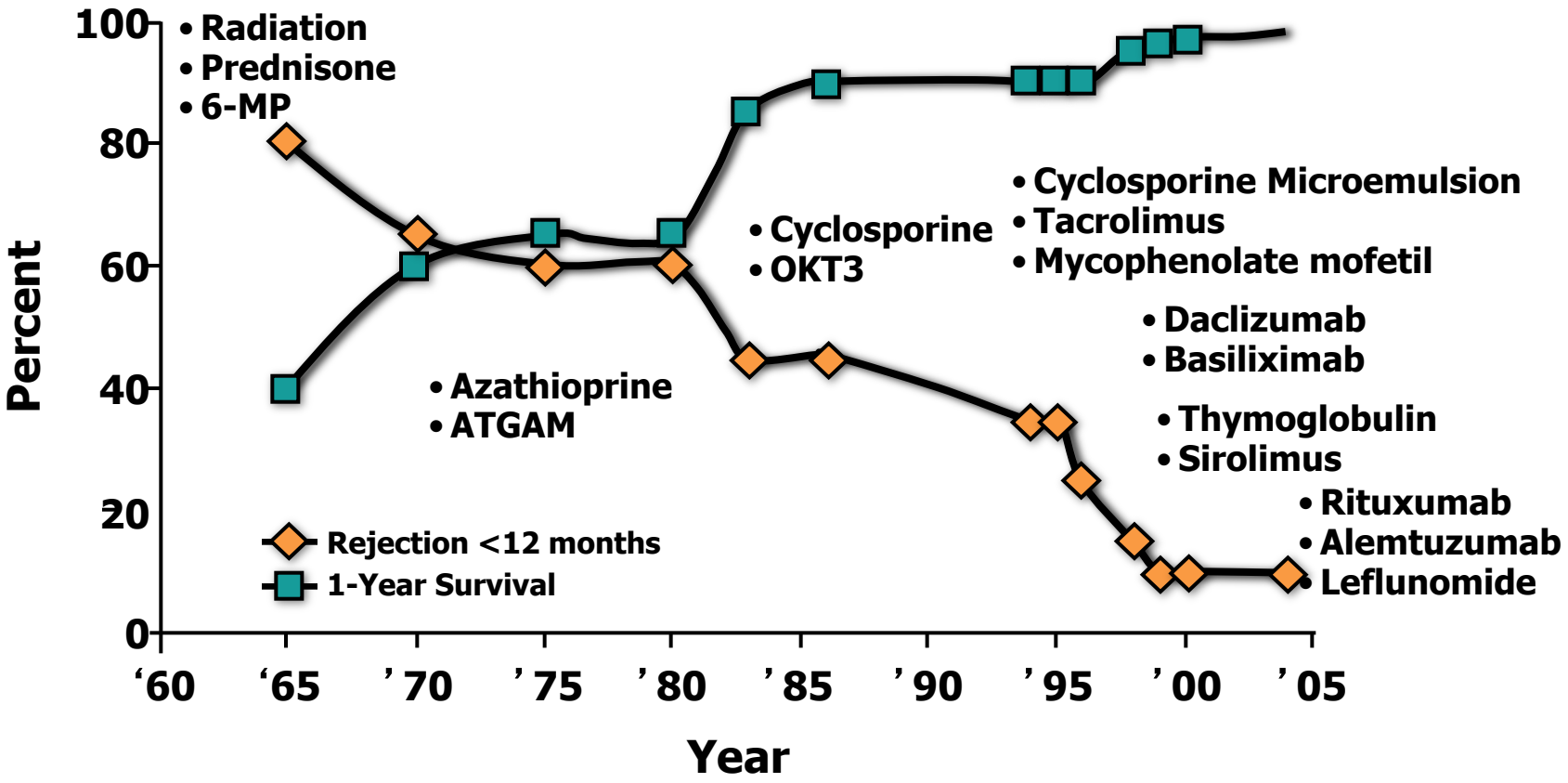
- Ischemia induces endothelial and renal tubule epithelial cells injury.
- Increased production of chemokines, cytokines, and oxygen free radicals.
- Activates antigen-presenting cells (APCs)
- Increases MHC molecules expression.



Gandolfo et al. Kidney International (2007) 71, 1193–1195

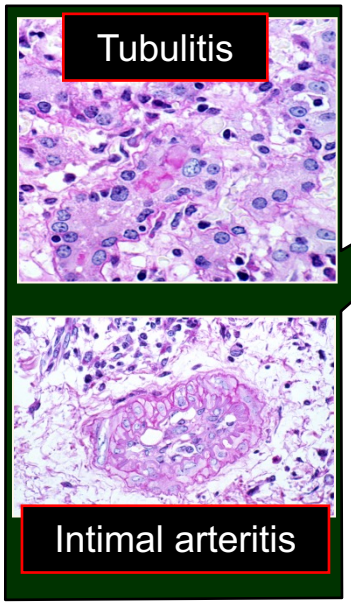

Direct Allorecognition
Ischemia-Reperfusion injury
ROS
ROS
The longer the ischemia time, the higher the risk of rejection
Influences our choice of induction therapy
Time
Induction therapy:
Depletional
Non-depletional
Maintenance therapy:
Created in BioRender.com
bio

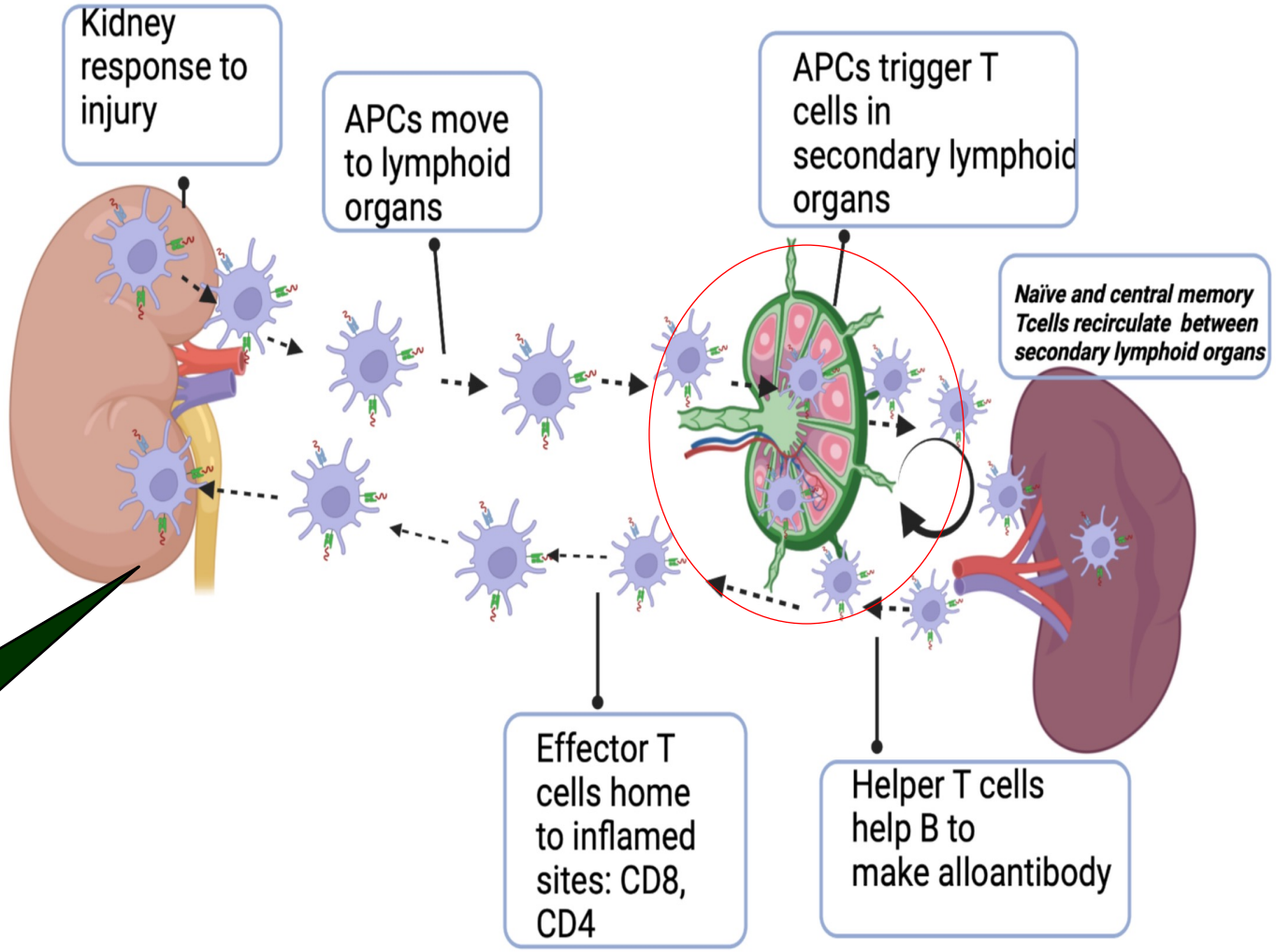
Introduction of Immunosuppressive Agents



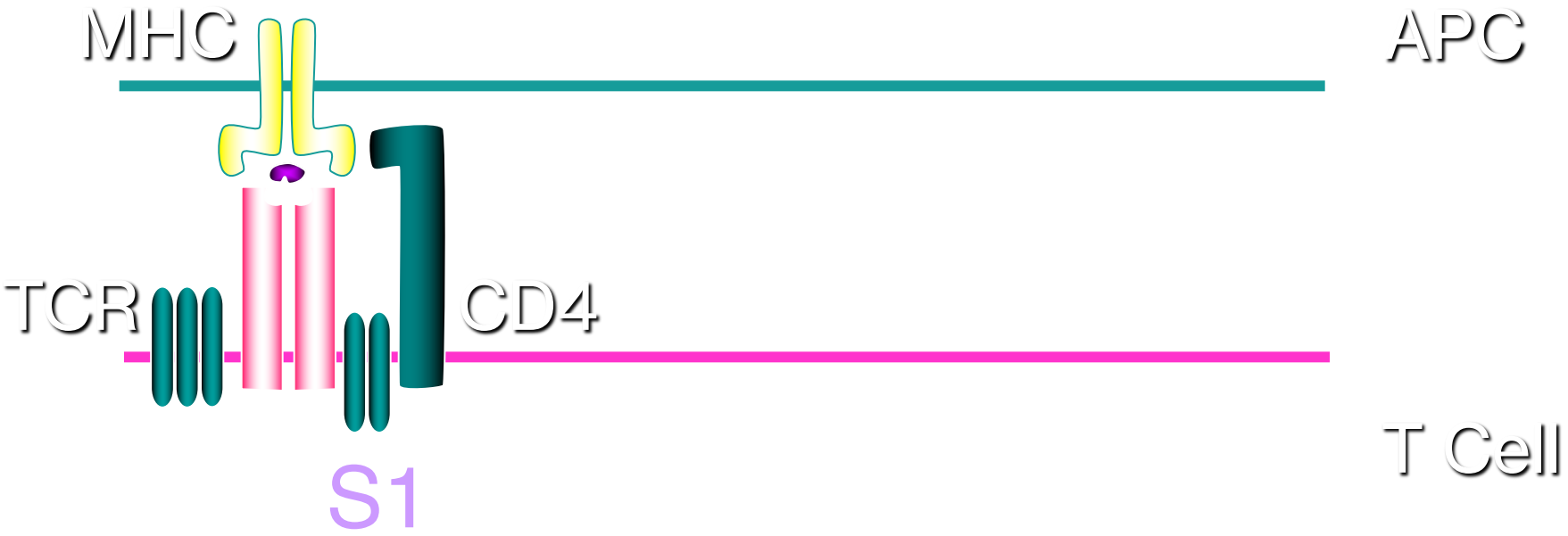
Adapted from Zand MS. *Semin Dial*. 2005;18:511-519.

Allograft rejection



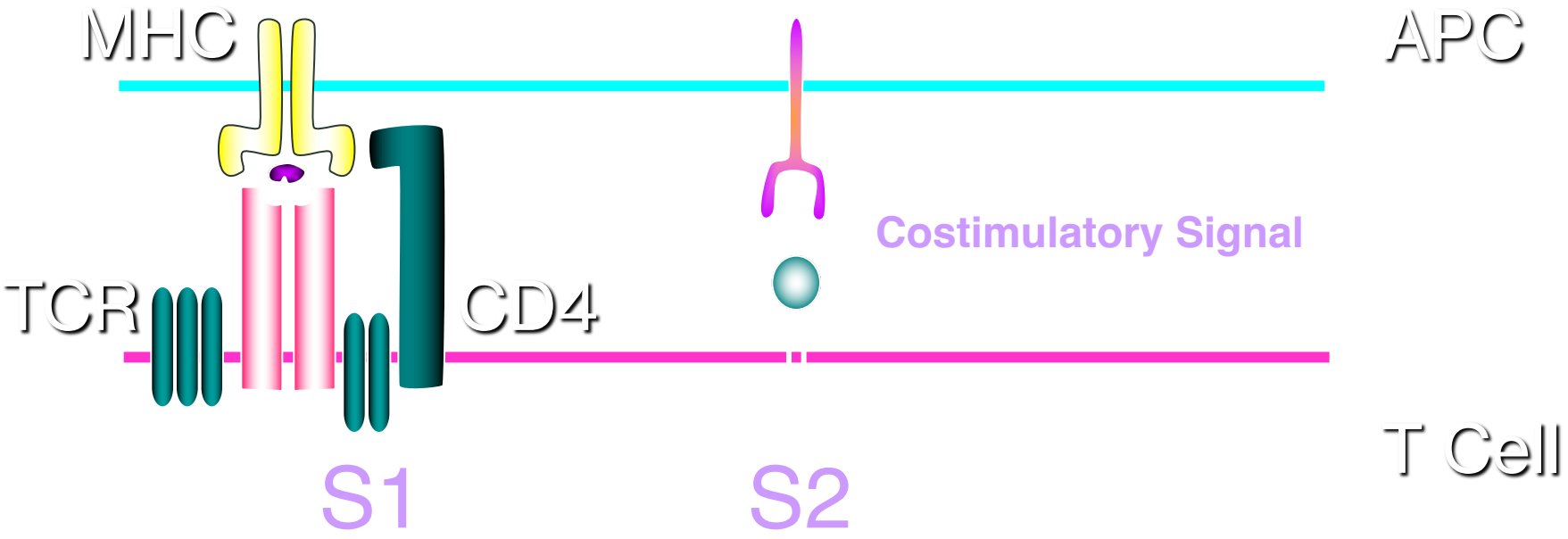


T Cells Require 2 Signals



IL-2 etc.

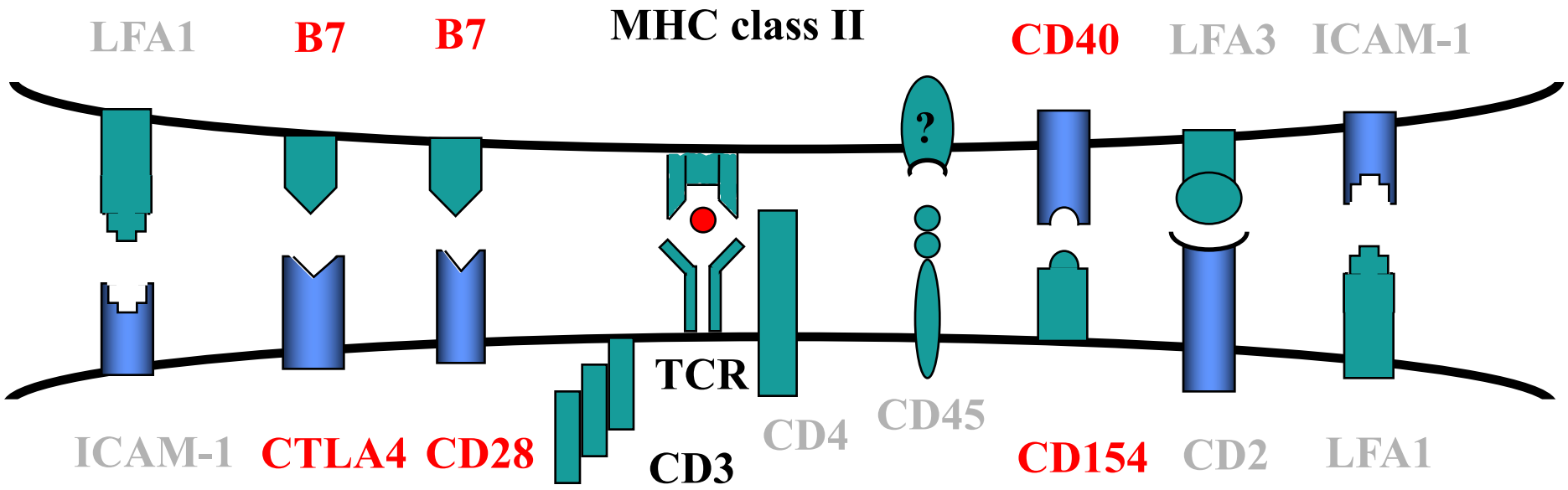
T Cells Require 2 Signals



IL-2 etc.

