INTRODUCTION TO
TRANSPLANT IMMUNOLOGY

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OUTLINES

- Introduction
- Immunology of graft rejection
- Antigen recognition
- Graft Rejection and Graft Tolerance
- Type of Rejection
- Immunology tests in transplantation
- Services in Transplantation Immunology Unit, AIRC, IMR
Efforts to transplant organs or tissues from one human subject to another had been unsuccessful for many decades until the discovery of the human MHC in 1967.

Identification of this genetic region launched the field of clinical organ and tissue transplantation.

In 1968, the World Health Organization Nomenclature Committee designated that the leukocyte antigens controlled by the closely linked genes of the human MHC be named HLA (for human leukocyte antigen).
Transplants are either:
- autografts (transplanting tissue within the recipient), isografts (transplanting tissue between genetically identical recipients)
- allografts (transplanting between genetically non-identical individuals but of the same species)
- Xenografts (transplanting tissue between different species).

Transplant Immunology:
- When a foreign organ, such as a kidney, is transplanted into a non-identical individual of the same species, the organ is called an allograft.
- The immune response from the recipient to the allograft is termed an alloimmune response, which is initiated by T-cell recognition of alloantigens (commonly known as allore cognition).
- Allore cognition is the first step of a series of complex events that leads to T-cell activation, antibody production, and allograft rejection.
Liver transplant recipients generally require less immunosuppression and their rejection episodes are relatively easy to treat.
The immune system is a highly complex biological system whose function is to differentiate self from non-self, and subsequently eliminate substances recognized as foreign.

It is characterized by its complexity, multiplicity of effector mechanisms, and (in higher organisms) its immunological memory.

Although these processes have evolved primarily to detect and destroy microorganisms (and cancer), the same mechanisms result in the rejection of foreign tissue or transplants.

In order for an allograft to be rejected, the immune system of the recipient must be able to recognize the transplanted tissue as foreign, and then have effector mechanisms by which the graft can be destroyed.

In clinical transplantation the recognition process is known as allore cognition, and the entire immune response is known as the allore sponse.

There are essentially three types of tissue antigens that can be recognized by the immune system and subsequently provoke an alloimmune response:

1. ABO blood group antigens
2. HLA antigens
3. Non-HLA antigens (minor histocompatibility antigens).
ABO blood group antigens are glycoproteins present on the cell membranes of red blood cells, and a wide variety of tissues including endothelium.

There are four major ABO phenotypes: A, B, O and AB.

In addition to surface antigens, individuals develop antibodies against the A and/or B antigens that are not present on the individual’s tissue.

Transplantation of an abo-incompatible organ will lead to rapid deposition of antibodies directed against non-self ABO antigens, followed by massive activation of the complement system, and other downstream effector mechanisms, leading to hyperacute rejection and graft loss in almost all organ types.
HLA antigens are a significant hurdle to clinical organ transplantation as they are a major target for the immune response.

HLA antigens are cell surface proteins whose biological function is to present small portions of proteins (peptides) for recognition by receptors of cells of the immune system.

Genes that encode for HLA antigens are found in the Major Histocompatibility Complex (MHC), a genetic region located on the short arm of chromosome 6 in humans.

MHC and HLA are terms that are often used interchangeably, though strictly MHC refers to the area of the genome, and HLA refers to the human protein structure.
## HLA ANTIGENS AND THEIR CHARACTERISTICS

<table>
<thead>
<tr>
<th>HLA Antigen</th>
<th>Structure</th>
<th>Function</th>
<th>Distribution</th>
<th>Major Subtypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA Class I</td>
<td>Single heavy chain (α chain) associated with a smaller molecule (β2 microglobulin)</td>
<td>Presentation of intracellular peptide to CD8 T cells</td>
<td>Almost all cell types</td>
<td>HLA-A, HLA-B, HLA-C</td>
</tr>
<tr>
<td>HLA Class II</td>
<td>Pair of non-identical protein chains (α chain and β chain)</td>
<td>Presentation of extracellular peptides to CD4 (helper) T cells</td>
<td>Antigen-presenting cells (APCs), e.g. dendritic cells, B cells, and macrophages*</td>
<td>HLA-DP, HLA-DQ, HLA-DR</td>
</tr>
</tbody>
</table>

