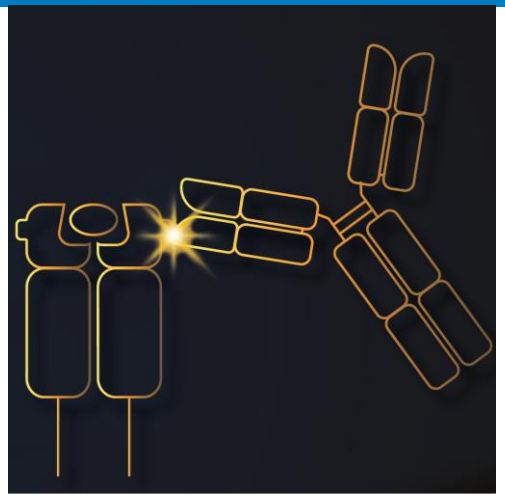
Leiden University
Medical Center

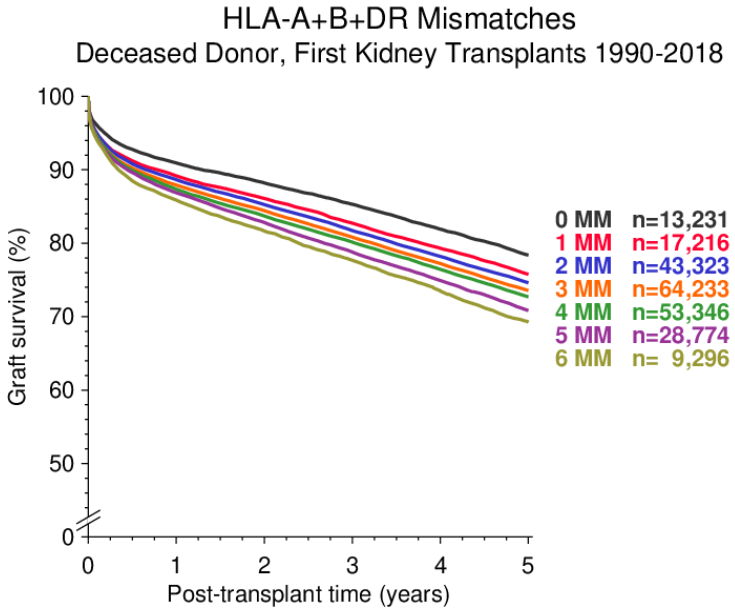
HLA epitopes: from theory to practice



Cynthia Kramer, PhD
Department of Immunology

ET EXTRAMURAL MEETING 2020

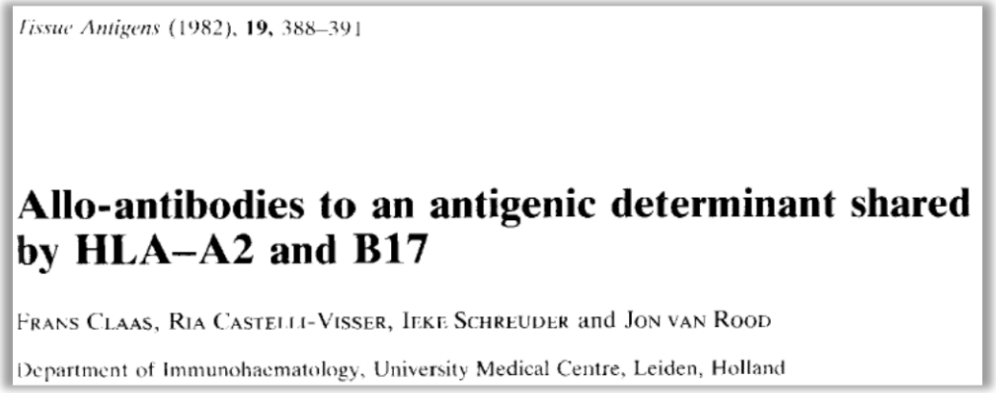
HLA matching is still relevant in kidney transplantation



Collaborative Transplant Study 2019

HLA antigen mismatches can lead to an antibody response

- HLA antigens share antigenic determinants
- Not every HLA mismatch leads to an antibody response