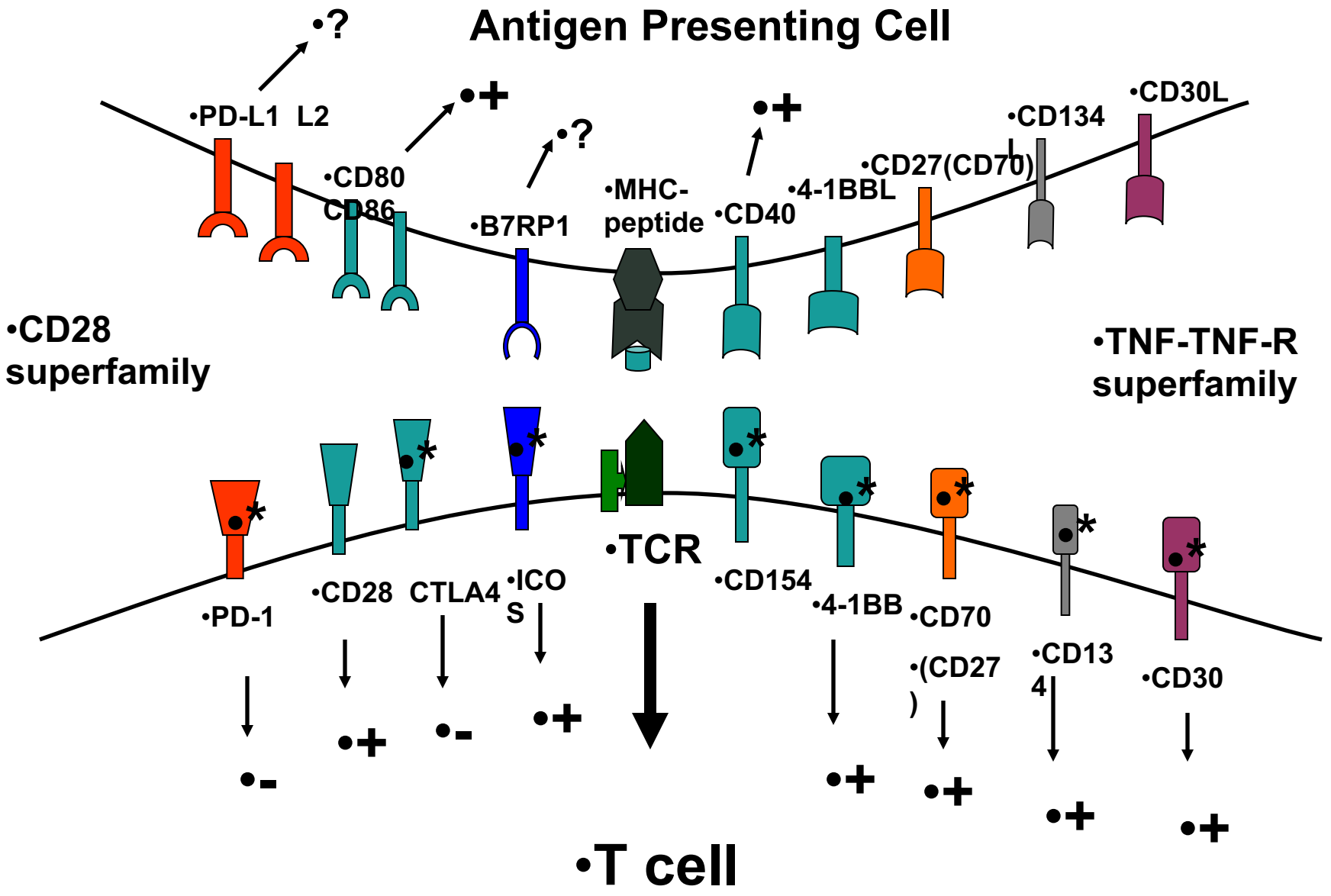
T CELL COSTIMULATION
"The Happy days"

APC

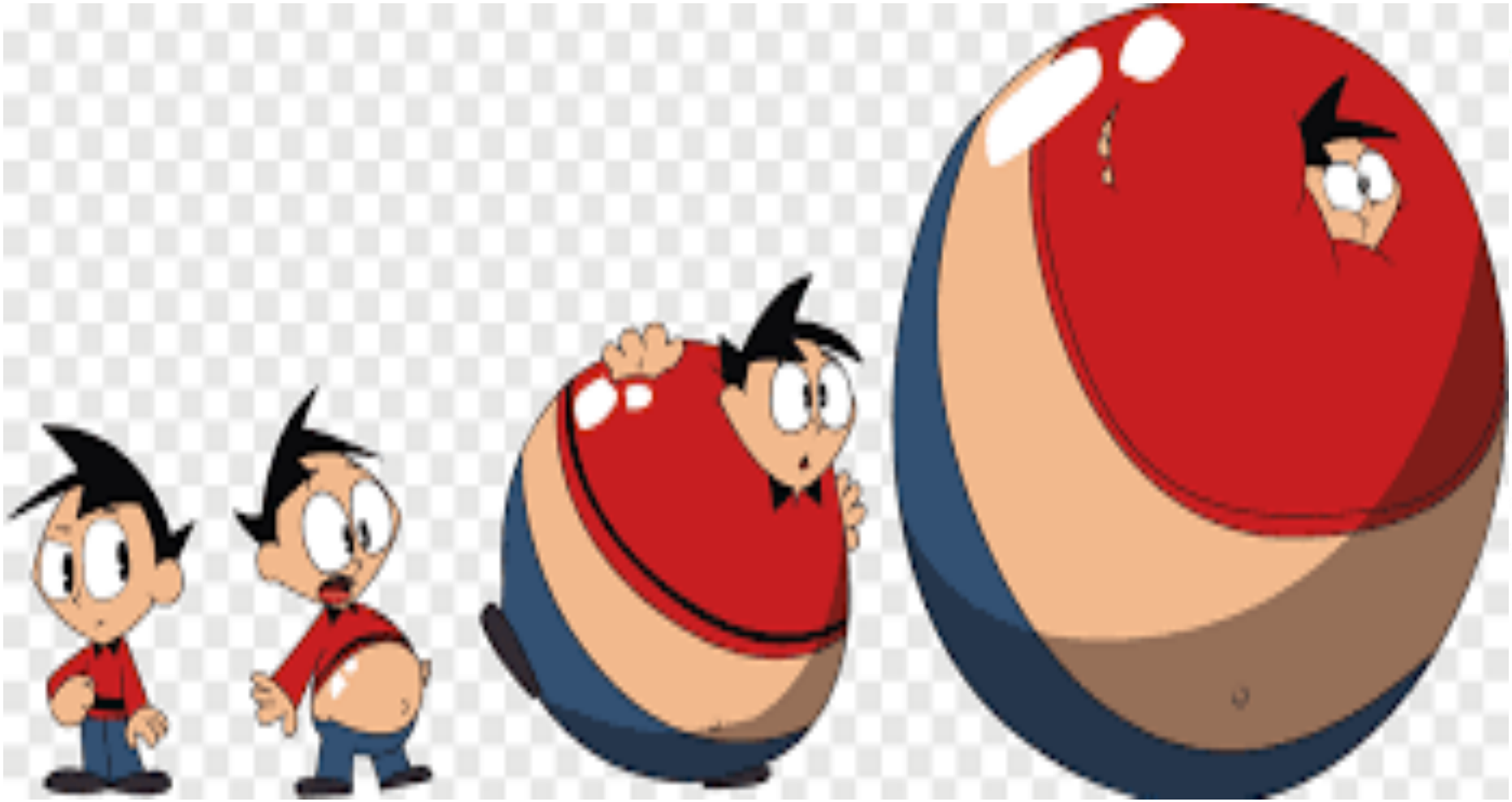


T Cell

Denton et al., Lancet 1999

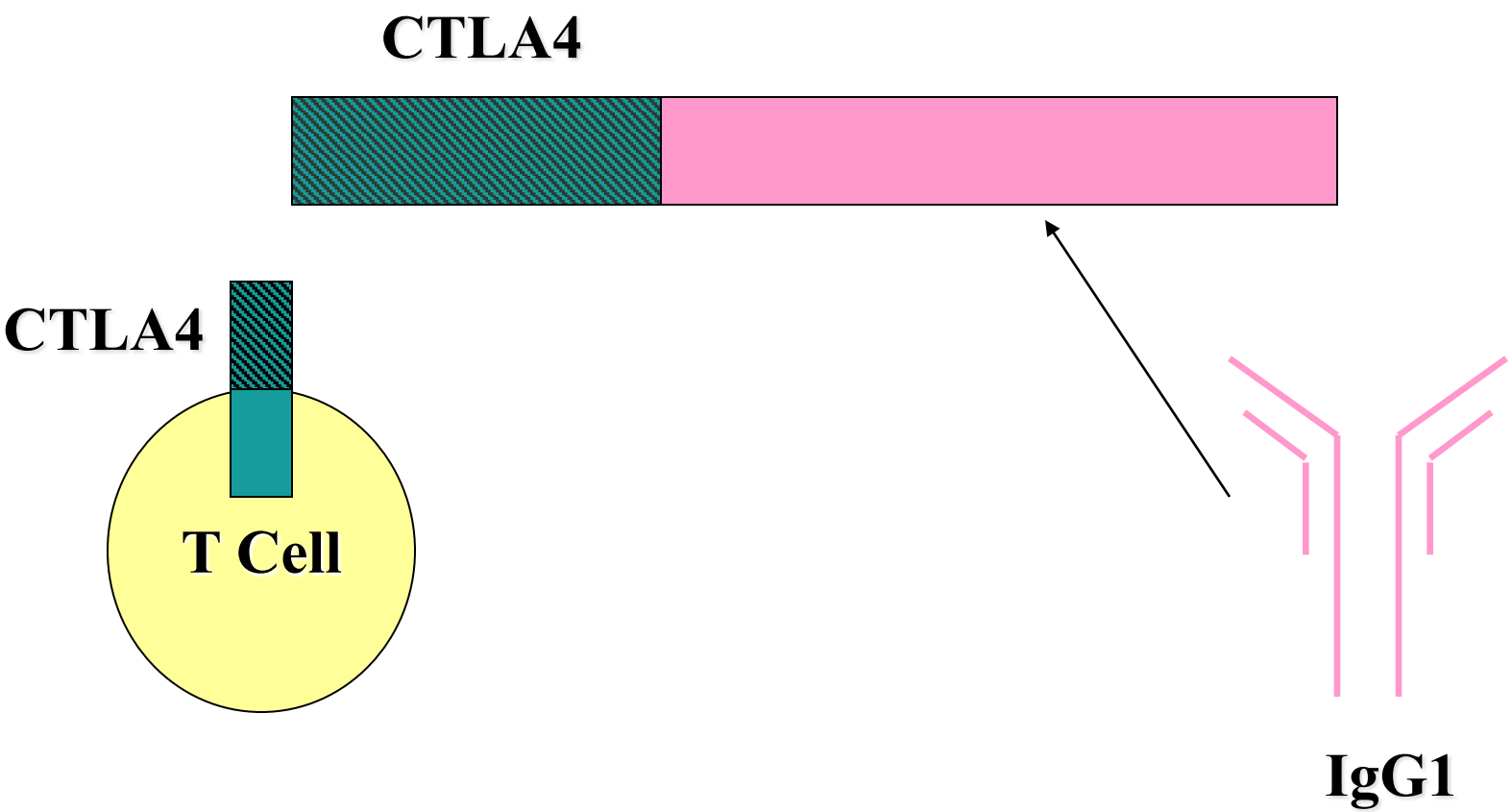

Antigen Presenting Cell
•?
•PD-L1 L2
•+
•CD80
CD86
•?
•B7RP1
•MHC-
peptide
•+
•CD40
•4-1BBL
•CD27(CD70)
•CD134
•CD30L
•CD28
superfamily
•TNF-TNF-R
superfamily
•TCR
•PD-1
•CD28
CTLA4
•ICO
S
•CD154
•4-1BB
•CD70
•(CD27
)
•CD13
4
•CD30
•-
•+
•-
•+
•+
•+
•+
•+
•T cell

CD28/CTLA-4 molecules: A prototype

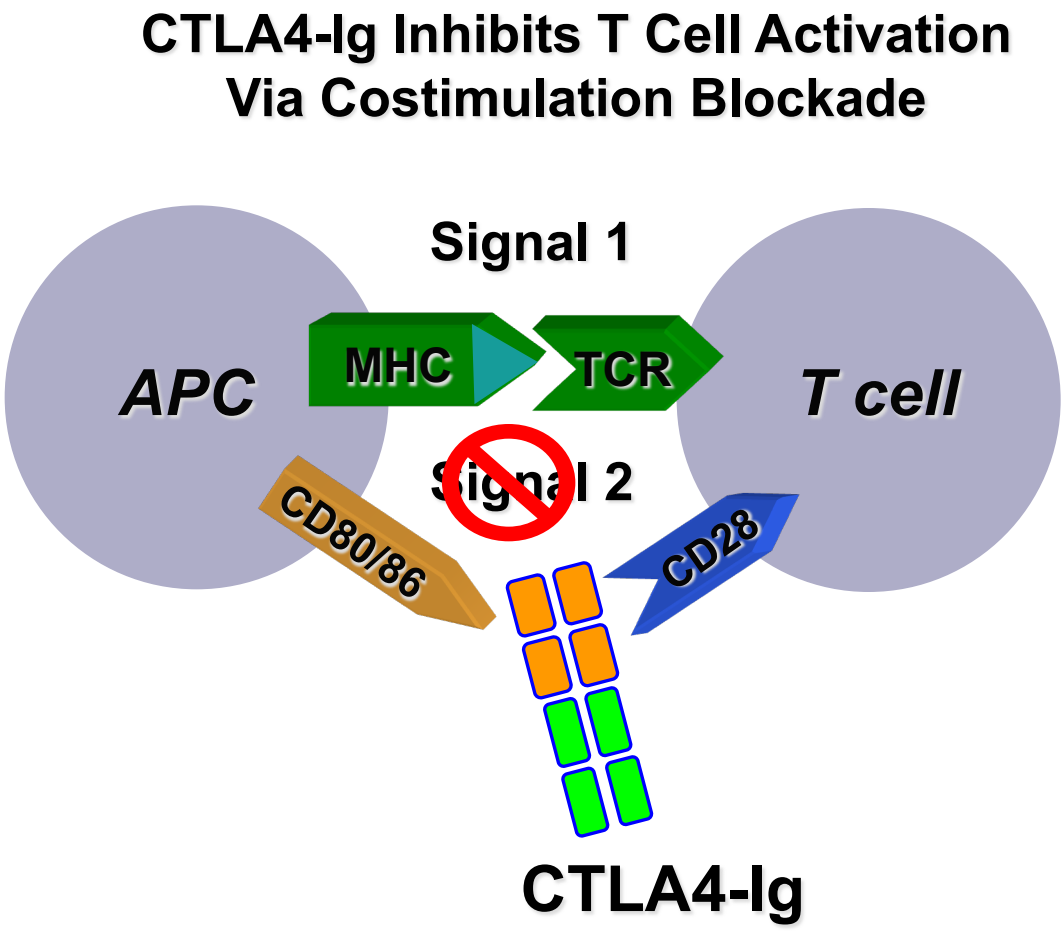


June et al., *Mol. Cell. Biol. 7, 4472–4481 (1987)*
Mueller et al. Annu. Rev. Immunol. 7, 445–480 (1989).