*HLA Class II molecules can also be expressed on other cell types (e.g. endothelium and some epithelial cells) under the influence of pro-inflammatory signals, e.g. interferon-γ.
MINOR HISTOCOMPATIBILITY ANTIGEN

- Small endogenous peptides, known as the minor histocompatibility antigen (MiHA), which occupy the antigen-binding sites of MHC molecules and are recognised by Cd8+ T-cells in the context of self-MHC leading to graft rejection.

- MHC class 1 related chain A and B (MICA and MICB) are expressed in endothelial cells.

- Other antibodies such as anti-angiotensin-2 receptor, antiglutathione S-transferase T1 and anti-endothelial antibodies.

- Anti-endothelial antibody can be detected by using donor monocyte for cross-match.

- Some minor transplant antigens may come from mitochondrial proteins and enzymes.
In order for the recipient’s immune system to respond to foreign (non-self) antigens and reject the tissues on which they are expressed, the immune system must be able to recognize these antigens.

Recipient CD4 T cells (T-helper cells) play a central role in activating and maintaining the alloimmune response.

Recognition of foreign HLA molecules by recipient CD4 T cells (allorecognition) occurs in host lymphoid tissue through two main pathways:

- indirect pathway
- direct pathways
Indirect allorecognition occurs when recipient T cells recognize and bind to donor HLA peptides that are being presented within self-HLA on the surface of recipient APC.

The indirect pathway is analogous to the physiological pathway by which T cells normally recognize foreign protein antigens, e.g. from microbes.

The indirect pathway may be more important in promoting chronic rejection once donor APC have been destroyed.

Direct allorecognition occurs when recipient T cells recognize and bind to intact donor HLA antigens that are being presented on the surface of donor APCs.

This pathway of immune system activation is unique to transplantation as T cells only encounter donor APCs when an organ is implanted into the recipient.

The direct pathway is thought to be dominant early after transplantation.
Recognition of non-self HLA by itself is not enough to activate a naive CD4 T helper cell; co-stimulatory signals are also required, e.g. Through binding between B7-CD28 and CD40-CD154.

If this ‘second signal’ is also delivered, the CD4 Thelper cell becomes activated.

This activated CD4 Thelper cell produces cytokines and divides further. Effector pathways are then activated.

There are three main effector pathways in the **adaptive alloimmune response**:

1. Cellular cytotoxicity
2. Humoral response
3. The delayed type hypersensitivity (DTH) reaction

All three arms are initially dependent on cytokines secreted by CD4 T helper cells.

Factors such as the degree of activation of the innate immune system, antigen dose, and previous immune stimuli determine which patterns of cytokines are produced by t helper cells and therefore the effector mechanism that predominates.
- CD8 cytotoxic T lymphocytes (CTLs) are antigen-specific, lysing graft cells that bear non-self HLA class I.
- CTLs cause tissue destruction through the release of perforins and granzymes, and through fas-mediated apoptosis.

- CD4 T cells within the spleen and lymph nodes provide activatory signals to alloantigen-specific B cells, leading initially to an IgM response.
- With continued B cell stimulation, alloantigen-specific B cells alter the class of immunoglobulin (antibody) that they produce, leading to the production of IgG antibody with high antigen affinity.
- Activation of the complement cascade by antibodies bound to target antigens leads to generation of the membrane attack complex (C5b-9).
- This complex can perforate cell membranes leading to cell lysis and death.
- Antibodies can damage donor organs through complement-independent mechanisms also; antibodies bound to target cells interact with natural killer cells and macrophages leading to release of cytotoxic granules and cell apoptosis.