Tissue Antigens (1982), **19**, 388–391

Allo-antibodies to an antigenic determinant shared by HLA–A2 and B17

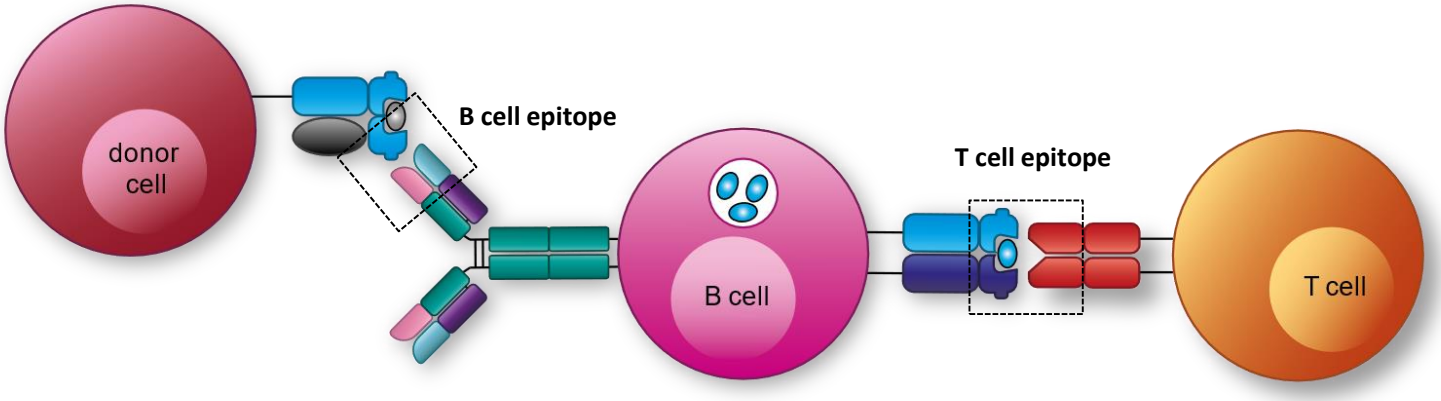
FRANS CLAAS, RIA CASTELLI-VISSER, IEKE SCHREUDER and JON VAN ROOD

Department of Immunohaematology, University Medical Centre, Leiden, Holland

Immunization by pregnancy: antibodies induced by HLA-A2 react also with HLA-B17

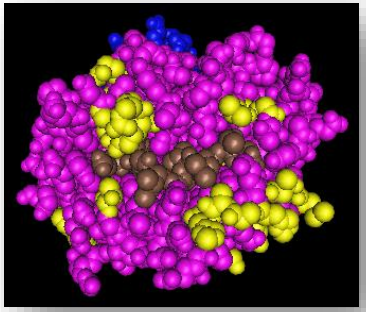
What is an epitope?

- **Epitope:** part of an antigen that can be recognized by receptors of the adaptive immune system
- Polymorphic amino acid residues of an antigen

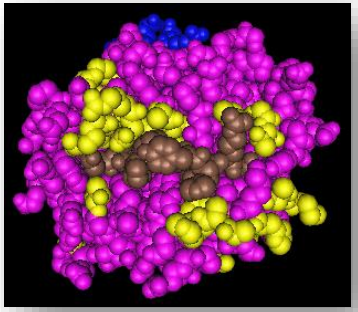


Immunogenicity of an HLA molecule is defined by the polymorphic amino acid residues on antibody accessible positions