CTLA4-Ig: Therapeutic Fusion Protein



Costimulation Blockade
Mechanism of Action of CTLA4-Ig



From Abatacept to Belatacept (LEA29Y)
Rational design of a drug

provides more potent immunosuppression required for transplantation

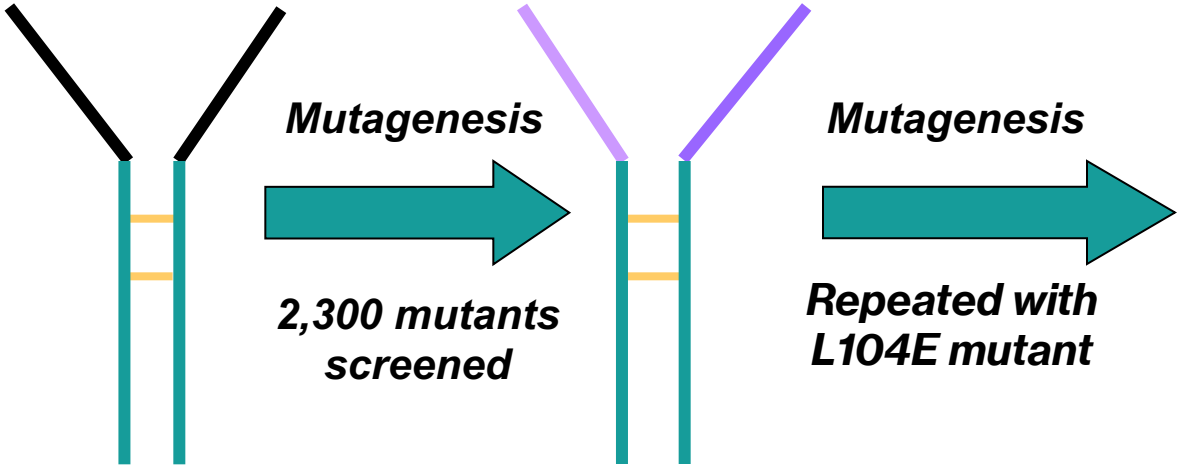
CTLA4Ig

L104E (CDR3) mutant identified

Belatacept (LEA29Y)

Leucine$_{104}$ → GlutamatE

Alanine$_{29}$ → TYrosine



Mutagenesis

2,300 mutants screened

Mutagenesis

Repeated with L104E mutant

Results:

2–fold slower dissociation from CD80 **+** **4–fold slower dissociation from CD86** **=** **~10-fold more potent inhibition of T-cell activation *In vitro***

Larsen CP et al. *Am J Trans 2005;5:443–453*

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Costimulation Blockade with Belatacept in Renal Transplantation

Flavio Vincenti, M.D., Christian Larsen, M.D., Ph.D., Antoine Durrbach, M.D., Ph.D., Thomas Wekerle, M.D., Björn Nashan, M.D., Ph.D., Gilles Blancho, M.D., Ph.D., Philippe Lang, M.D., Josep Grinyo, M.D., Philip F. Halloran, M.D., Ph.D., Kim Solez, M.D., David Hagerty, M.D., Elliott Levy, M.D., Wenjiong Zhou, Ph.D., Kannan Natarajan, Ph.D., and Bernard Charpentier, M.D., for the Belatacept Study Group*

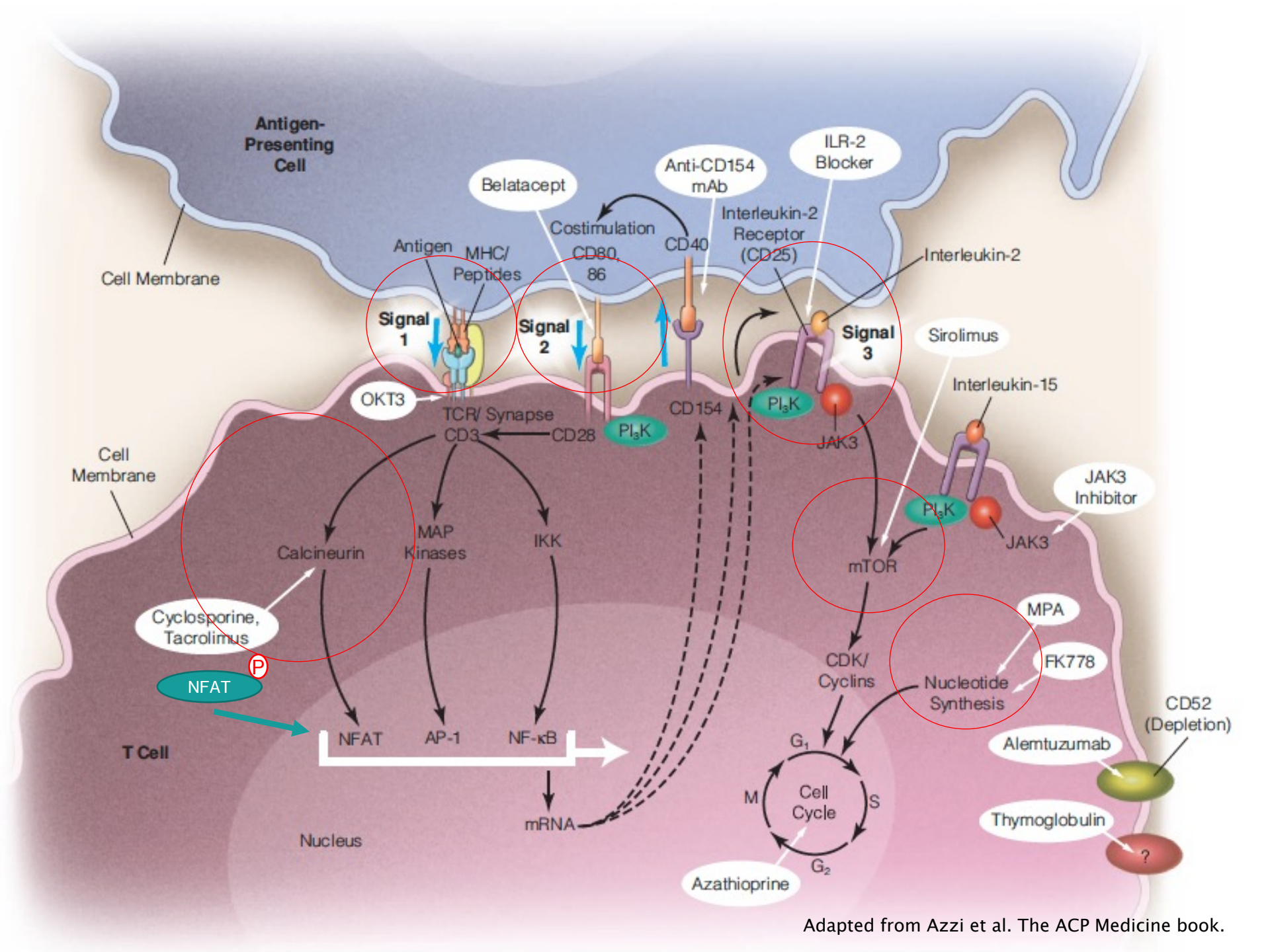
N ENGL J MED 353;8 WWW.NEJM.ORG AUGUST 25, 2005

Yes, but…

N Engl J Med. 2016 Jun 30;374(26):2599-600.

Belatacept and Long-Term Outcomes in Kidney Transplantation.

Riella LV, Gabardi S1, Azzi J.
Brigham and Women's Hospital, Harvard Medical School, Boston, MA



Adapted from Azzi et al. The ACP Medicine book.

Maintenance Immunosuppression

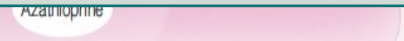
- Co-Stimulation Blockade
  - Belatacept (Nulojix®)
- Calcineurin Inhibitors (CNI)
  - Cyclosporine (Sandimmune® / Neoral® / Gengraf®)
  - Tacrolimus (Prograf® / Astagraf XL® Envarsus XR®)
- Mammalian Target of Rapamycin (mToR) Inhibitors
  - Sirolimus (Rapamune®)
  - Everolimus (Zortress®)
- Inhibitors of T-cell Proliferation
  - Azathioprine (Imuran®)
  - Mycophenolate mofetil (CellCept®)
  - Enteric-Coated Mycophenolic Acid (Myfortic®)
- Non-specific immunosuppressants
  - Corticosteroids

Targeted to the immune system so less metabolic side effects

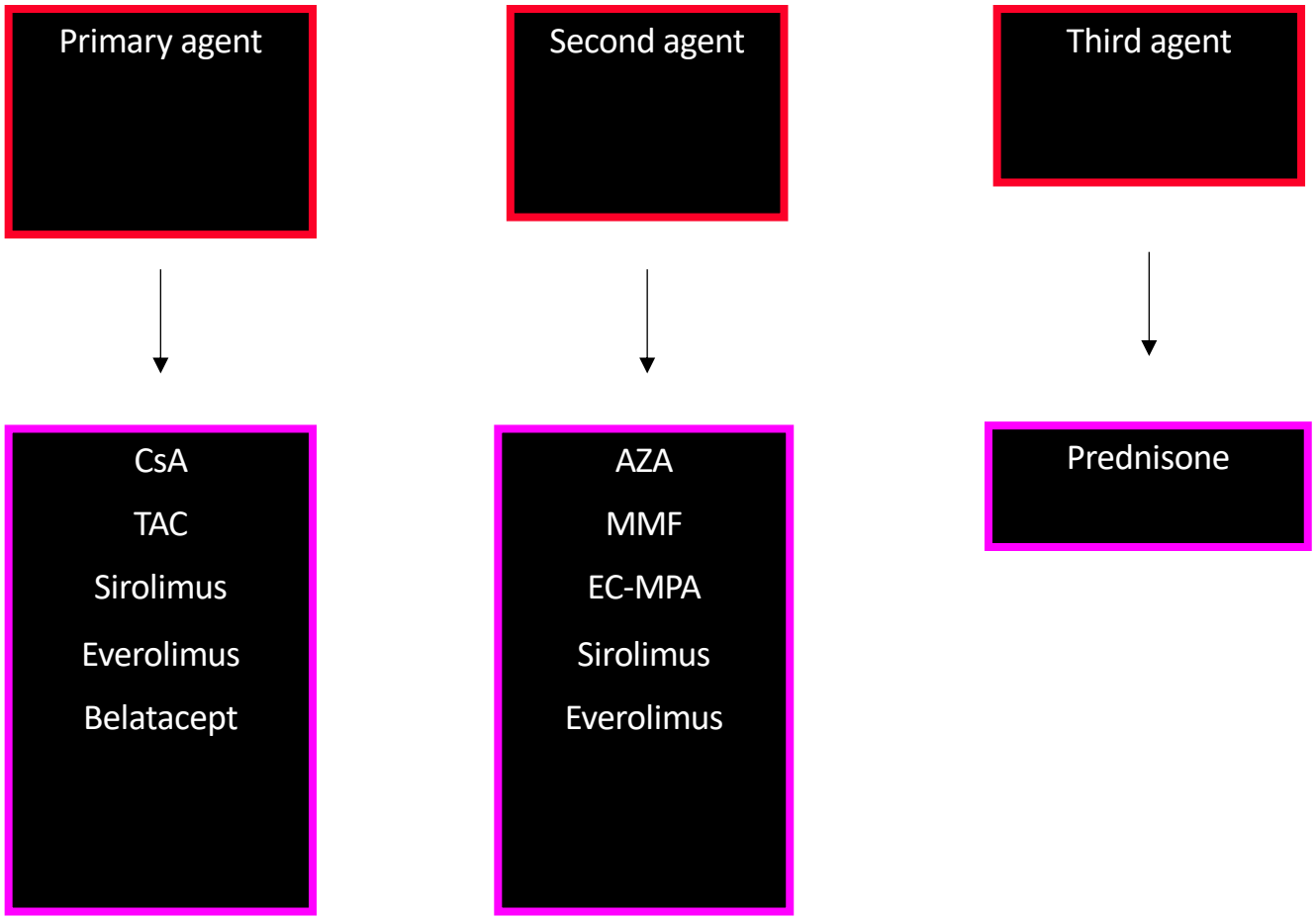
Calcineurin pathway ubiquitously expressed in tissues
-Insulin signaling->Diabetes
-Neurons -> Neurotoxicity
-Kidney epithelial and endothelial cells-> Nephrotoxicity

PI3K-mTOR pathway ubiquitously expressed in tissues and Important in cell proliferation and survival
->Inhibits wound healing
->Leukopenia and GI symptoms
->Preferred drug in patients who develop cancer

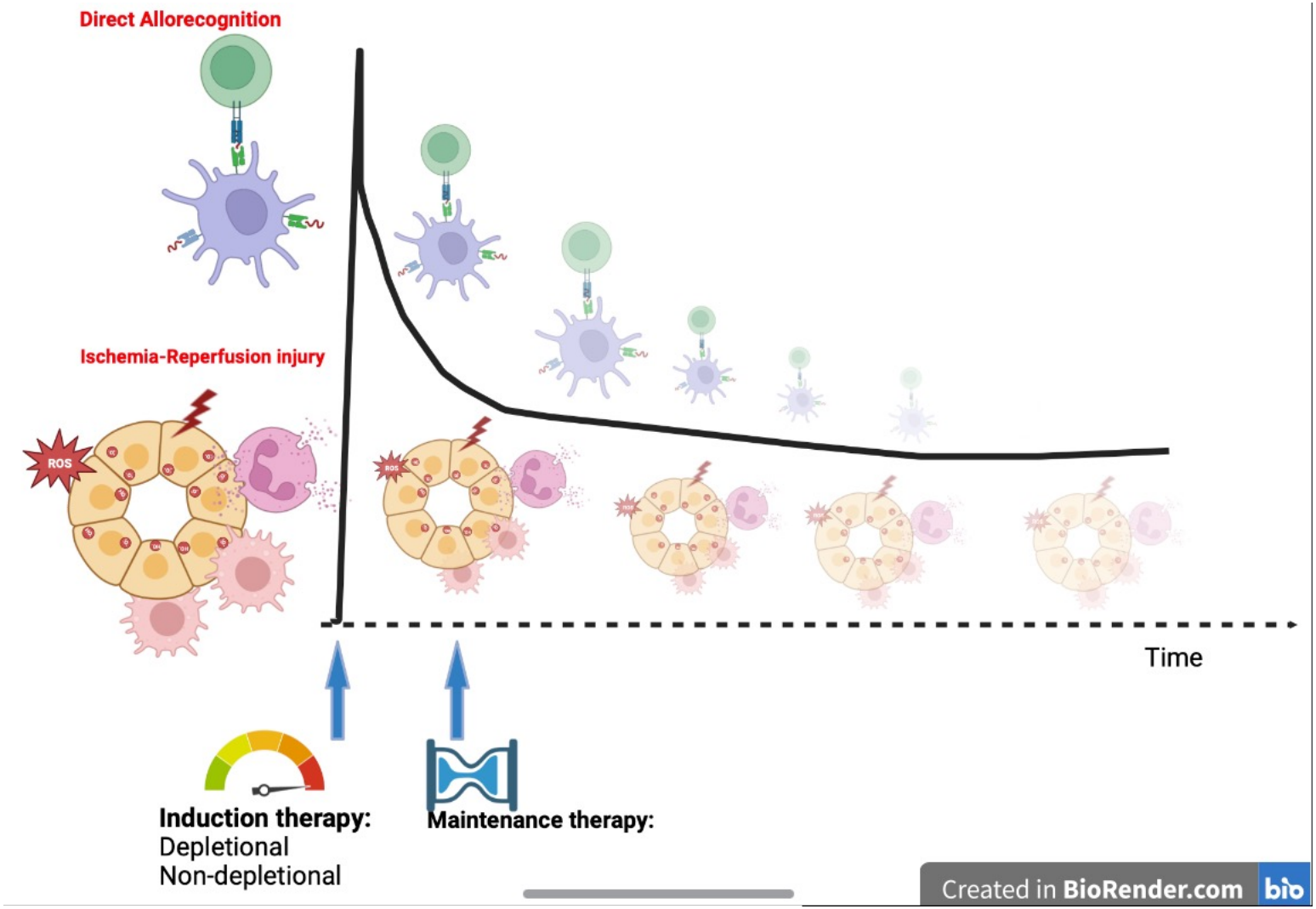
Nucleotide synthesis ubiquitously expressed in tissues and Important in cell proliferation
->Leukopenia and GI symptoms
->Inhibits wound healing



Common Combinations of Immunosuppressive Regimens



AZA = azathioprine, CSA = cyclosporine, EC-MPA=enteric-coated mycophenolate sodium,
MMF = mycophenolate mofetil, TAC = tacrolimus


Direct Allorecognition
Ischemia-Reperfusion injury
ROS
ROS
Time
Induction therapy:
Depletional
Non-depletional
Maintenance therapy:
Created in BioRender.com
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Question 1:

A 25 year old woman with ESRD secondary to reflux nephropathy is s/p 0 antigen mismatch LRT from her twin brother.
Which one of the following is true:

A- No need for maintenance therapy in this perfect match case
B- No risk for acute or chronic rejection
C-Induction therapy should be with depletional therapy
D- This match is perfect for major and minor transplantation antigens
E-None of the above

Question 2:

58 year old caucasian female with history of end stage kidney disease due to reflux nephropathy, on PD for 10 years, was called in for a cadaveric kidney transplant (A2B0DR0 mismatch). cPRA Class I is 0 % and Class II is 0 %. CDC AHG-T cell crossmatch negative . CIT:24 hours, WIT: 45 minutes.

