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REVIEW ARTICLE

A review of HLA matching to improve clinical outcome in bone marrow transplants and the role of haplotyping in unrelated donor transplants

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ABSTRACT

When the first allogeneic bone marrow transplants in humans was attempted, little was known of the major histocompatibility complex (HLA) and its role in ensuring success of the graft. Therefore, many of the early attempts resulted in failure. During the 1970s just a small number of alleles were detected serologically and the mixed lymphocyte reaction (MLR) served as a surrogate for class II matching, the DR series of alleles not being formally serologically defined until 1978. Nevertheless, selection of nuclear family members who were two HLA haplotype matched became possible using serology and the MLR and transplant successes were reported.

The breakthrough in technology was undoubtedly the introduction of DNA techniques, particularly DNA sequencing. This enabled fully matched unrelated individuals to be found as potential bone marrow donors. Genetic phasing or haplotyping of unrelated donors who are found on bone marrow registries has remained elusive until recently. The development of individual chromosome sequencing will permit haplotype matching in the absence of family studies and will expedite studies aimed at identifying other genes which are important matching factors.

AIM: The aim of this review is to outline the technological advances that have been made in HLA genotyping over the last 50 years. The progress from serology to DNA sequence as part of the matching algorithm is one of the reasons clinical results have improved so much during this period. This review outlines the DNA techniques that have been utilised for this purpose, concluding with a discussion of the value of haplotype matching in unrelated donor transplants, where the donor has been sourced from a bone marrow registry. Haplotype matching represents one of the last remaining hurdles to be overcome in unrelated donor bone marrow transplants.

Introduction

The terms haematopoietic stem cell transplantation (HSCT) and Bone Marrow Transplantation (BMT) are often used interchangeably in the literature. This dual terminology arose from the fact that originally BMT was performed almost exclusively, until the 1990s, when cord blood and growth factor mobilised peripheral adult stem cells provided an alternative source for transfusion in patients requiring bone marrow re-constitution. In this paper to avoid confusion on the part of the reader I will use the acronym BMT throughout, but the reader should keep in mind that this term may not be always accurate post the year 2000.

The first attempts at bone marrow transplantation (BMT) for patients with various blood cancers or aplasia were universally a failure. In 1970 Mortimer Bortin[1] published results of all the BMTs performed between 1959 and 1970. At the time of writing only 3 patients were alive, 125 had failed to engraft, 49 relapsed to their original disease and only 1 showed evidence of blood chimerism. Disappointingly, none of the 73 patients with aplastic anaemia displayed any clinical benefit from having a BMT. These disappointing results led to a moratorium on this form of treatment to treat leukemia or other conditions which were thought to benefit from BMT. What should be kept in mind is that the HLA system was not discovered until 1958 by Jean Dausset[2] who was a haematologist working in Paris, and the laboratories that were applying HLA to their matching armamentarium during this period were dealing with a small number of serologically defined specificities coded for by the HLA class I genes (now known as HLA-A, -B, and -C). The HLA class II system was not defined until the 7th Histocompatibility Workshop held in Oxford, UK, in 1978. Likewise, the first immunosuppressant, Imuran, a derivative of 6-mercaptopurine which was developed by Gertrude Elion and George Hitchings at the Burroughs Wellcome laboratories in New York (U.S.A)[3,4] (later to become Glaxo, Smith and Kline), which was originally used to treat childhood leukemia and was not freely available until the mid-1960s. From the perspective of HLA matching and control of alloresponses. it is not surprising these early attempts at BMT were failures.

As HLA typing technology improved and more serological specificities were defined, matching in BMT became a possibility. The first data that suggested MHC matching was important in BMT was shown in dogs by Epstein and Donall Thomas using littermates to restrict the genetic differences[5]. The closeness of MHC type was confirmed by immunization with buffy coat. A donor was selected from each litter and marrow injected into a matched and non-matched recipient after 1500r of radiation. None of the mismatched group lived beyond 14 days compared to the matched group who all lived for 40 days and 4/6 lived for greater than 100 days. While the tissue matching in retrospect was basic, the results demonstrated the importance of MHC matching in improving outcome in BMT.