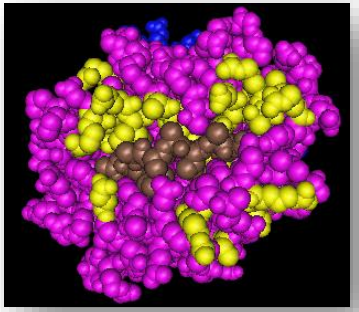
HLA-A2



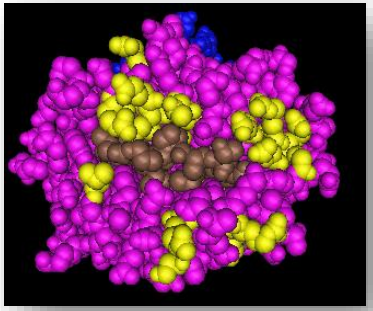
HLA-A68



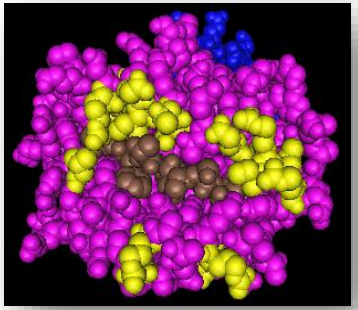
HLA-B27



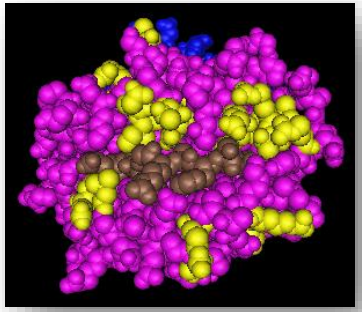
HLA-B35



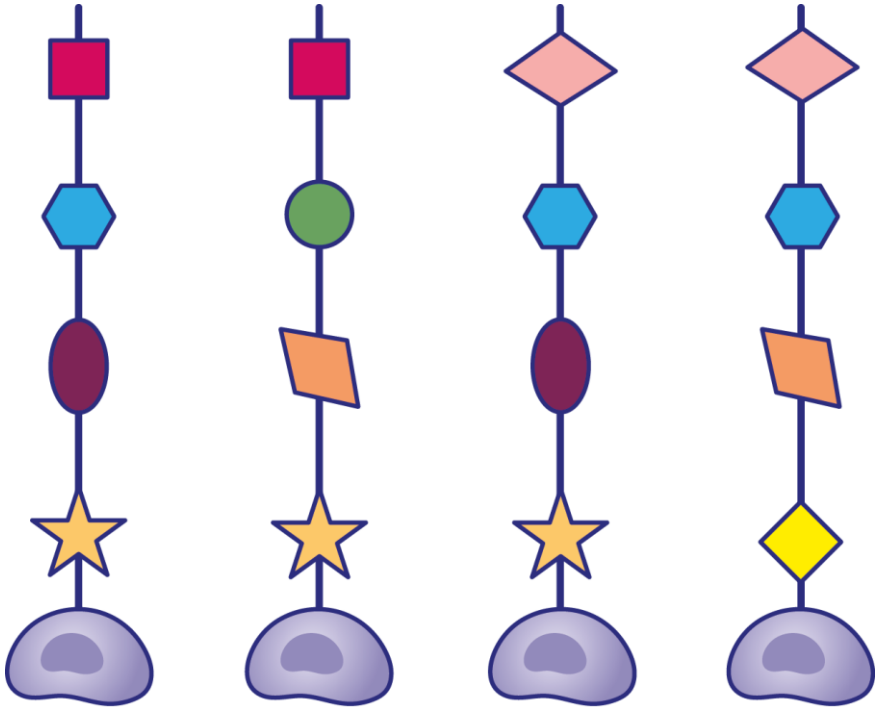
HLA-B51



HLA-B44



Each HLA antigen has a unique set of epitopes, but individual epitopes can be present on other HLA antigens



