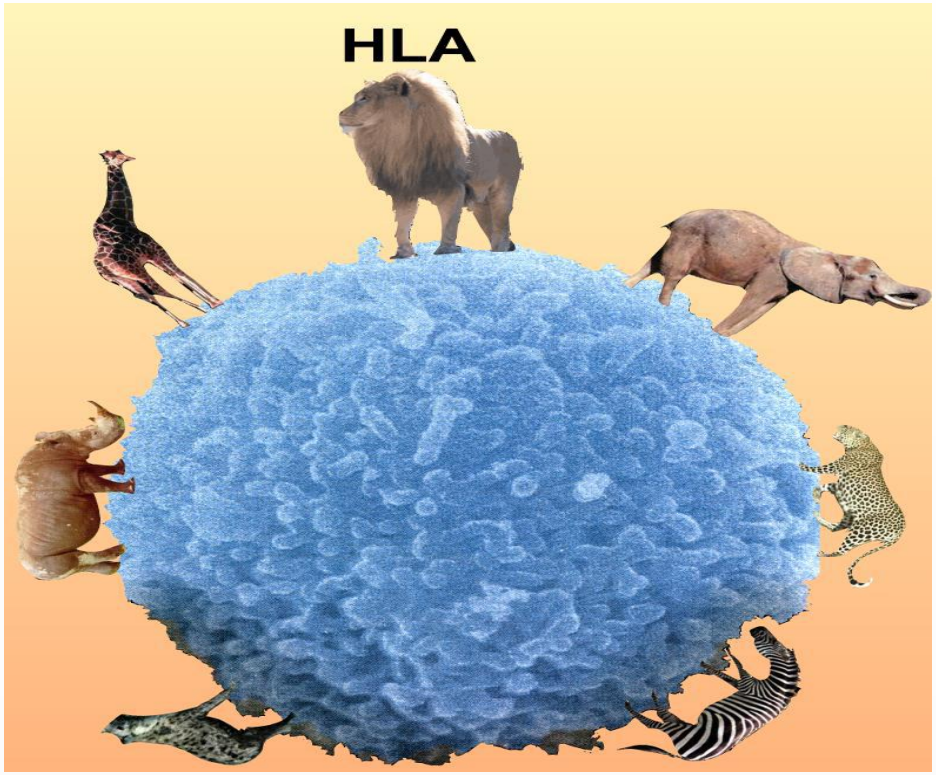
SAPIENZA
UNIVERSITÀ DI ROMA

HLA, Histocompatibility Testing and Transplantation

Dr U. La Rocca
ursula.larocca@uniroma1.it

13 November 2023

The cell surface is a jungle !



Major histocompatibility complex (MHC),
group of genes that code for **proteins** found on the **surfaces of cells that help the immune system to recognize foreign substances.**

In humans the complex is also called the human leukocyte antigen (HLA) system.

MHC - Major histocompatibility complex (MHC)

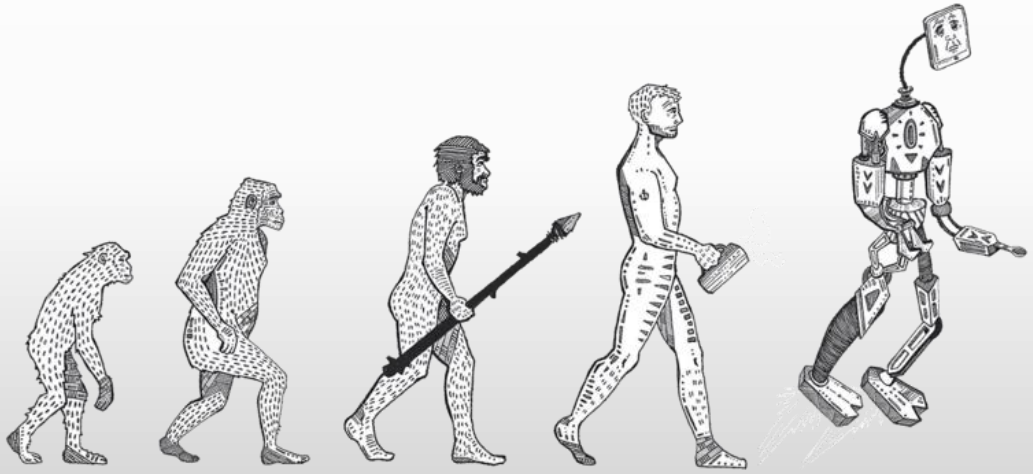
MHC proteins are found in **all higher vertebrates.**

A set of genes encoding proteins essential to immune response with a major role in histocompatibility and protection against pathogens.

The **gene composition and arrangement vary between species**, but there is a **high correspondence**

The HLA system

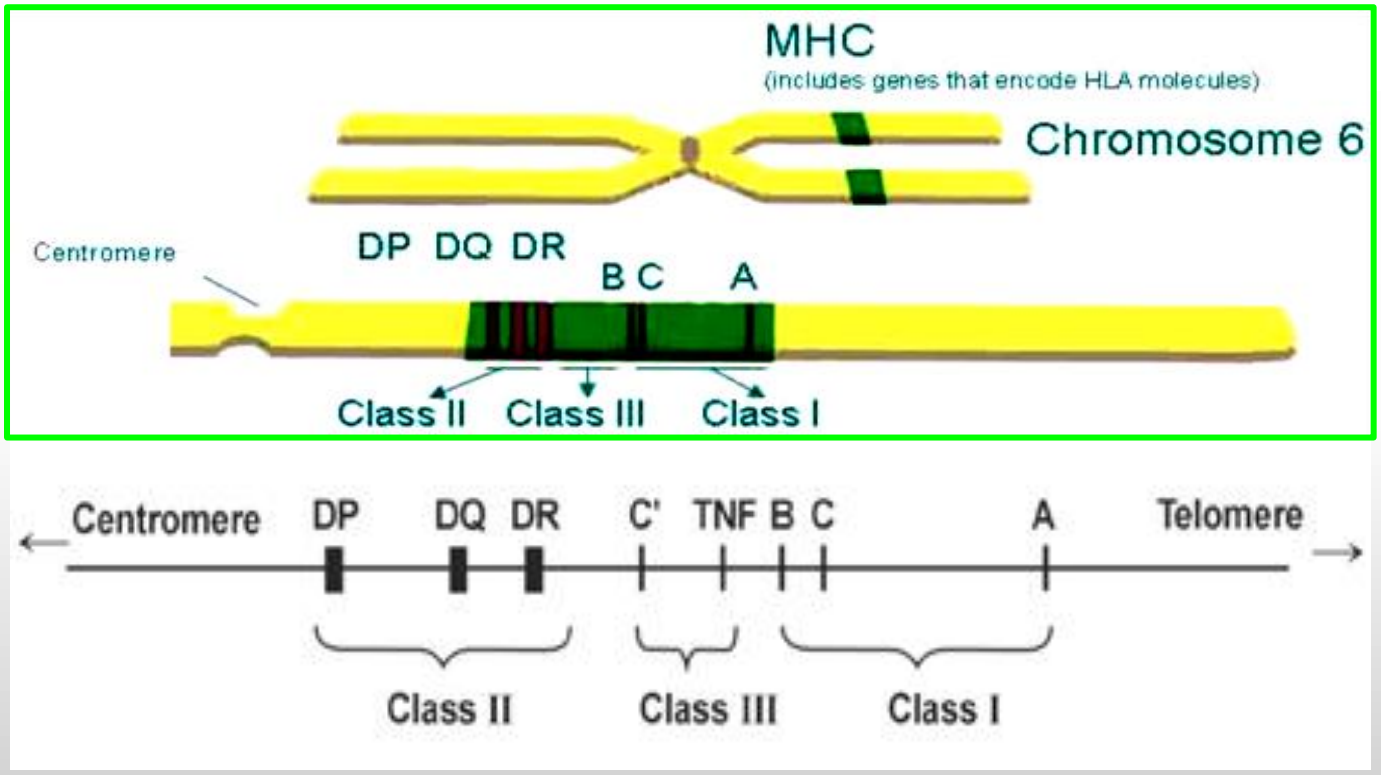
The human MHC is called the **HLA (Human Leukocyte Antigen)** system because these antigens were **first identified and characterized using alloantibodies obtained from multiparous women and previously transfused patients, against leukocytes.**



Where?
Genomic organization of the HLA system

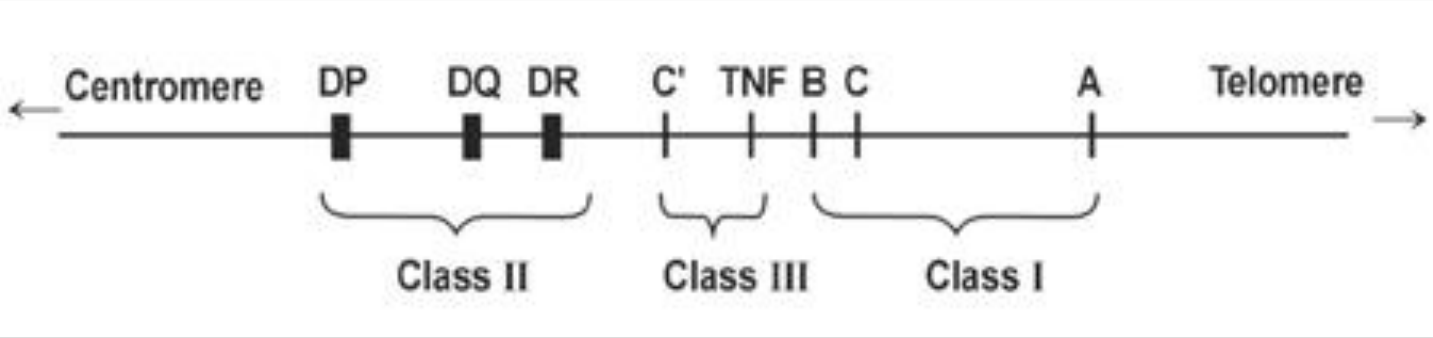
The HLA system maps to the short arm of chromosome 6 (6p21) and spans approximately 3,600 kb of DNA.

It is divided into three regions.

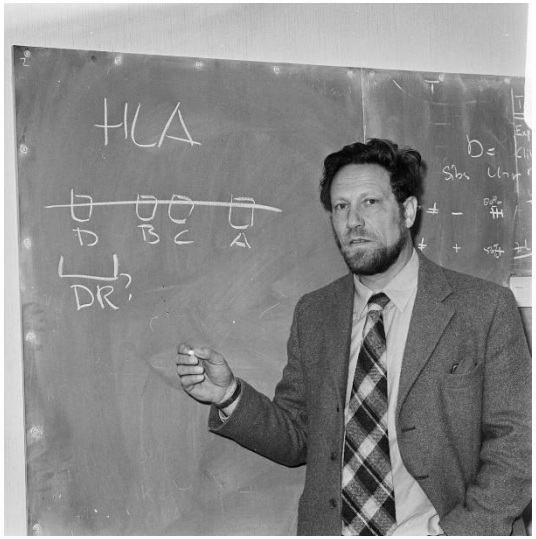


Genomic organization of the HLA system

- ✓ The class I region contains the classical HLA-A, HLA-B, and HLA-C genes that encode the heavy chains of class I molecules.
- ✓ The class II region consists of a series of sub-regions (A and B), accounting for six main genes: HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA and HLA-DRB1 .
- ✓ The class III region contains genes for complement components (C2, C4, factor B), 21-hydroxylase, TNF and others.



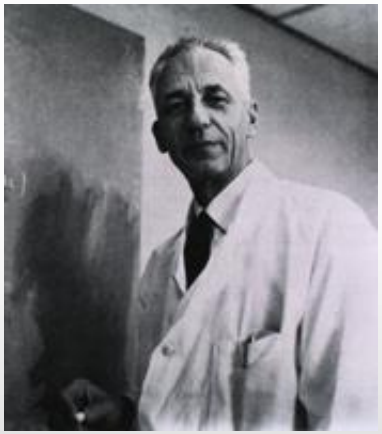
A short history of HLA



Jon van Rood

Three papers appeared **in 1958 by Jean Dausset, Jon Van Rood and Rose Payne**, all describing **antibodies in human sera from multitransfused patients or multiparous women, reacting with leucocytes from many individuals who were tested.**

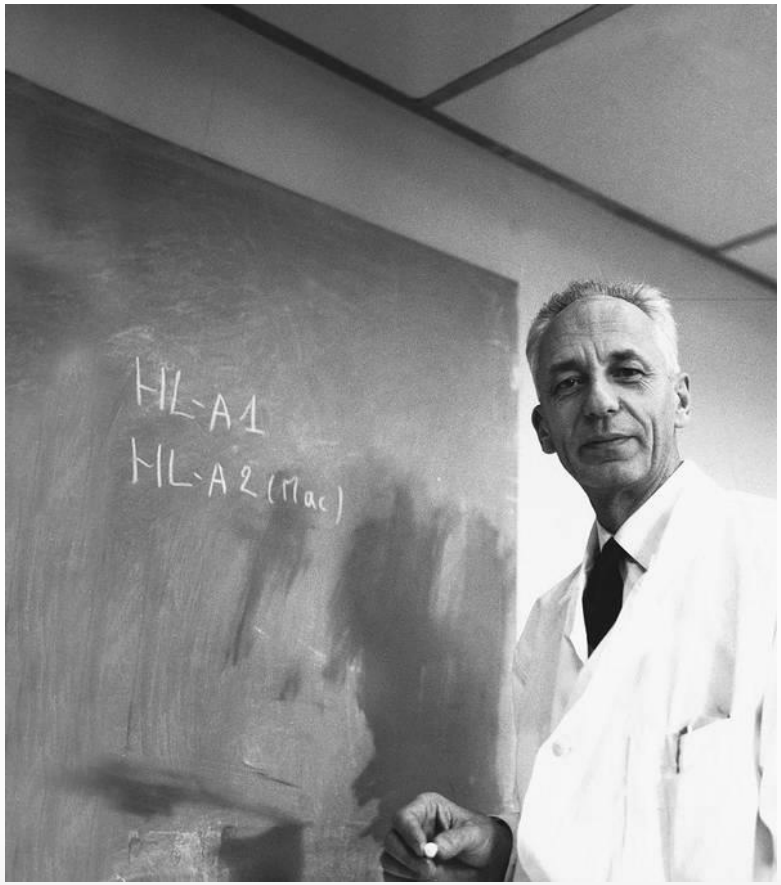
Antibodies in this sera detected a polymorphic system of antigens on human leucocytes.



Jean Dausset 1916-2009
1980 Nobel Prize

Rose Payne
1909- 1999

A short history of HLA



Jean Dausset 1916-2009
1980 Nobel Prize

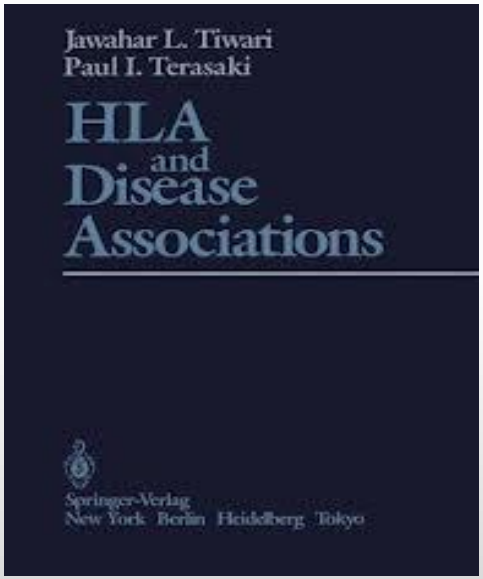
Jean Dausset
described 1958
the first HLA antigen named MAC
(an acronym made up of the initials of the first three donors with whom the serum he was testing did not react)
For his discovery,
he received
the Nobel Prize in 1980

'*Finally, in a more long-time perspective, the study of leucocyte antigens might become of great importance in tissue transplantation, in particular in bone marrow transplantation*'

The Major Histocompatibility Complex

In 1964, Terasaki developed the microcytotoxicity test, a tissue-typing test for organ transplant donors and recipients to identify HLA

The test was adopted to detect routinely HLA antigens as the international standard for tissue typing.



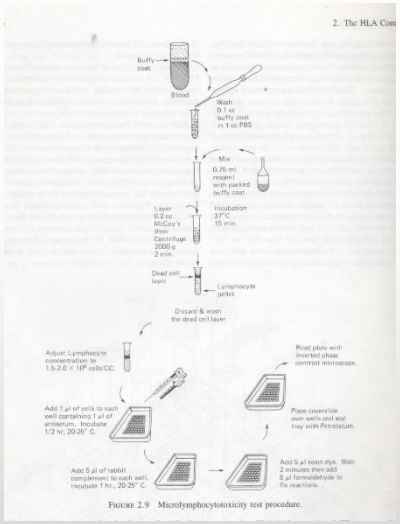
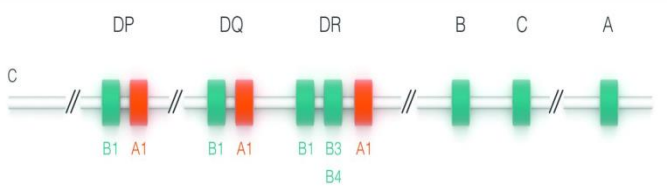


FIGURE 2.9 Microlymphocytotoxicity test procedure.

This test is still used nowadays even if the overall tendency is to abandon serology

HLA

- **HLA CLASS I -> SURFACE OF ALL NUCLEATED CELLS**
- **HLA CLASS II -> B LYMPHOCYTES, ANTIGEN-PRESENTING CELLS (MONOCYTES, MACROPHAGES AND DENDRITIC CELLS) AND ACTIVATED T LYMPHOCYTES**



	DP	DQ	DR	B	C	A
serotypes (~100)	6	9	19	46	10	21
alleles (>14,000)	673	955	1,977	4,179	2,902	3,356
proteins (>10,000)	539	648	1,444	3,095	2,067	2,372

JANUARY 2016 … MORE THAN 14,000 HLA ALLELES HAVE BEEN ASSIGNED, ACCOUNTING FOR MORE THAN 10,000 DIFFERENT HLA PROTEINS

Tiercy, Haematologica , 2016

2023

- HLA Class I Alleles 25,844
- HLA Class II Alleles 10,970

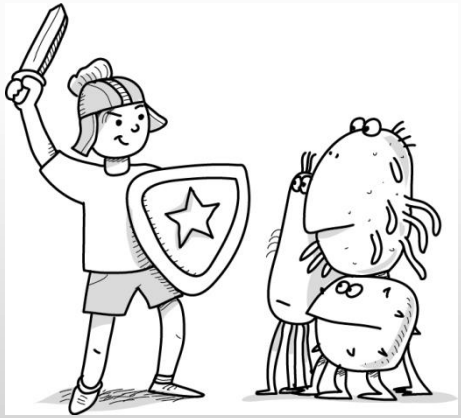
September 2018: more than 18.000 HLA class I and II

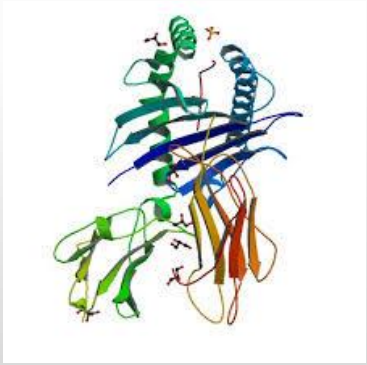
WHAT do they do? … HLA role

- The primary role is the regulation of the immune response, helping in the best way the immune system in recognition of foreign molecules and antigens

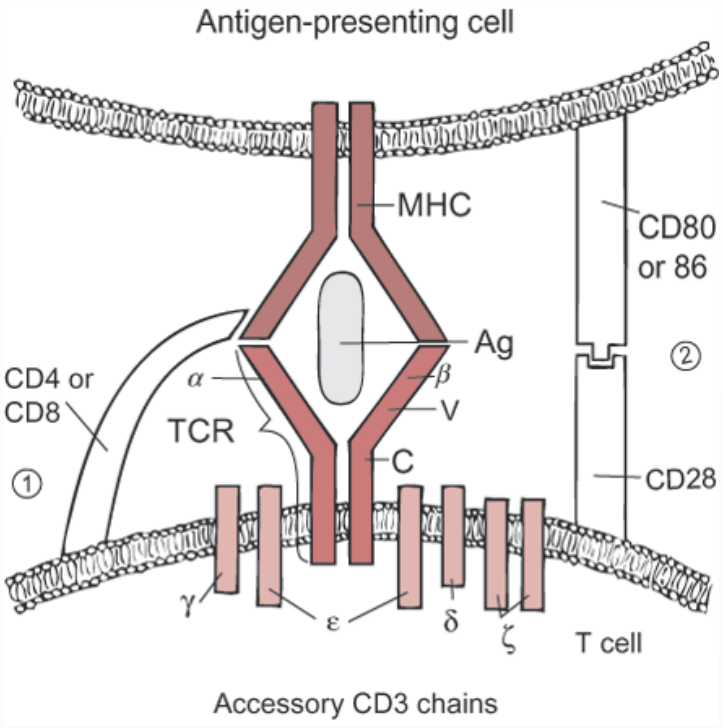
- HLA proteins are able to capture and present antigens of every type.

- To do it efficiently, each cell has a complement of slightly different HLA molecules, each of which is specialized in interacting with different types of antigens.





HOW



MHC RESTRICTION:

PEPTIDES ARE BOUND TO MHC MOLECULES AND THESE COMPLEXES ARE RECOGNIZED BY T-CELL RECEPTOR

The α and β chains of the T-CELL RECEPTOR (TCR) bind to Antigen (AG)–MHC complex on an antigen-presenting cell (APC), and CD4 or CD8 interacts with the MHC

Both actions stimulate the T CELL (1st signal) through the accessory CD3 chains

HLA IN DETAILS … Why ?