Viral serologies:

- HBsAB positive: HbsAg negative; HbcAg negative
- Hep C: negative
- HIV Ab: negative
- CMV: positive (Donor: positive)
- EBV: positive (Donor : negative)
- Recipient ABO: O
- Donor ABO: O

Patient was induced with thymoglobulin and started on Steroid and MMF. The surgery was uneventful. Post-op Day 1, urine output is 5-10cc/hour, Post-op Day 7, urine output around the same and she remains dialysis dependent.

She is on MMF 1gm BID and FK level is 8.5 mg/dl

Which one of the following statements is correct?

A- Thymo induction was not appropriate as she had a good matched kidney and no preformed HLA antibodies.

B- Patient has no increased risk for rejection and delayed graft function is simply explained by the prolonged cold ischemia time

C- Biopsy is needed ASAP to rule out acute rejection.

D- Prograf should be stopped as it is contributing to the delayed graft function

Question 3:

28 year old caucasian female with history of end stage kidney disease due to presumed hypertension, on PD for 4 years, is s/p deceased kidney transplant (A2B2DR0 mismatch) 3 years ago. cPRA Class I is 0 % and Class II is 0 %. CDC AHG-T cell crossmatch negative. Patient's creatinine stable at 1.1. mg/dl. No episodes of rejection or infections post transplant. However, patient developed recurrent squamous cell carcinoma over the last 2 years. Patient's immunosuppressive therapies: MMF 1gm BID, tacrolimus 1 mg BID (level 6) and prednisone 5 mg.

Which one of the following statements is correct?

A- Switching tacrolimus to rapamycine is associated with decreased risk of recurrence of squamous cell carcinoma in a randomized controlled trial.

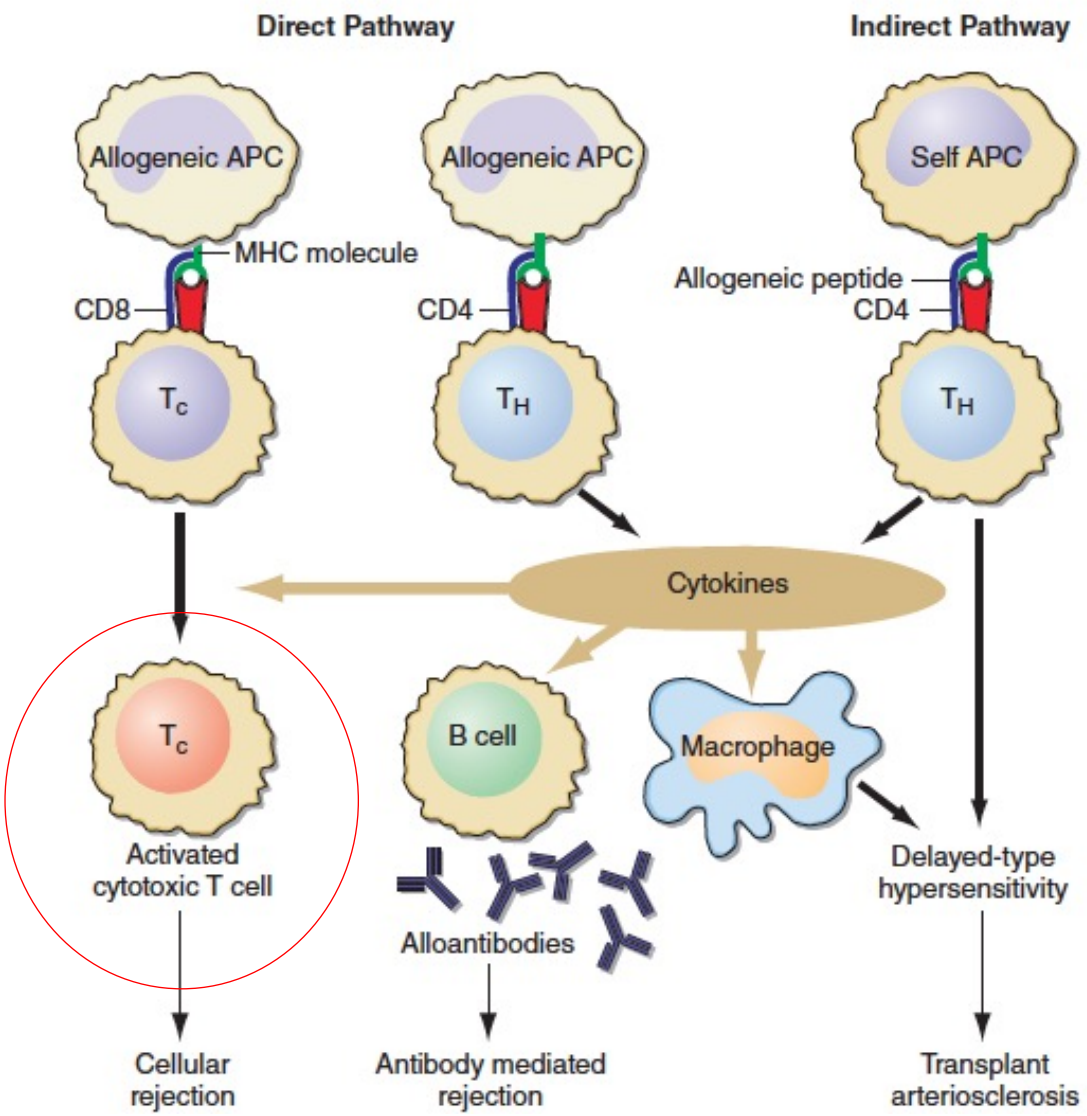
B- PI3K-mTOR pathway is highly activated in anti-cancer T cells and mTOR inhibitors should be avoided.

C- Patient remains at high risk for rejection due to her young age but tacrolimus should be switched to rapamycine.

D- Prednisone should be stopped to reduce the risk of skin cancer recurrence.

E- Tacrolimus should be switched to rapmycine and MMF reduced to 500 mg BID within 2 weeks.

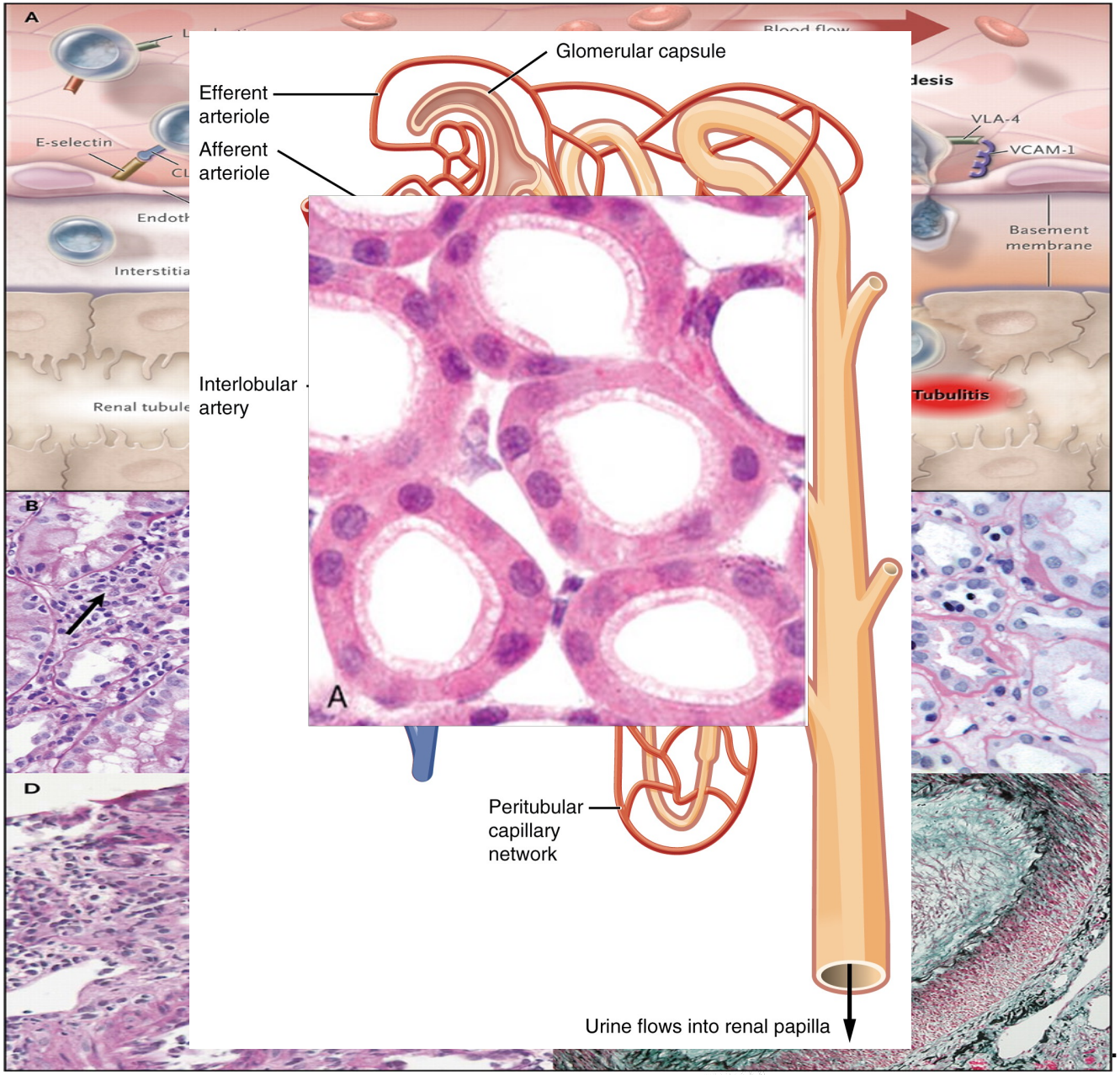
Step Three: Effector mechanisms

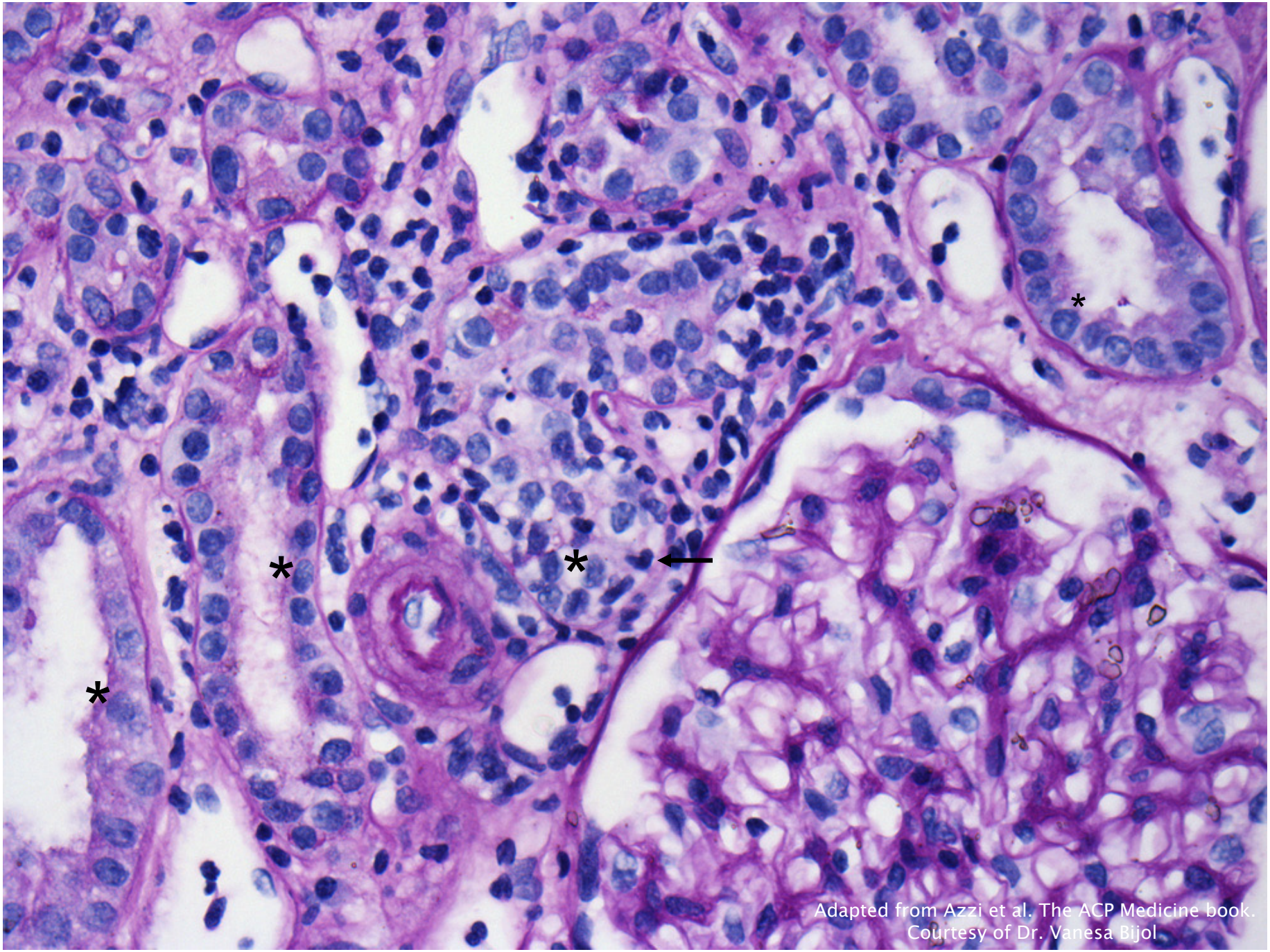


Adapted from Azzi et al. Harrison's principles of internal medicine.