- The DTH reaction is a strong local inflammatory response within the rejecting allograft, dependent on the production of pro-inflammatory cytokines (e.g. TNF-a and IFN-g) by CD4 T cells.
- These CD4 T cells also activate macrophages, leading to further tissue damage and phagocytosis of dying graft cells.
Graft Rejection

- Rejection of transplanted kidney involves both adaptive (T and B cells) and innate (dendritic cells, macrophages, neutrophils, mast cells and natural killer cells) immune system.

- The innate immune system is activated by DAMPS, cytokines and chemokines released due to ischaemia-perfusion injury and microbial products, which once activated, triggers the adaptive immune system leading to cellular rejection.

- On the other hand, the activation stimulus to adaptive immune system is the alloantigens.

Graft Tolerance:

- In the transplant setting, tolerance can be defined as the absence of a damaging alloantigen-specific immune response without the need for ongoing exogenous immunosuppression and with an intact response to other antigens.
<table>
<thead>
<tr>
<th>Types of rejection, their characteristics and treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rejection type and usual time of onset</strong></td>
</tr>
</tbody>
</table>
| Hyperacute rejection (minutes to hours) | - Presence of pre-formed antibodies (either anti-ABO or anti-HLA) in high volumes directed against the graft  
- Rapid deposition of antibodies on the graft activates the complement cascade, leading to cell destruction, graft thrombosis and necrosis | - If it occurs it is irreversible  
- Can be prevented by transplanting ABO-compatible organs into recipients without significant volumes of anti-donor HLA antibodies |
| Acute rejection (weeks to months) | - Cell-mediated: macrophages, CD8 T cells and neutrophils within the graft causing organ damage  
- Antibody-mediated: anti-donor antibodies produced by B cells causing complement deposition, etc. | - Cell-mediated: mild-moderate episodes can be treated with high-dose IV methylprednisolone; severe episodes require additional anti-thymocyte globulin (ATG) to deplete T cells  
- Antibody-mediated: IV methylprednisolone + plasmapheresis + IVIG (consider splenectomy, eculizumab, ATG also) |
| Chronic rejection (months to years) | - Chronic, indolent alloimmune responses (cell- or antibody-mediated) | - Optimize immunosuppressive therapy  
- Control other long-term risk factors for graft dysfunction (BP, lipids, smoking) |

BP, blood pressure; IV, intravenous; IVIG, intravenous immunoglobulin.
TRANSPLANT IMMUNOLOGY TESTS

- HLA molecular typing
- Donor Specific Antibodies and non-specific panel reactive antibodies (PRA)
- Crossmatching (CXM): Complement-dependent Cytotoxicity (CDC) & Flowcytometry CXM
- HLA class I-specific memory B cell ELISPOT assay
- Antigen-specific assays for measuring tolerance
- Phenotypical studies of circulating immune cells
1. **PCR-sequence specific primers (PCR-SSP):** only primers whose sequences are complementary to that of the target sequence of a DNA samples present will bind to this DNA (need higher number of DNA and limited number of test)

2. **PCR-sequence-specific oligonucleotide probes (PCR-SSO):** 100 types microspheres (beads) are dyed to create 100 distinct colors each microsphere type is coated with different sequence specific oligonucleotide (HLA allele)- larger number of test, still have HLA ambiguity

3. **Sequence-based typing (SBT):** polymorphic regions are amplified by PCR and then sequenced (gold standard)- expensive equipment

4. **Next generation sequencing (NGS):** PCR-based aiming at amplifying either multiple exons at once as large DNA fragments, or selected polymorphic exons separately -able to solve HLA ambiguity in SBT, very expensive equipment
PCR-SEQUENCE-SPECIFIC PRIMER (PCR-SSP)

• Only primers whose sequences are complementary to that of the target sequence of a DNA samples present will bind to this DNA

• Amplification will take place in the polymerase chain reaction (PCR)

• Noncomplementary primers do not bind to the DNA → no amplification
PCR-SSP: PRINCIPLE

- The amplified DNA is determined using agarose gel electrophoresis.
- Successful amplification will appear as a band in the gel.