THE MHC REGION THE MOST POLYMORPHIC REGIONS OF THE HUMAN GENOME

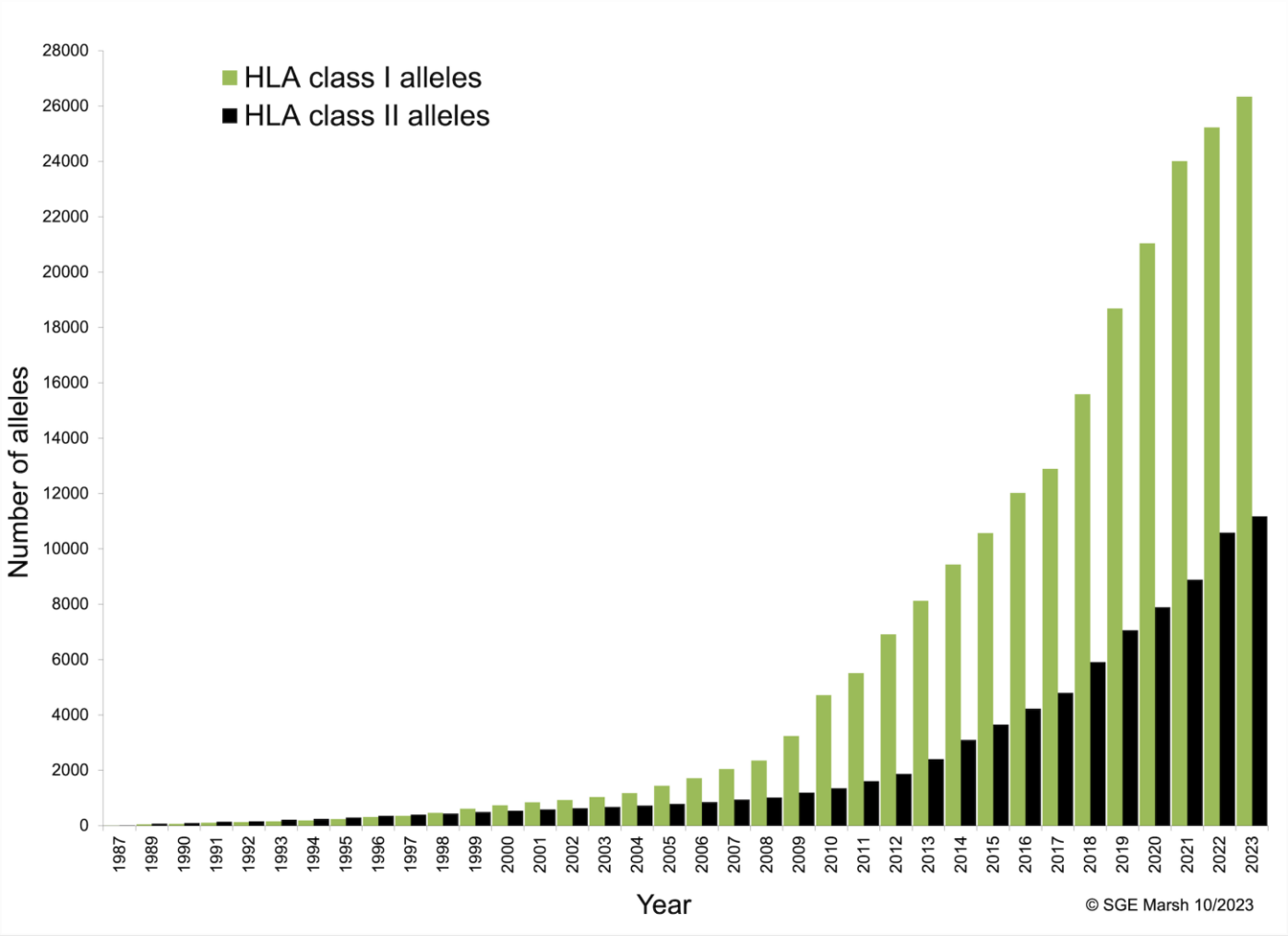
HLA Class I Alleles	25,844
HLA Class II Alleles	10,970
Other non-HLA Alleles	805

	DP	DQ	DR	B	C	A
serotypes (~100)	6	9	19	46	10	21
alleles (>14,000)	673	955	1,977	4,179	2,902	3,356
proteins (>10,000)	539	648	1,444	3,095	2,067	2,372

Polymorphism is expressed in the antigen-binding groove
It represents the consequence of selective pressure, related to the role of HLA molecules in the presentation of infectious agents in the different areas of the world

POLYMORPHISM HELP TO ENSURE THE SURVIVAL OF THE SPECIES AGAINST MAJOR PATHOGEN EPIDEMICS

Graph showing numbers of alleles named by year from 1987 to 2023



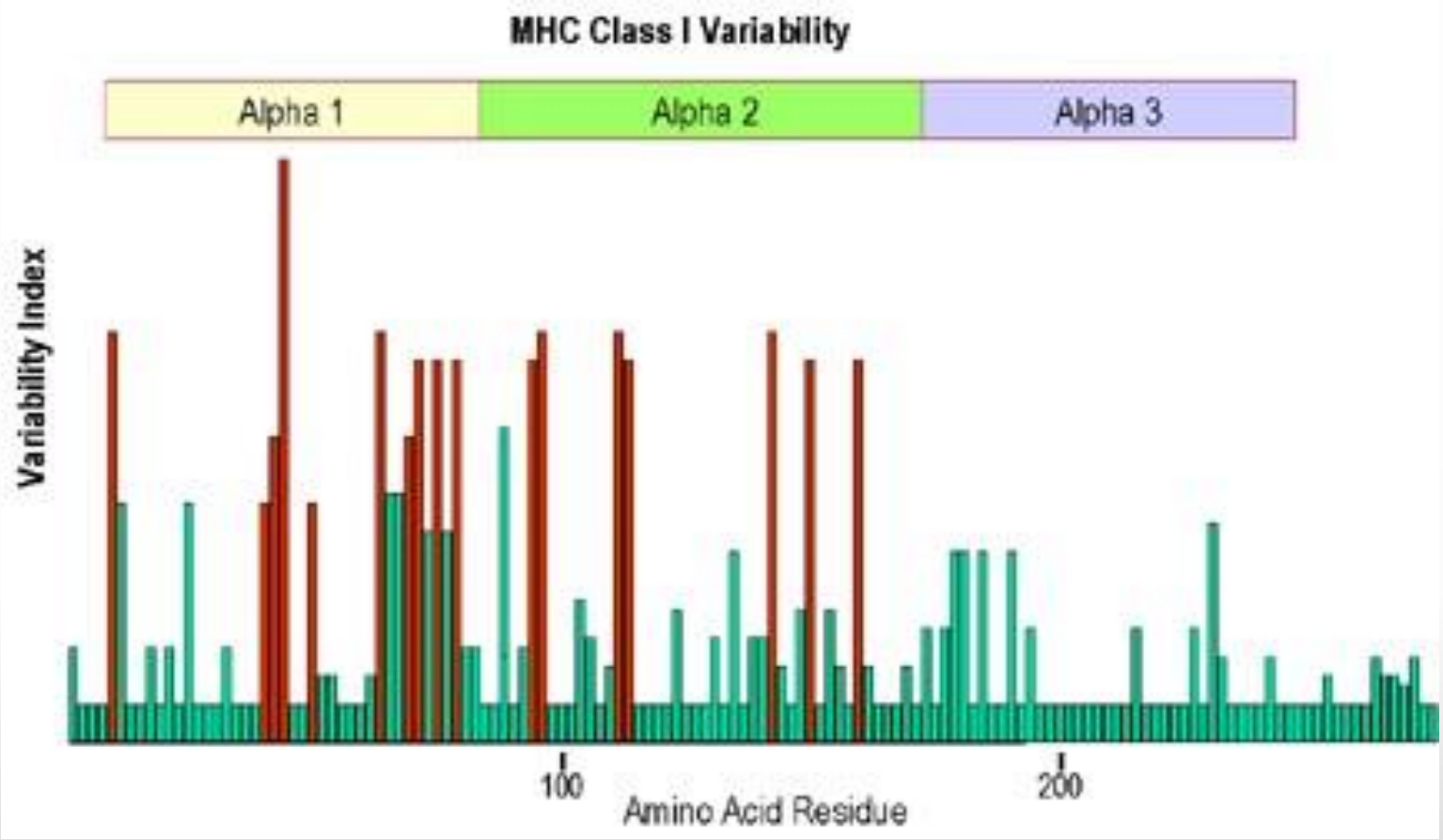
And the numbers are growing…

As of September 2023

There are currently 36814 HLA and related alleles described by the HLA nomenclature and included in the IPD-IMGT/HLA Database

MHC Class I polymorphism

The high polymorphism characterizes especially the I class of HLA molecules



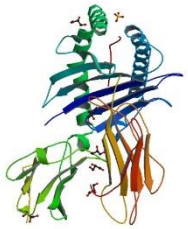
The **distribution and frequency of HLA antigens vary greatly among different ethnic groups**.

It has been postulated that **this diversity of HLA polymorphism** has evolved **under unique selective pressure** in **different geographic areas**.

This could be related to the **role of the HLA molecules in the presentation of prevalent infectious agents** in the different areas of the world.

The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications
Sung Yoon Choo, 2007

HLA Keypoints!



HLA Keypoints!

Some key point concepts! There are two groups of MHC genes structurally and functionally distinct:

Class I → recognition by CD8+ T cells
of endogenous antigens synthesized within the target cell
(cellular, transformed or virus-induced proteins)

Class II → recognition by CD4+ T cells
extracellular exogenous proteins are endocytosed
and undergo degradation in the acid endosomal compartment

Both HLA classes, I and II, are responsible for the compatibility of the tissues of genetically different individuals and for the rejection of the transplant

MHC genes are codominantly expressed in each individual
Monozygotic twins have the same histocompatibility molecules on their cells

HLA expression

Tissue	MHC class I	MHC class II
T cells	+++	+/-
B cells	+++	+++
Macrophages	+++	++
Other APC	+++	+++
Thymus epithelium	+	+++
Neutrophils	+++	-
Hepatocytes	+	-
Kidney	+	-
Brain	+	-
Erythrocytes	-	-

The pattern of expression reflects the function of MHC molecules:

- **Class I** is involved in the regulation of anti-viral immune responses
- **Class II** is involved in regulation of the cells of the immune system

Erythrocytes can not support pathogens replication - hence no MHC class I !!!

But, some pathogens exploit this - e.g. *Plasmodium* species.

Structure of HLA molecules

Glycoproteins, heterodimers (two chains)

Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors

- ✓ **Polymorphic (predominantly in the cleft)**
- ✓ **Non polymorphic part of the molecule contain binding sites for theT cell molecules CD4 and CD8**

HLA class I molecules

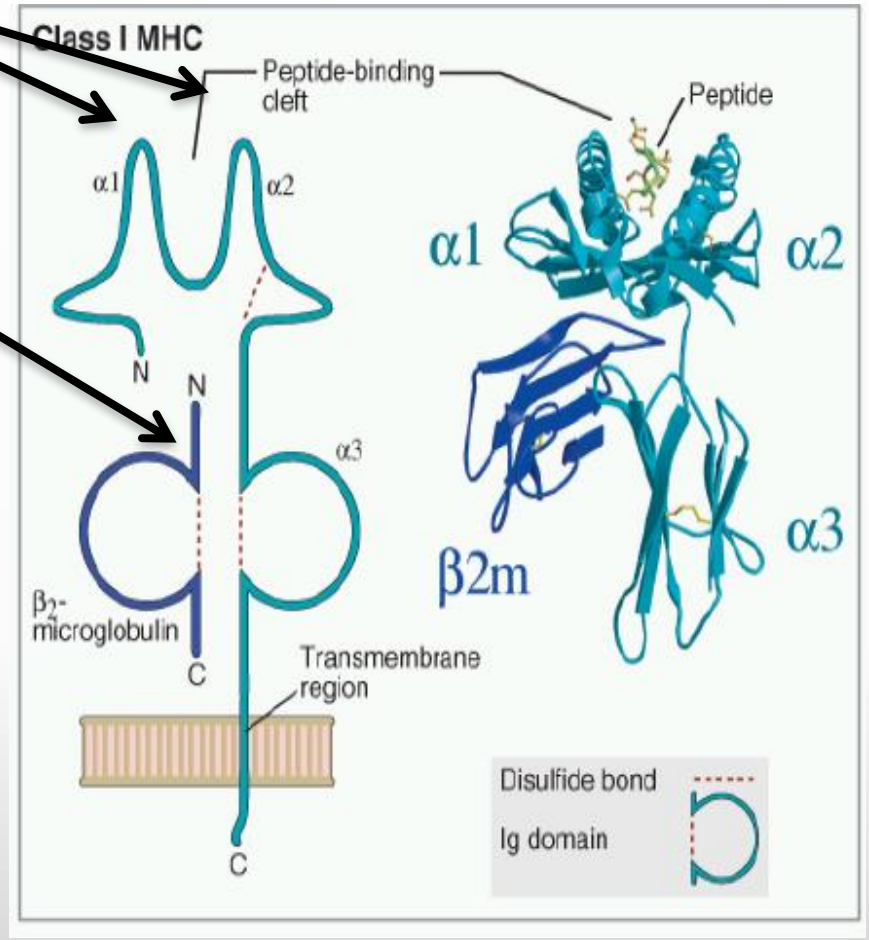
Glycoproteins, heterodimers (two chains)

Structure of HLA molecules of both classes enables antigen binding and contact with T cell receptors

Polymorphic sites

Binding site of CD8

α3 domain & β2m have structural & amino acid sequence homology with Ig C domains Ig GENE SUPERFAMILY



Class I molecules consist of glycosylated heavy chains non-covalently bound to β2 mycroglobulin (extracellular).

Human b2m is invariant and its gene was mapped to chromosome 15.

The class I heavy chain has three extracellular domains (α1, α2, α3), a transmembrane region and an intracytoplasmic domain.

The a1 and a2 domains contain variable aminoacid sequences and these domains determine the antigenic specificities of the HLA class I molecules.

Structure of MHC class I molecules

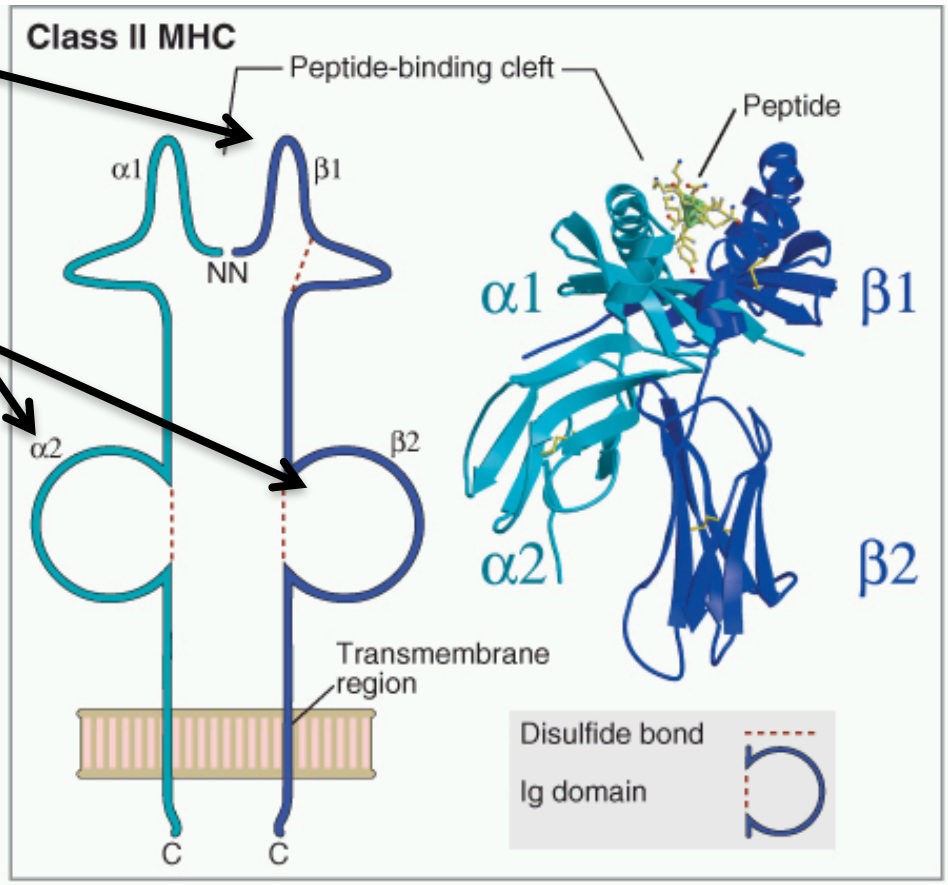
α1 and α2 domains form two segmented α-helices on eight anti-parallel β-strands to form an antigen-binding cleft.

HLA class II molecules

Polymorphic sites

Binding site of CD4

α2 & β2 domains have structural & amino acid sequence homology with Ig C domains Ig GENE SUPERFAMILY



These are heterodimers of two non covalently associated glycosylated polypetide chains; α and β.

The α and β chains are transmembrane and they have the same overall structure.

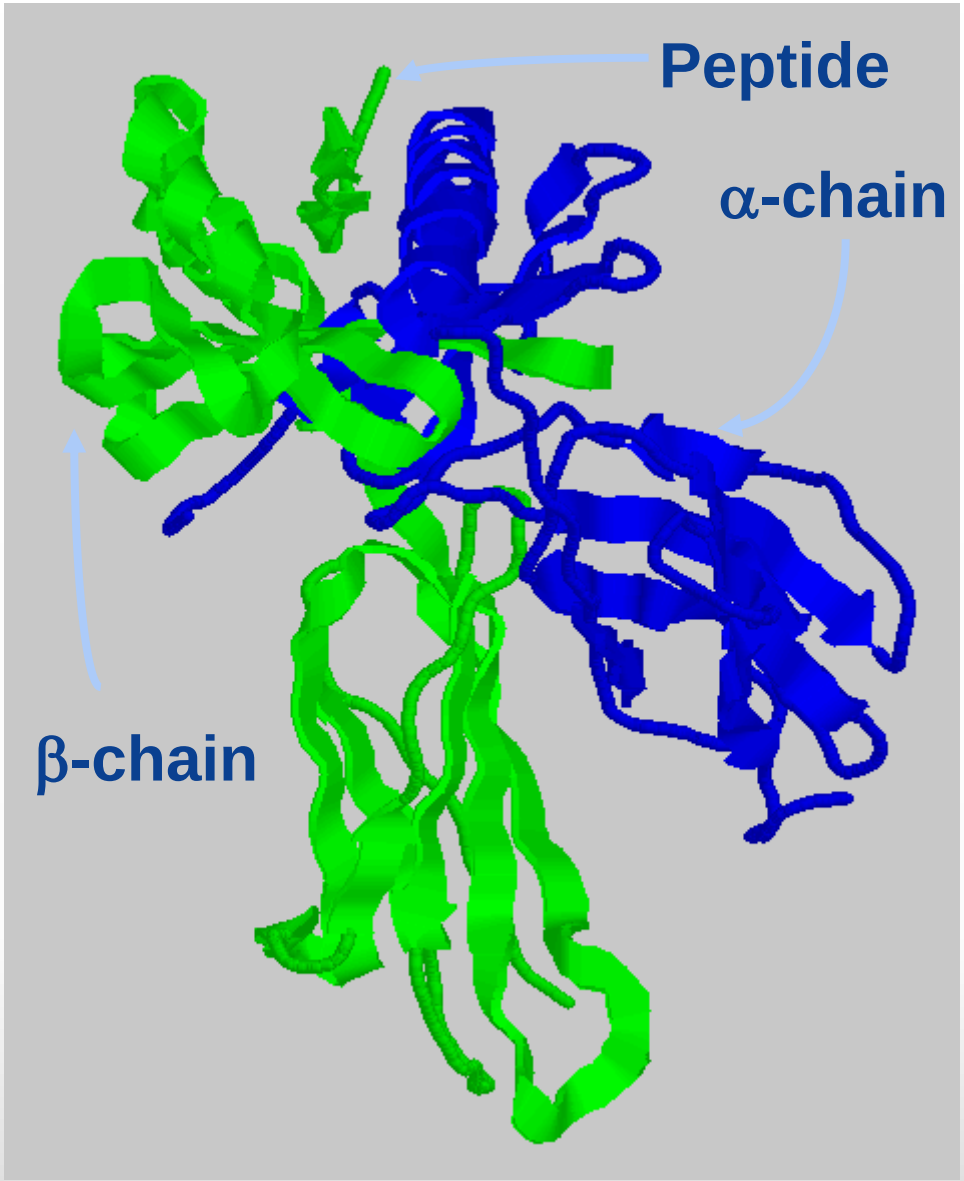
An extracellular portion composed of two domains is anchored on the membrane by a short trasmembrane region and a cytoplasmic domain.

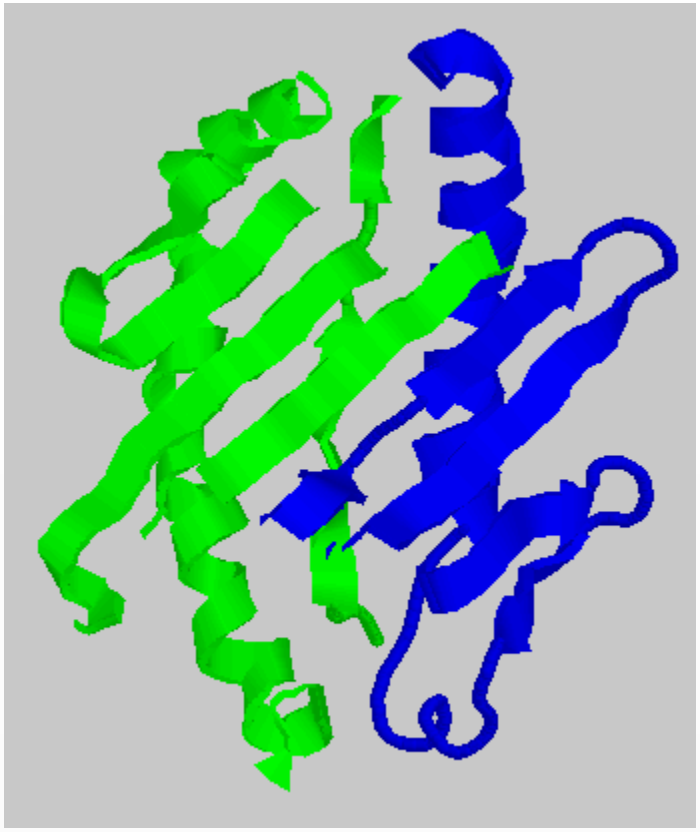
The α1 and β1. domains form an antigen binding groove.

The both ends of class II groove are more open and longer peptides can be accomodated

Polymorphism of class II molecules occur in the first aminoterminal β 1 domain.