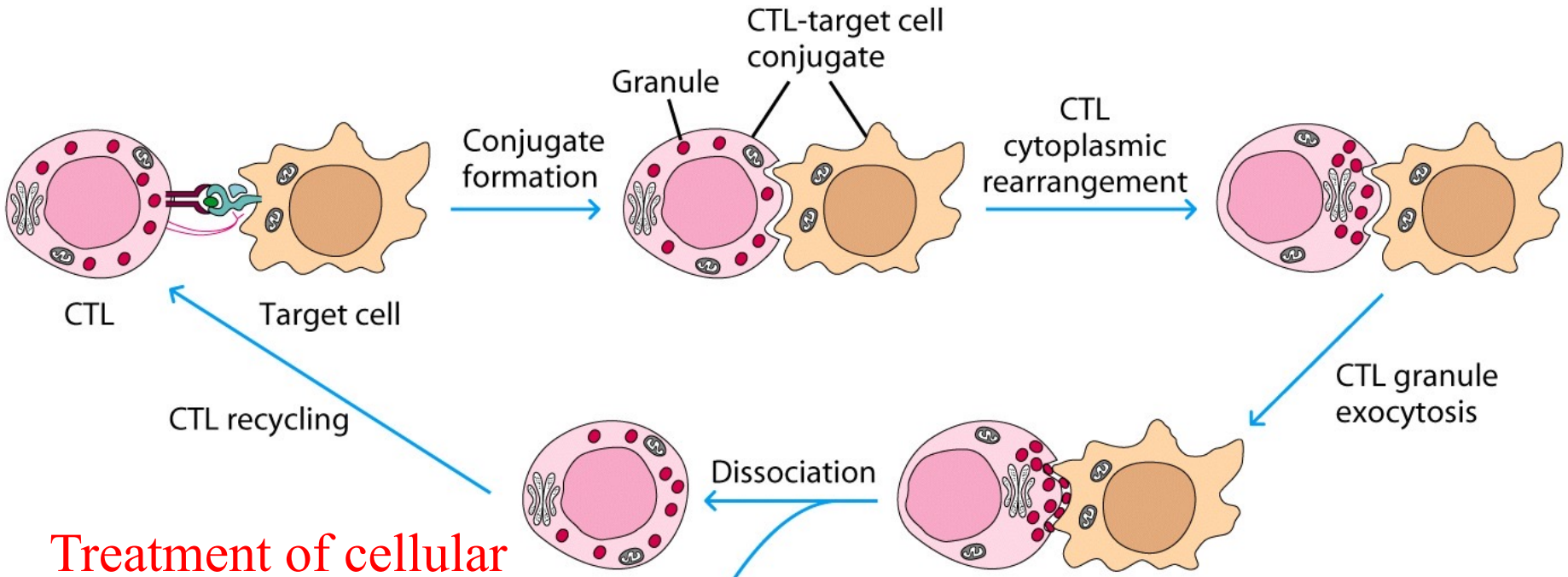
Acute T-Cell–Mediated Rejection.

- T cell transport into the allograft after activation in the lymphoid organs
- T cells invading tubules causing T cell mediated rejection.

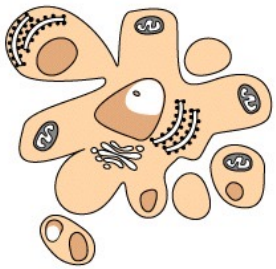


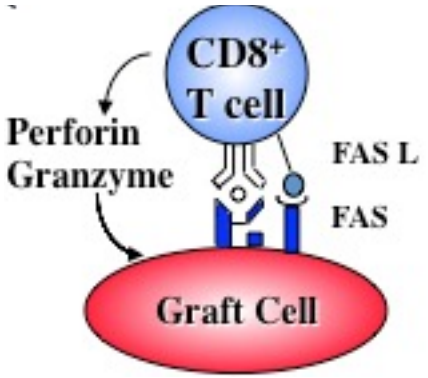

Adapted from Azzi et al. The ACP Medicine book.
Courtesy of Dr. Vanesa Bijol

CD8 cytotoxicity

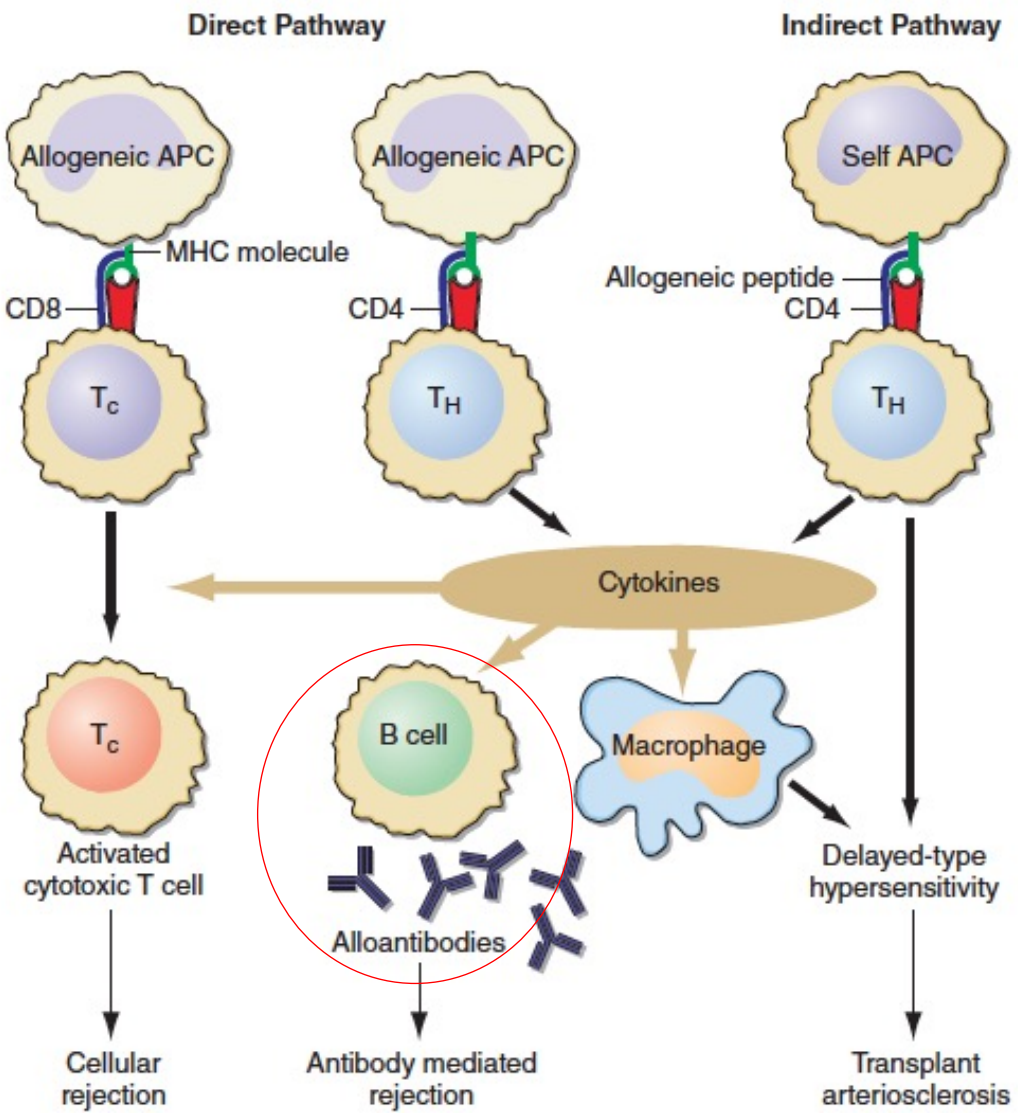


Treatment of cellular rejection is to deplete the cell infiltrate:
High dose solumedrol
or
Depletional therapy



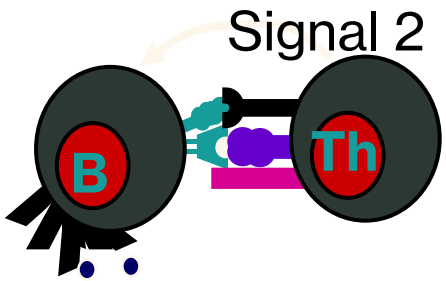


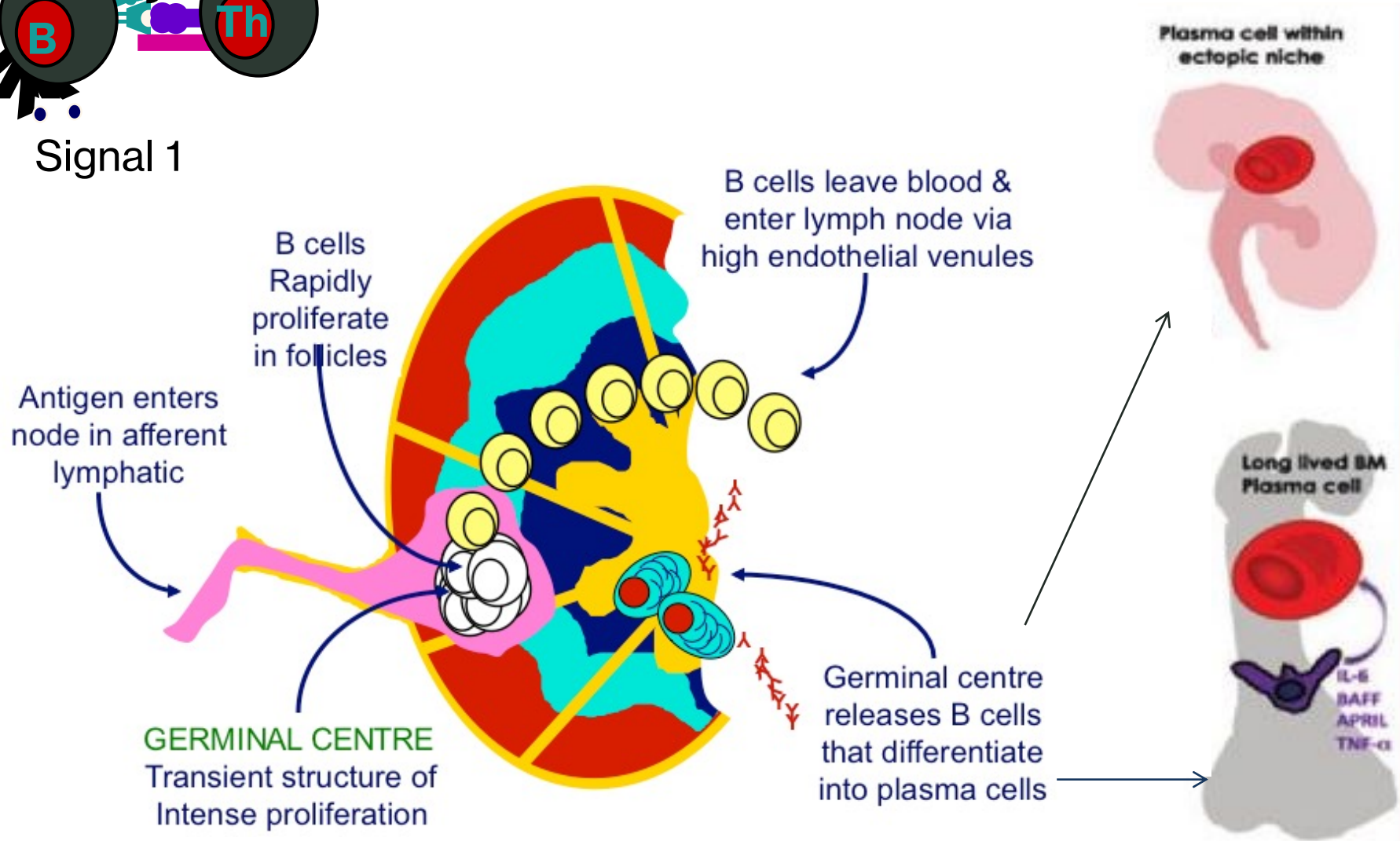
Step Three: Effector mechanisms



Adapted from Azzi et al. Harrison's principles of internal medicine.

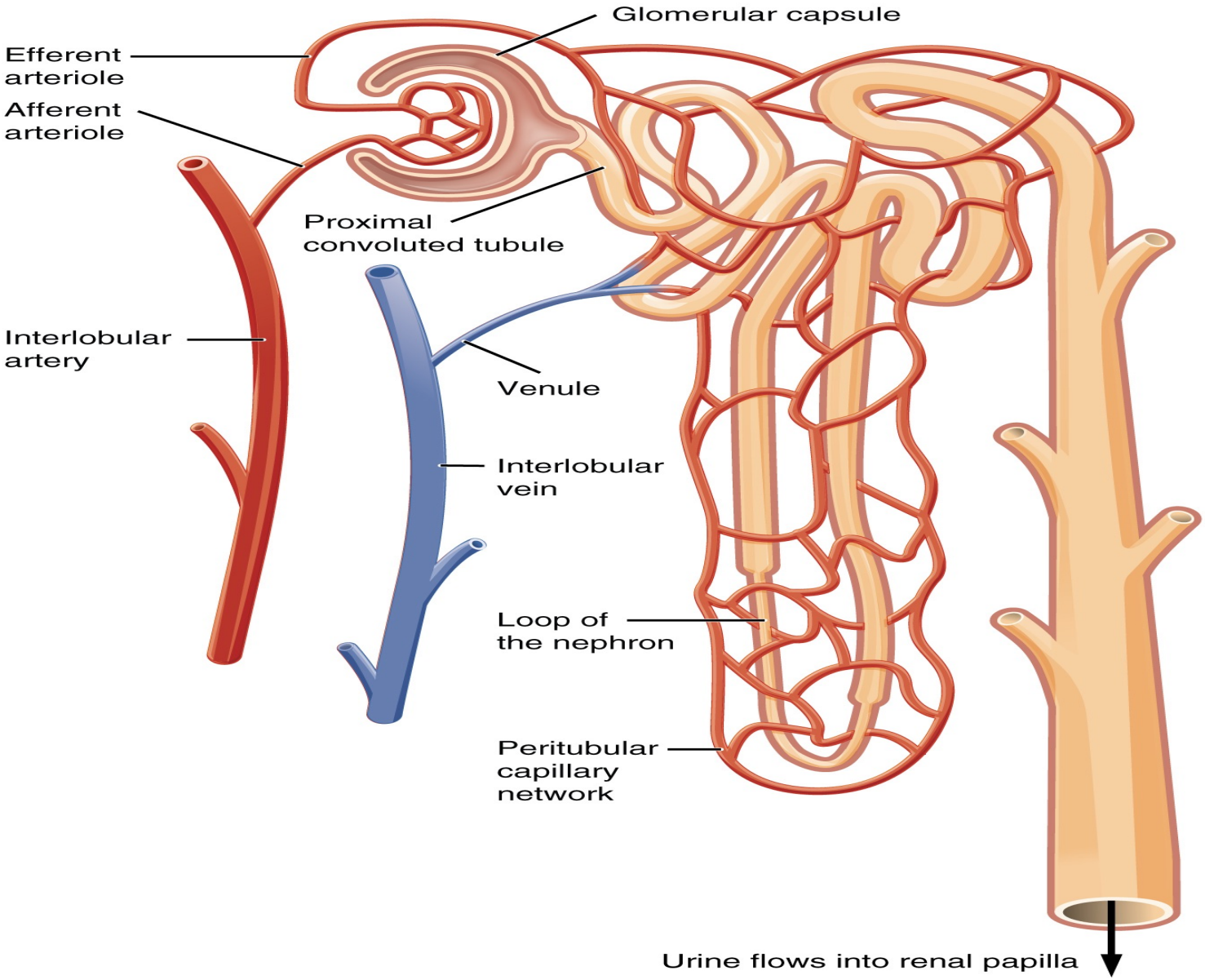
B cell activation





- Antibodies recognize polymorphic regions of the MHC molecules
- May develop upon exposure to alloantigens through pregnancy, blood transfusion, previous transplant or by cross reactivity

