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Major technological advances will enhance Australian donor- recipient matching and improve transplant outcomes

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Abstract

In recent times, there has been numerous significant technological and supportive changes in Australian transplantation. These changes are often deployed without the wider clinical community in full understanding of what has brought about these changes and impact they bring. Here we aim to clarify the reasoning behind these changes and shed light on potential future endeavors to improve patient outcomes.

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Introduction

A major concerted national overhaul of solid organ transplant donor-recipient matching practices is underway. Advancements in Human Leukocyte Antigen (HLA) typing through next generation DNA sequencing technology and antibody detection, coupled with a large federal government and Australian Lifeblood investment in data management, are dramatically changing the face of Australian solid organ transplantation. We summarise the background to these initiatives and their expected immediate impact on clinical practices.

The avoidance of HLA *incompatibility*

HLA molecules are cell surface proteins that are considered the antigen of concern in transplantation. Donor-recipient differences in HLA subtypes (*HLA-A, B, C, DQ, DP* and *DR*) are responsible for the recipients' immunological responses to the transplanted allograft. Therefore, matching the HLA of the donor-recipient pairing, or conversely, defining HLA mismatching of this pairing, are central to the subsequent clinical course and success of the transplanted organ(1). HLA mismatching can lead to increased risk of development of antibodies post-transplant. Pre-existing or de-novo recipient anti-HLA donor-specific antibodies (DSA) are the major cause of antibody-mediated allograft rejection (AMR) and chronic graft loss(2), and the avoidance of HLA mismatching at time of transplant improves graft success.

Historically, HLA typing was defined by serological reactive patterns of lymphocytes(3) against known antibodies (e.g. an antibody against HLA A24). Although this serological definition allowed a certain level of variation amongst HLA types, it could only define a handful of HLA types accurately. However advancements in molecular DNA sequencing now define variations between HLA types to amino acid level, therefore improving the differentiation of HLA mismatching at HLA allele level (e.g. *A**24:02). The recent advancement of Next Generation Sequencing (NGS), whereby entire genes are sequenced, has enabled an even higher resolution of HLA typing to be performed. Australian Tissue Typing laboratories now routinely report to 2-field or even 3-field NGS (e.g. *A**24:02:01)

(Figure 1). Notwithstanding, time constraints at the time of transplant, often mandate the use of faster real-time PCR (RT-PCR) molecular *HLA* typing at a lower resolution to exclude problematic carefully predefined clinically important DSA. Although, the naming of HLA allele can be confusing to the uninitiated (Figure 1), they do represent logical real-world genetic polymorphisms that need to be taken into account when considering the avoidance of HLA mismatches.

Knowledge of HLA *compatibility*

Optimising transplant outcomes is not purely related to the avoidance of known DSA, as donor-recipient HLA mismatching can result in post-transplant AMR due to development of *de novo* DSA. HLA compatibility can potentially now be clinically enhanced by use of computer algorithms based on crystal or neural network modelling. HLAMatchmaker is the most extensively studied computer algorithm and it defines structural B-cell eplet mismatching between recipient and donor. Eplets are the true immunological target sites on the HLA surface (Figure 2), and eplet mismatching leads to immune activation and the subsequent development of anti-HLA DSA. As B-cells are principal cells involved in humoral immunity including antibody development, much of the earlier work was defining these B-cell eplets. These studies demonstrated that lower HLAMatchmaker eplet mismatching associates with better outcomes in kidneys(4), heart(5), liver(6) and lung transplantation(7). More recently, HLA-EMMA was developed which like HLAMatchmaker, also defines B-cell epitopes. The principal difference is HLA-EMMA defines the solvent accessible amino acids by inter locus by neural network modelling which allows each HLA sub-type to be scored independently. Therefore, potentially limiting miscounting of mismatching as not all HLA crystal structures are actually immunologically ‘seen’. Lower HLA-EMMA scores are similarly associated with improved outcomes in transplantation(8).

Although not yet routinely available for all transplant matching, some Australian centres, in conjunction with their local Tissue Typing laboratories, do characterise eplets to reduce de-novo DSA development(9), improve long-term graft survival and increase access to

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subsequent transplants. Similarly, other centres are using careful eplet evaluation to increase donor offers for sensitised patients(10).

Improved Crossmatch technology

Historically a cell-based complement dependent crossmatch (CDC-XM) has been used in pre-transplant assessments in Australia to identify DSA. This CDC-XM involves mixing recipient serum with donor lymphocytes. However, it has become evident that not all clinically relevant DSAs are identified through a CDC-XM due to lower sensitivity. Therefore, CDC is being actively phased out. Going forward, Australian Tissue Typing labs have progressed to the more sensitive flow cytometry crossmatch (FXM) or a virtual crossmatch (VXM) using single antigen bead Luminex defined antibody results for transplant assessments, marking a very significant change in how transplant risk is evaluated.