Perfect match $\rightarrow$ Amplification by Thermal cycler (Specific Allele)

Mismatch $\rightarrow$ no Amplification (unspecific Allele)
Negative control well

Negative reaction

Positive reaction
PCR-SSP:

Advantages:
- A very rapid and sensitive method

Limitations:
- Need higher DNA concentration/ high volume of DNA
- Limited number of tests
- Need Gel preparation for determination of the amplified DNA.
- Exposed to Ethidium Bromide (staining step)
SEQUENCE- SPECIFIC OLIGONUCLEOTIDE PROBES (PCR-SSOP)

- 100 types Microspheres (beads) are dyed to create 100 distinct colors
- Each microsphere type is coated with different sequence specific oligonucleotide (HLA allele)

red laser for bead classification
green laser for assay result

Tells the instrument which bead is being examined
Tells the instrument how much DNA is bound to the bead
BEAD REACTION AND SOFTWARE ANALYSIS

Distribution of particular bead from other labs

Distribution of bead for the tested samples in our lab

A*02, A*24
SSOP: PRO AND CONS

- Fairly rapid
- Higher number of test/run
- High(er) resolution
- Less DNA concentration/volume DNA needed
- No gel preparation & exposure to Ethidium Bromide

- Expert and well trained staff to analyse the results
- Expensive equipment
SEQUENCE-BASED TYPING (SBT)

- Sequence-based typing (SBT) is high resolution.
- Polymorphic regions are amplified by PCR and then sequenced.
- Gold standard
Sequences are compared to reference sequences for previously assigned alleles.
Advantages

• Gold standard for HLA typing
• Detect novel alleles

Limitations

• Expensive
• Sequencers are costly
• Requires high skilled technologists
• Cis/trans polymorphisms – ambiguous result – require additional testing
NEXT-GENERATION SEQUENCING (NGS)

- PCR-based aiming at amplifying either multiple exons at once as large DNA fragments, or selected polymorphic exons separately.

- NGS – able to overcome the limitations of SBT

<table>
<thead>
<tr>
<th>SBT</th>
<th>NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA genotyping derived from <strong>few exons</strong> of HLA class I &amp; II genes</td>
<td>HLA genotyping derived from <strong>all exons</strong> of HLA class I &amp; II</td>
</tr>
<tr>
<td><strong>Fails to solve</strong> cis/trans chromosomal polymorphic positions of HLA alleles</td>
<td><strong>Able to solve</strong> cis/trans chromosomal polymorphic positions of HLA alleles</td>
</tr>
</tbody>
</table>
(A) Sanger sequence-based HLA-typing

Sample

DNA extraction

PCR amplification of a single HLA gene

Non-clonal sequencing (Sanger method)

Allele-specific PCR or allele-specific sequencing primers

2-3 X

Ambiguous typing result

Analysis

Genotype for one HLA gene

(B) NGS-based HLA-typing

Sample

DNA extraction

PCR amplification of multiple HLA genes

Multiplex barcoded library preparation

HLA-sequence capture of multiple HLA genes

Sequencing template preparation (emulsion PCR or bridge amplification*)

Clonal sequencing

Analysis

Genotype for all HLA genes
Advantages

- the possibility to start with a low quantity of DNA (a few nanograms)
- With the large amount of reads, offering the possibility to sequence entire genes for several hundreds of samples at once.

Limitations

- Expensive
- Requires high skilled technologists
- Difficult to design primers that amplify the vast majority of the known HLA alleles
In deceased donor kidney transplantation, differences between the donor and recipient HLA types (HLA ‘mismatches’) are minimized, especially at the HLADR, -B, and -A loci (in descending order of importance).

Large studies have shown that kidney transplants in recipients with minimal HLA mismatches to their donor have lower rates of acute rejection and longer graft survival.

Because there are potentially two different HLA molecules expressed at each locus (one paternal-derived, one maternal-derived), and three important loci (-DR, -B, and -A), there are between 0 and 6 possible HLA mismatches between the donor and the recipient.