Pathophysiology of Antibody-Mediated rejection

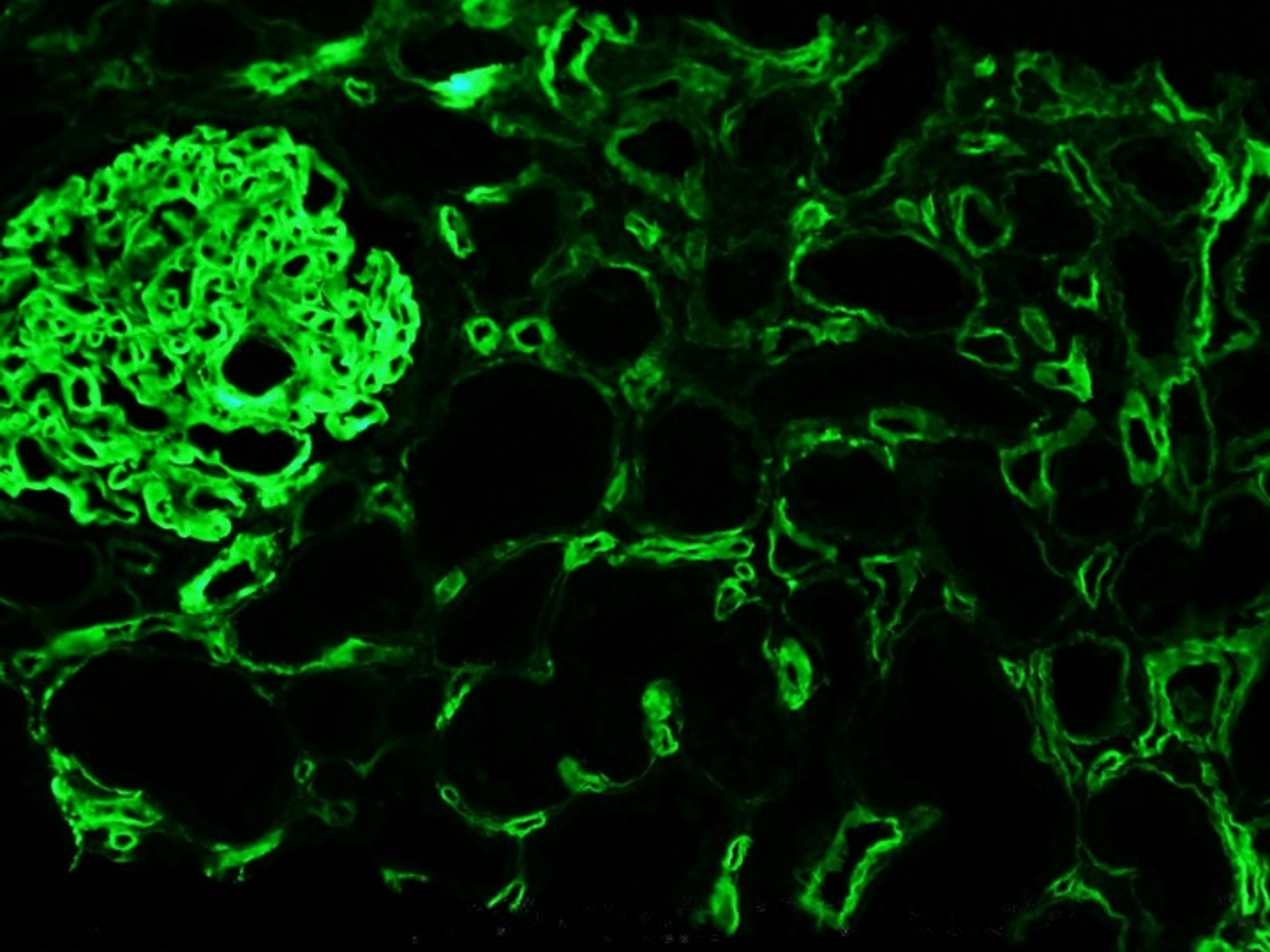


ex

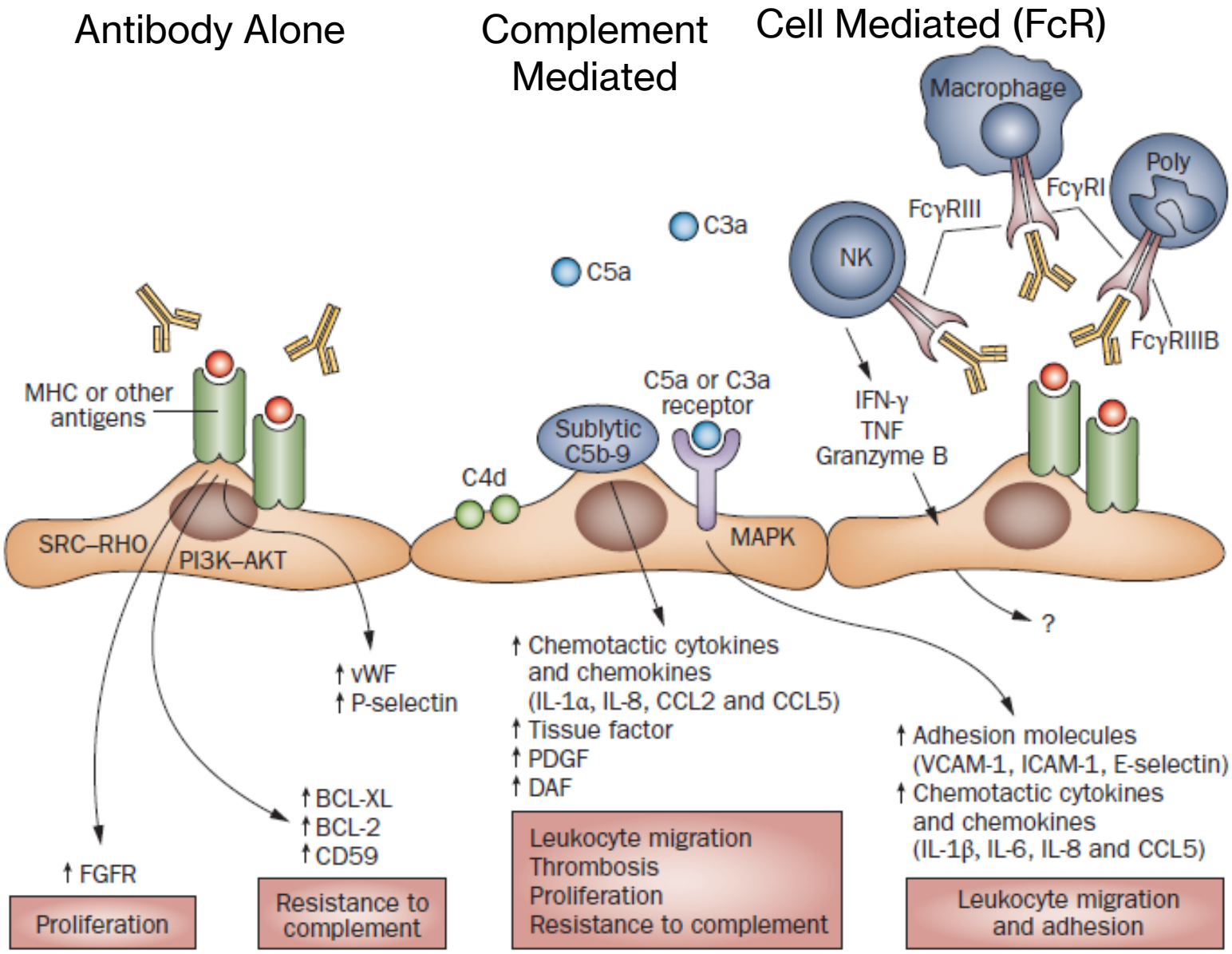
C4d

ker

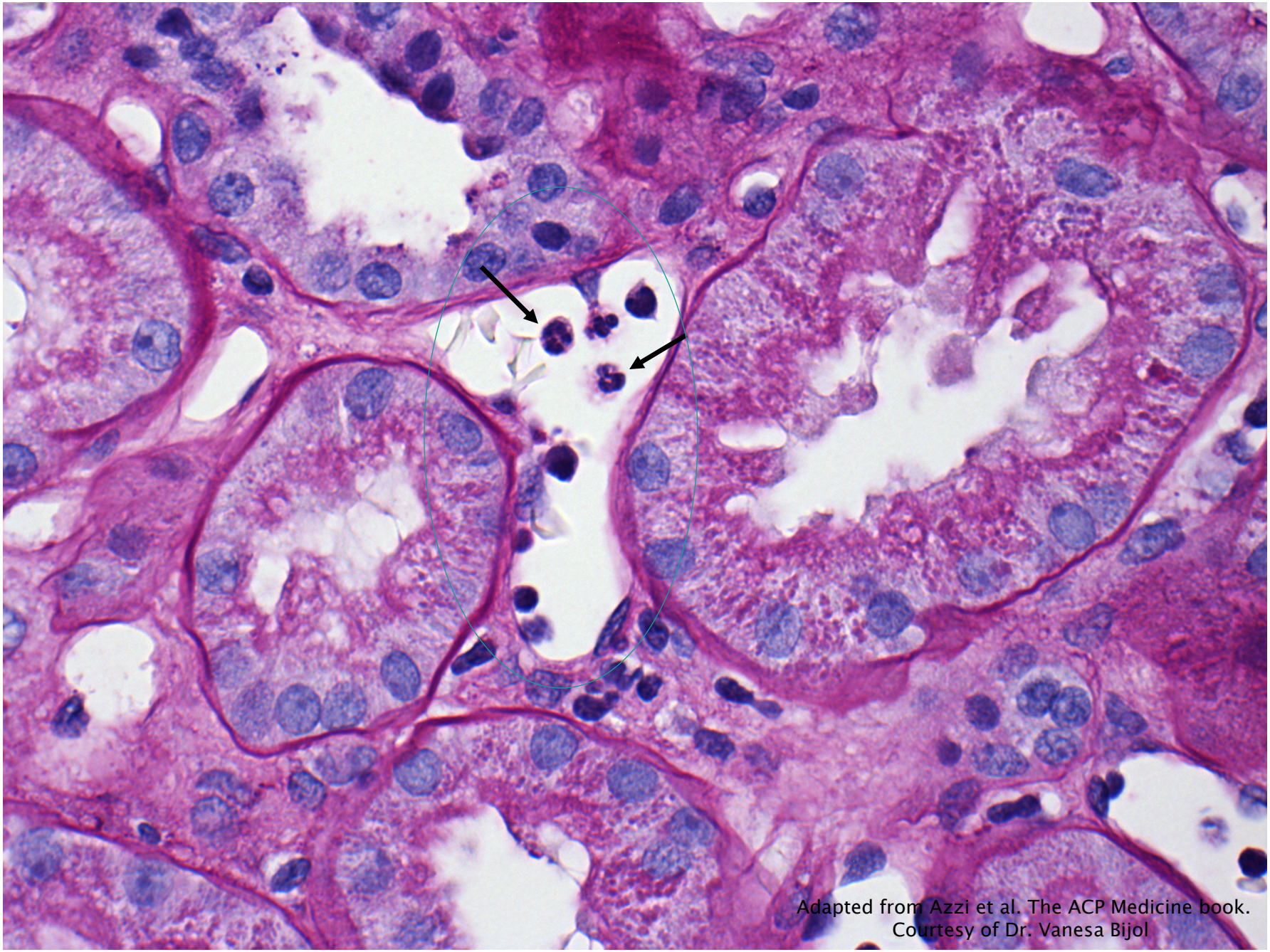
Schwartz et al. , NEJM 2010



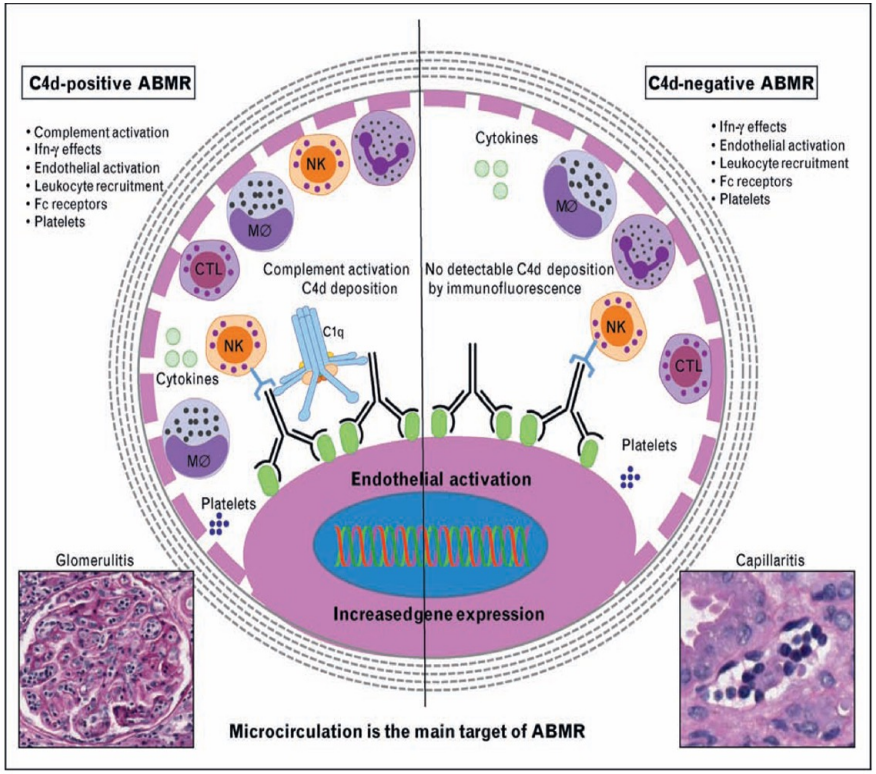
Three Pathways to Antibody-Mediated Injury



Farkash and Colvin, Nat Rev Nephrol 8:255, 2012


Adapted from Azzi et al. The ACP Medicine book.
Courtesy of Dr. Vanesa Bijol

Acute B-cell-mediated rejection



Sis B; Halloran P.
Endothelial transcripts uncover a previously unknown phenotype: C4d-negative antibody-mediated rejection.
Current Opinion in Organ Transplantation. 15(1):42-48, February 2010.

Anti-donor Abs such as those directed at MHC antigens can trigger AMR.

However, the ***absence of DSA cannot be taken to exclude AMR***.