The FXM detects IgG antibodies directed towards donor by mixing patient serum, donor lymphocytes (T- & B-cells) and specific fluorochromes and measuring fluorescence on a flow cytometry analyser. Recently the Halifaster-FXM (HFXM) has evolved from historically time-consuming prior versions to enable a FXM as part of a pre-transplant assessment (11) (Table 1). One concern with FXM is the potential for a false positive result due to non-HLA auto-antibodies which interact with the assay(12). The clinical relevance of these autoantibodies is not understood, and a positive FXM in this circumstance may inappropriately exclude patients from transplant who would not actually have an increased risk. To overcome this, centres now have access to a VXM, whereby donor-recipient compatibility is assessed on donor HLA and the pre-determined recipient antibody profiles (Table 1). A VXM has the benefit of being faster and potentially offered to a greater number of waitlist patients as it does not require cell numbers. However, the accuracy of any VXM is reliant on the frequency of screening patients for antibodies, as missing antibodies would render the pre-transplant VXM redundant. Australian testing labs have recently increased the frequency of screening to allow any changes in antibody profiles to be detected and improve the accuracy of the pre-transplant risk assessment.

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An Improved information-technology platform

An effective transplant IT system is crucial to enable quick and accurate retrieval of stored transplant data. In Australia, the historical outdated state based National Organ Matching System (NOMS) was recently replaced with OrganMatch (OM). OM is a national state-of the art web based, high capacity, high security platform that connects the Australian Tissue Typing Services, Australian DonateLife Network and transplant teams in all states(13). OM has the ability to store all clinical matching data, assist with organ allocation, distribution and auditing. The OM system is future-proofed with capacity to model potential adjustments or algorithms assist in image transfer (de-identified radiology or echocardiography) and explore eplet compatibility (Table 2).

Improved professional collaboration

Underpinning all of the above, the acquisition, utilisation and deployment of these new technologies in Australia has required an impressive collaborative effort on the part of numerous Governmental Agencies and clinical Transplant Services across all organs and all jurisdictions. For the first time national standards and consistency are being instituted across all states. As these novel process and workflows roll out, education, clinical risk management, audit, evaluation and revision will be key to its success.

The promise of further technological enhancements

Technological advances in the wings hold promise of further efficiencies and success too. Enhanced nanopore sequencing technology is reported as a faster real-time NGS technology as a step towards high resolution HLA typing for deceased donors(14) While most computer based matching studies are looking at B-cell epitopes, PIRCHE defines T-cell epitopes and lower PIRCHE scores associate with improved outcomes(15, 16). Algorithms are also being created to look at electrostatic differences between recipient-donor and amino acid mismatching(17).

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Our understanding of immunological responses to targets on the transplanted organ is improving, and algorithms defining these targets as either T- or B-cell eplets, electrostatic or amino acid differences are allowing us to better define immunological compatibility. These concepts could soon be incorporated into the transplant risk assessment and be used to direct recipient selection towards structurally matched transplants. Therefore, leading to a 'personalised medicine' approach to targeted immunosuppressive regimes, reducing transplant complications and improving outcomes. There are emerging opportunities for the development of on-line 'big-data' international collaborative platforms to link transplant centres to enable such advances. OpenTransplant is one such free-access platform that may enable data sharing and access to simultaneous epitope algorithms.

However, even HLA matched transplants suffer rejection, suggesting non-HLA factors contribute to transplant success. Although T- and B-cell lymphocytes have historically defined recipient-donor compatibility, Natural-Killer (NK) cells, may also be relevant to transplant success(18). The role of NK cells and their immunological targets on donor organs is not fully understood, however the studies to determine their role are currently been undertaken and potential inclusion in transplant risk maybe imminent. As we broaden our understanding of the targets in transplantation, and the complexity of the immunological interactions experienced in transplantation, the increased availability of commercial test kits and technology to sequence numerous genes simultaneously may warrant the inclusion of multiple immunological markers in the pre-transplant assessment in the future.