### Examples of donor and recipient HLA mismatches

<table>
<thead>
<tr>
<th>Donor-recipient pair 1 (mismatch 0-0-0)</th>
<th>Donor HLA type:</th>
<th>A5, A2, B13, B7, DR1, DR3</th>
<th>Recipient HLA type:</th>
<th>A5, A2, B13, B7, DR1, DR3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor-recipient pair 2 (mismatch 0-1-2)</td>
<td>Donor HLA type:</td>
<td>A5, A2, B13, B7, DR1, DR3</td>
<td>Recipient HLA type:</td>
<td>A5, A2, B13, B7, DR8, DR9</td>
</tr>
<tr>
<td>Donor-recipient pair 3 (mismatch 2-2-2)</td>
<td>Donor HLA type:</td>
<td>A5, A2, B13, B7, DR1, DR3</td>
<td>Recipient HLA type:</td>
<td>A1, A10, B16, B37, DR11, DR12</td>
</tr>
</tbody>
</table>
Sensitisation is defined by the presence of antibodies in the recipient blood against a panel of selected HLA antigens representing donor population, which is reported as percentage panel reactivity antibodies (PRA).

PRA estimates the likelihood of positive crossmatch to potential donors.

The higher is the PRA level; the lower becomes the chance of receiving compatible kidney and longer the waiting time on the waitlist.

Sensitisation is caused by exposure to HLA antigens through failed previous transplants, pregnancy and blood transfusion.

The percent PRA in an individual may vary from time to time due either to a change in the antibody titre, or a change in the usage of HLA antigens in the assay.

The technology of PRA assay has advance significantly from the initial CDC assay to solid phase based enzyme linked immunosorbent assay (ELISA), to a current multiplexed particle-based flow cytometry (Luminex).

Single antigen beads are increasingly used to characterise the preformed HLA antibodies before renal transplant (RT) and to detect development DSA after RT.
Patients can generate anti-HLA antibodies if they receive blood transfusions, have had previous organ transplant that have subsequently failed, or have been pregnant. These are known as ‘sensitizing events’.

Patients on the kidney transplant waiting list have blood samples taken at least every 3 months to check for new anti-HLA antibodies.

High volumes of anti-HLA antibodies against a large number of different HLA molecules make it less likely that a suitable donor organ will be found.

The degree to which a potential recipient is sensitized can be described using calculated reaction frequency (CRF).

This calculates the percentage of the last 10,000 blood-group compatible deceased donors in the UK that the potential recipient would have had significant HLA antibodies against.

A CRF of 0% implies that the patient has no anti-hla antibodies, while a CRF of 100% implies that they have anti- hHLA antibodies against all 10,000 previous donors.
Donor-specific antibodies (DSA) are measures routinely post-transplantation to detect the reactivity of the recipient B cells against the donor antigens.

DSA: HLA or non HLA antigen (eg. MHC class I polypeptide-related sequencea or B (MICA and MICB), smooth muscle antigen (vimentin), collagen-v and cell surface receptor such a type I angiotensin II receptor are reliably detected by currently available techniques.

If the titre of the specific DSA rises suggesting inadequate immunosuppression, several therapeutic options, including plasmapheresis, thymoglobulin, intravenous immunoglobulin (IVIG), and anti-cd20 antibody (rituximab) can be attempted.

Emerging therapies include proteasome inhibitors such as bortezomib.

Several studies have suggested that DSA to HLA antigens and endothelial antigens may be a driver not only of acute antibody-mediate rejection but also chronic rejection.
HLA ANTIBODIES TEST

- Recipient serum potentially containing anti-HLA antibodies is added to a mixture of synthetic beads.

- Each bead is coated with a set of antigens (screening beads) or for more precise detail, with a single antigen (single antigen beads).

- A unique dye signature (up to 100) specifies the identity of each bead (A).

- If anti-HLA antibodies are present these will bind to the appropriate bead (B) and

- a detection antibody can subsequently bind and capture a reporter dye (C).

- Each unique bead can then be interrogated for the presence of the reporter dye on its surface using a dual beam laser (D).