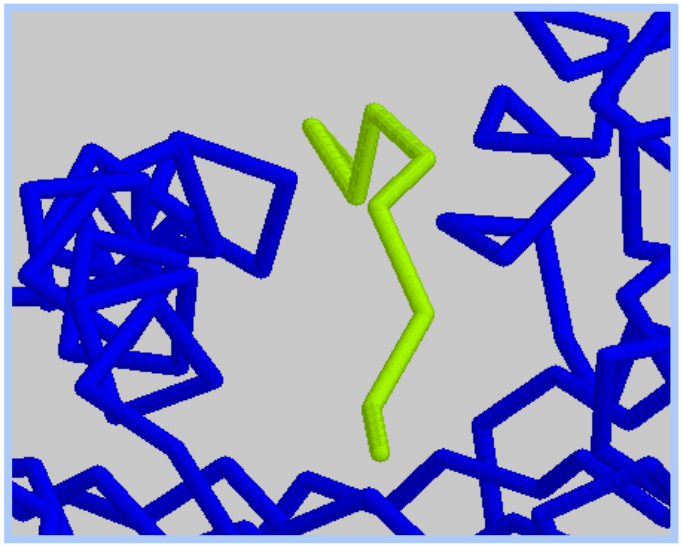
MHC class II molecule structure

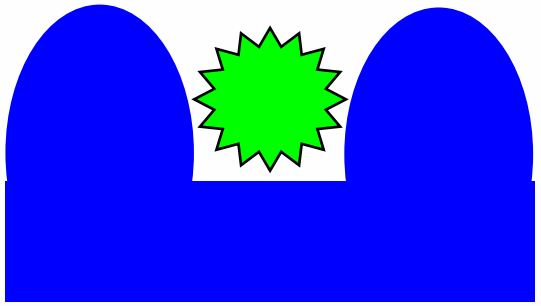




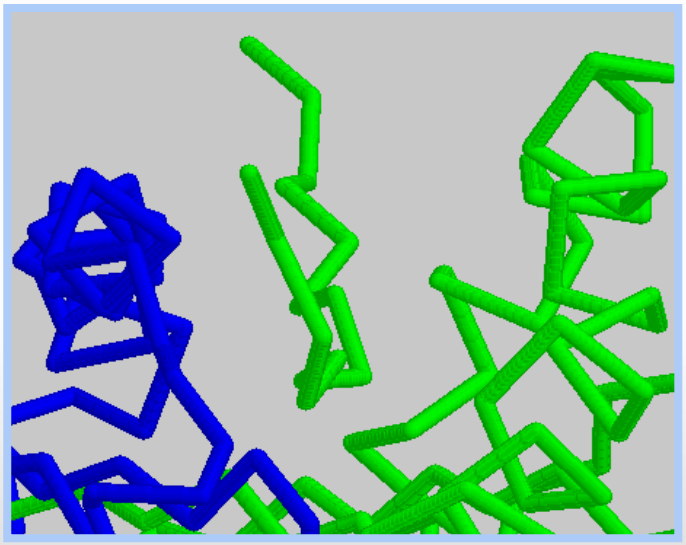
Cleft is made of both α and β chains

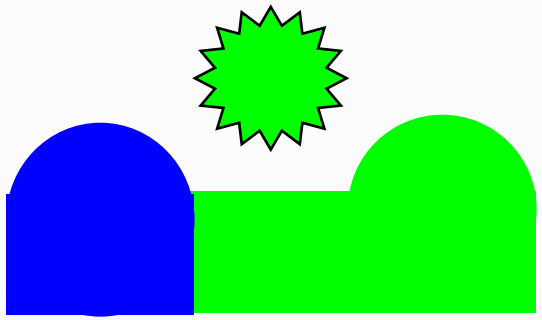
Cleft geometry





MHC class I

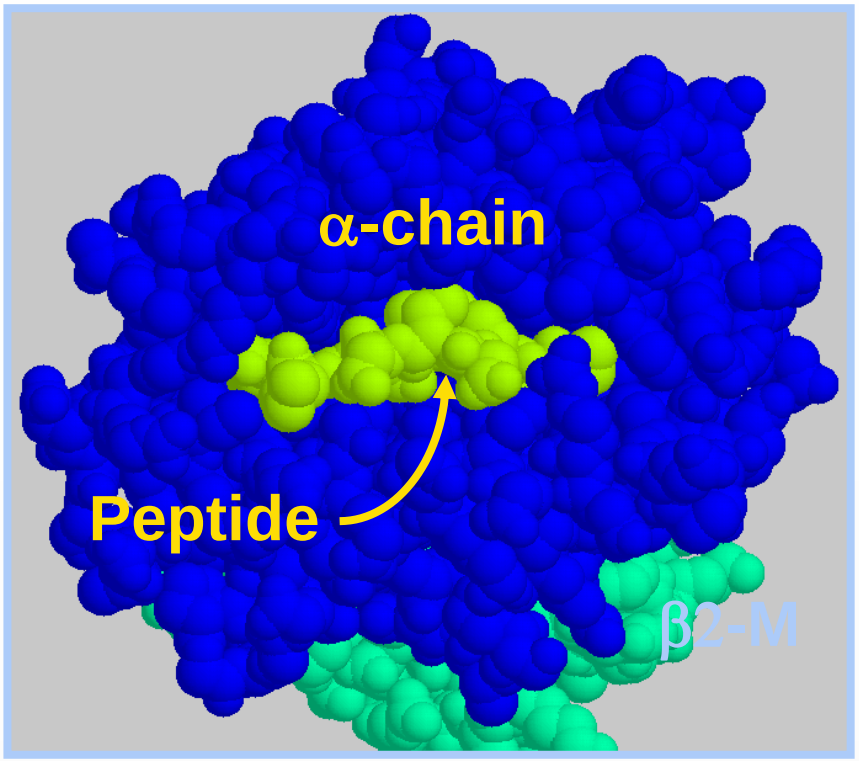


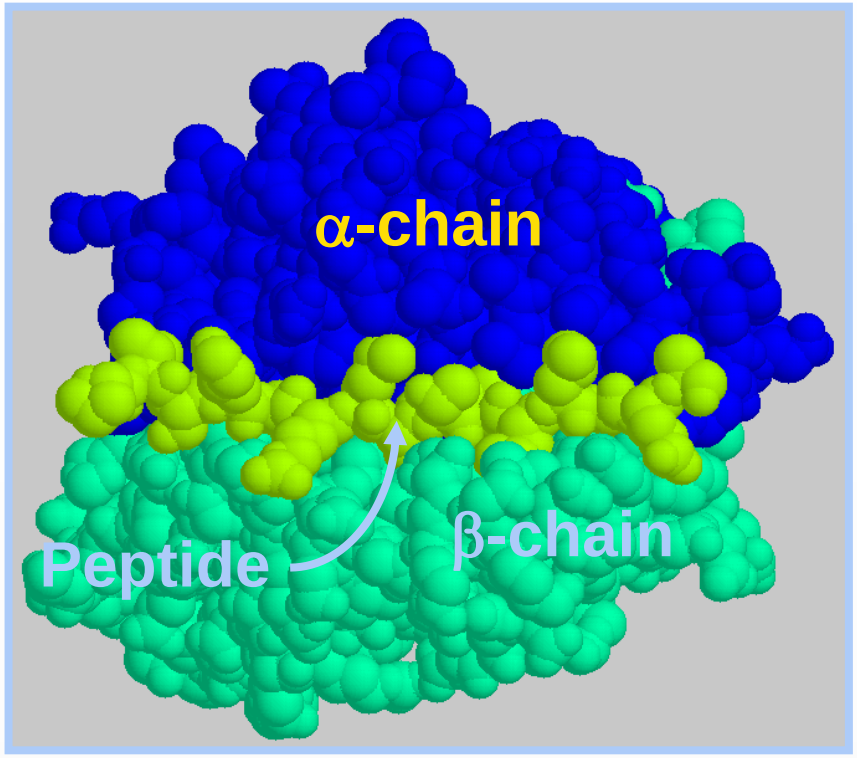


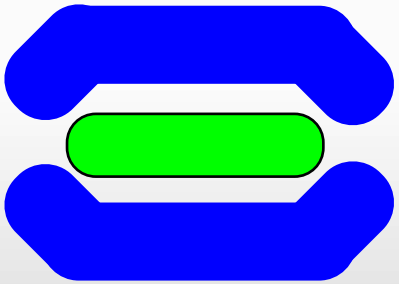
MHC class II

Peptide is held in the cleft by non-covalent forces

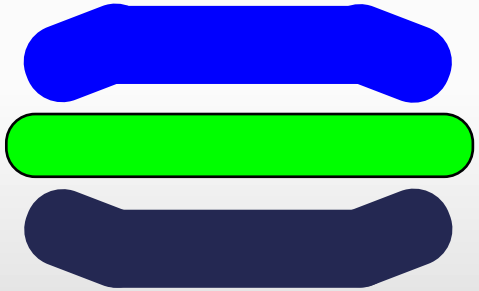
Cleft geometry





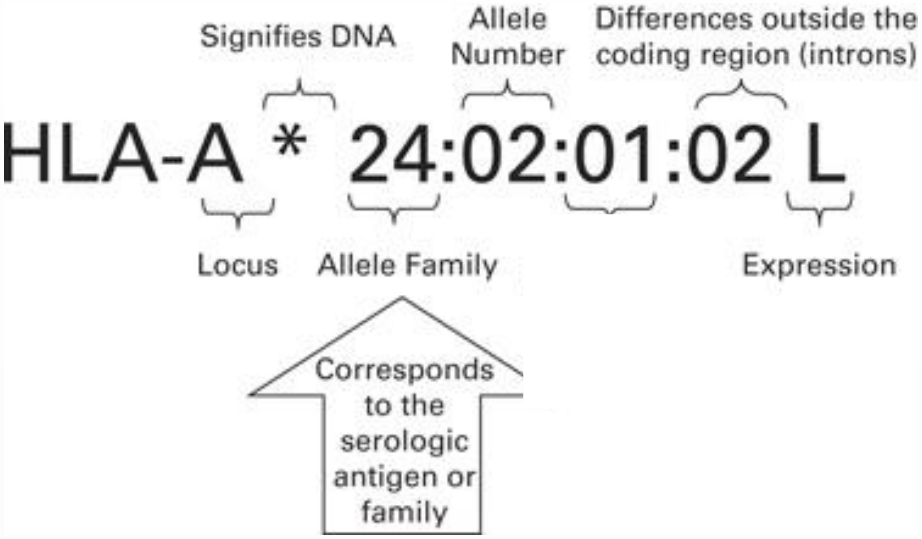


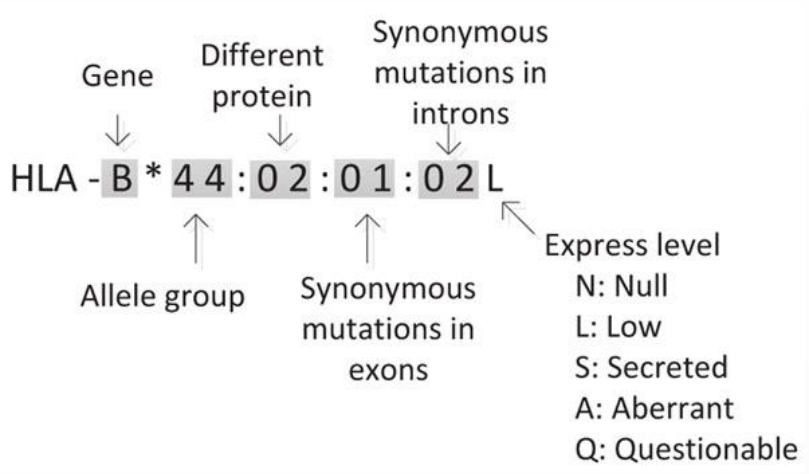
MHC class I accommodate peptides of 8-10 amino acids



MHC class II accommodate peptides of >13-30 amino acids

HLA nomenclature





Low Resolution

HLA-A*68:02:01:02

High Resolution

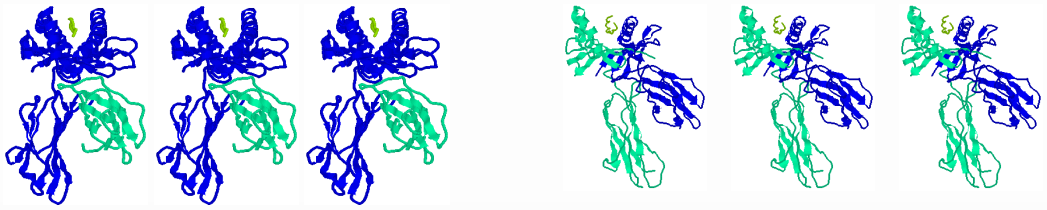
Nomenclature

Antigene	Low Resolution		High Resolution	
	1° haplotype	2° haplotype	1° haplotype	2° haplotype
Class I				
A	26	2	26:01	02:01
B	7	35	07:02	35:01
C	7	4	07:02	04:01
Class II				
DRB1	15	4	15:01	04:05
DQB1	6	3	06:02	03:02
DPB1	2	4	02:01	04:01

MHC-binding peptides

Each human usually expresses:

- ✓ **3 types of MHC class I (A, B, C)**
- ✓ **3 types of MHC class II (DR, DP,DQ)**



The number of different T cell antigen receptors is estimated to be

1,000,000,000,000,000

Each of which may potentially recognise a different peptide antigen

How can 6 molecules have the capacity to bind to 1,000,000,000,000,000 different peptides?

Eluted peptides from MHC class I molecules have different sequences but contain motifs

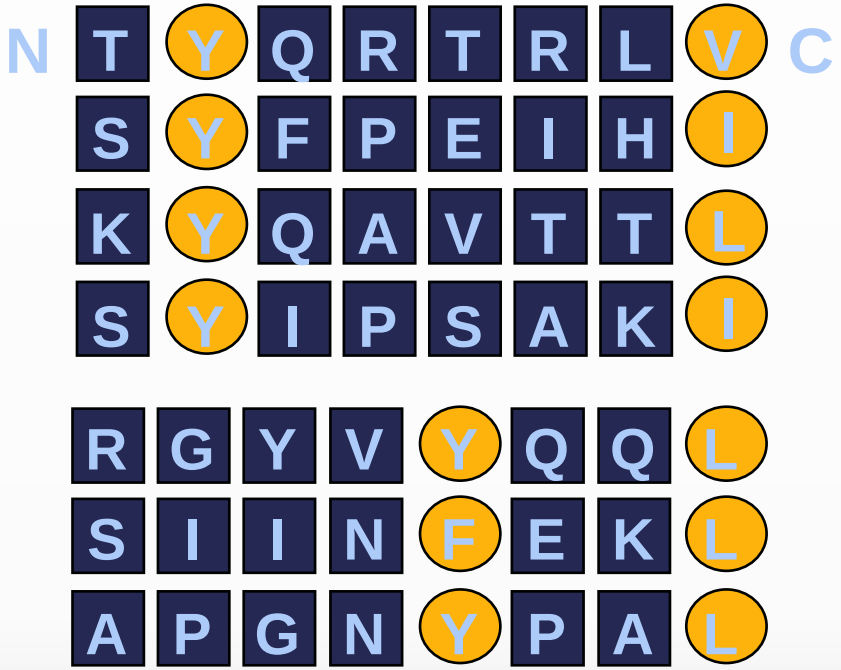
The answer is the polimorphism!!!!

Several studies showed that there are common sequences in a peptide antigen that binds to an MHC molecule and are called MOTIF like a key
They share common sequences, motif, to tether anchor on the MHC!!!!!

Peptides bound to a particular type of MHC class I molecule have conserved patterns of amino acids

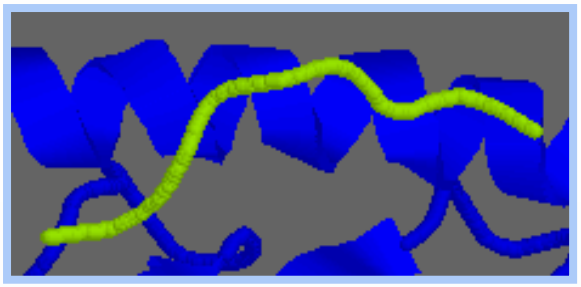
A common sequence in a peptide antigen that binds to an MHC molecule is called a MOTIF

Amino acids common to many peptides tether the peptide to structural features of the MHC molecule
ANCHOR RESIDUES

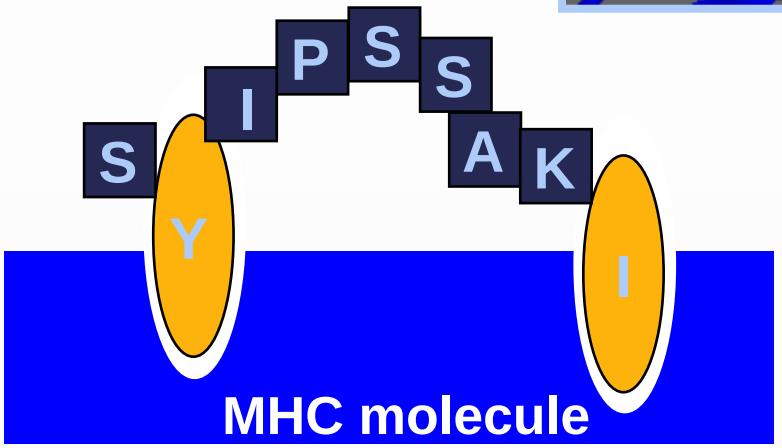


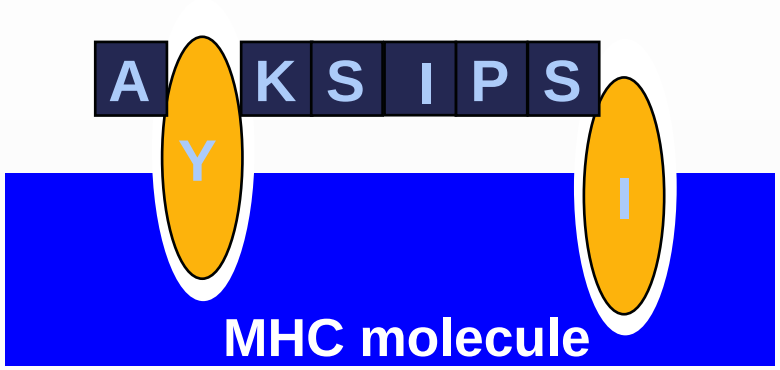
Different types of MHC molecule bind peptides with different patterns of conserved amino acids

MHC molecules can bind peptides of different length



Arched peptide

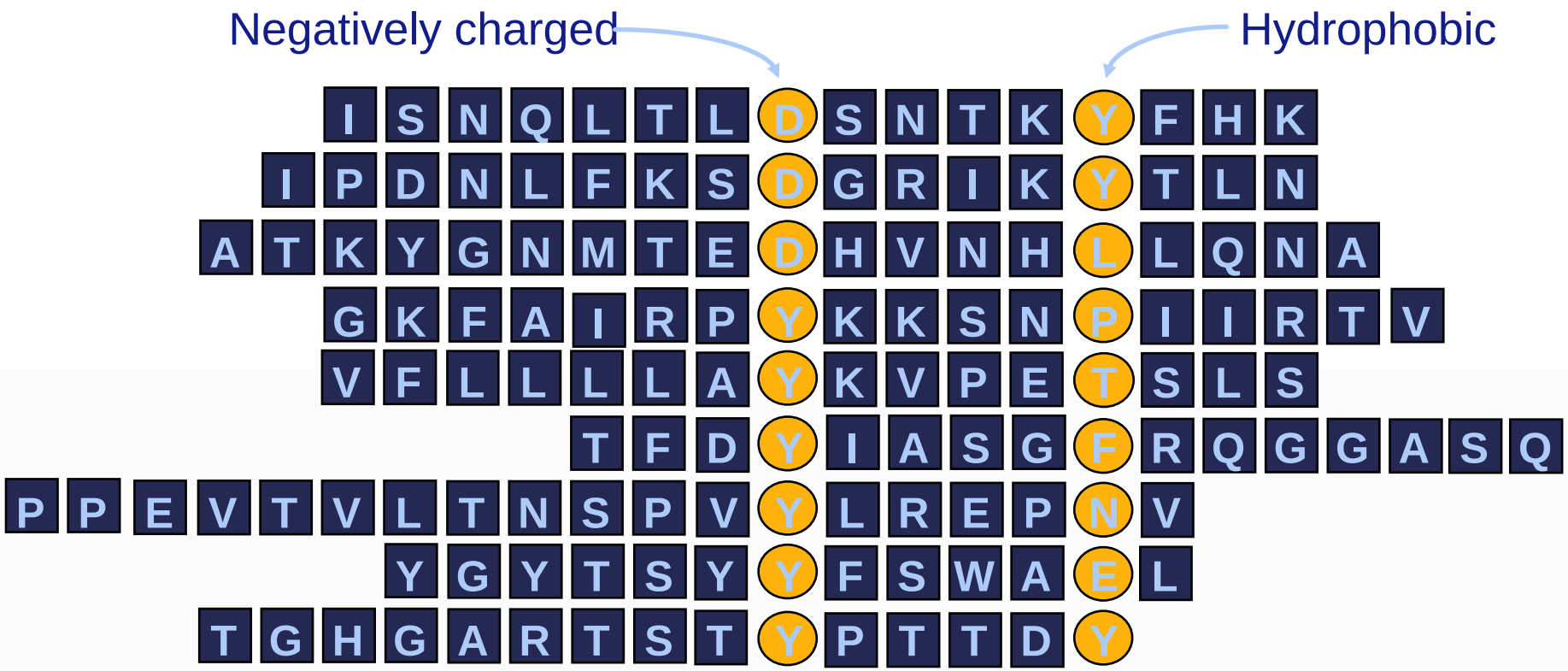




Complementary anchor residues & pockets provide **the broad specificity** of a particular type of MHC molecule for peptides

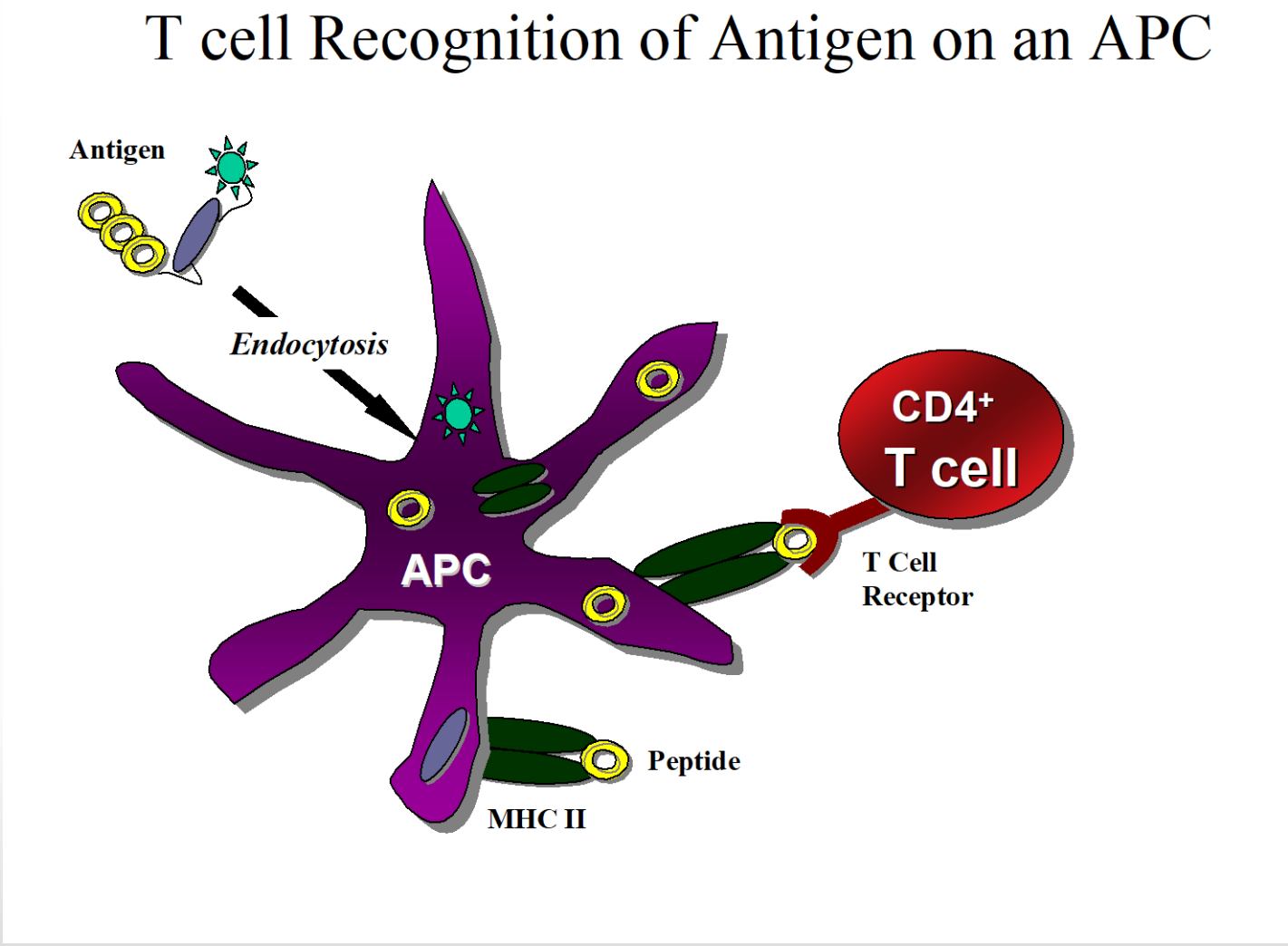
Peptide sequences between anchors can vary
Numbers of amino acids between anchors can vary