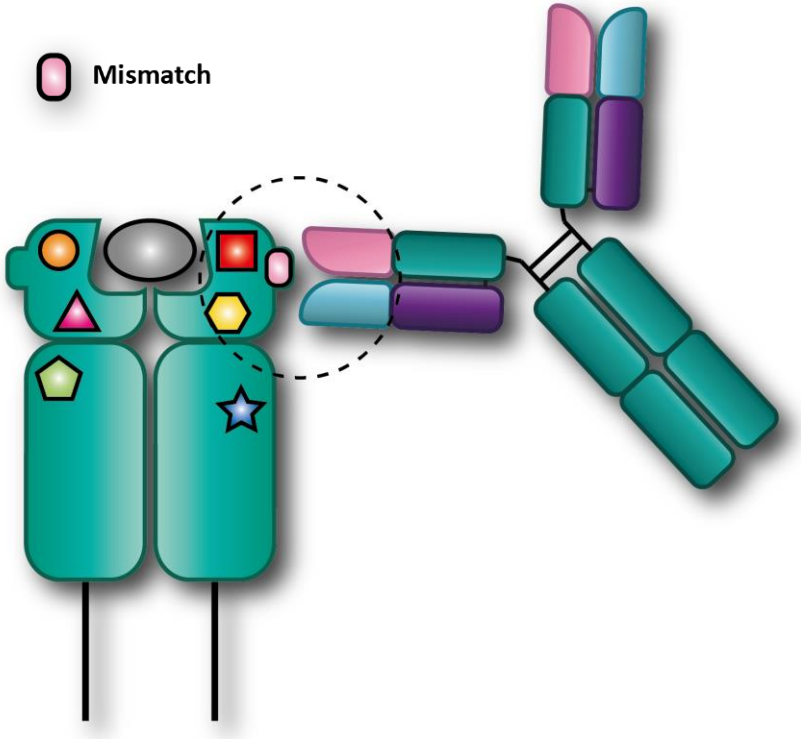
Tambur & Claas AJT 2015

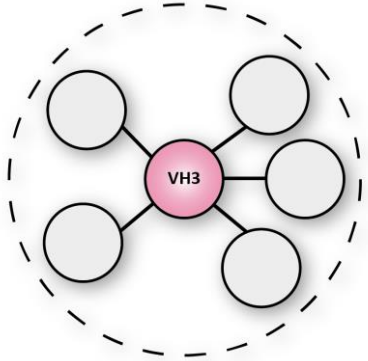
B cell epitope has two important properties

- **B cell epitope** is the complete surface area of antigen that interacts with paratope of an antibody, also known as **structural epitope**
  - After antibody forming, multiple amino acid configurations within 15Å radius can be involved in antibody binding

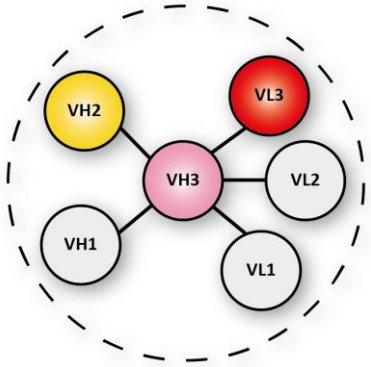
- An antibody is induced by a (number) polymorphic amino acid residue(s) within a B cell epitope, which are often referred to as **immunogenic** or **functional epitope** or **eplet**
  - Polymorphic amino acids within 3-3.5Å radius
  - Defines the specificity of an antibody via its interaction with CDR-H3 of an antibody

B cell epitope has two important properties





Functional or Immunogenic epitope (eplet)