Cases with: **C4d+, DSA+**

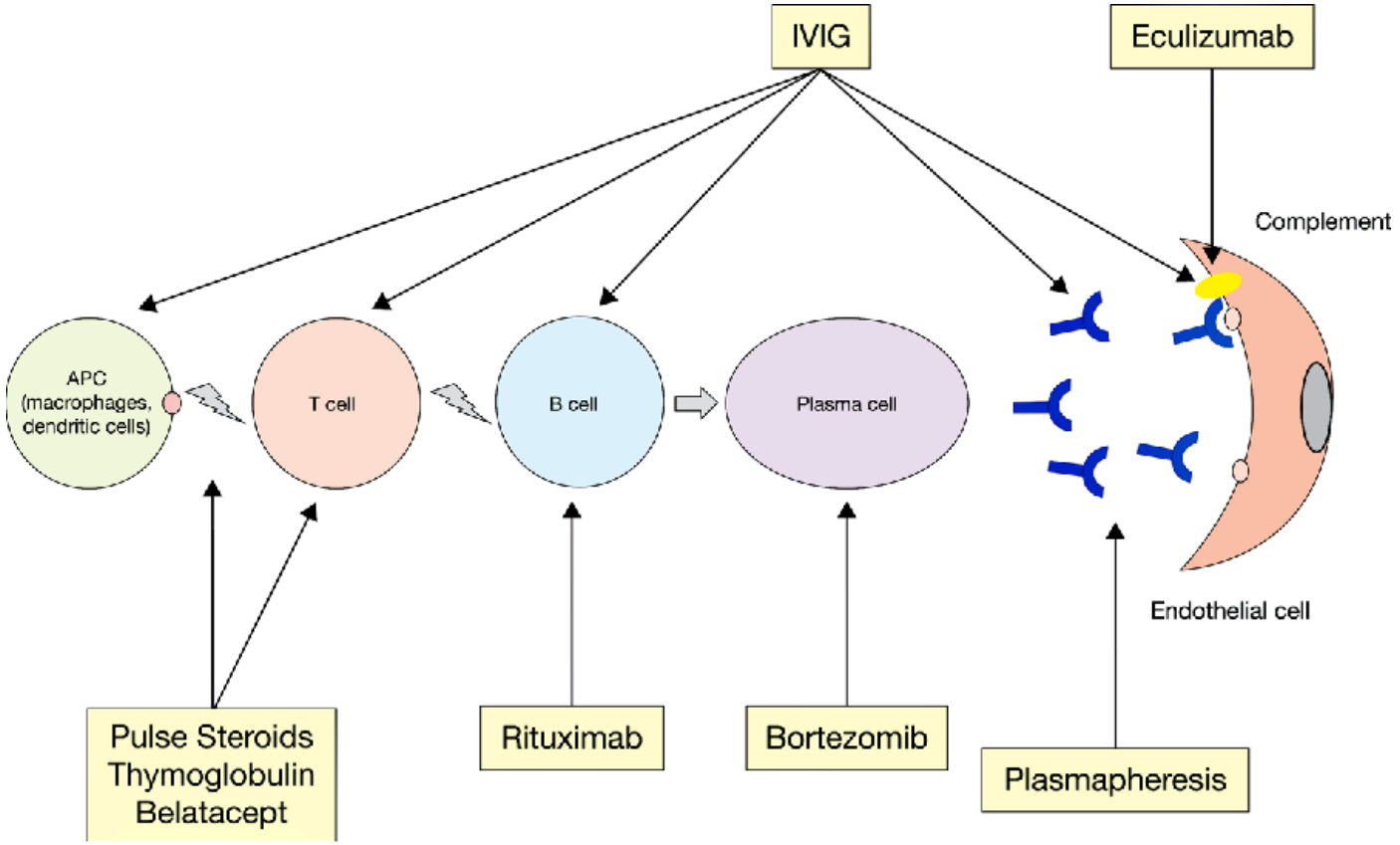
Cases with: **C4d+, DSA-**

- some antigens may be expressed on the endothelial cells and not on lymphocytes, which are typically used for the test (MICA).
- graft can absorb huge amounts of antibodies from blood and Abs can be below the level of detection.

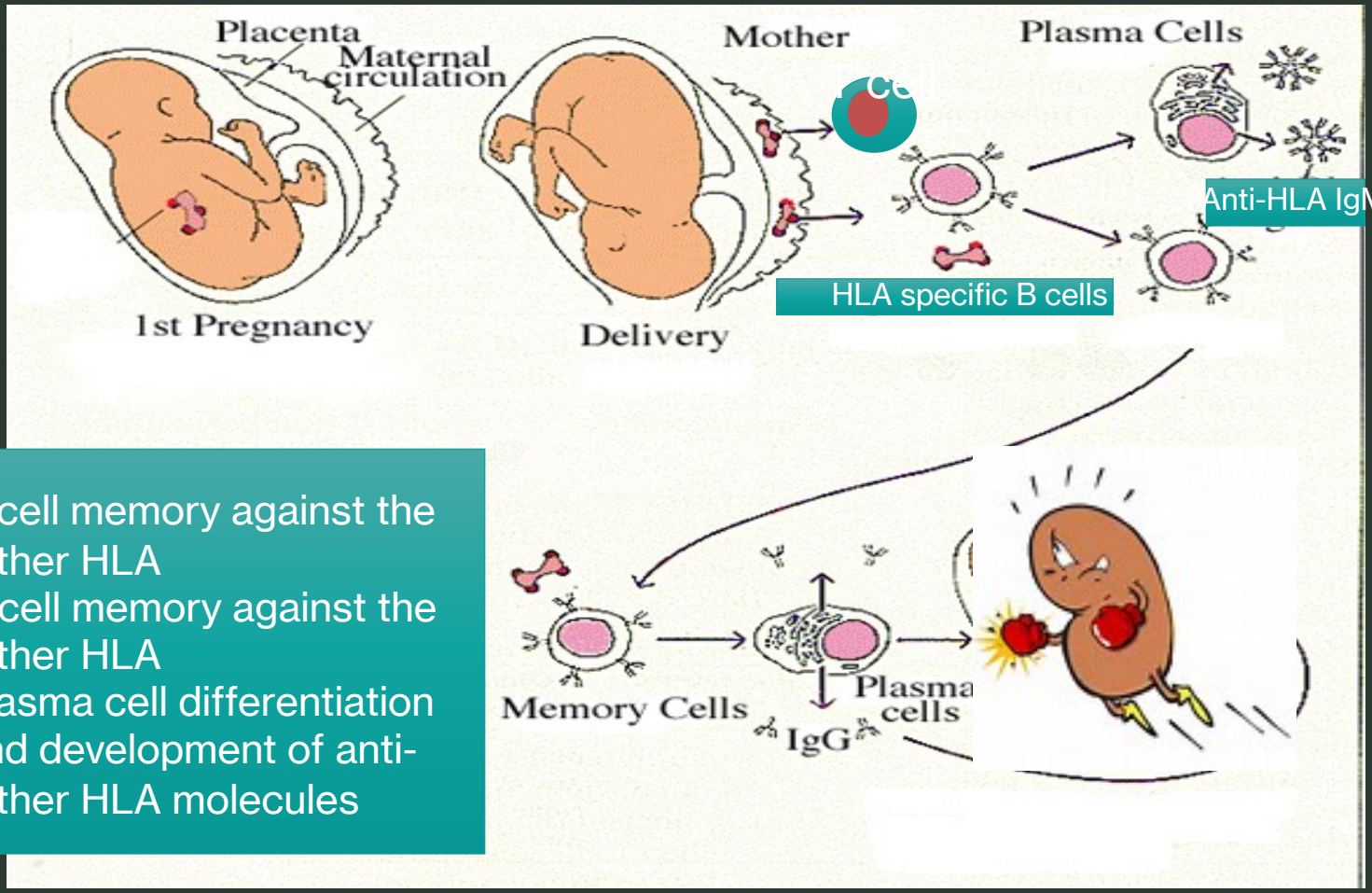
Cases with: **C4d-,DSA+**

- Negative staining may result from non-complement fixing antibodies, low reactivity of Abs.

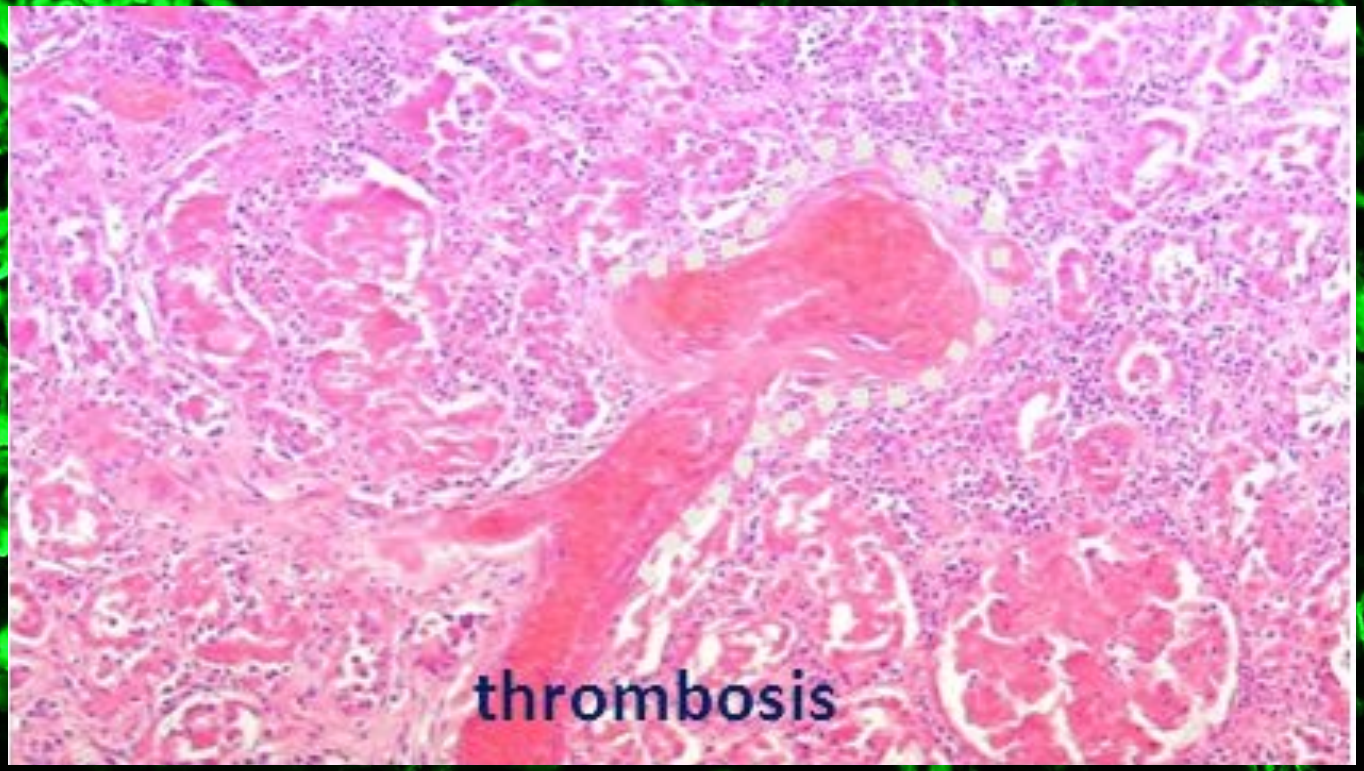
Treatment of Acute Antibody Mediated rejection



Immune sensitization during pregnancy

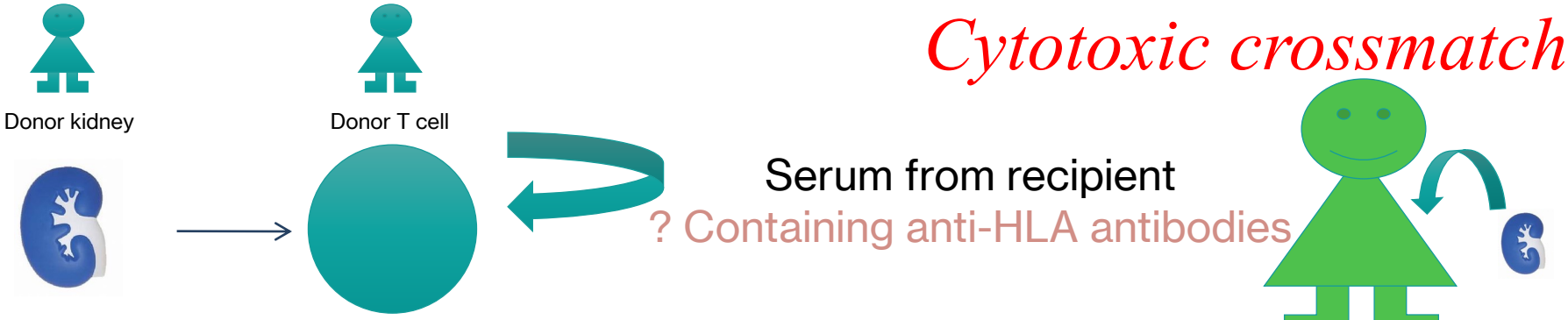


- T cell memory against the father HLA
- B cell memory against the father HLA
- Plasma cell differentiation and development of anti-father HLA molecules

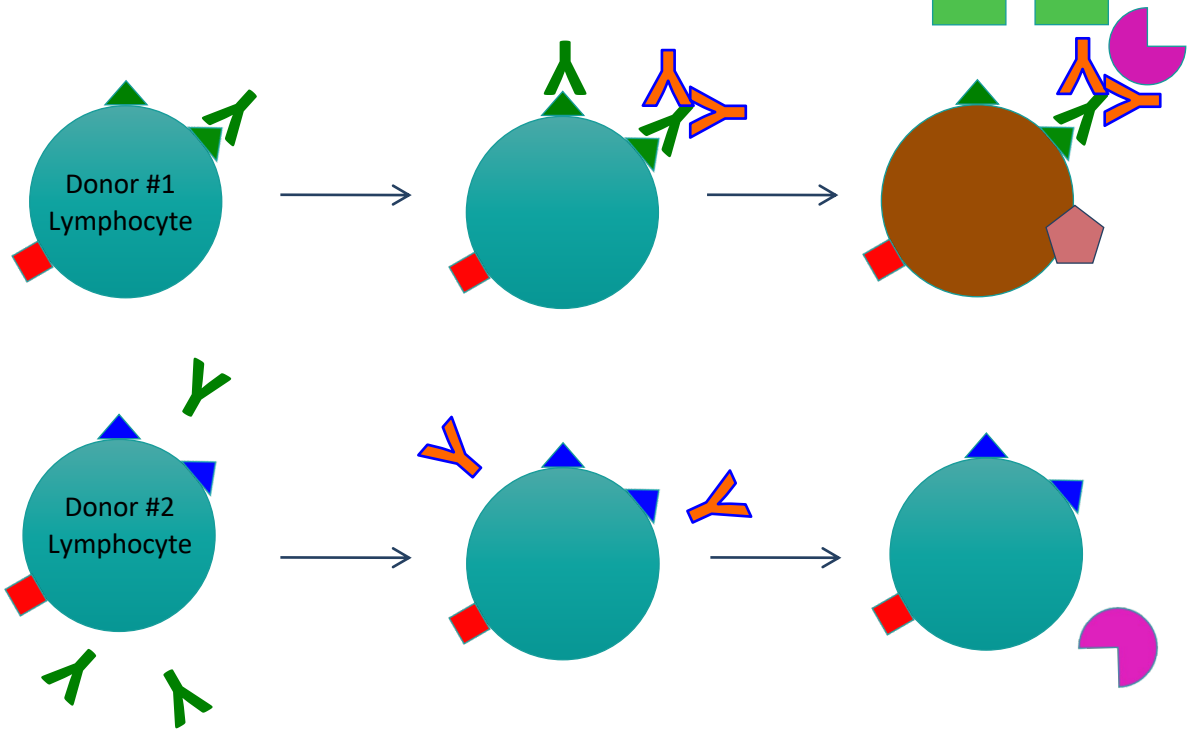


Adapted from Azzi et al. The ACP Medicine book.
Courtesy of Dr. Vanesa Bijol

Cytotoxic crossmatch



	Donor Lymphocyte
Y	Anti-HLA antibodies in recipient serum
Y	Anti-human immunoglobulin
	Complement factors
	Membrane Attack Complex
	Lysis of cell



{ Detect preformed DSA that may induce hyperacute rejection
The only absolute contraindication for transplantation }