While it is clear HLA antibodies are the main driver of rejection, the clinical relevance of non-HLA autoantibodies poses a challenge, as little is known outside of a few targets in renal transplantation. Pre-transplant Angiotensin II receptor type 1 (AT1R) antibodies in waitlisted renal patients, have demonstrated increase risk of early graft loss and rejection(19). Therefore, desensitisation measures are used to lower the AT1R levels in renal patients to improve outcomes. Importantly though, the recent development of kits with numerous non-HLA auto targets going forward provides the potential to include a wider range of antibody

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monitoring in wait-list patients. As we improve our understanding of these autoantibodies, the possibility of an autoantibody profile in the pre-transplant assessment is nearing, which may again help us to better define true immunological risk.

Although here we have discussed recent changes to Australian transplantation, some of these changes could also be beneficial to centres worldwide. Improved high resolution HLA typing, improved database and IT system management and increased communication channels with clinical teams all allow for better service delivery. The use of a virtual crossmatch for risk assessment relies on more regular screening which may not be feasible in some centres, where a cell-based assay is still possible with fewer recipients to assess. Therefore, a move away from this cell-based approach must consider the increased testing frequency and associating costs. Furthermore, while using eplets to define HLA compatibility is gaining traction, there is still much work needed to form a consensus on the best use of these eplet mismatch scores in renal allocation, although the use in the thoracic organs could be more easily implemented, the move to an eplet approach maybe a few years away.

Conclusion

With the increasing research in the organ transplant space coupled with improving technology, Australia is well placed to deliver on a strategy that targets improved donor organ utility and clinical transplant outcomes. Scientists, transplant clinicians and governmental bodies must continue to communicate, cooperate and support one another to enable the progression to world's best practice.

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Figure Legend

Figure 1: HLA nomenclature and level of HLA resolution with typing methods used in LTx. HLA nomenclature defines how amino acid variations organised in groups. Quicker low resolution RT-PCR used for deceased donor processing has limited gene coverage, while higher resolution typing by NGS has greater gene coverage but increased testing times.

Figure 2: HLA Eplets located on the cell surface. The HLA antigen is made up of several eplets found within the antibody accessible regions, as seen with 76ANT and 65RNA. Mismatches to these eplets can elicit immunological responses post-transplant, such as antibody development. Illustration provided by Dr Philippa Saunders from Microbiology and Immunology, University of Melbourne at the Peter Doherty Institute for Infection and Immunity.

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Tables

	Cell based	Sensitivity	Analysis	Limitation	TAT
Complement dependant (CDC)	Yes	Poor	Microscope	Sensitivity Cell Quality	4-6 hours
Flow cytometry (FXM)	Yes	Good	Flow analyser	Time dependant Cell Quality/numbers	6-8 hours
Flow cytometry-Halifaster (HFXM)	Yes	Good	Flow analyser	Cell Quality/numbers	4-6 hours
Virtual (VXM)	No	Great	Virtually	Antibody Detection accuracy & frequency	2 hours

Table 1: Crossmatching methods used in Australian LTx. Summary of the routinely used crossmatches used in transplantation within Australian centres. CDC crossmatching has a poorer sensitivity when compared to other assays, while cell quality also determines effectiveness. While FXM and HFXM both have better sensitivity, but cell quality and numbers restrict routine use. The VXM is not cell-based but the frequency of antibody testing determine its accuracy as to sparse of testing may miss newer antibodies for the VXM.

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	Old	New
Platform	Networked	Cloud
Matching capabilities	Limited	Future proof
National system	State based	National
Self-service unit reporting	No	Yes
Access for clinicians	No	Yes
HLA reporting	Limited	Future proof
Compatible for epitope use	No	Yes
Allocating highly sensitised patients	Difficult	Personalised (UAs)
CDC	Yes	Yes
FXM	No	Yes
VXM	No	Yes
Use of donor demographics to define donor quality	No	Yes
Live data	Not accessible	Accessible

Table 2: Differences between NOMS and OrganMatch Australian IT systems. The differences between NOMS (older) to OM (Newer) database of solid organ transplantation in Australia.