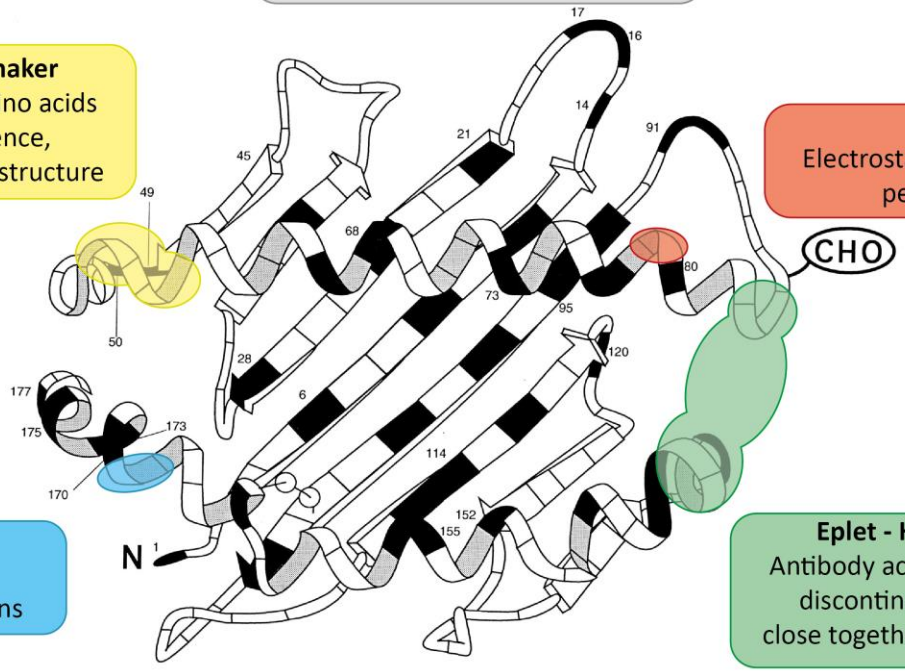
B cell or Structural epitope

Various approaches based on the polymorphic amino acid residues have been introduced to predict the immunogenicity of HLA molecules

EMS-3D
Surface electrostatic mismatch score based on HLA molecule

Triplet - HLAMatchmaker
Antibody accessible amino acids continuous in sequence, close together in folded structure

EMS
Electrostatic mismatch score per amino acid

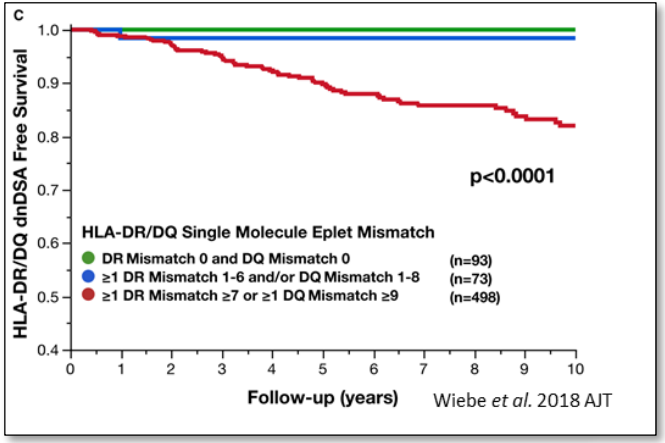


HLA-EMMA
Single amino acid on solvent accessible positions

Eplet - HLAMatchmaker
Antibody accessible amino acids discontinuous in sequence, close together in folded structure

Eplet mismatches have been associated with sensitisation risk

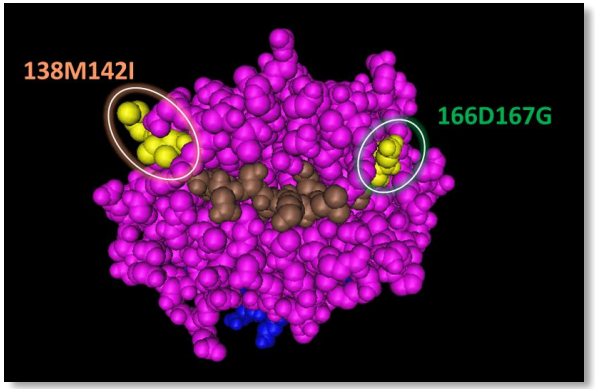
- The number of eplet mismatches between donor and recipient can be determined with HLAMatchmaker
- Eplet mismatches have been correlated with *de novo* donor-specific antibody formation, transplant glomerulopathy, and antibody-mediated rejection
- Eplet loads have been introduced as indicator of sensitisation risk
- **However, not every eplet mismatch is immunogenic**



Eplets require experimental verification to establish that an antibody can actually bind to it

- Eplets are **theoretically** defined single or configuration of polymorphic amino acids on antibody accessible positions within 3-3.5 Å radius

- Experimental **antibody-verification** of eplets is essential
  - Human HLA monoclonal antibodies (mAbs)
  - Absorption/elution studies



Eplets	HLA-ABC	HLA-DRB	HLA-DQB + DQA	HLA-DPB + DPA
Theoretical	224	123	83	62
Antibody-verified	72	36	27	11

https://www.epregistry.com.br/ accessed on 14-11-2020

Antibody-verification of eplets by human monoclonal HLA antibodies

- LB_DR7_A mAb reactivity analysis identified **14K 25Q** as unique amino acid configuration on reactive HLA alleles, which antibody-verifies eplet **25Q (25Q 14K 30L)** provided that the eplet is redefined to **25Q 14K**