Peptide antigen binding to MHC class II molecules

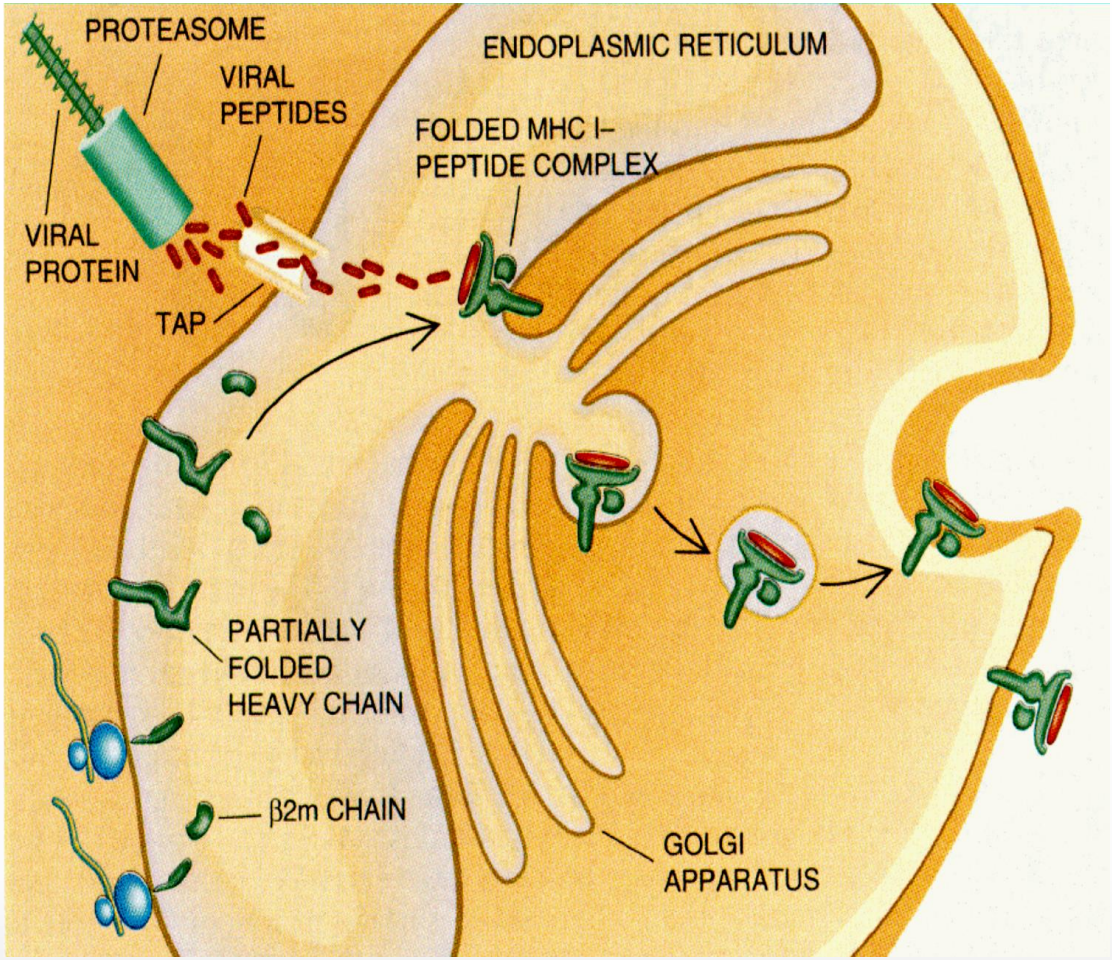


- Anchor residues *are not* localized at the N and C termini
- Ends of the peptide are in extended conformation and may be trimmed
- **Motifs are less clear than in class I-binding peptides**

HLA in immune response



INTRACELLULAR ANTIGENS ARE PRESENTED BY MHC CLASS I MOLECULES



Class I molecules present intracellular antigens that are processed in the cytoplasm and pumped into the endoplasmic reticulum, where new HLA molecules are being assembled. Processing of the antigens is performed by the proteosome

Peptides that are processed in this manner are transported into the endoplasmic reticulum by TAP (transporter associated with antigen).

The newly synthesized HLA molecule is maintained in a partially folded conformation by *calnexin* (not shown). When β_2-microglobulin binds to the HLA molecule, the complex dissociates from calnexin and binds a complex of *calreticulin* and *tapasin*, which then bind to TAP-1.

The proper binding of a peptide to the HLA molecule dissociates the complex, and the HLA molecule is then transported through the Golgi complex to the cell surface.

MHC Class I pathway

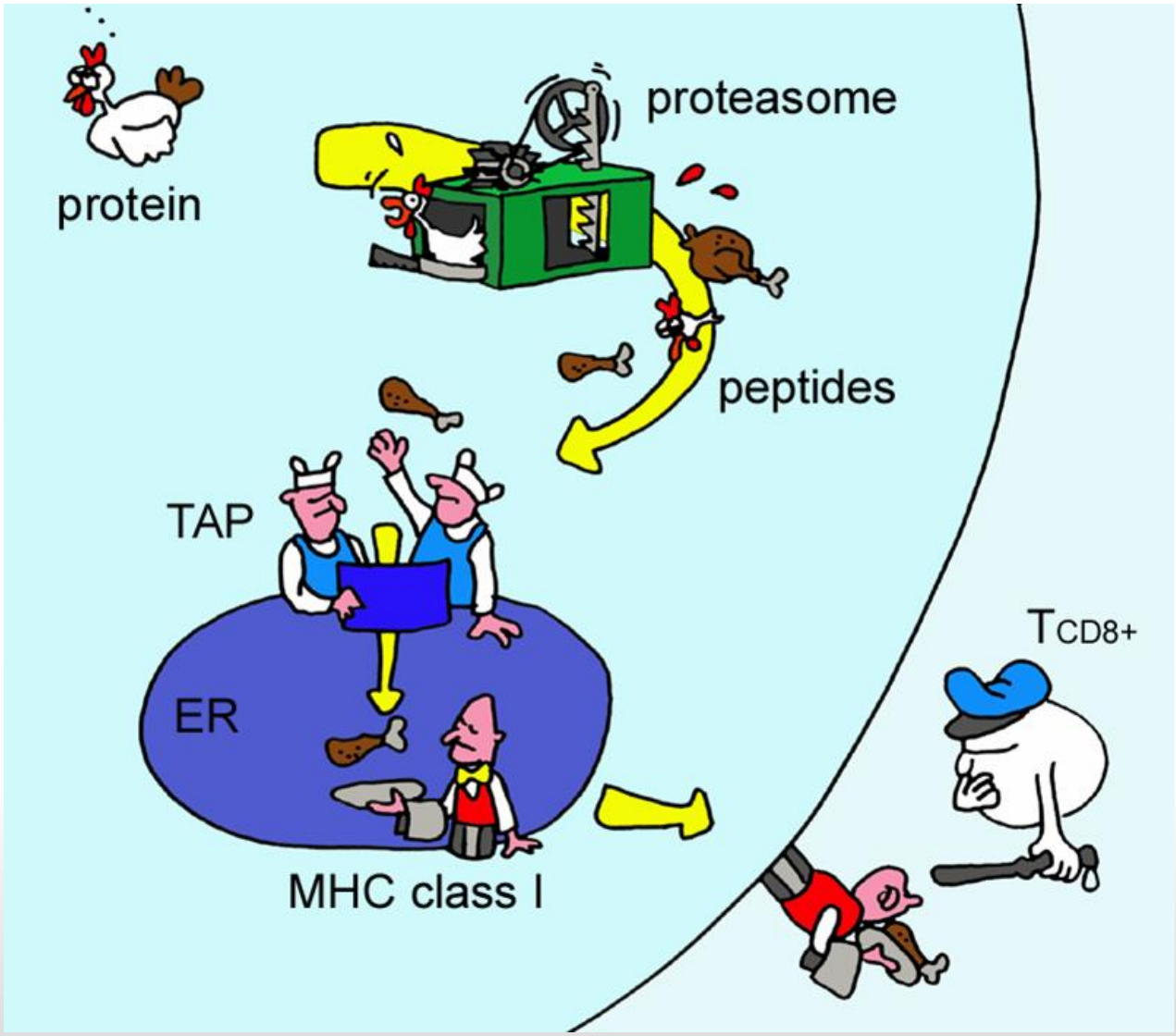
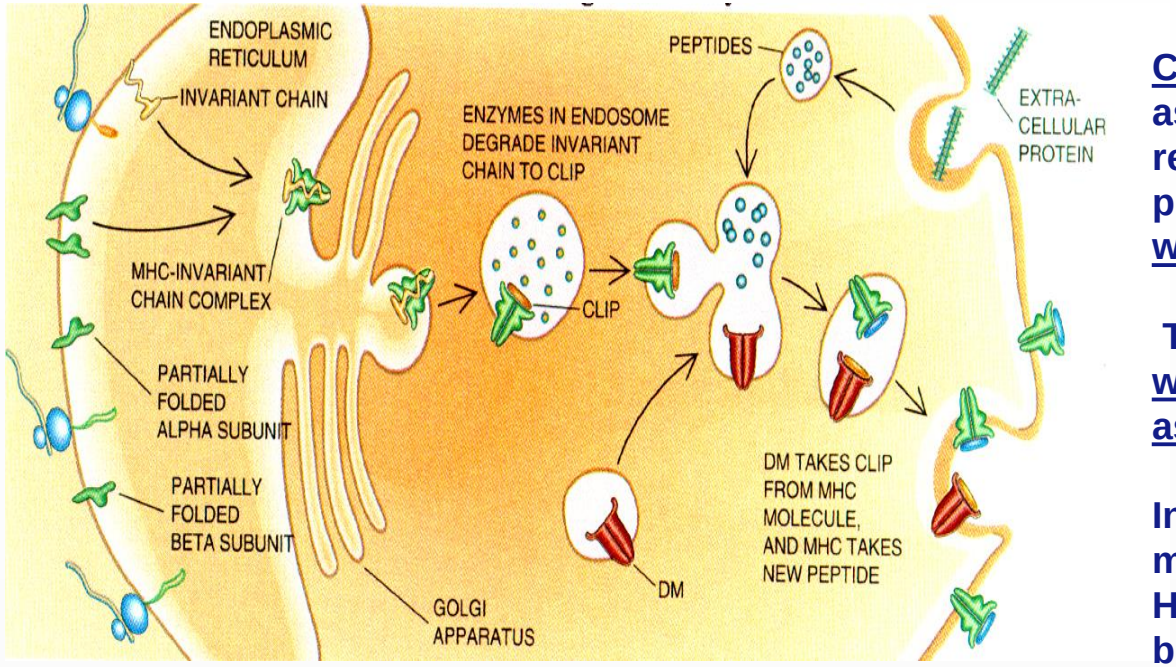


Figure by Eric A.J. Reits

Extracellular antigens are presented by MHC class II molecules



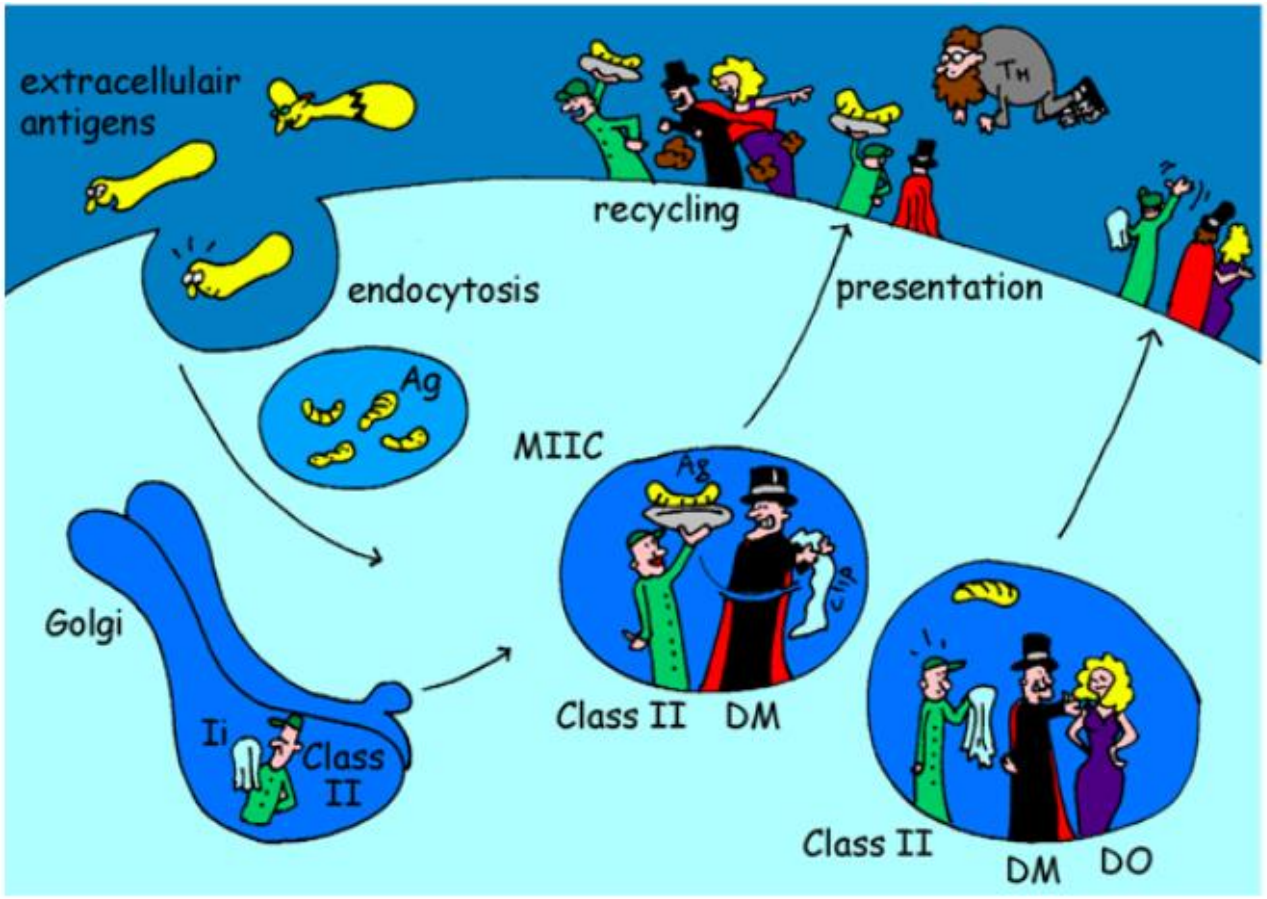
Class II molecules are also assembled in the endoplasmic reticulum, but associate with a third protein known as the *invariant chain*, which prevents peptide binding.

The invariant chain is processed within endosomes to *CLIP* (class II-associated invariant chain peptide).

In the presence of HLA-DM molecules, CLIP dissociates from HLA-DR or DQ molecules and allows binding of new peptides that have been endocytosed from the extracellular environment.

Thus, MHC class II molecules differ from MHC class I molecules because they preferentially present extracellular antigens rather intracellular ones. HLA-DM serves to 'edit' peptide binding, promoting association with high affinity peptides over lower affinity peptides.

MHC Class II pathway

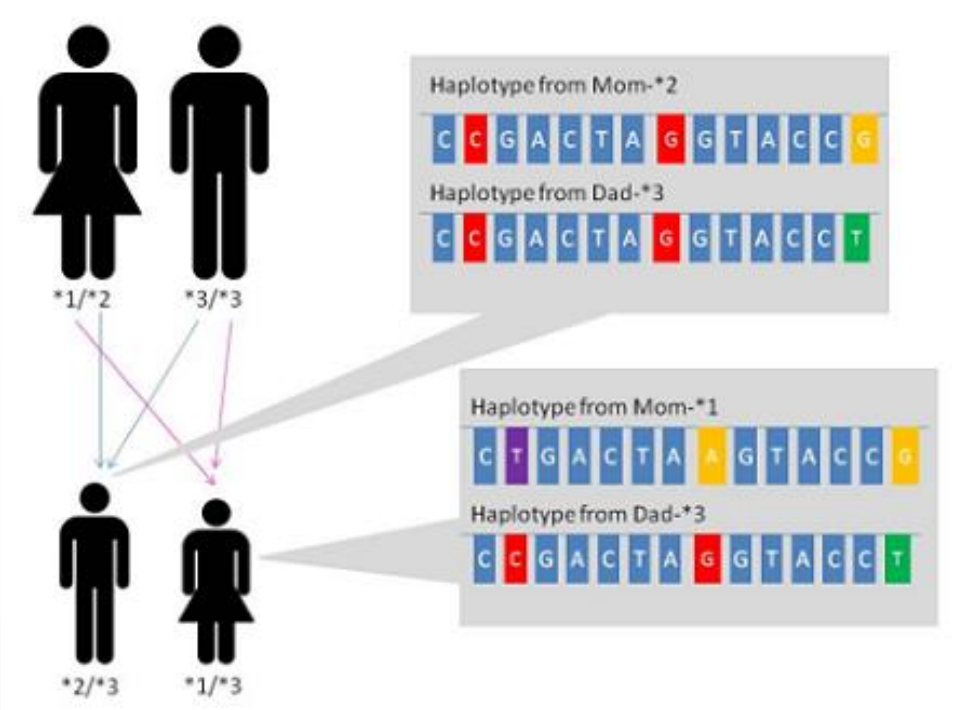


What's a *haplotype?*

Genes in the MHC are tightly linked and usually inherited in a unit called an MHC *haplotype.*

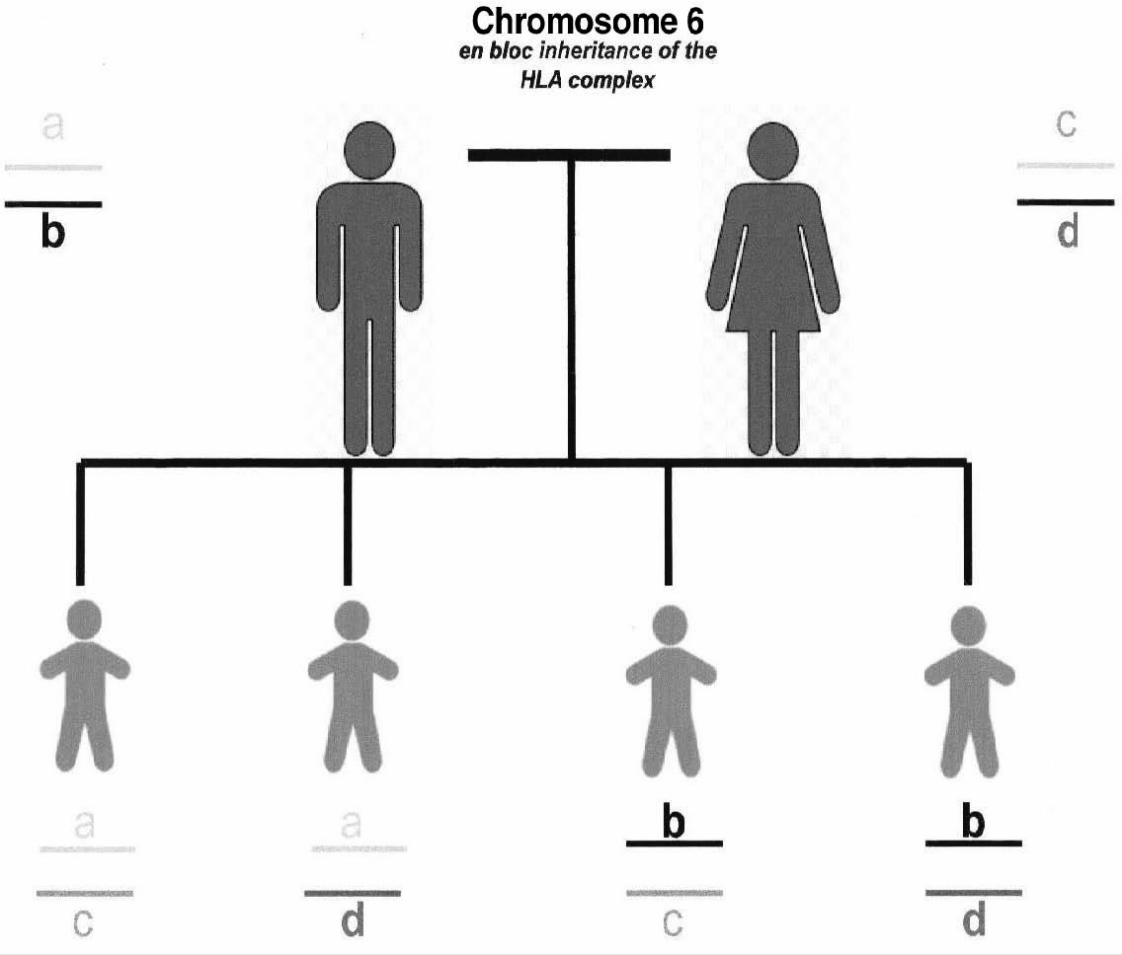
Each individual inherits in a mendelian fashion one haplotype from his/her father and one haplotype from his/her mother

What's a *haplotype?*

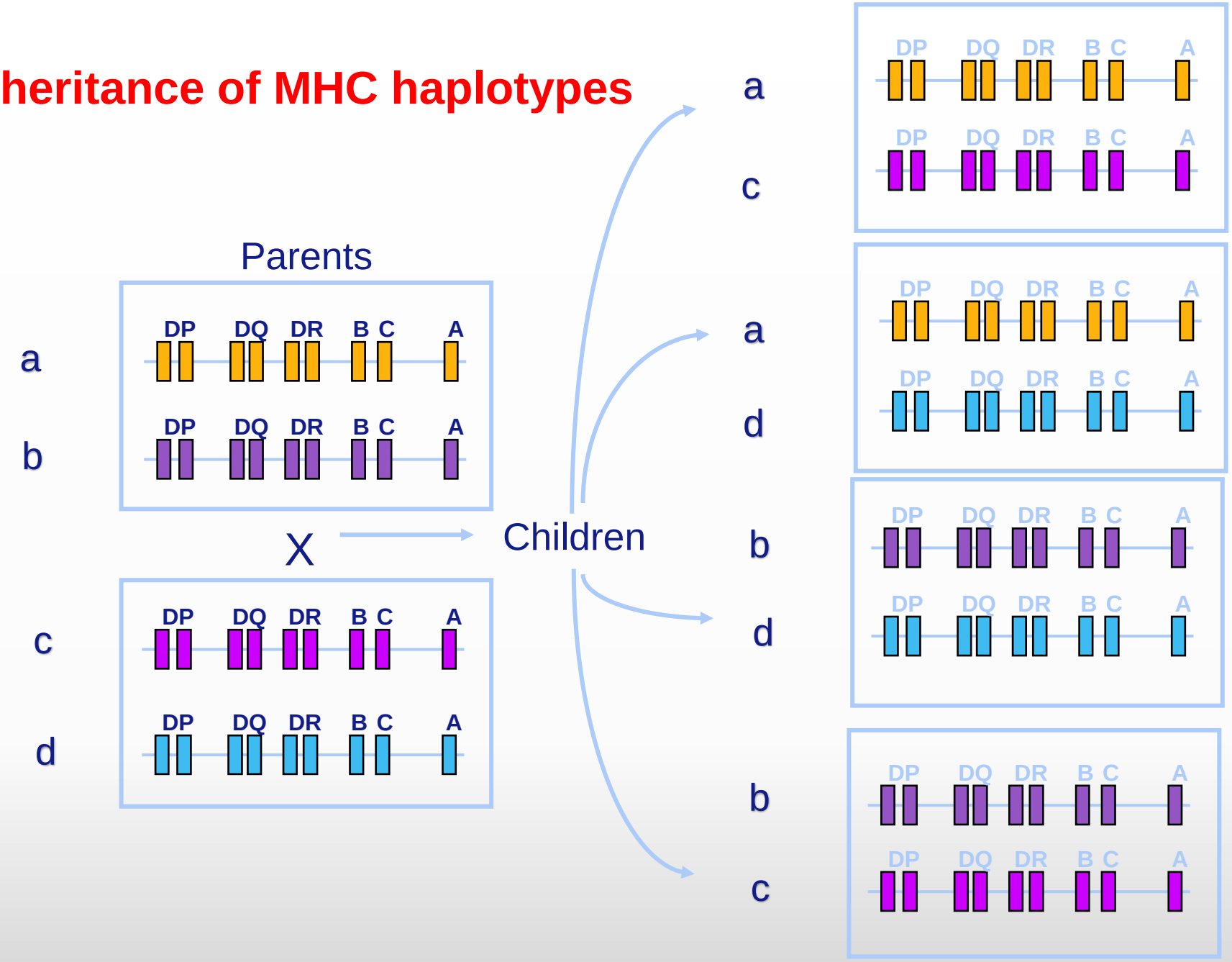


Genes in the MHC are closely linked and the HLA haplotype is inherited in a mendelian fashion from each parent

Since a person has two copies of each gene – one from their mother and one from their father – they will inherit a haplotype from each parent. Each person's haplotype is written as two haplotype numbers separated by a slash.