- A profile of antibodies can thus be identified in the recipient and compared with the known HLA identity of any potential donor, allowing a prediction of the crossmatch result.
LABScreen Panel Reactive Antigens (PRA)

LABScreen Single Antigen Beads (SAB)
PRA CLASS I - PATIENT: A68; A02
DONOR HLA : A02
DONOR SPECIFIC ANTIBODY: A02 (MFI: 1602.78)
Single Antigen Beads (SAB)

DSA = Cw7
When a potential donor is identified, an HLA crossmatch with fresh serum from recipient and lymphocytes from donor has to be performed to exclude preformed DSA, which can lead hyperacute AMR.

The final crossmatch must be negative to proceed with renal transplant.

Complement dependent cytotoxicity (CDC-XM) crossmatch and flow cytometry crossmatch (FC-XM) are two commonly used methods, although the choice is dependent on individual centre experience and their availability.

T cells express HLA class I antigens only, while B cells express both HLA class I and class II antigens.

Furthermore, there is significantly increased expression of HLA class I antigens in B cells compared to T cells.

A positive T cell crossmatch is considered as true and significant sensitisation with DSA against HLA class I antigens.

A negative T cell crossmatch and a positive B cell crossmatch may represents presence of either HLA class II antibodies or low titre of HLA class I antibodies.

A positive T cell crossmatch associated with a negative B cell crossmatch is likely to be due to the presence of non-HLA antibodies.
Lymphocytes from the donor are isolated and separated into T and B cells.
Serum from the recipient is mixed with the lymphocytes in a multi-well plate.
Complement is then added (usually derived from rabbit serum).
If donor-specific antibody is present and binds to donor cells, the complement cascade will be activated via the classical pathway resulting in lysis of the lymphocytes.
## CDC-XM Scoring System

<table>
<thead>
<tr>
<th>% of Dead Cells per Well</th>
<th>Score</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>11-20</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>21-50</td>
<td>4</td>
<td>Weak Positive (++)</td>
</tr>
<tr>
<td>51-80</td>
<td>6</td>
<td>Positive (+++)</td>
</tr>
<tr>
<td>81-100</td>
<td>8</td>
<td>Strong Positive (+++)</td>
</tr>
</tbody>
</table>

- **Positive CDC at 4°C**: Presence of IgM in patient’s serum
- **Positive CDC at room temperature (25°C)**: Presence of IgM and IgG in patient’s serum
- **Positive CDC at 37°C**: Presence of IgG in patient’s serum

**Negative Reaction**
Cells appear translucent and do not absorb the Eosin Y stain.

**Positive Reaction**
Cells are enlarged and opaque due to the Eosin Y stain penetrating the cell membrane.
Flow Cytometry Crossmatch (FCXM)
Flow Cytometry Crossmatch (FCXM)

- Performed using the same initial base ingredients as CDC-XM & was first described in 1983
- Flow crossmatching is performed using the same initial base ingredients as CDC crossmatching (i.e. donor lymphocytes and recipient serum)
- The two are mixed to allow antibody binding and after washing, a CD marker for T (CD3) and B (CD19) cells with different fluorochromes AHG is added to bind attached DSAbs and hence allow detection by flow cytometry.
- Mean Flourescence Intensity (MFI) correlating with the amount of antibody binding to the cells is measured by a flow cytometer
- FCXM is much more sensitive than CDC in detecting low level antibodies.
FCXM Results

Negative FCXM

Positive FCXM
The antigens corresponding to preformed HLA antibodies are considered unacceptable for that patient.

So a patient will not be offered a kidney from a deceased donor, who expresses an unacceptable HLA antigen (positive virtual crossmatch).

Only those patients whose hla antibodies are not donor directed will appear on the matching run (negative virtual crossmatch).