These experiments in dogs gave clinicians the courage to re-embark on human BMT. The first successful BMT for leukemia was performed by Mathé at the Department of Hematology, Institut Gustave-Roussy, and Institute Cancerologie et d' Immunogénétique, Villejuif, Seine, Paris, France, in 1965.[6] The recipient, a 26yo male clinician was diagnosed with ALL (acute lymphocytic leukaemia). After

initial treatment with cortisone and 6-mercaptopurine he was diagnosed as being in remission in November 1961. However, in December 1962 lymphoblasts were seen in his peripheral blood. In April a decision was made to proceed with a BMT. A crude test for compatibility was used in which skin grafts from the patient to a selected family member were performed[7]. After rejection of the skin the volunteer received grafts from a number of family members and rejection times noted, varying from 12 to 22 days. Reading the paper, it is not clear how the information was used. However, on the 23rd of April, 1963 the patient was infused with 2 litres of bone marrow which consisted of a mixture of marrow derived from all nuclear family members (both parents, 3 brothers and a sister). Myeloid restoration was observed at 2 weeks and 6 months and phenotyping of the engrafted red cells confirmed a male donor. Subsequent skin graft from the engrafted donor onto the recipient was still intact after 7 months. The patient suffered a setback which appeared to be graft versus host disease but remained alive and well 12 months post infusion. In a follow up of the patient's progress by the authors, it was reported that the patient died on the 17th of December 1964 after a convulsive episode. In retrospect, one of the interesting features of the case was the fact that only one donor engrafted, a situation observed 40 years later with the use of multiple cord blood infusions, a rich source of haematopoietic stem cells, to treat leukemias.

Gatti and co-workers[8], from Robert Good's unit at the University of South Florida were the first to perform a transplant in a patient using an HLA matched sibling. The patient was a 5-month-old boy with a sex linked acquired immunodeficiency. The donor was selected on the basis of serology and a negative mixed lymphocyte reaction, which in retrospect was indicative of HLA class II matching. Although HLA serology was in its infancy at the time, family studies plus the negative MLR results indicated an HLA identical sibling who was selected as the donor. The patient developed mild GVHD which resolved in the absence of treatment. At 2 months post-transplant donor cells were observed in biopsies of the skin and rectum. The patient recovered and remained alive and well. The transplant by any measure must be considered a success.

The first attempt at a one HLA haplotype transplant was described by Buckner from Donall Thomas's group in 1970.[9] A 46-year-old male with Chronic Granulocytic Leukemia was transplanted with marrow from his one HLA haplotype mismatched sister. Because the other siblings lived interstate it was decided to proceed with the mismatched transplant to save time as the patient was in blast crisis. HLA typing of the parents confirmed the one haplotype match of the sister.

The patient was transplanted in March 1969 and the post-transplant course could be viewed as consisting of 3 phases. For the first 14 days the recipient was lymphopenic and was supported by platelet transfusions and whole blood transfusions. Between days 14 and 41 the patient's cell count rose. He developed diarrhea and a skin rash which was interpreted as GVHD. On day 42 the patient developed a fever which progressed with a fall in his platelet count. Penicillin treatment was commenced on day 54 due to infection with Neisseria Catarrhalis and the patient unfortunately died on day 56 post-transplant.

Fritz Bach and his team from Harvard University transplanted bone marrow from an HLA

serologically compatible and MLR negative sister to a male patient with Wiskott-Aldrich syndrome, an X linked immunodeficiency syndrome.[10,11] Two attempts at this transplant took place, the first without pre-conditioning with the graft failing to take, the second involving the use of cyclophosphamide which resulted in the patient achieving a state of chimerism with no evidence of GVHD. At the time of writing, he was 6 weeks post-transplant and was doing well.

Meuwissen reported on a 15 year follow up on this patient.[12]On discharge his T cell compartment was of donor origin while his B cell compartment displayed evidence of chimerism leading the authors to conclude that some form of myeloablative treatment was needed for complete engraftment. Despite these findings, the young boy's immune system appeared normal and he suffered no fungal, bacterial or viral infection. These findings demonstrated that successful bone marrow transfer from an HLA identical sibling could be used as a successful treatment for blood disorders. It seemed the 1970s ushered in the new era of bone marrow transplantation.

HLA serological matching and the mixed lymphocyte culture (MLR)

During the 1970s the accepted method of determining HLA compatibility was using serological methods, employing antibodies to HLA determinants expressed on the cell surface. This method determined HLA class I (HLA-A -B and -C), while the still undiscovered class II loci compatibility was measured by the MLR.

There were technological advances in the 1970s. Serology became more sophisticated as new alleles were discovered and by the end of the decade most HLA matching laboratories had access to well characterized sera which could identify HLA class II specificities (HLA-DR and -DQ). Serology for HLA-DP remained unachievable, as it is even today.

Transplanting for leukemia became an additional challenge over that for aplastic anaemia and immunodeficiency, because myeloablation was necessary to remove all leukemic cells, it increased the risk of GVHD and in some cases engraftment did not occur. In fact, Glucksberg from Donnall Thomas's group [13] analysed their experience with GVHD and found that it was increased in those with haematological malignancies compared with aplastic anaemia patients (32/42 -76.2% and 11/19 -57.9%). During the 1970s HLA matched donors were sought within the nuclear family. i.e., HLA haplotype matched. Jean Dausset who was awarded the Nobel Prize for his discovery of the HLA system[14], was the first to suggest a registry of unrelated donors for use in the case where an HLA donor is not available within the nuclear family. Given the chance of receiving the same paternal and maternal HLA haplotypes is one in four and the average number of children in a family in the UK is now 1.9, the lack of an HLA matched donor is approximately 70% and rising, given the current birth trends.