Chromosome 6
en bloc inheritance of the
HLA complex
a
b
c
d
a
c
a
d
b
c
b
d

Inheritance of MHC haplotypes



Linkage Disequilibrium

The occurance of some combination of alleles in a population more often or less often than would be expected from a random formation of haplotypes from alleles based on their frequencies

Linkage disequilibrium can be caused by evolutionary factors such as natural selection and genetic drift.

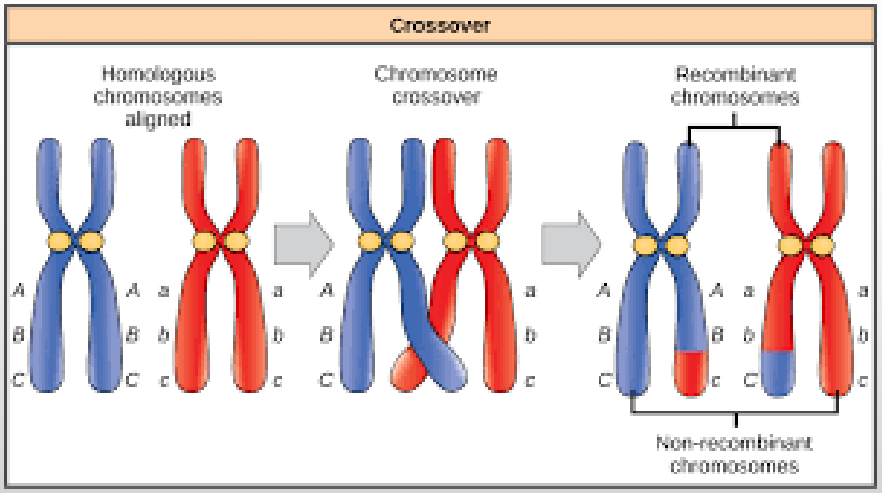
In Caucasian HLA-A1, B8, DR17 is the most common HLA haplotype (frequency 5%)

Errors in the inheritance of haplotypes generate polymorphism in the MHC by gene conversion and recombination

CROSS OVER: The genes of the HLA region occasionally demonstrate chromosome crossover, in which segments containing linked genetic material are exchanged between the two chromosomes during meiosis or gametogenesis.

The recombinant chromosomes are then transmitted

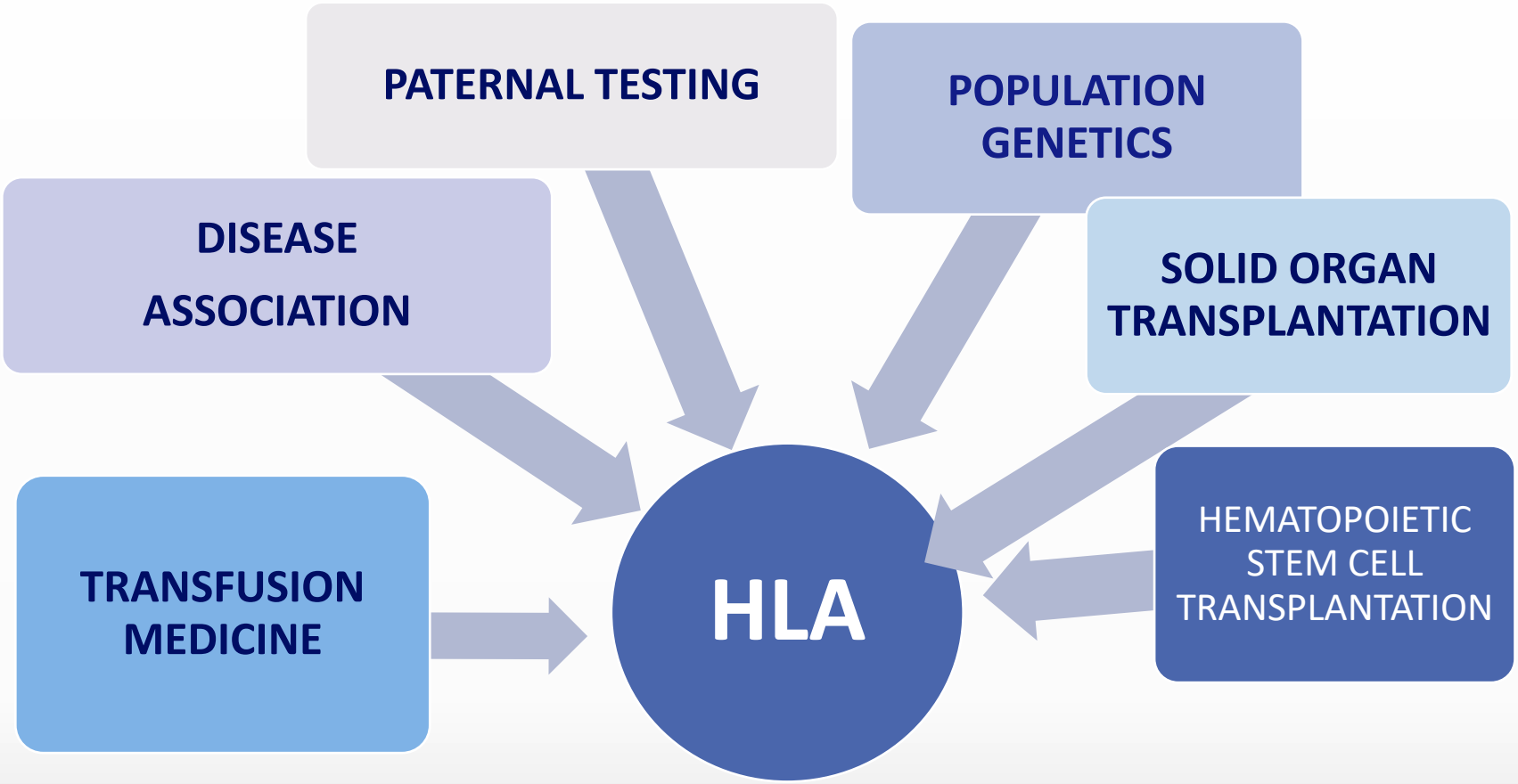
as **NEW HAPLOTYPES** to the offspring.



The crossover **frequency is related partly to the PHYSICAL DISTANCE between the genes** and partly to the **RESISTANCE OR SUSCEPTIBILITY of specific A, B, and DR antigens to RECOMBINATION.**

Haplotype Inheritance

The HLA system in clinical practice



The HLA system

PATERNAL TESTING

HLA system testing does not provide certainties when the case involves a paternal HLA haplotype that is common in the particular ethnic group.

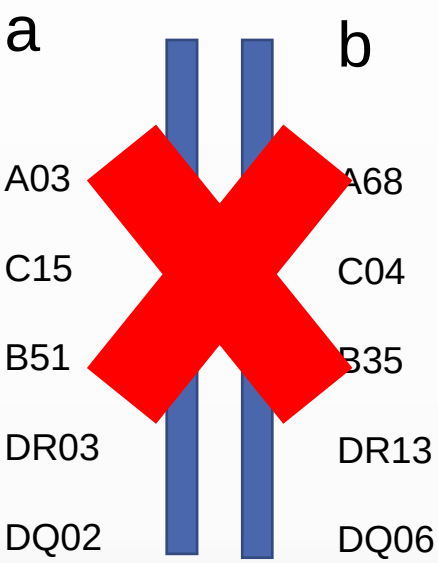
During the past decade, *SHORT TANDEM REPEATS* (STRs), also known as microsatellites or simple sequence repeats loci, became a valuable tool in paternity testing because of their *high polymorphism* and *heterozygosity .*

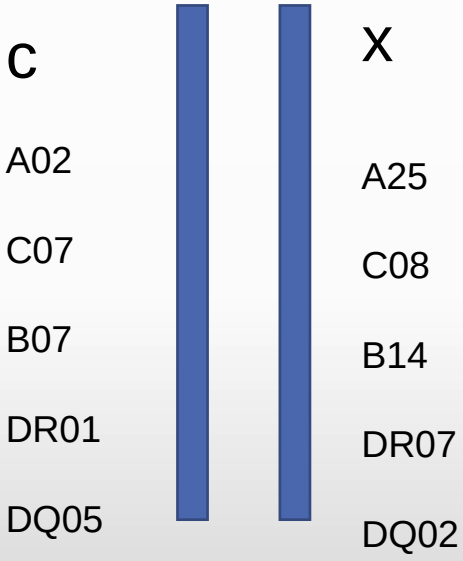
Grubic et al. International Congress Series 1261 (2004) 535–5

The HLA system

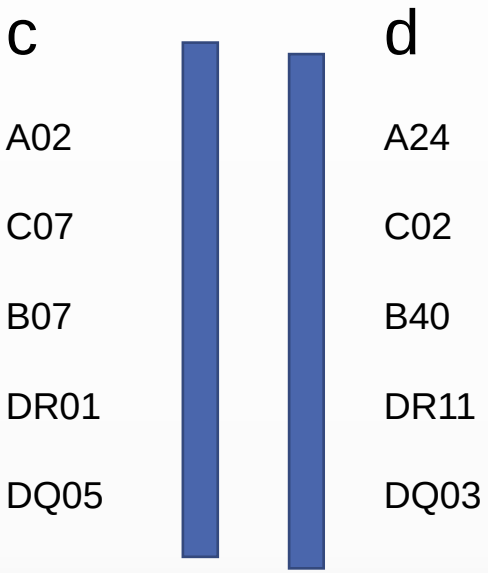
PATERNAL TESTING : EXAMPLE

FATHER





MOTHER



The HLA system in clinical practice

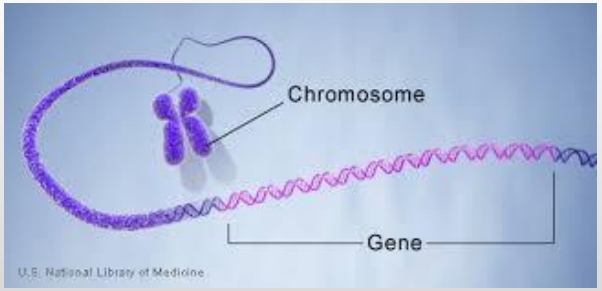
DISEASE ASSOCIATION

Genetic tests in genetic diseases

Single gene → Diagnostic

Major gene + Additive → Genetic risk Complex trait

(none of the genes is necessary or sufficient for the developing of the disease)



HLA and disease association

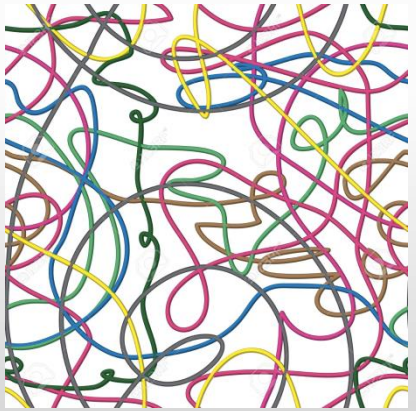
Population studies carried out over the last several decades have identified a long list of human diseases that are significantly most common among individuals that carry particular HLA alleles.

Some examples:

- ✓ Ankylosing spondylitis, 90% patients, B*27:02, B*27:05
- ✓ Narcolepsy, 90% patients, DQB1*06:02
- ✓ Rheumatoid Arthritis, 90% patients, DRB1*01:01, *04:01/04/05
- ✓ Coeliac Disease 80% patients DQA1*05,DQB1*02

Is HLA typing diagnostic?

- No diseases associated 100% with HLA antigens
- HLA typing has a limited diagnostic value, and only assesses the risk of a person to develop the disease.
- **Analysis of HLA** susceptibility genes has mostly **negative predictive value, since the absence of risk alleles makes highly unlikely the development of the disease (but does not exclude it!)**



What about the mechanism?

The exact mechanisms underlying the most HLA-disease association are not well understood and other genetic and environmental factors may play roles as well

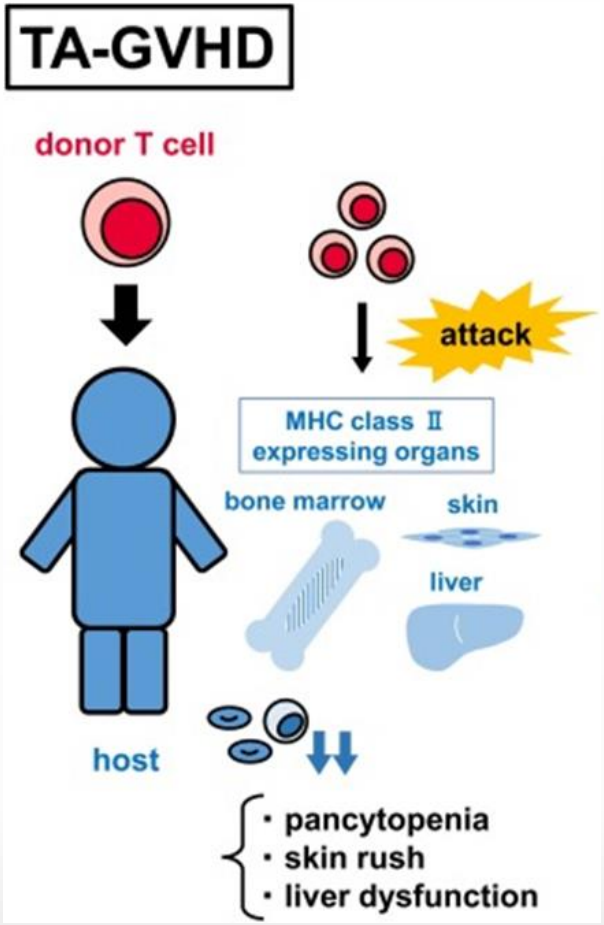
The HLA system in clinical practice

Significance of HLA
in Blood Transfusion and Transplantation

Significance of HLA in Blood Transfusion and Transplantation

HLA and Transfusion, Alloimmunization

- Refractoriness to platelets
- Transfusion-Associated Graft-Versus-Host Disease (TA-GVHD)
- Transfusion-Related Acute Lung Injury (TRALI)



Transfusion and Apheresis Science, Volume 61, Issue 2, 2022

THE HLA SYSTEM IN TRANSFUSION THERAPY

Antibodies directed against → HLA Antigens

CAUSES OF IMMUNIZATION:

- PREGNANCIES
- BLOOD TRANSFUSIONS
- PREVIOUS TRANSPLANTATION

CONSEQUENCES OF IMMUNIZATION:

- SOLID ORGAN TRANSPLANTATION (hyperacute, acute and chronic graft rejection)
- HSCT (primary graft failure and delayed engraftment)
- TRANSFUSION MEDICINE (transfusion refractoriness; TRALI)

THE HLA SYSTEM IN TRANSFUSION THERAPY

Platelet transfusion therapy plays a major role in the management of patients with haematological and oncological disorders.

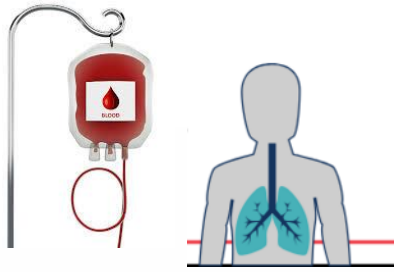
HLA antibodies → **Platelets Transfusion Refractoriness**

IMMUNOLOGICAL DESTRUCTION CAUSED BY ANTIBODIES DIRECTED AGAINST HLA CLASS I ANTIGENS

Approximately 30–50% of transfusion dependent patients become refractory to platelet transfusion

IF THE SPECIFICITY OF THE PATIENT'S ANTIBODIES CAN BE DETERMINED, PLATELETS DONORS WHO ARE NEGATIVE FOR CORRESPONDING HLA ANTIGENS CAN BE SELECTED

Transfusion related acute lung injury (TRALI)



THE PRESENCE (IN PLASMA PRODUCTS) OF DONOR ANTIBODIES DIRECTED AGAINST RECIPIENT HLA ANTIGENS COULD CAUSE THE TRALI.

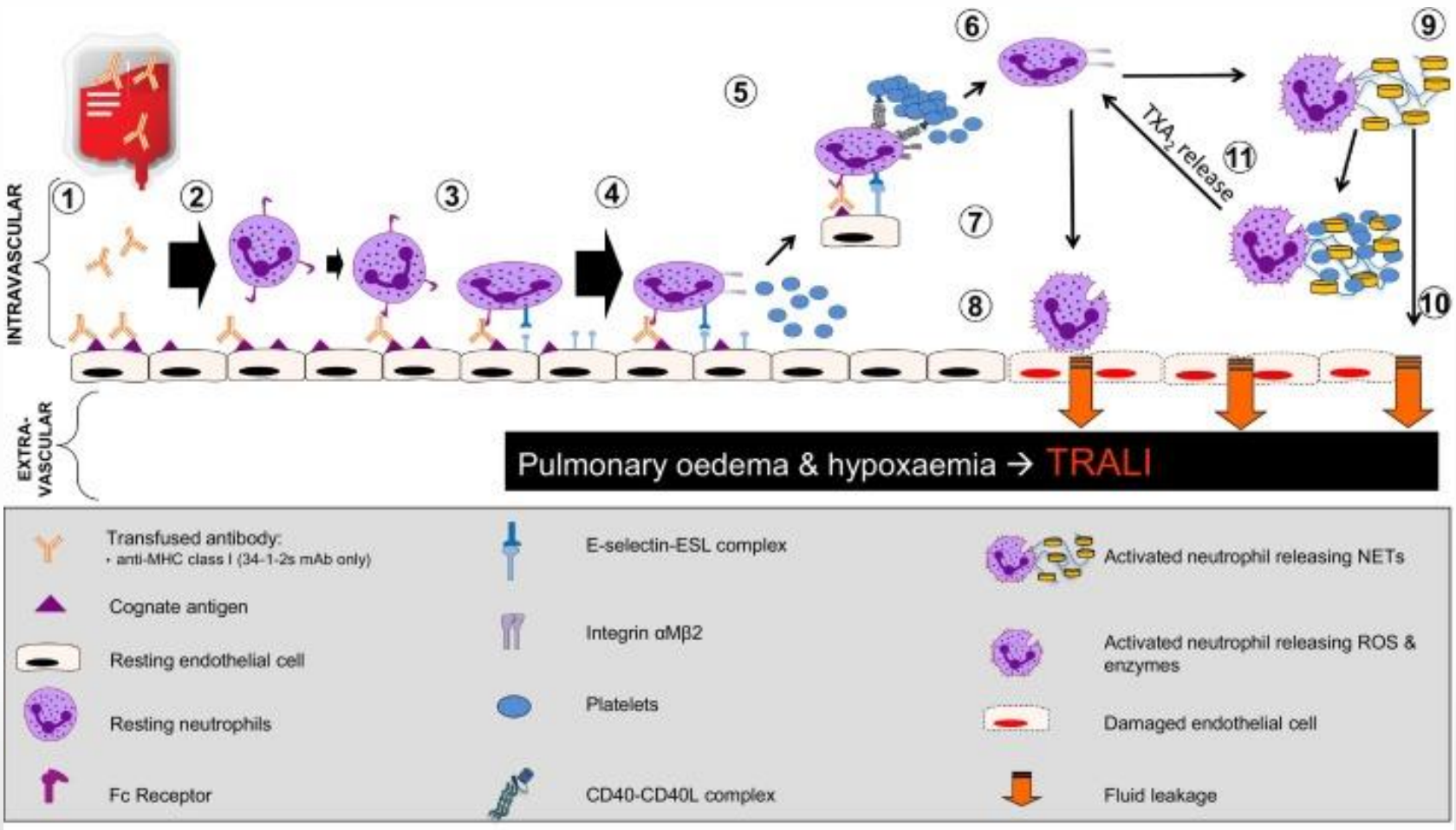
TRALI is a rare but life threatening complication of blood transfusion and can be clinically indistinguishable from adult respiratory distress syndrome.