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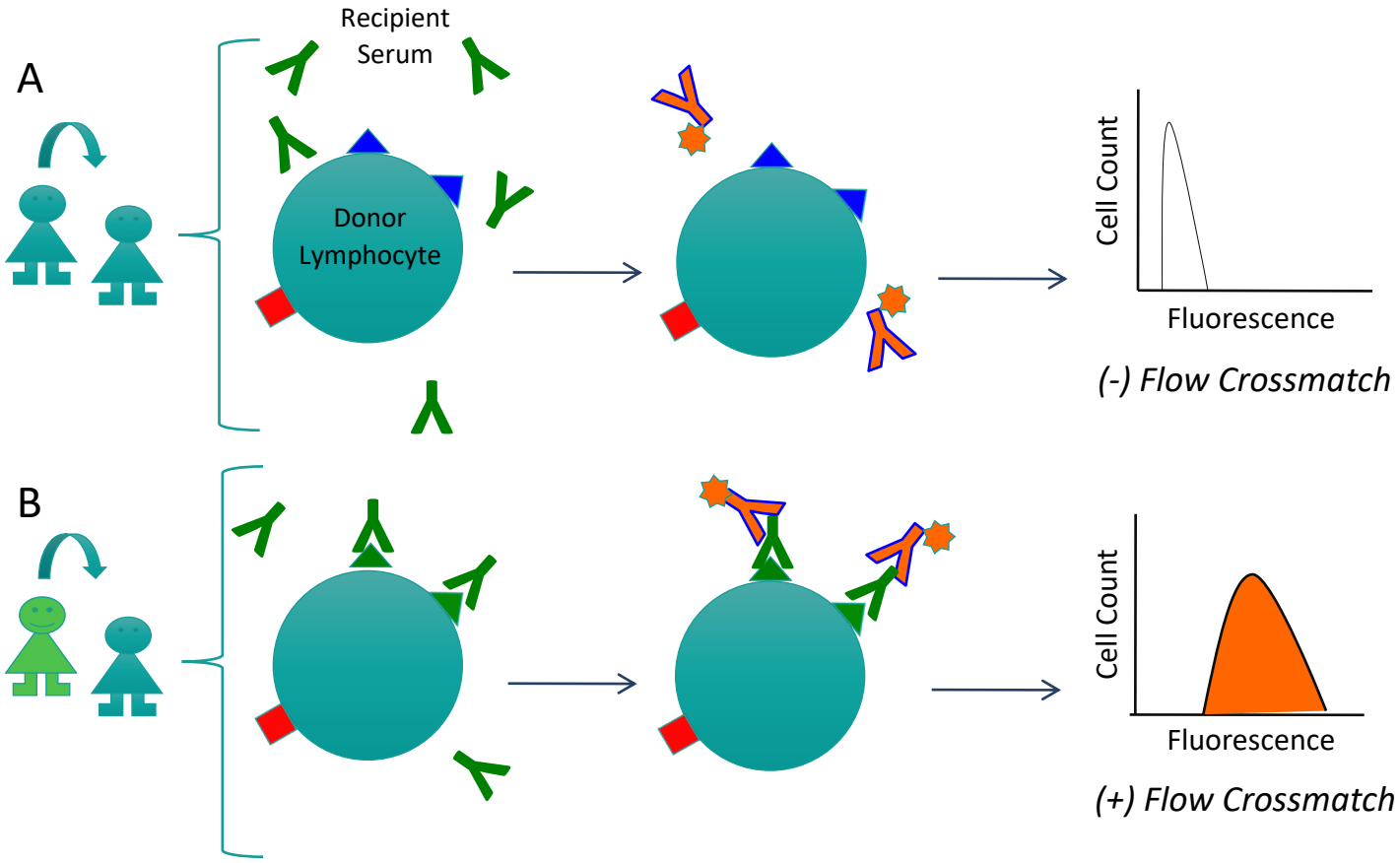
Flow Crossmatch

	Donor Lymphocyte
	Anti-HLA antibodies in recipient serum
	Anti-human immunoglobulin
	Anti-human Ig conjugated to fluorescent marker



Positive flow crossmatch is not an absolute contraindication for transplantation

Increases risk of rejection: cellular and antibody mediated rejection

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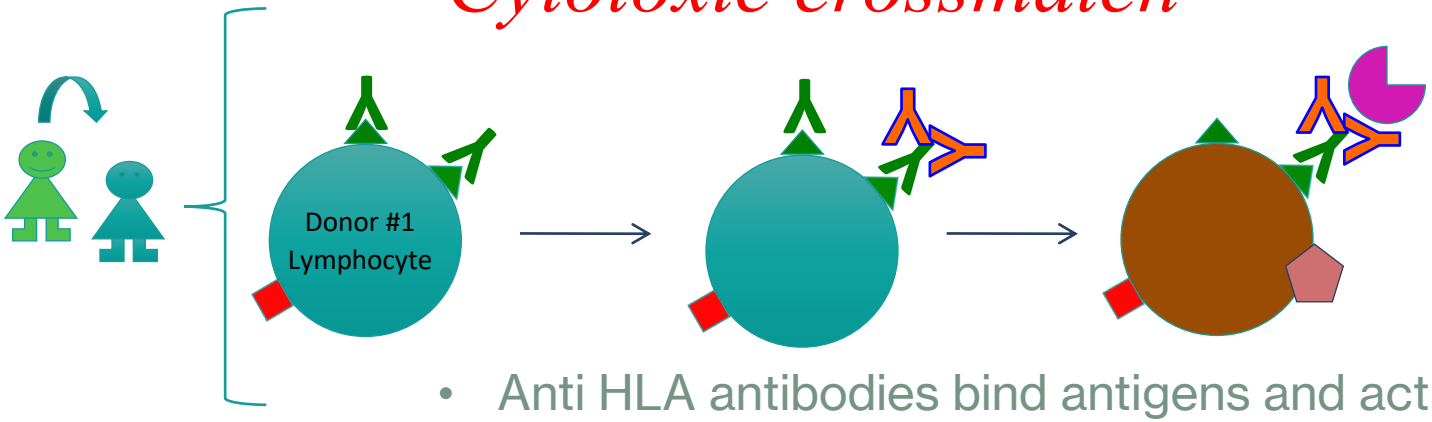
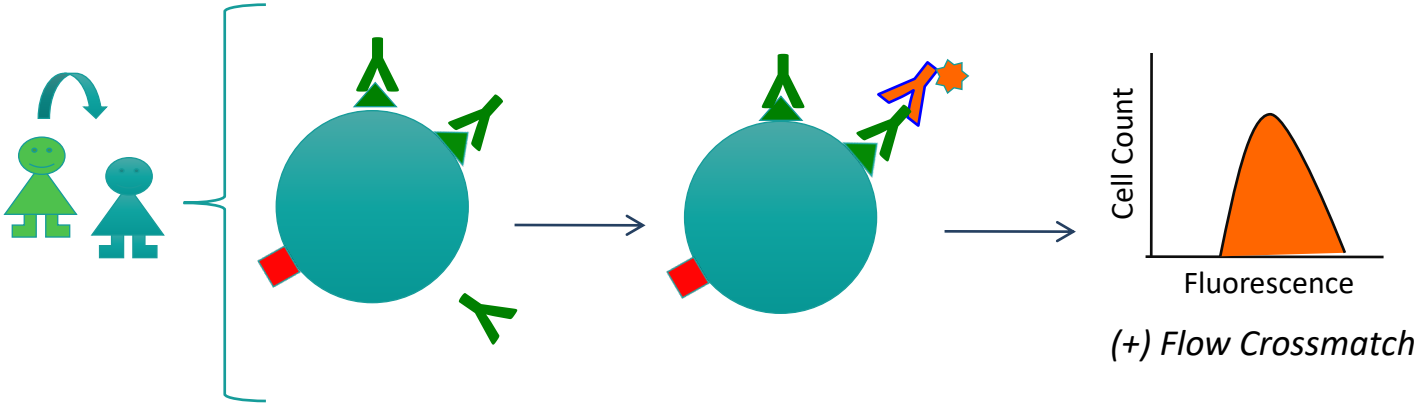
Cytotoxic crossmatch



- Anti HLA antibodies bind antigens and activate complements
- Predict Hyperacute rejection

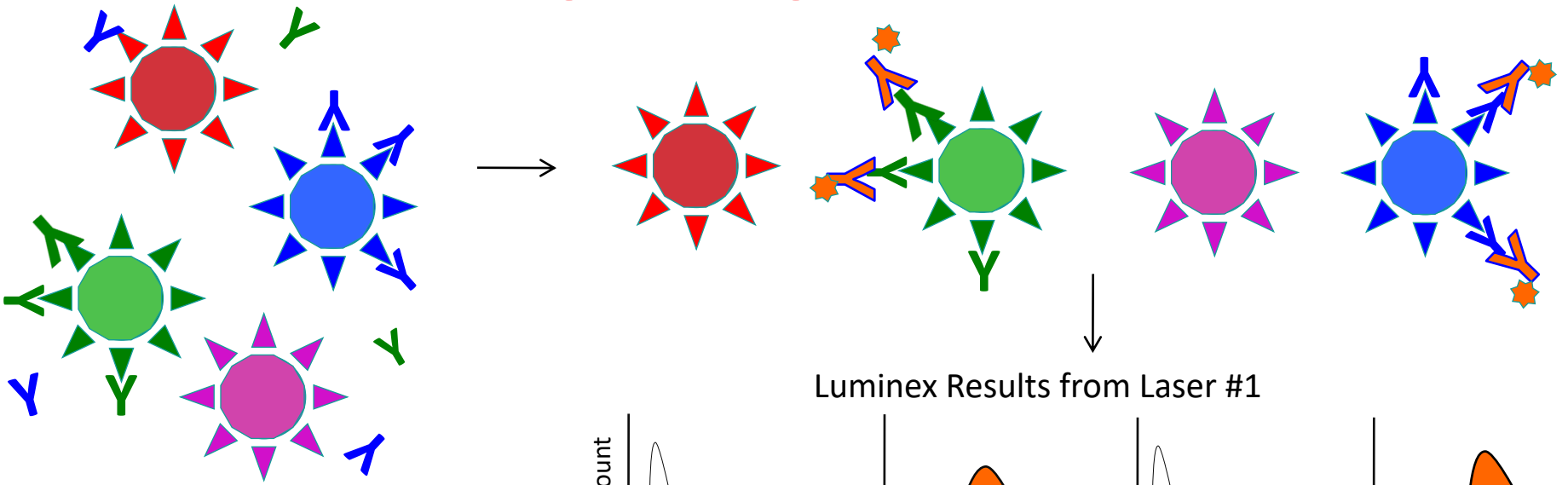
Flow Crossmatch



(+) Flow Crossmatch

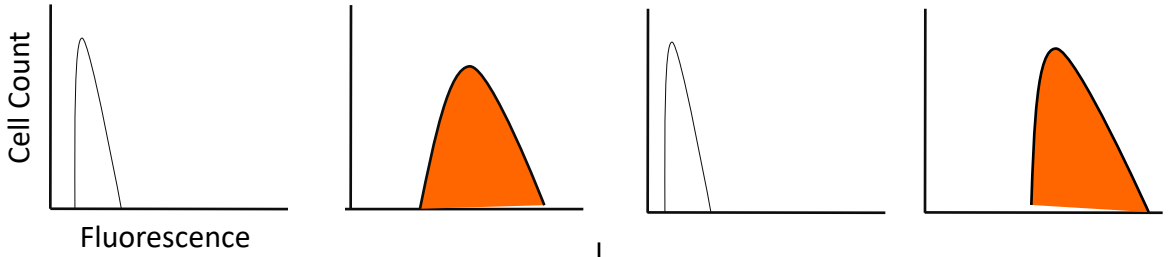
- Anti HLA antibodies bind antigens
- Does not predict Hyperacute rejection

Single Antigen Bead

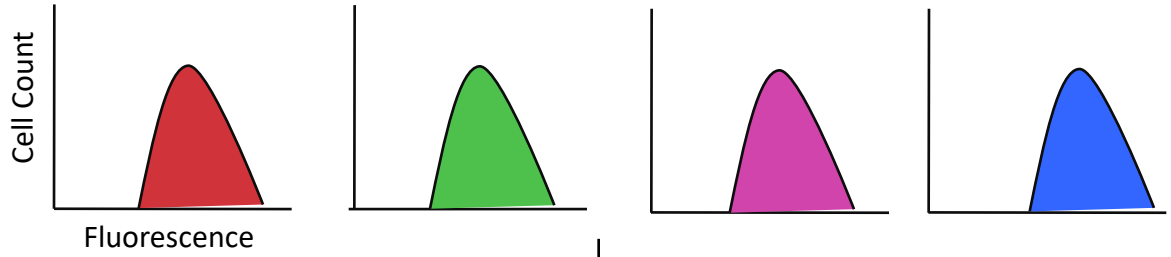


	Microbeads with impregnated dye and defined HLA
	HLA-A201
	HLA-A202
	HLA-A301
	HLA-A302
	Anti-human immunoglobulin with conjugated fluorescent marker

Luminex Results from Laser #1



Luminex Results from Laser #2



Serum of individual contains Abs to HLA-A202 and HLA-A302

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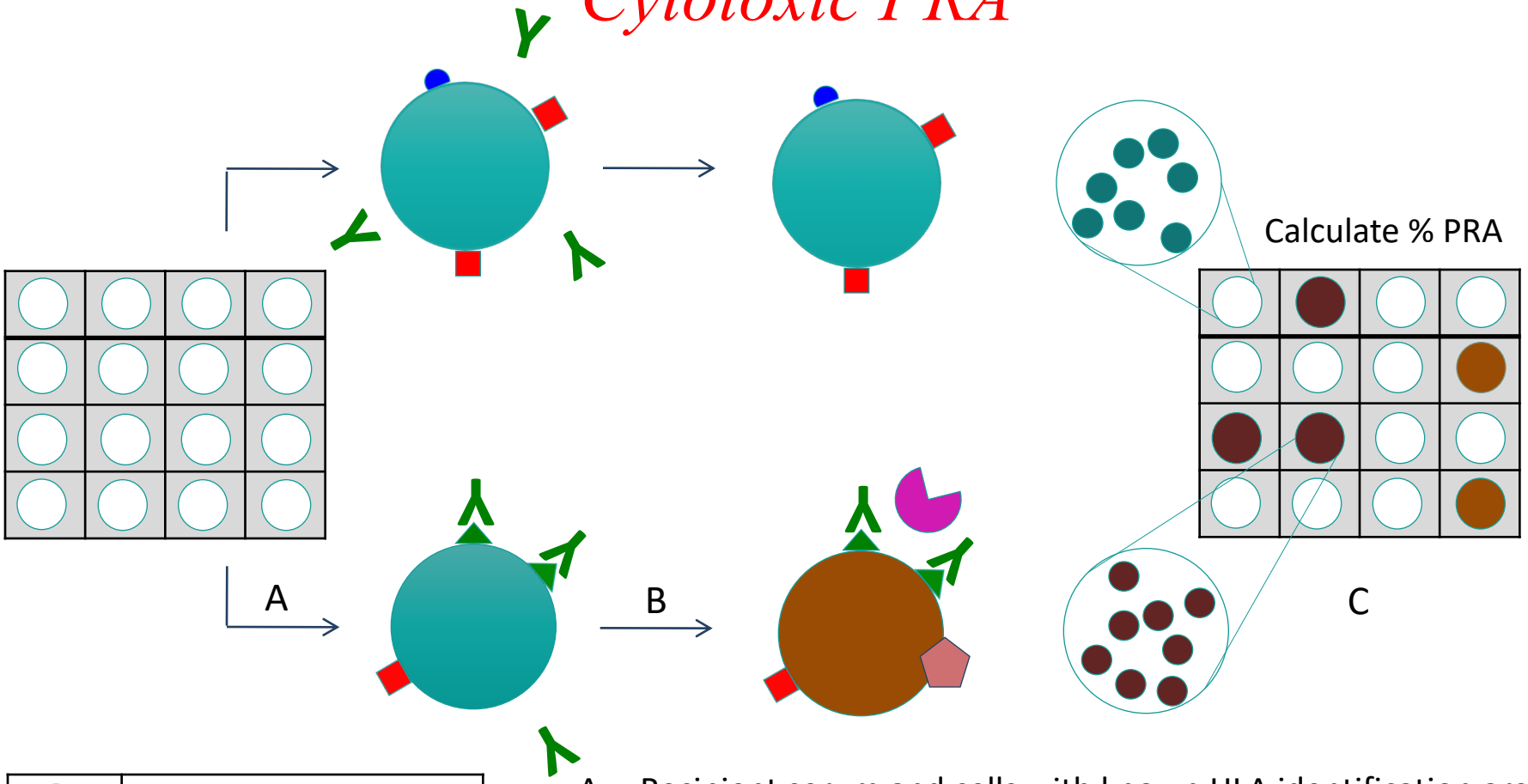
Cytotoxic PRA



	Lysed Cell
Y	Anti-HLA Ab
	Complement factors
	Membrane Attack Complex

A. Recipient serum and cells with known HLA identification are combined in individual wells of a multi-well plate
B. A wash step washes away any unbound anti-HLA antibodies. Complement factors are added and conjugate with bound anti-HLA initiating the formation of the membrane attack complex and causing cell lysis
C. Lysed cells in individual wells of the plate are visualized after addition of a vital dye

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Autumn,
the season that teaches us that change can be beautiful

Thank you

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