Dausset's concept involved storing lymphocytes from prospective donors and then using them as a database against which patients could search for an HLA class I matched donor. In the event a match was found, cells would be collected from the putative donor and an MLR performed. If a match was found the donor would travel to the transplant centre rather than sending harvested marrow.

Matching for HLA -A, -B, -C, -DR, -DQ (Class I and II) matching in bone marrow transplantation

With the advent of serological typing for HLA class II alleles in 1978 most transplant centres introduced DR and DQ into the matching algorithm. For several years this was done exclusively by serology but in the late 1980s DNA techniques were introduced which heralded the third era of BMT. There were several method developed during the 1980s for class II DNA typing which are summarized below:

Restriction fragment length (RFLP)

This method uses restriction enzymes to cleave the DNA at specific sequence sites. A P^{52} probe is then used to hybridize with the fragments to produce a specific pattern. Kohonen-Corish working in Sue Serjeantson's laboratory at the Australian National University in Canberra, demonstrated that generic DR typing could be achieved by using one enzyme, Taq1, and utilising a DR probe. [15]

Roy Howell and co-authors from the Kings College London group, examined HLA matching in 10 leukemic patients and their potential related marrow donors and family members using serology, RFLP and MLR for class II typing.[16] The results were in good agreement, demonstrating the usefulness of RFLP as a class II typing tool. Of interest was the observation that serological class II typing of 4 patients and 2 healthy individuals resulted in ambiguous results. All were typed successfully by RFLP, the results being confirmed by MLR. These results demonstrated a tangible role for RFLP in cases where class II typing was not obtainable in patients due to sub-optimal blood samples. Marilyn Pollack who worked in Bo Dupont's department at the Sloan Kettering Institute in New York published a paper in 1983 [17] showing that recipient/donor combinations with serological identities known to be in linkage disequilibrium were more likely to be MLR negative than other specificity combinations. A negative MLR prediction could be made more readily with DR1, DR2 and DR3 combinations. However, positive MLRs were commonly observed in combinations with the highest LD. The authors invoked the SB locus as being responsible, which later was designated HLA-DP and was shown to be responsible for positive reactions in the MLR. Smith and Belcher from the Wright University in Dayton, Ohio[18] warned that alleles of DR and DQ were not detected by serology at the time and that even in the case of apparent HLA-A, B, C, DR, DQ matching between recipient and donor that an MLR was still required to confirm compatibility. The general consensus at the time was that due to some uncertainty as to the genes which comprised the HLA class II region and the additional problems with accurate identification of HLA-DP alleles that there was still a role for the MLR in the matching armamentarium in BMT.

Sequence specific oligonucleotide (SSO) hybridization and Sequence Specific Primers (SSP) in BMT.

Polymerase Chain reaction.

The discovery and development of the polymerase chain reaction (PCR) by Kary Mullis, who worked at Cetus Corporation in Emeryville, California and who received the Nobel prize in 1993 for his discovery, revolutionised the field of molecular biology in a fashion that had never been seen before[19]. It enabled the amplification of small amounts of DNA, sufficient to allow interrogation of gene sequences,

revolutionising genetic testing. This impacted on forensic testing[20,21], genetic testing for disease[22], testing for the presence of infectious agents[23,24] and transplantation matching both for solid organ and bone marrow [25,26], to mention just a few of the fields that benefited from this breakthrough.

The PCR test relies on the selection of primers complementary to the 3` and 5`sequence of the gene fragment under consideration. These fragments hybridise to the gene sequence and in the presence of Taq polymerase the sequence of the DNA strand is "read" and duplicated to produce another identical strand. After one cycle two copies of the sequence are obtained and after two cycles 4 copies and then 8 copies after three cycles. The length of the fragment is determined by the position of the primers within the gene under study. With this exponential growth 2^{30} copies are produced after 30 cycles, sufficient to use in most genetic testing, including sequencing. The power of this test is illustrated by the fact that if one strand of DNA was put into an Olympic size swimming pool, after 30 rounds of PCR there would be at least one strand present in a 1 ml sample taken from the pool.

The following outlines how incorporation of the PCR technique has been used in the HLA field to move the study of HLA genetics from serology to sequence and specifically how it has transformed matching of donors and recipients in BMT.