This reaction normally develops within 2h following the administration of **plasma-containing blood components.**

ANTIBODIES ARE MOST COMMONLY FOUND IN THE DONATIONS OF MULTIPAROUS WOMEN

USE OF MALE DONORS FOR PLASMA CONTAINING PRODUCTS

Transfusion related acute lung injury (TRALI)

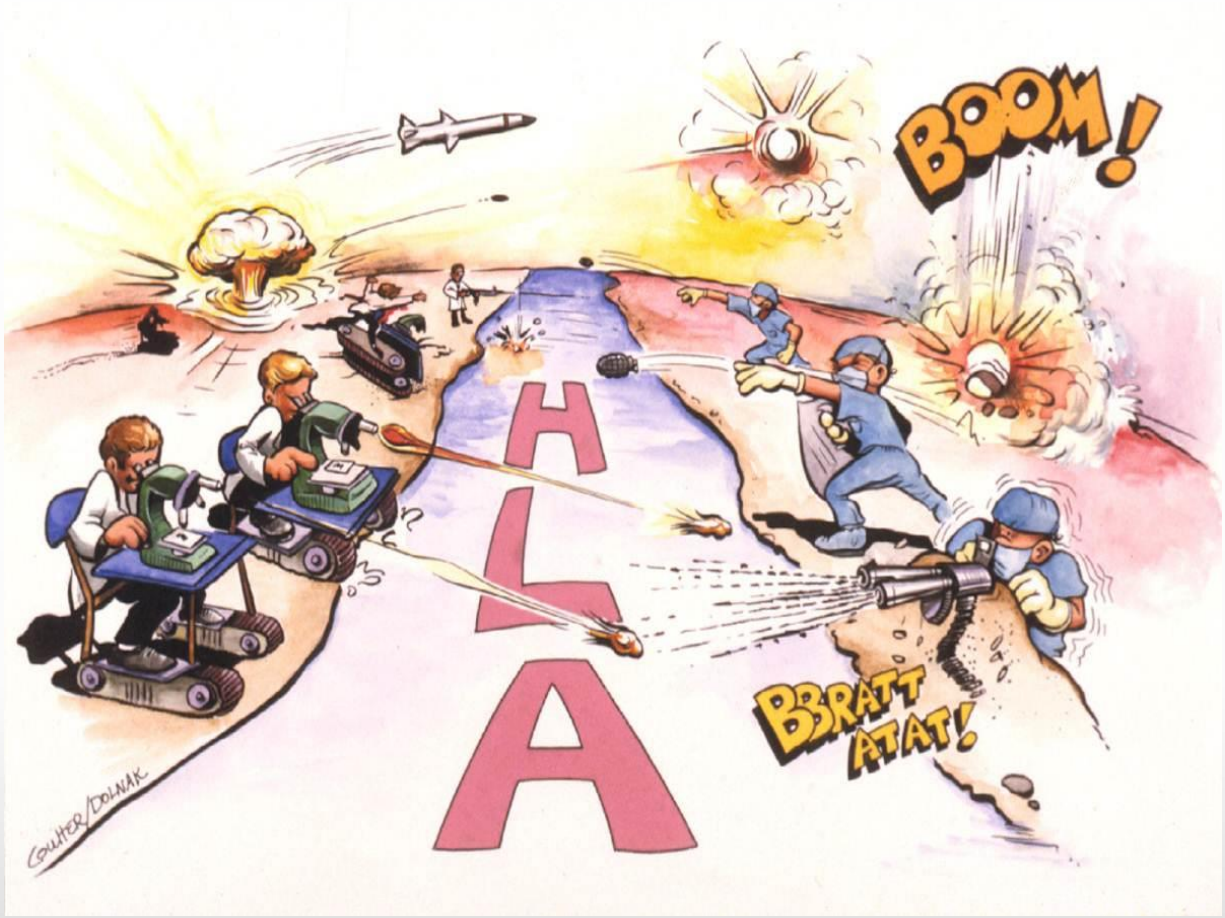


How to reduce HLA immunization?

PREFERENTIAL USE OF MALE DONORS FOR PLASMA CONTAINING PRODUCTS

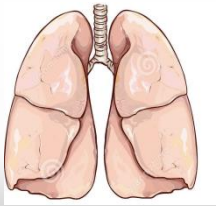
DEVELOPMENT OF CELLULAR COMPONENTS LACKING EXPRESSION OF THESE ALLOANTIGENS (LEUKOCYTE DEPLETION; UNIVERSAL LEUKOREDUCTION: PRE AND POST STORAGE)

HLA and transplantation



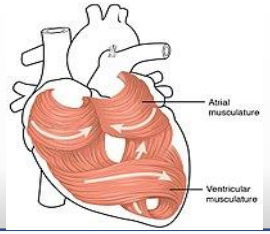
HLA and Transplantation Solid organ

Lung: Class II HLA match

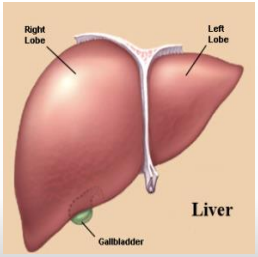


Heart: Class I HLA match on case-by-case basis, no class II

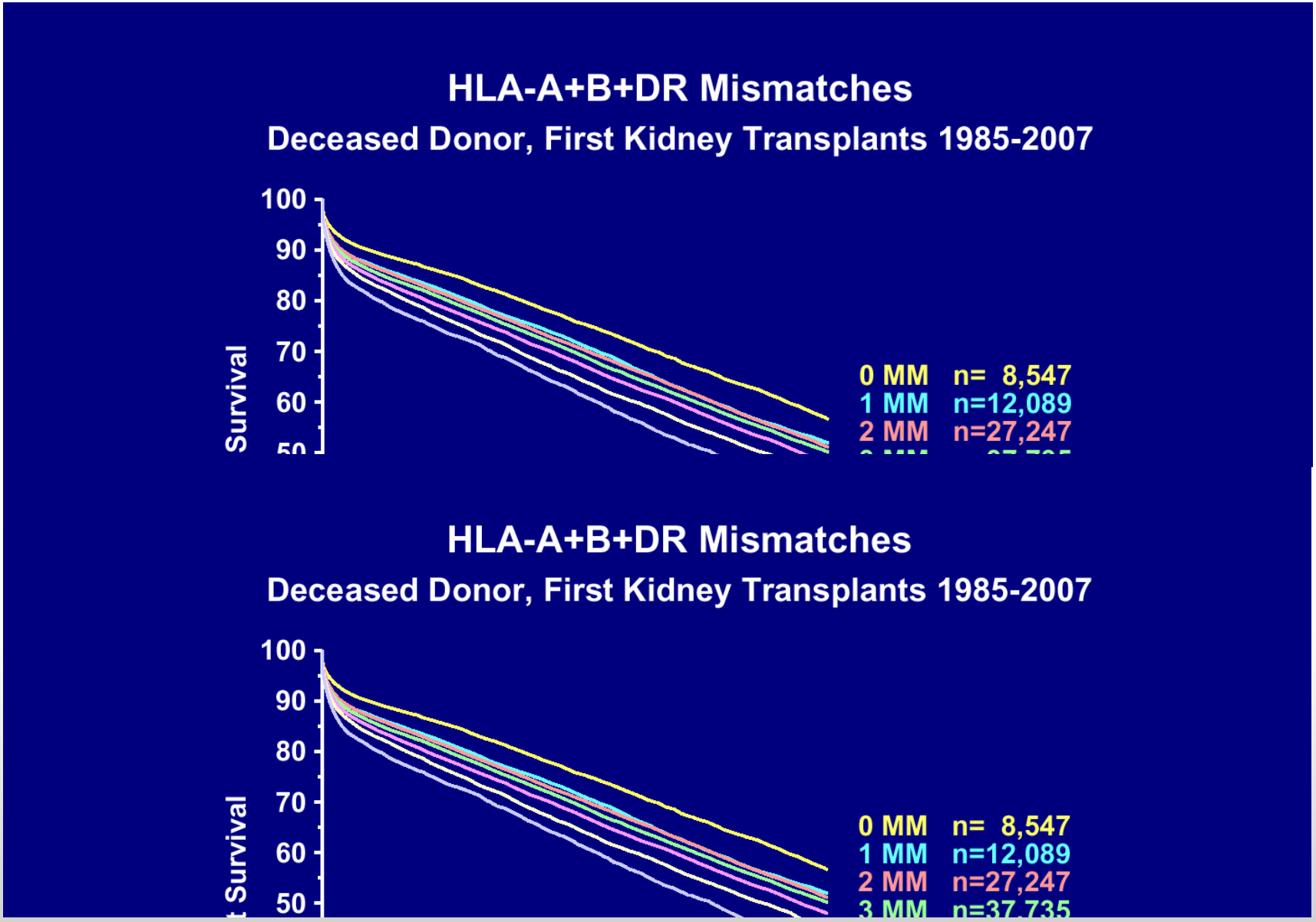
Liver: No HLA typing indicated



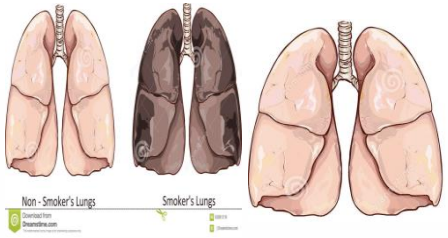
Kidney: Class I and II HLA matching but OK if only 1 or 2 out of 6 matching HLA alleles because prognosis depends more on timing of transplant

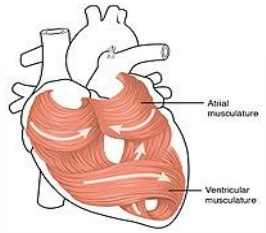


What is the magnitude of the HLA matching effect in kidney transplantation?



Heart, Lung and Liver Transplantation



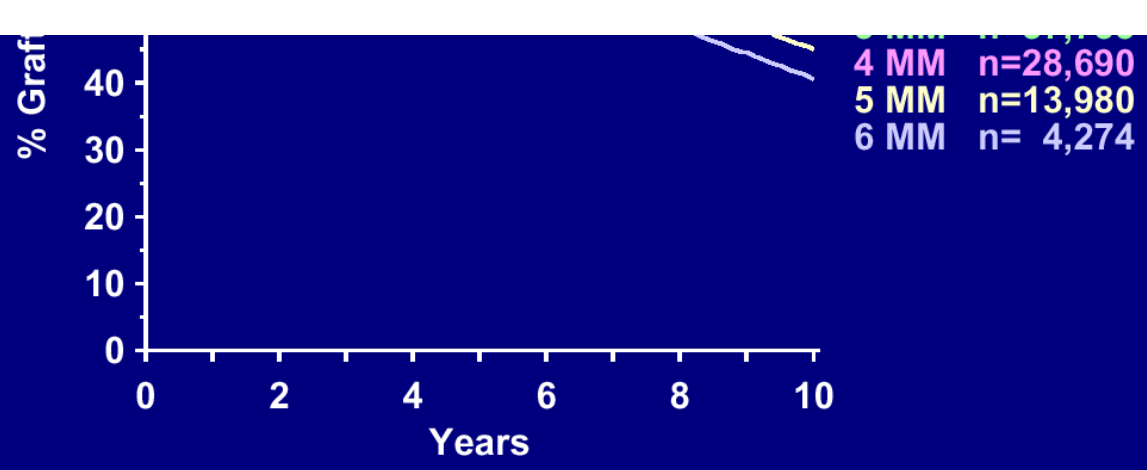


HLA matching is not applied to heart, lung and liver transplants for the following reasons:

- ✓**Patient pool is too small for matching.**
  Cold ischaemia time is too short. Hearts must be transplanted within **6-7 hours compared with 24 hours for kidneys**. Long distance organ sharing therefore is not feasible.

- ✓**Most heart, lung and liver transplants are in clinical urgence. This overides any matching considerations.**

….**But this does not mean HLA matching does not have an effect.**

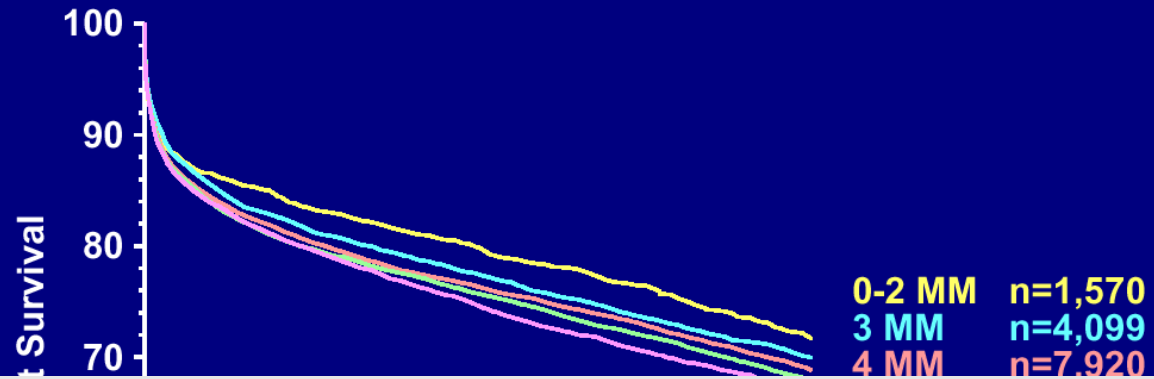


CTS Collaborative Transplant Study

K-21101-0209

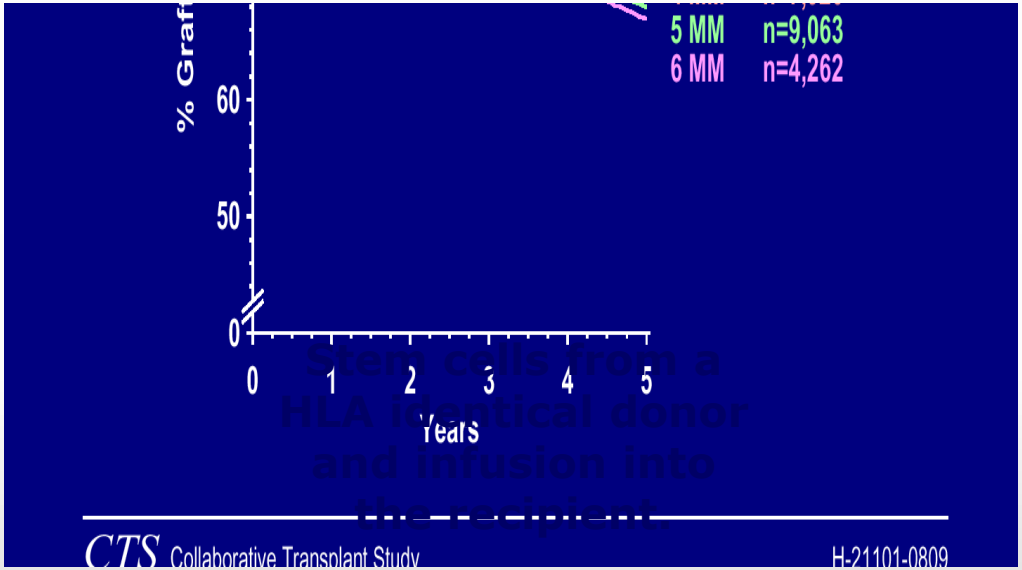
HLA-A+B+DR Mismatches

First Orthotopic Heart Transplants 1985-2007



Hematopoietic Stem cell Transplantation (HSCT)

- Replacement of patient **stem cell compartment** with one obtained from a healthy donor
- Reconstitution of a **new immune system** able to recognize recipient tissues as non-self.



Stem cells from a HLA identical donor and infusion into the recipient.

Hematopoietic Stem cell Transplantation (HSCT)

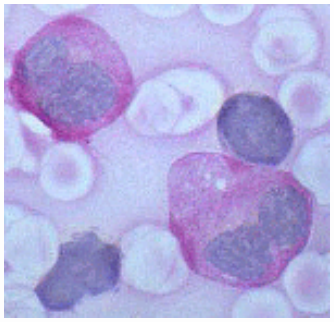
HLA genotypically identical sibling donors are the gold standard for transplantation purposes, but only 30% patients have such a donor.

For the remaining ***70% patients alternative sources of stem cells are a matched unrelated adult volunteer donor, a haploidentical donor or a cord blood unit.***

In most European populations a 10/10 matched donor can be found for at least 50% of patients and an additional 20-30% patients may have a 9/10 matched donor.

Tiercy, Haematologica, 2016

HSCT: Types of transplan



ALLOGENEIC

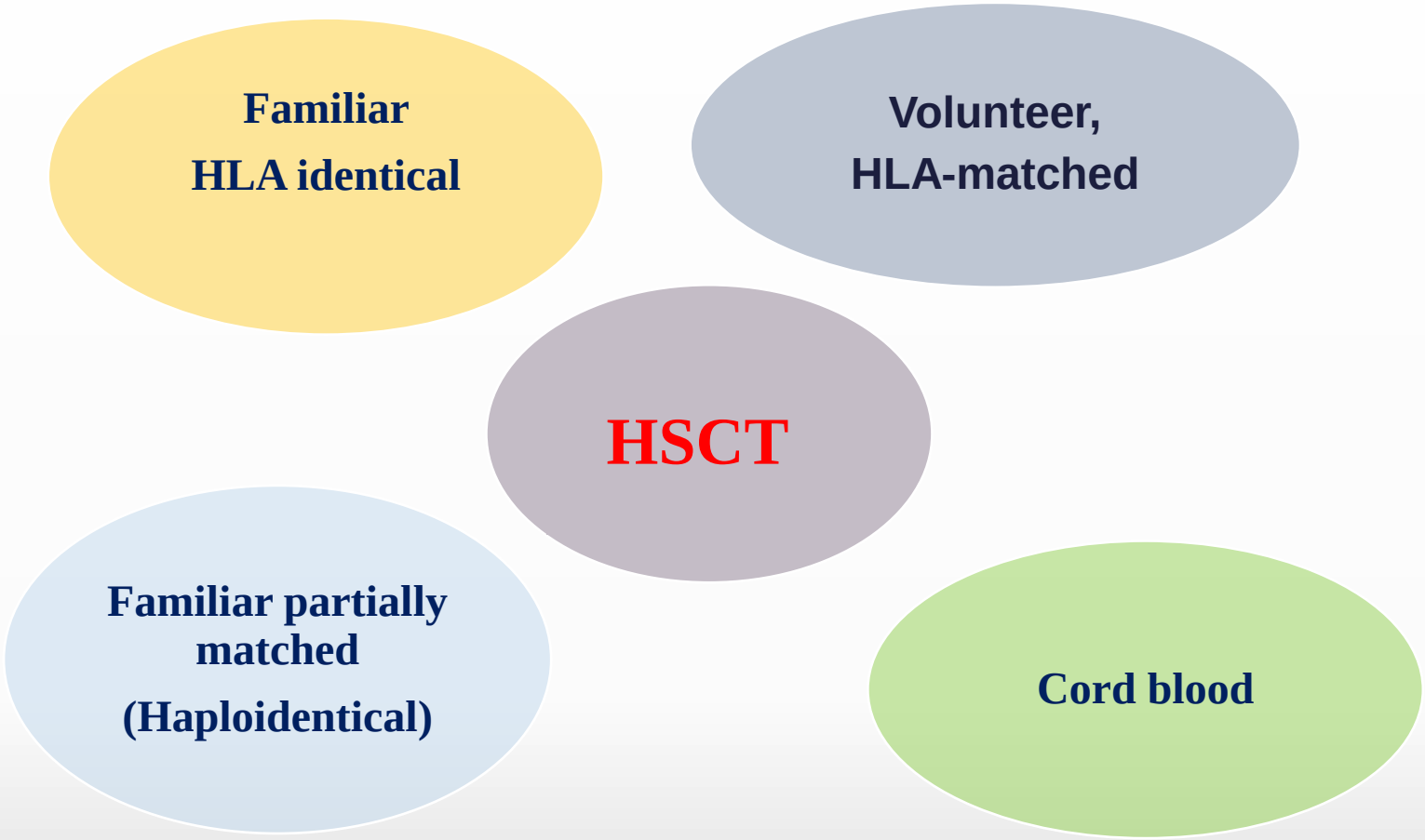
Related

Unrelated

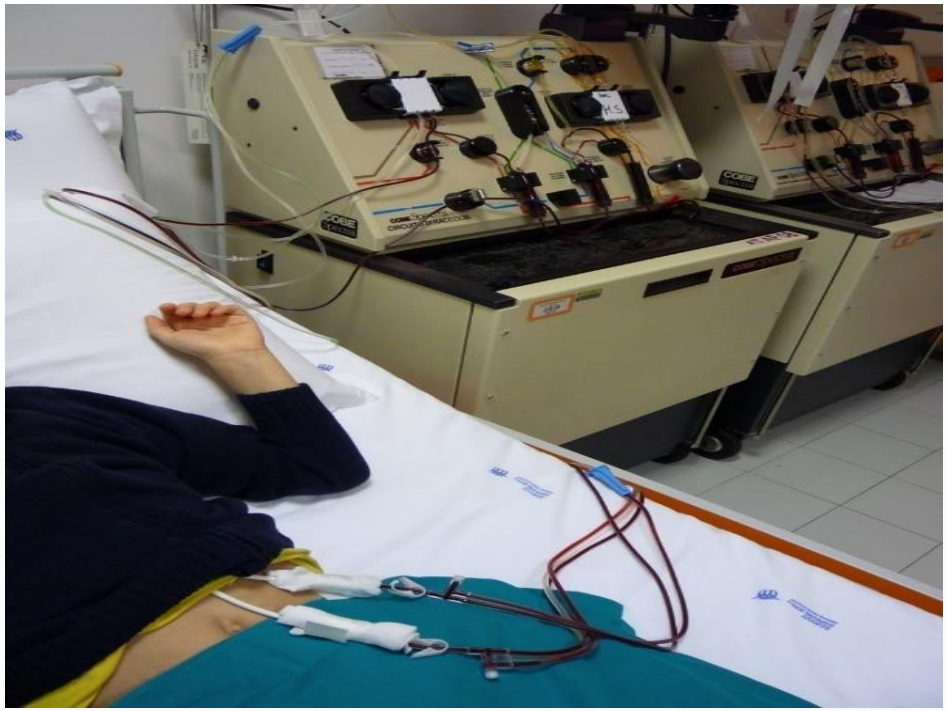
Sibling

Haploidentical

HSCT: Donor Sources



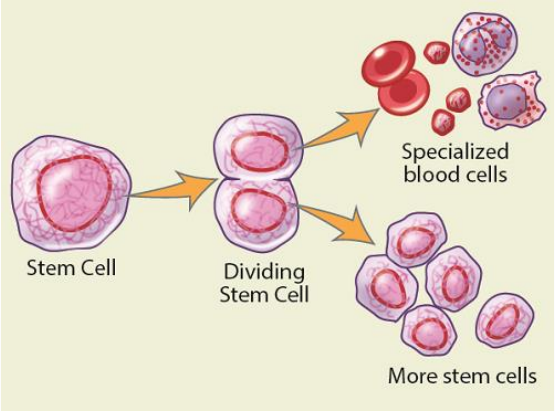
Haematopoietic Stem Cell Transplantation (HSCT) : STEM CELL SOURCES



Haematopoietic Stem Cell Transplantation (HSCT)

Types of transplants:

- Bone Marrow
- PBSC (GCSF mobilized stem cells)
- cord blood



From an immunological perspective what is the important difference between solid organ and HSCT?