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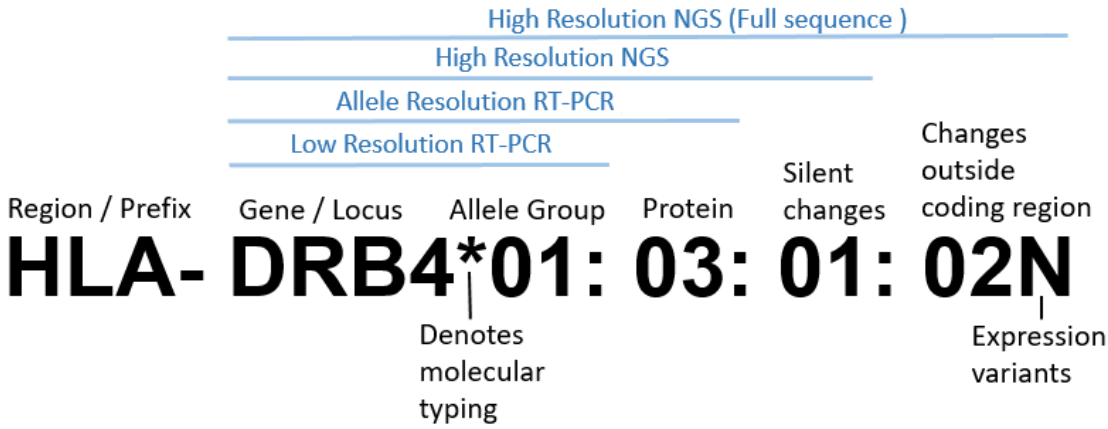

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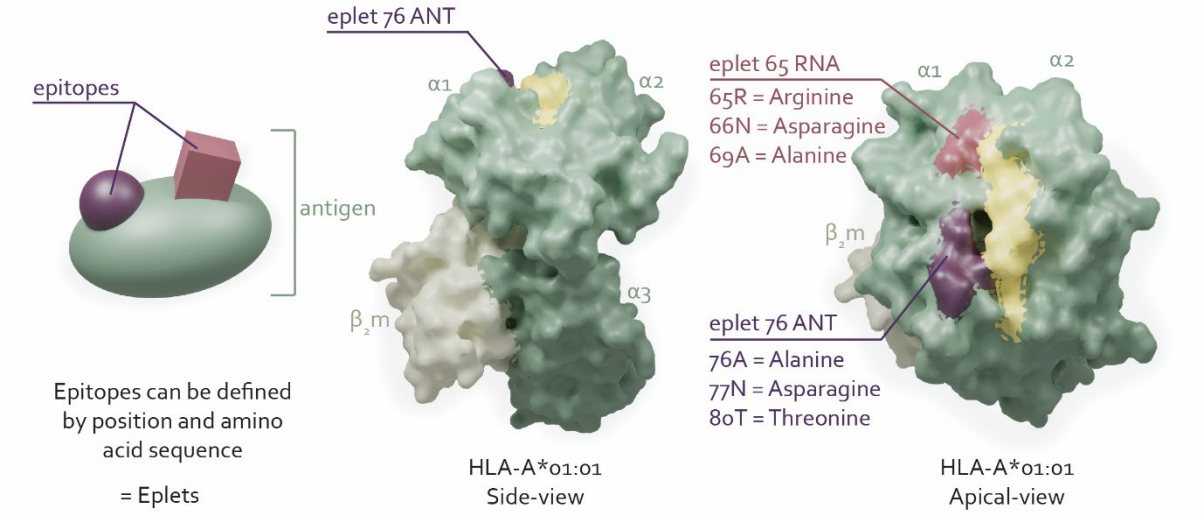

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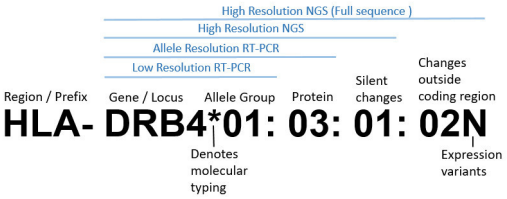

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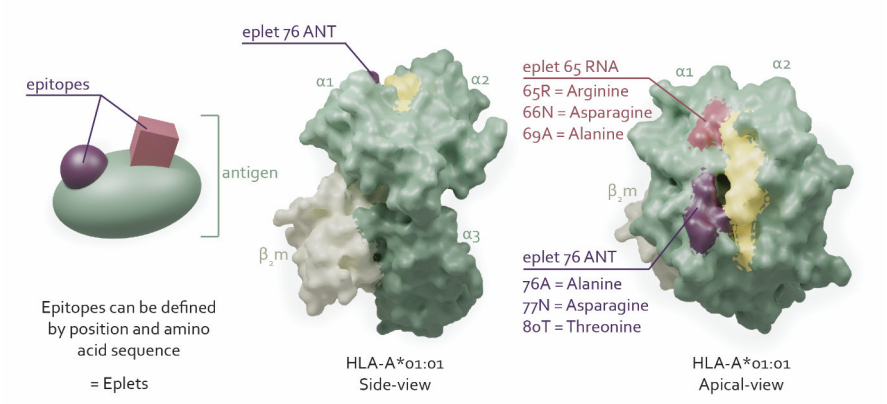

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