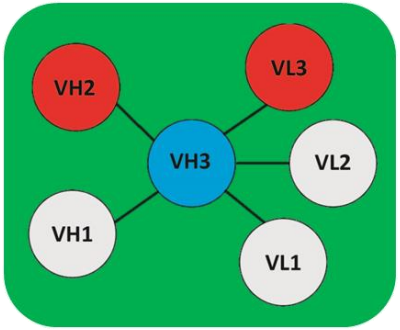
A

	BCM	Antigen	11	14	25	30
	19367	**DRB1*07:01**	**G**	**K**	**Q**	**L**
	0	DRB1*01:01	L	E	R	C
	0	DRB1*03:01	S	E	R	Y
	0	DRB1*04:01	V	E	R	Y
	0	DRB1*08:01	S	E	R	Y
	0	DRB1*09:01	D	E	R	G
	0	DRB1*10:01	V	E	R	R
S	0	DRB1*11:01	S	E	R	Y
	0	DRB1*12:01	S	E	R	H
	0	DRB1*13:01	S	E	R	Y
	0	DRB1*14:01	S	E	R	Y
S	0	DRB1*15:01	P	E	R	Y
	0	DRB1*16:01	P	E	R	Y
S	0	DRB3*02:02	L	E	R	H
	0	DRB4*01:01	A	E	W	Y
S	0	DRB5*01:01	D	E	R	D
	0	rest				

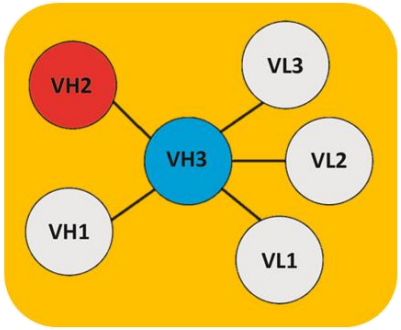
G: Glycine
K: Lysine
Q: Glutamine
L: Leucine

Kramer *et al* AJT 2020

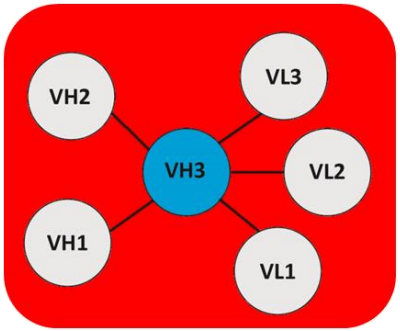
Crucial amino acid configurations determine the reactivity of an HLA antibody



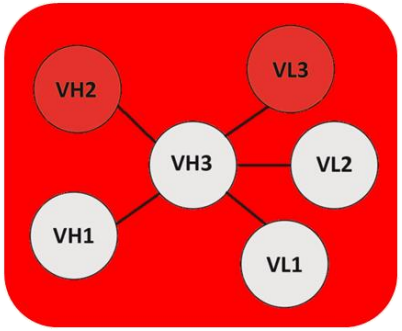
Strong binding



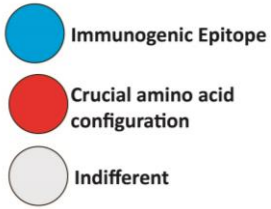
Weak/no binding



No binding



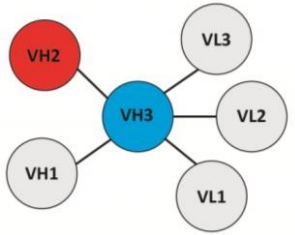
No binding



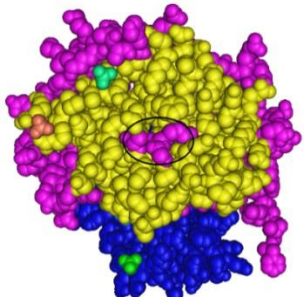
Antibody induced by 144TKR reacts only with some 144TKR carrying alleles