Haematopoietic Stem Cell Transplantation (HSCT)

DOUBLE BARRIER :

Unlike solid organ transplantation, HSCT involves **the transfer of donor immunocompetent cells** which are **able to recognize HLA differences in the recipient**.

This leads to a **rejection reaction** directed against the recipient.

This is called **graft versus host disease (GVHD)**. GVHD targets the skin, gut and liver and can be life-threatening.

HLA disparities may cause …

Host versus graft (HVG) reaction
Host T cells *attack transplanted* ***donor stem cells*** *(leads to graft failure)*

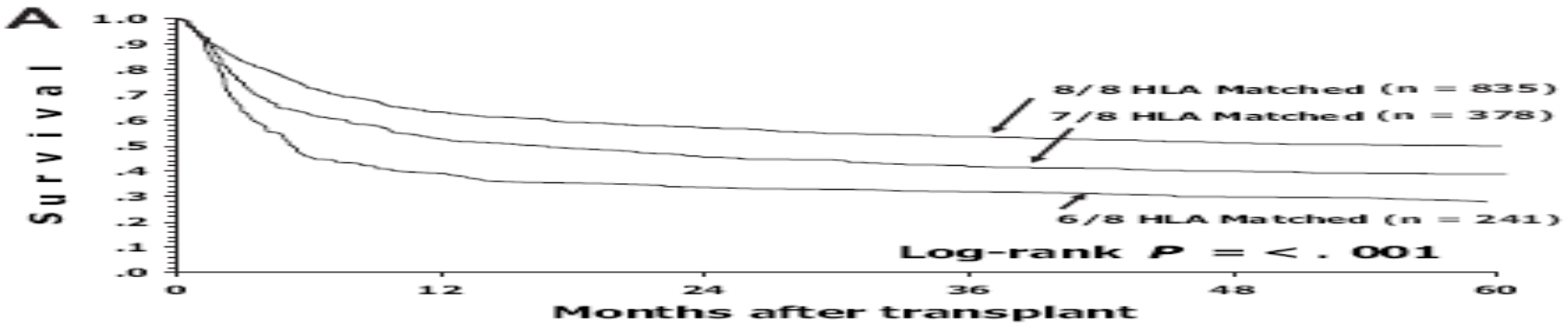
Graft versus host (GVH) reaction (30-60%)
Donor T cells *attack* ***cells of the host*** *(leads to host tissue damage, GvHD)*

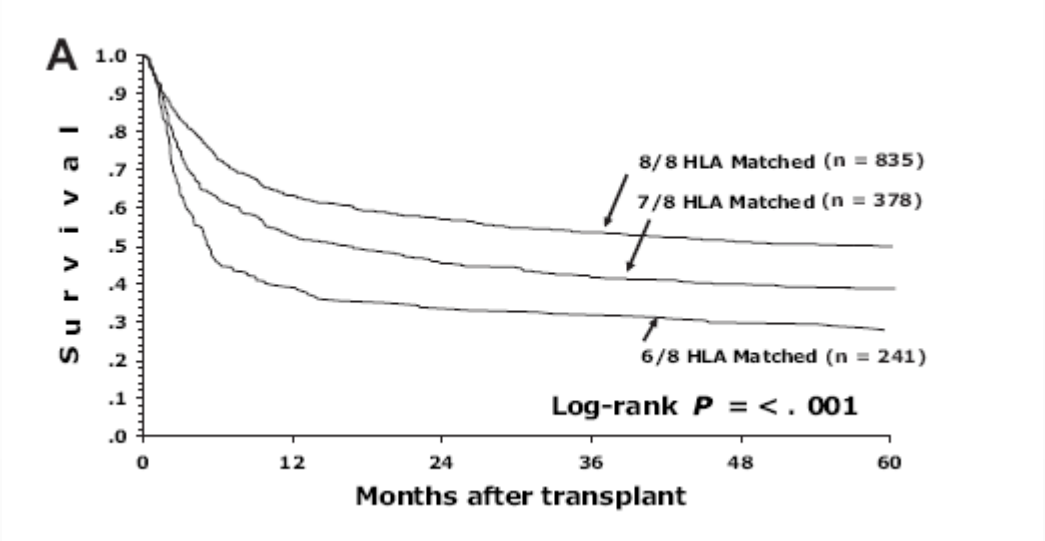
Graft versus leukemia (GVL) effect
Donor T cells attack host leukemia cells (may lead to cure of the malignancy)

Haematopoietic Stem Cell Transplantation (HSCT)

To minimize the risk of GVHD and HVG recipient and donor should be matched at the allele level at all HLA loci A,B,C,DR,DQ,DP.

If a compatible donor is not found within the family bone marrow registries are searched.

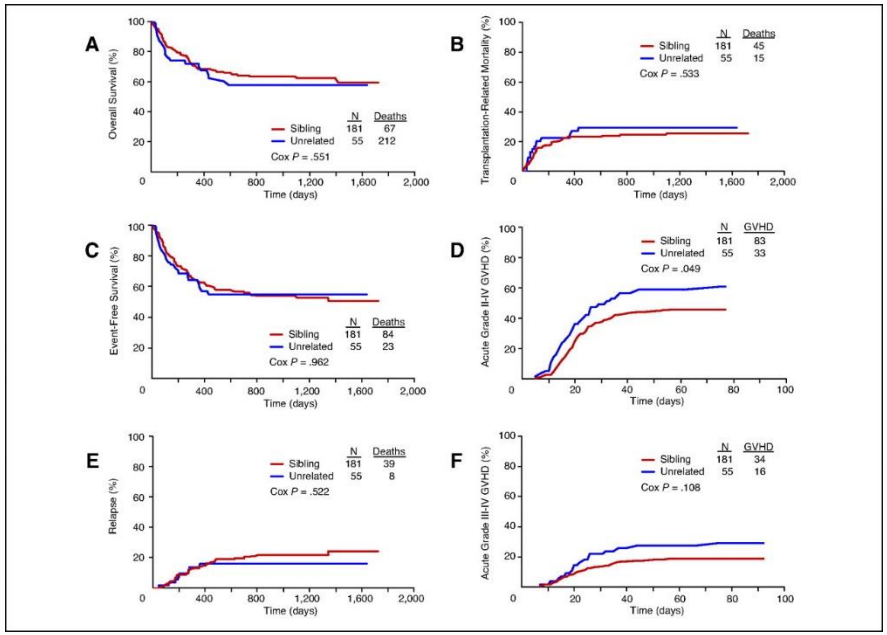




Increasing single or double HLA-A, -B, -C, and -DRB1 disparity (both antigenic and allelic) was associated with progressively higher mortality or reduced survival. A single HLA-B or -C mismatched locus was better than an -A or -DRB1 mismatch

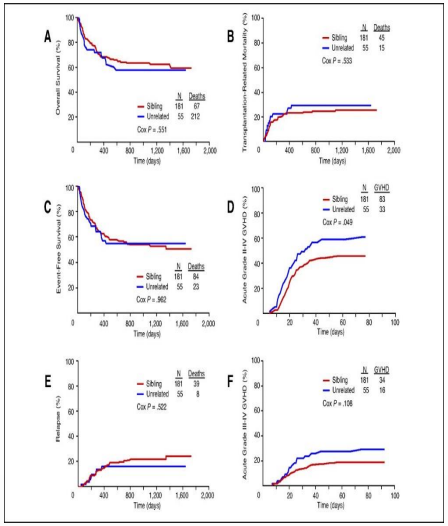
Blood 2007

HLA- HSCT



Yakoub-Agha I, et al.Clin Oncol. 2006

PROSPECTIVE In patients with standard-risk malignancy, transplantation from unrelated HLA-allellically matched donors led to outcomes similar to those from HLA-identical sibling donors

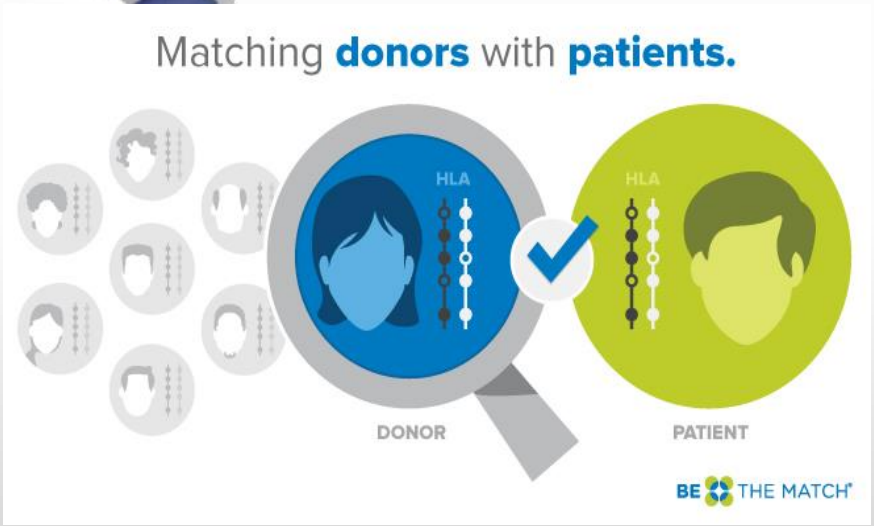


Gupta V, et al. Blood 2010

RETROSPECTIVE HLA-well-matched URD and MSD yielded similar LFS and overall survival.
LFS and OS were significantly inferior for HLA-partially-matched URD recipients, those with prior myelodysplastic syndrome, and those older than 50 years.

One prospective (Yakoub-Agha et al. 2006) and several retrospective analyses indicate that outcomes after MSD and fully MUD (8/8 or 10/10) HSCT are comparable.

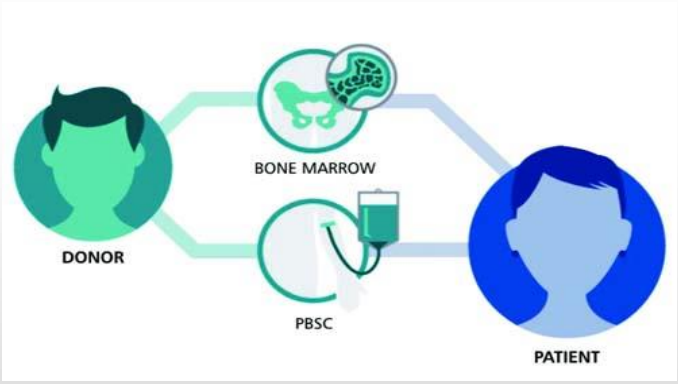
BONE MARROW REGISTRIES



Probability of finding HLA-identical Donor

- ✓Sibling 1:4
- ✓Extended family: cousin, uncle etc 1:10,000
- ✓Unrelated 1:1,000,000 Worldwide Registry of Bone Marrow Donors

2023: OVER 40 MILLION DONORS ARE NOW REGISTERED IN THE INTERNATIONAL DATABASE



Today

BMDW

Probability of finding HLA-identical Donor

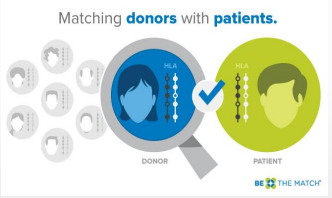
Bone Marrow Donors Worldwide (BMDW) was started in 1989, when the EBMT Immunobiology Working Party took the initiative to collect and inventory an easily accessible listing of all donors worldwide. The book was sent to transplant centers four times a year.

The first edition of the BMDW comprised the phenotypes of 8 registries from 8 countries with a total of 156,000 donors.

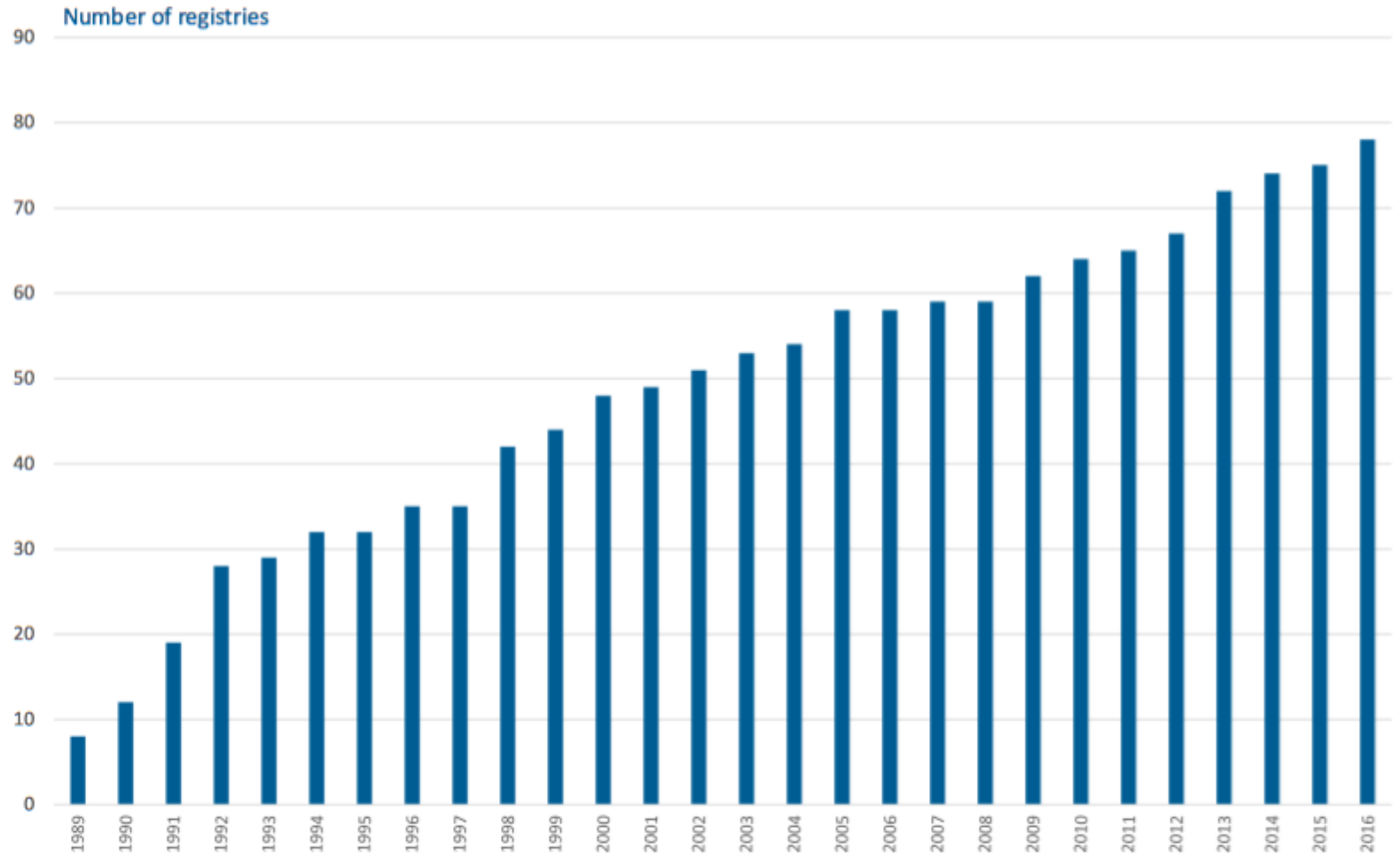
Today the BMDW comprises of more than 39,617,766 donors and 803,548 cord blood units

EBMT ORG

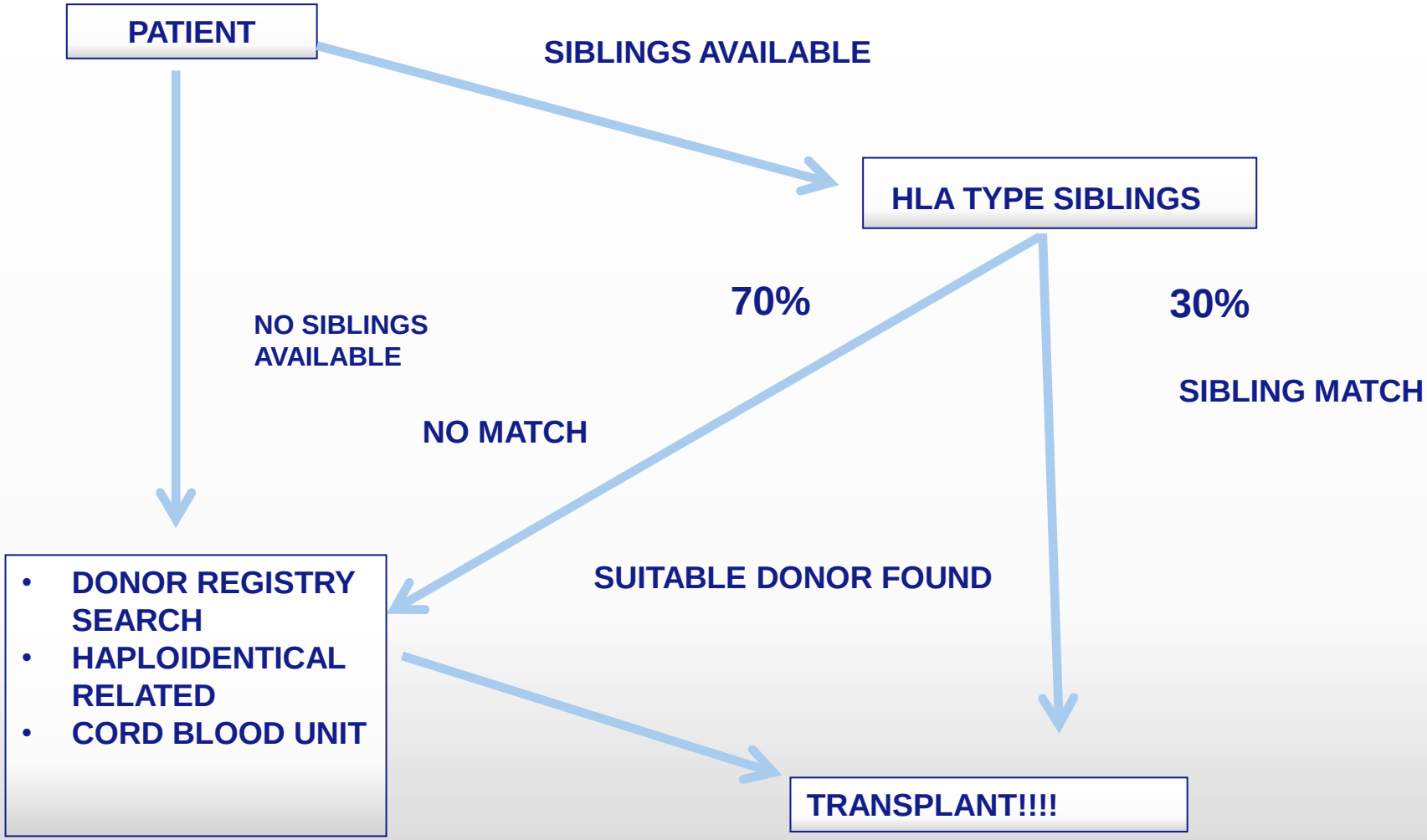
- 76 HEMATOPOIETIC CELL DONOR REGISTRIES FROM 53 COUNTRIES
- 53 CORD BLOOD BANKS FROM 36 COUNTRIES



PARTICIPATING STEM CELL DONOR REGISTRIES



Donor search approach



The path towards transplant

- All tests must be performed according to the **EFI/ASHI/ASEATTA** rules
- Only laboratories with EFI/ASHI/ASEATTA accreditation can perform immunogenetic tests for transplantation

HLA typing techniques

HLA typing techniques

Serology

Microlymphocytotoxicity Test

Molecular Biology

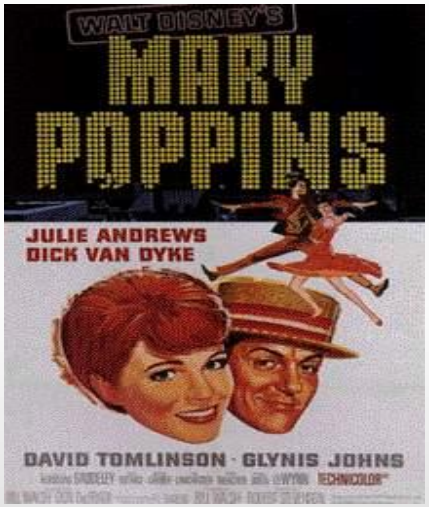
PCR-SSO

PCR-SSP

PCR-SBT

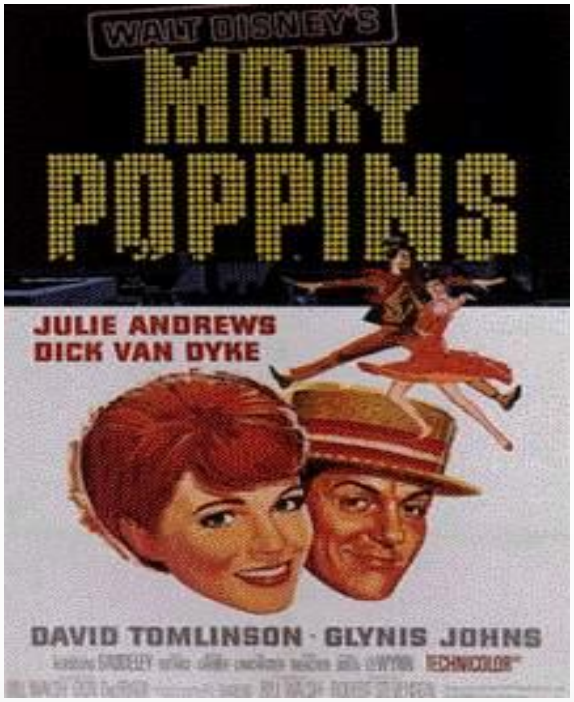
What happened in 1964 ?

- Vietnam war
- Nelson Mandela sentenced to life imprisonment
- Martin Luther King Nobel Prize for peace
- Jean Paul Sartre Nobel Prize for literature
- Tokyo Olimpic Games
- My fair lady best movie of the year
- And….



The microlymphocytoxicity test
P.I. Terasaki

Mary Poppins
W. Disney



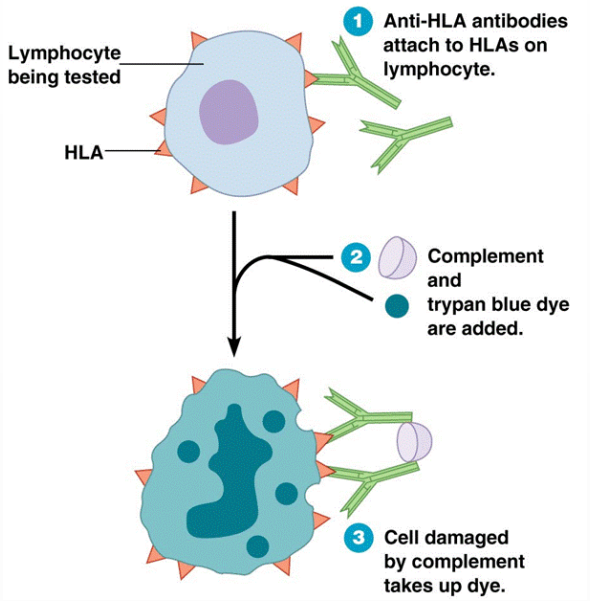
Principle of microlymphocytoxicity test

Lymphocytes are tested with a panel of sera containing well characterized **HLA-specific alloantibodies**.