Virtual crossmatch is increasingly used in clinical practice as this has improved the efficiency.
Predicting the outcome of compatibility between a donor and recipient without performing the actual crossmatch

Increases access to transplantation of highly sensitized patients

Requirements:

i. Pre-screening and profiling of HLA antibodies in candidates on waiting list (Luminex PRA & SAB)

ii. HLA typing of the potential donor (PCR-SSP or PCR-SSO)
Virtual Crossmatch (VXM)

- Virtual crossmatching refers to the comparison of the anti-HLA antibodies of the recipient (as defined by Luminex), with the HLA of the donor.
- If there is a DSAb present this would represent a positive virtual crossmatch.
- Antibodies are defined against HLA class I and II antigens.

**Prediction:** Antibody mediated rejection on allograft.
There also are situations in which patients have been previously immunized, but no serum HLA antibodies are detectable at time of transplantation.

In these patients, donor-reactive memory b cells may be present that can rapidly respond upon rechallenge with allo-antigens present on the transplanted organ.

Similarly, after desensitization strategies for those patients having high HLA antibody levels, there may be memory B cells present despite the reduction of HLA antibody titers.

It has been previously shown that drugs included in standard immunosuppressive protocols are not all directly targeting B cells.

Therefore, the clinical consequences of these pre-existing donor-reactive memory B cells upon transplantation are yet to be fully elucidate
HLA class I-specific memory B cell ELISPOT assay that allows for determining the level of donor-reactive memory B cells prior to transplantation.

This assay is based on polyclonal activation of peripheral blood B cells, followed by the detection of HLA class I-specific b cells by monomeric HLA molecules in ELISPOT format.

It can detect HLA class I specific memory B cells in pregnancy-immunized females, as well as in HLA-immunized patients on the transplant waiting list.
Ex-vivo assessments of donor antigen-specific T cell responses were explored early on as a way to detect or predict tolerance.

Those assays measured either:

1. Proliferation of recipient T cells in a mixed lymphocyte reaction (MLR)
2. Lysis of donor T cells in a cytotoxic T lymphocyte assay (CTL)
3. Production of interleukins with either an enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot assay [interferon (IFN)-γ ELISPOT] or intracellular staining.

Assays that are able to explore the indirect pathway over traditional MLR and CTL, and have tried to focus upon the most relevant memory T cells that are only weakly eliminated by traditional depleting agents.

Indirect allore cognition testing is feasible on condition that donor antigens be presented as protein lysates together with recipient antigen presenting cells.

In kidney transplantation, this is performed via elispot or trans-vivo delayed-type hypersensitivity (tvdth) assays.
As far as impact of rejection on kidney graft outcome is concerned, particularly steroid-resistant rejection episodes have a negative impact on graft survival rates.

With a combination of several activated T cell- (CD25, lag-3, granzyme-b) and macrophage-expressed (mannose receptor, S100A9) transcripts, it was possible to distinguish steroid resistant rejections from steroid-responsive rejections with an overall predictive value of 70%.

Inclusion of metallothionein expression levels further enhanced the predictive power of this model.

Micro RNAs in the urine, and the extent of cell-free donor DNA in the recipient's plasma, are diagnostic and/or predictive for acute rejection and long-term prognosis.
Tests conducted in Transplantation Immunology Unit at Allergy & Immunology Research Centre IMR

i. Tests for organ transplantation:
   - Complement dependent cytotoxicity crossmatch (CDC-XM)
   - Flow cytometry crossmatch (FC-XM)
   - HLA Antibody (molecular method – PCR Luminex)
   - HLA typing

ii. Tests for haematopoetic stem cell transplantation (HSCT): HLA Typing, HLA Antibody

iii. HLA typing for associated diseases eg.:
   - HLA B*27 for Ankylosing spondylitis
   - Drugs (HLA B*15:02 for carbamazepine)
Number of Diagnostic tests performed by the Transplantation Immunology Unit, AIRC, IMR