Immunogenic epitope

Allele	Immunogenic Epitope
A*11:01	144TKR



Reactivity is specific for 144TKR+151H

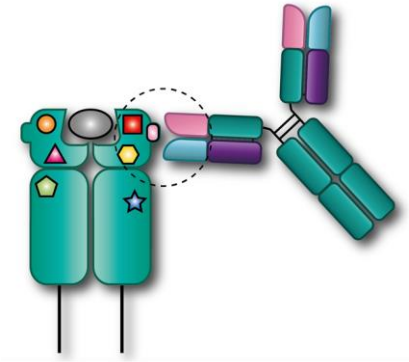


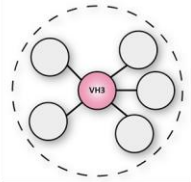
T: Threonine
K: Lysine
R: Arginine
H: Histidine

Duquesnoy, Mulder et al. 2013

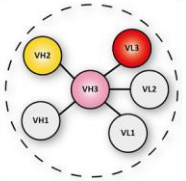
HLA epitopes: from theory to practice

- Antibody-verification of the theoretically defined eplets
  - Some eplets require redefinition
- Complex reactivity patterns cannot be explained only by immunogenic epitope/eplet
  - Additional amino acid configurations are crucial for HLA antibody binding
  - Binding strength and release of free energy
- Distinguish between immunogenic epitope (eplets) and B cell epitope





Functional or Immunogenic epitope (eplet)

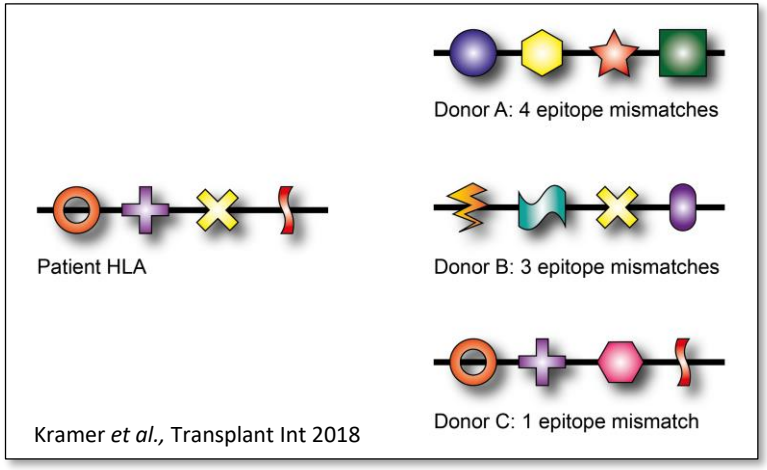


B cell or Structural epitope

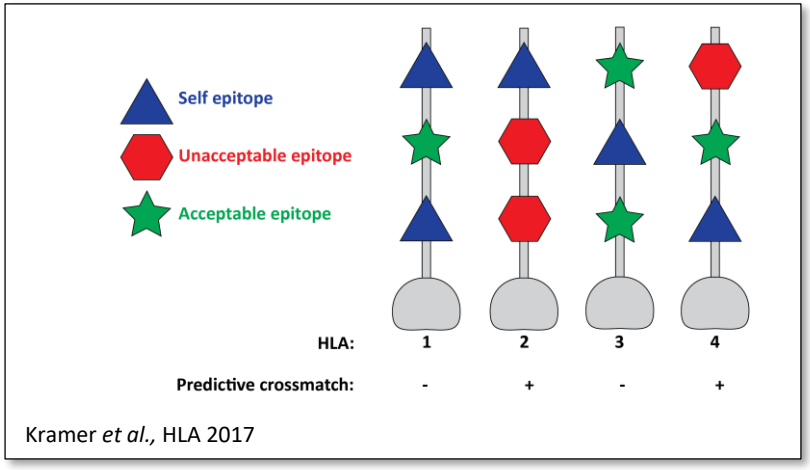
Towards HLA epitope matching

1. Prevention of *de novo* donor-specific formation after solid organ transplantation by limiting the number of potential immunogenic epitopes on mismatched HLA molecule

2. Prediction of acceptable and unacceptable mismatches for highly sensitized patients



Kramer *et al.,* Transplant Int 2018



Kramer *et al.,* HLA 2017

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Thank you for your attention!

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