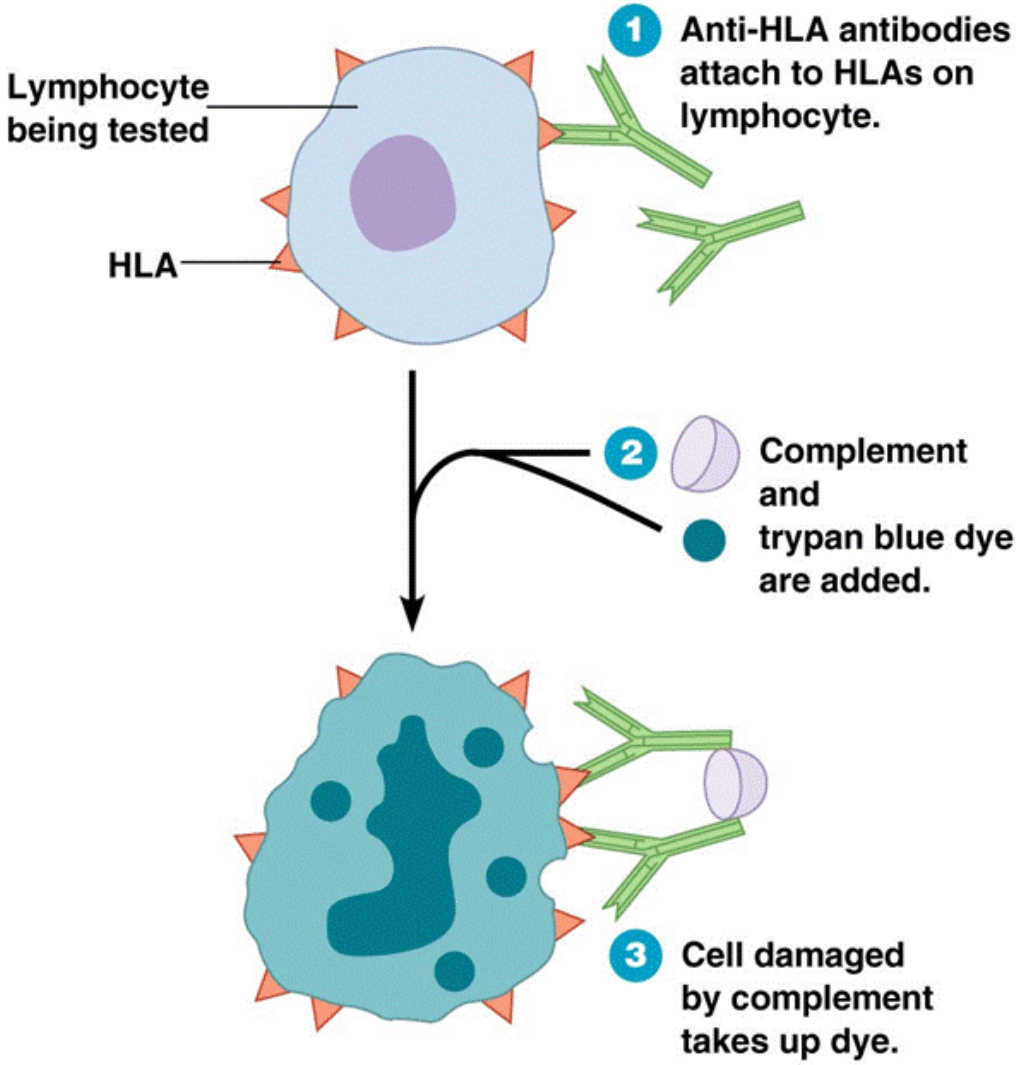
After a short incubation, rabbit serum is added as a source of complement; the complement cascade is activated through the membrane attack complex, leading to lymphocytotoxicity.

Cells that have no attached antibody, activated complement, or damage to the membrane keep the vital dyes from penetrating; cells with damaged membranes allow the dye (fluorochrome ethidium bromide) to enter.

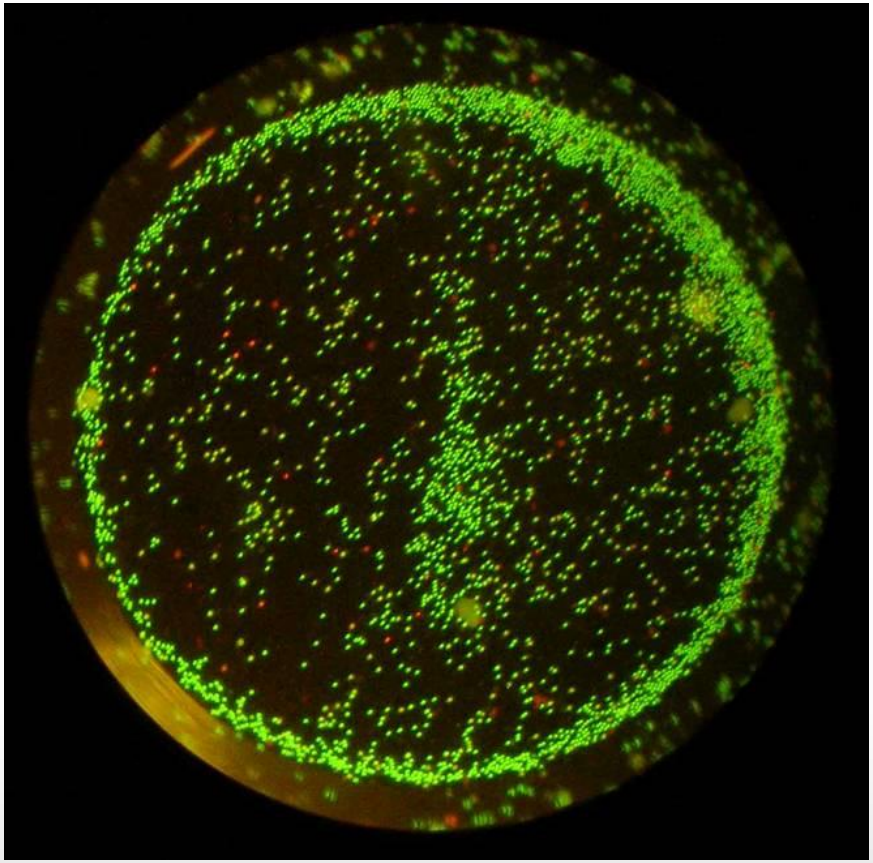
The cells are examined for dye exclusion or uptake under phase contrast microscopy. If a fluorescent microscope is available, fluorescent vital dyes can also be used.



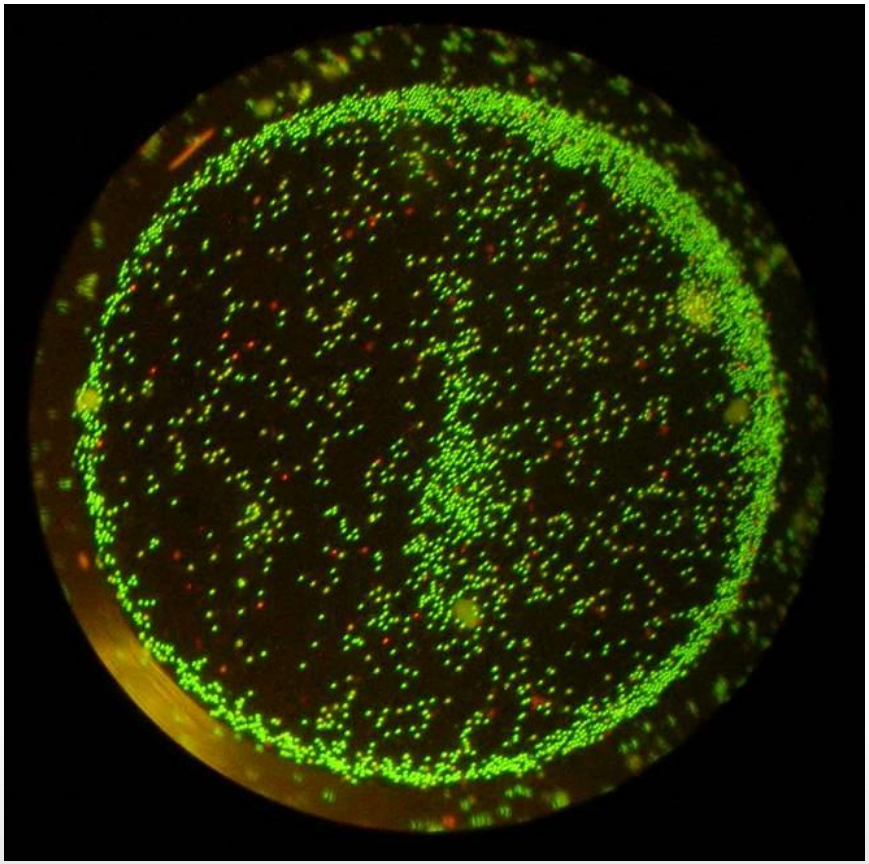
Principle of microlymphocytoxicity test



The microlymphocytoxicity test



Negative

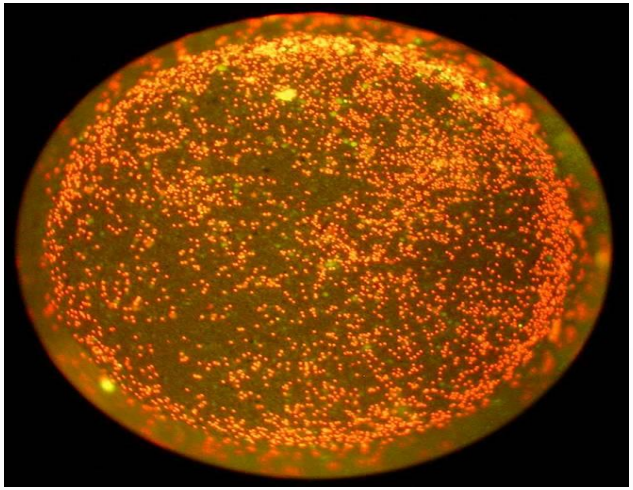


Positive

Molecular Biology

PCR (polymerase chain reaction): is a molecular genetics technique allowing the analysis of any short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA.

PCR is used to reproduce (amplify) selected sections of DNA or RNA for analysis



PCR

PCR-SSP

Sequence-Specific-Primers

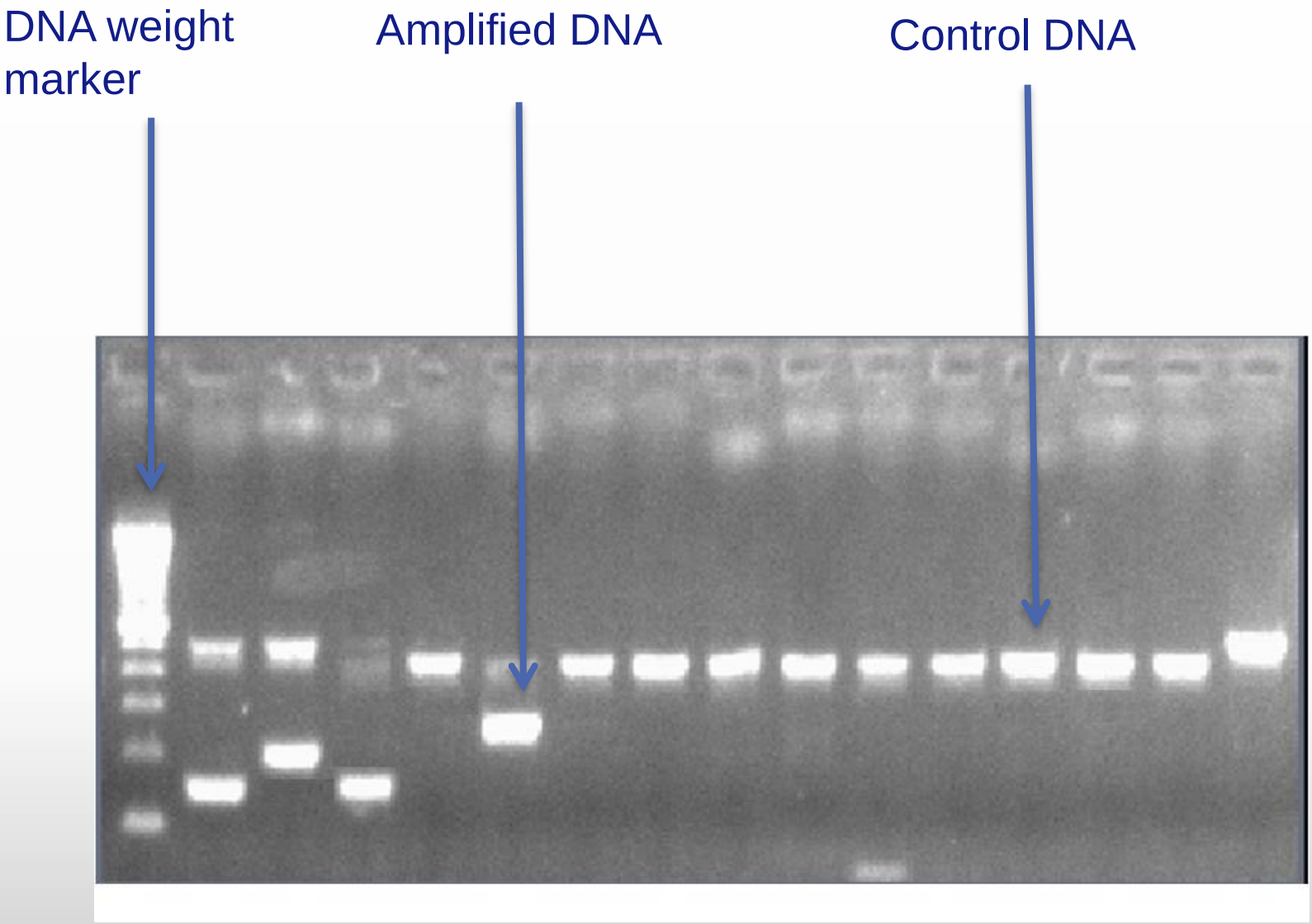
Oliglonucleotides are complementary to specific sequences of a certain allele or groups of alleles

PCR-SSO

Sequence-Specific-Oligotyping

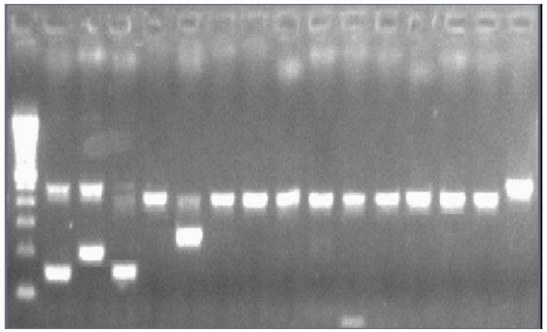
Oligonucleotides recognize common sequences to different alleles of a certain locus

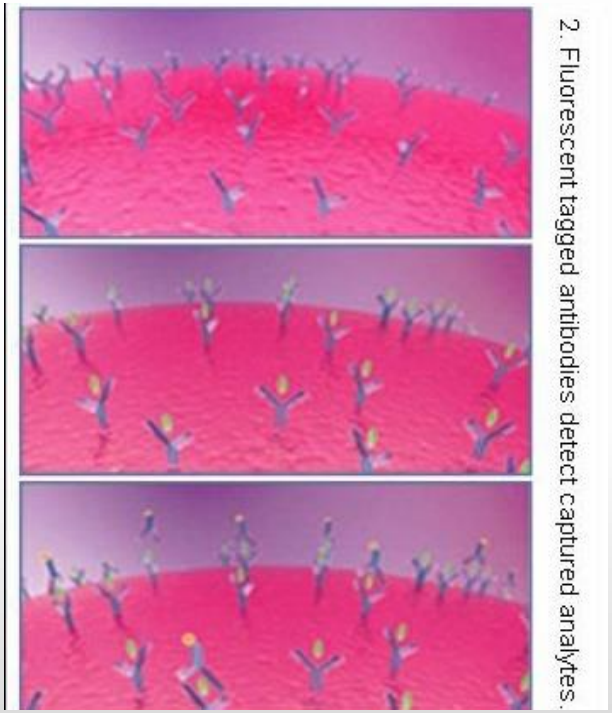
DNA picture

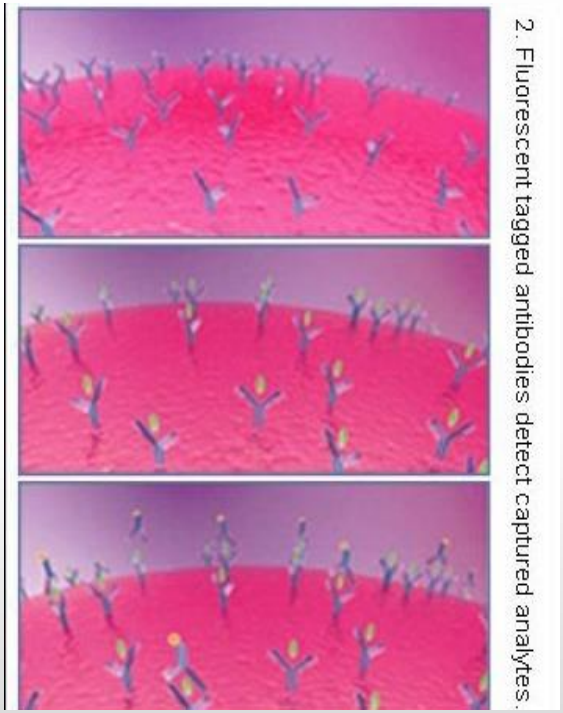


THE Luminex Technology

Assays based on the xMAP® technology use a liquid suspension array with up to 100 uniquely colour-coded bead sets. Each of the 100 beads are internally labelled with a specific ratio of two fluorophores to assign it a unique spectral address. **The beads are then conjugated with different biomolecules (including RNA, DNA, enzyme substrates, receptors, antigens, and antibodies), allowing the capture of specific analytes from the sample.** A fluorescently-labelled reporter molecule is then added to the sample in order to detect and quantitate each captured analyte. The beads are drawn through a flow cell where two lasers excite each bead. Fluorescent signals are recorded, translating the signals into data for each bead-based assay.

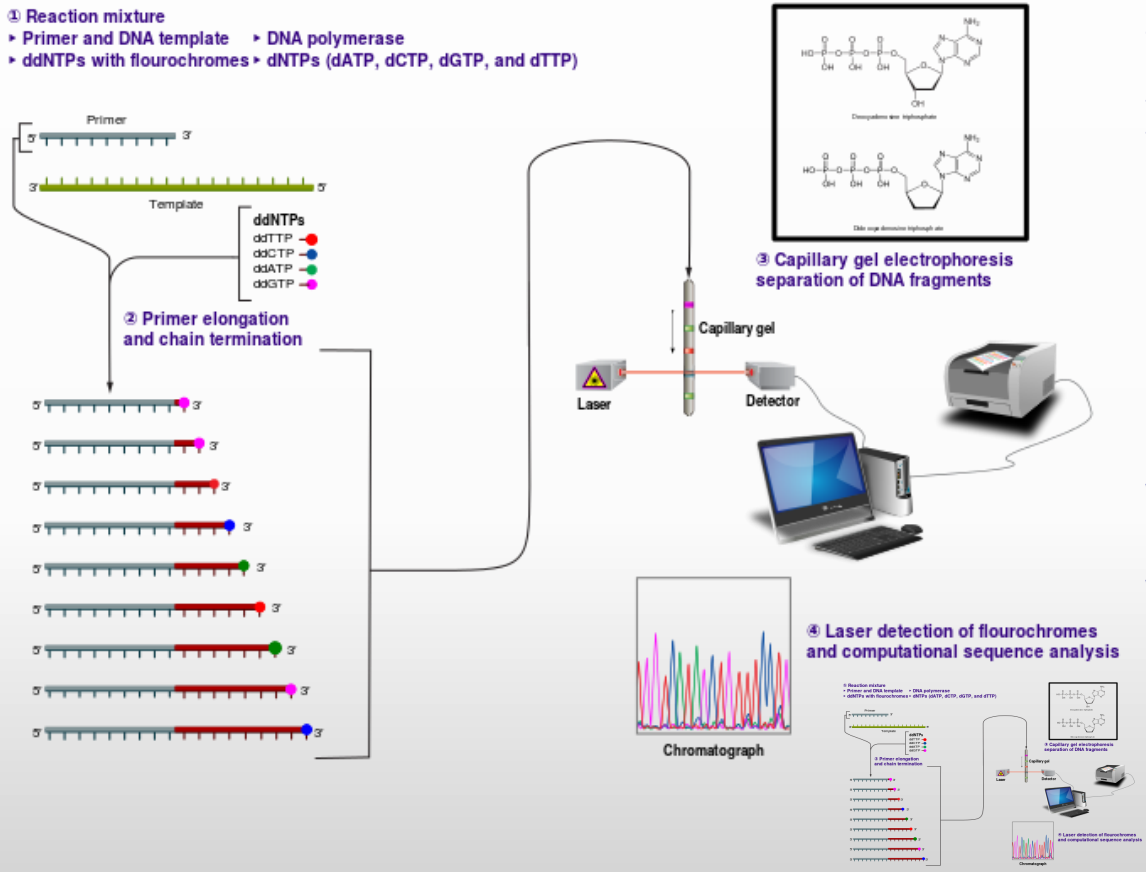






HLA Sequencing

Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides (instead of deoxynucleotides) by DNA polymerase during in vitro DNA replication.



The Sanger (chain-termination) method for DNA sequencing. (1) A primer is annealed to a sequence, (2) Reagents are added to the primer and template, including: DNA polymerase, dNTPs, and a small amount of all four dideoxynucleotides (ddNTPs) labeled with fluorophores. During primer elongation, the random insertion of a ddNTP instead of a dNTP terminates synthesis of the chain because DNA polymerase cannot react with the missing hydroxyl. This produces all possible lengths of chains. (3) The products are separated on a single lane capillary gel, where the resulting bands are read by a imaging system. (4) This produces several hundred thousand nucleotides a day, data which require storage and subsequent computational analysis.


The beauty
of the world lies
in the diversity
of its people.
- Unknown

The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications

Sung Yoon Choo

Yonsei Med J. 2007 February 28; 48(1): 11–23.

HLA TESTING IN THE MOLECULAR DIAGNOSTIC LABORATORY

Kathleen Madden. Devon Chabot-Richards

Virchows Arch 474, 139–147 (2019)

https://doi.org/10.1007/s00428-018-2501-3

YOUTUBE

ARMANDO HASUDUNGAN

IMMUNOLOGY

35 VIDEOS

https://youtu.be/6OUYvleM68M