<table>
<thead>
<tr>
<th>No</th>
<th>Name of Diagnostic Test</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HLA crossmatching + Cadaveric (Complement Dependent Cytotoxicity)</td>
<td>681</td>
<td>716</td>
</tr>
<tr>
<td>2</td>
<td>HLA crossmatching (Flow cytometry)</td>
<td>486</td>
<td>406</td>
</tr>
<tr>
<td>3</td>
<td>AB/DR HLA typing (PCR-SSP)</td>
<td>216</td>
<td>159</td>
</tr>
<tr>
<td>4</td>
<td>Class I HLA typing (PCR-SSP)</td>
<td>384</td>
<td>555</td>
</tr>
<tr>
<td>5</td>
<td>Class II HLA typing (PCR-SSP)</td>
<td>384</td>
<td>555</td>
</tr>
<tr>
<td>6</td>
<td>ABDR HLA typing (PCR-SSO)</td>
<td>5,923</td>
<td>5,932</td>
</tr>
<tr>
<td>7</td>
<td>Class I/Class II HLA typing (PCR-SSO)</td>
<td>4,002</td>
<td>3,365</td>
</tr>
<tr>
<td>8</td>
<td>HLA B27 typing + B5 + B1502 and other typing (Disease Association)</td>
<td>689</td>
<td>836</td>
</tr>
<tr>
<td>9</td>
<td>HLA antibody detection – PRA Class I &amp; II Screening</td>
<td>449</td>
<td>453</td>
</tr>
<tr>
<td>10</td>
<td>HLA antibody detection – PRA Class I</td>
<td>273</td>
<td>259</td>
</tr>
<tr>
<td>11</td>
<td>HLA antibody detection – PRA Class II</td>
<td>230</td>
<td>194</td>
</tr>
<tr>
<td>12</td>
<td>HLA antibody detection – DSA Class I</td>
<td>132</td>
<td>96</td>
</tr>
<tr>
<td>13</td>
<td>HLA antibody detection – DSA Class II</td>
<td>114</td>
<td>84</td>
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<td><strong>Total</strong></td>
<td><strong>13,963</strong></td>
<td><strong>13,610</strong></td>
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ROADMAP OF IMMUNOLOGICAL SERVICES IN MALAYSIA

2018
1. T-cell HLA crossmatch (CDC & flow)
2. HLA antibodies (luminex/sab)
3. HLA typing (PCR-SSO & PCR-SSP)

2019
1. B-cell HLA Crossmatch (cdc & flow)
2. Virtual crossmatch

2020 onwards:
Other immunological markers

Require: Financial, Personnel, Equipment, Software
MSCR is a database/registry of Malaysians who volunteers to donate their hematopoietic stem cells (HSC) to the patients in need.

It is based at the Institute for Medical Research (IMR).

Founded in December 2000, it is a collaborative project between Ministry of Health (Institute for Medical Research) and the National Cancer Council (MAKNA).

OBJECTIVES

- To educate public about hematopoietic stem cell (HSC) donation
- To recruit and maintain database of potential MUD
- Perform potential MUD search for patients in need of HSCT
MSCR carries out the following activities:

1. Organizes public and professional educational and awareness campaigns on HSC donation

2. To recruit and maintain database of potential MUD

3. Conduct HLA typing tests (Low and High resolution)

4. Involved in MUD search as requested from clinician via NSCCC

CURRENT MALAYSIAN STEM CELL REGISTRY MEMBERS: 31,900
### Number of MCSCR Donors who donated Peripheral Blood Stem Cell / Bone Marrow (Year 2010 – 2018)

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of PBSCT/Bone Marrow</th>
<th>Race of Patients</th>
<th>Race of Donors</th>
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<tbody>
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<td>2010</td>
<td>1</td>
<td>i) Malay</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>16</strong></td>
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</tbody>
</table>

Malays = 11
Chinese = 5
Successful story of patient treated with HSCT from MSCR MUD
HSCT from MSCR MUD in 2013

MSCR Donor:
• SM/29/Female/Malay
• Bone Marrow donation

Patient:
• MAZ/13/Male/Malay
• ALL
• Under Paeds Institute HKL
Six years later ............. Now age 19 years old
ACKNOWLEDGEMENT:
TRANSPLANTATION IMMUNOLOGY UNIT, AIRC, IMR
ACKNOWLEDGEMENT:
MALAYSIAN STEM CELL REGISTRY, AIRC, IMR
THANK YOU