The patterns of polymorphisms of both HLA class 1 and II became clear when the sequences of the HLA alleles were first reported. The majority of variation in the HLA class I genes were located in the second and third exons. In contrast, the variation in sequence in the HLA class II genes were mainly confined to the second exon and appeared to be more ordered than that observed in the class I genes. This explains why many of the first reports of DNA typing by PCR based methods were restricted to HLA class II.

Sequence Specific Oligonucleotide Hybridisation (SSO)

SSO involves the amplification of class 1 (2^{nd} and 3^{rd} exons) and class II genes (2^{nd} exon) by PCR using primers specific for the sequences which are the boundaries of the known polymorphisms. The amplified DNA is bound to a membrane and then probed with short length (approx. 20mer) oligonucleotides specific for the variable regions.

In a 1991 paper published in the European Journal of Immunogenetics, Ivinson showed the importance of standardising conditions for the optimal use of the assay[27]. In an effort to move away from a radioactive labelling of the probe, a biotin avidin system was developed for use with SSO. The probe was labelled with biotin and probe/target binding was detected using streptavidin horse radish peroxidase with a substrate that changed colour with binding. The assay was introduced by Willem Verduyn and colleagues to study DR matching in a Eurotransplant cohort of renal transplant recipients.[28] They demonstrated the efficacy of the technique by comparing it with conventional class II serology. A variation of this technique was described by Buyse et al[29] who fixed the chemiluminescence probes onto a series of paper strips. The reagents for the chemiluminescent step which again involved biotin/streptavidin reagents were provided by a commercial company (Chemiluminescence kit Prolix Inc. Bedford MD). DNA was amplified

by PCR for the HLA gene of interest and then hybridised to complementary probes, which was essentially the reverse of the first iteration of the SSO technique.

SSP

This method relies on selection of primers for a PCR reaction. The assay can therefore be used for generic or allele typing depending on the primers selected. The SSP assay has the advantage of speed. Once the PCR reaction is complete, the product can be run on a gel with the appropriate positive and negative PCR controls. A positive amplification indicates the presence of the allele for which the primers were selected.

Jordan from Madrigal's group at the Royal Free Hospital in London, conducted a comparative study of PCR-SSP, PCR-SSO and RFLP as methods for HLA class II genotyping in BMT.[30] While there was good agreement between the methods in 21 out of 200 potential bone marrow donors genotyped, there was some disagreement between the RFLP technique and the PCR based techniques manly involving the DRB3 locus. This group recommended using the SSO technique for low resolution typing involving large numbers of samples, while the SSP method be used for small numbers or for urgent cases, or where higher resolution was required, a recommendation adopted by many laboratories at the time. In more recent times direct sequencing of the HLA genes has become the gold standard for matching in BMT. However, on a routine basis the PCR based techniques still have their place particularly the SSP technique, when quick genotyping is required.

Direct DNA sequencing for definitive matching in BMT

With the introduction of routine sequencing the true polymorphism of the HLA class I and II genes became apparent. As of June 2023, there were 25,844 recognised class I sequences, or alleles and 10,970 class II alleles. (https://hla.alleles.org/nomenclature/stats.html.

These numbers have to be considered in the context of Bone Marrow Registries and efforts to try and find a compatible donor for a patient. The figures quoted, for example for class 1 include alleles at HLA-A, -B and -C, therefore multiplying the class I number with the class II number will give an underestimation of the possible number of HLA genotypes. Nevertheless, the figure obtained, 283 $x10^6$ is far in excess of the $41x10^6$ registrants and the 804,000 cord blood samples currently on the World Marrow Donor Association website. (https://statistics.wmda.info/).

The fact is that many of these genotypes have not been observed despite 20 plus years of searching. This is largely due to a phenomenon called linkage disequilibrium (LD). In the hypothetical situation when two neighbouring alleles occur with a frequency of 50% on a haplotype, one would expect them to co-occur with a frequency equal to the product of the frequency of the individual alleles i.e., 50%x50% =25%. Any statistically significant deviation from this frequency is termed linkage disequilibrium (LD). LD in the MHC can either be positive (alleles occur together more often than expected) or negative (alleles occur together less often than expected). LD was first described and characterised in the

HLA system but has since been demonstrated across the genome.

Anne Spurkland from Erik Thorsby's laboratory in Oslo, Norway published a paper in Tissue Antigens in 1993, describing the use of direct sequencing of both DR and DQ genes in a group of BMT recipients and donors.[31] Making the point that PCR-SSO and PCR-SSP are techniques which require a large amount of preparation, they introduced direct sequencing as a method which in time would become the gold standard and routine method in most HLA typing centres. The technique they introduced involved the use of biotin labelled primers for the initial PCR step, followed by adherence to streptavidin coated magnetic beads. The amplified DNA was incubated with NaOH which rendered the DNA single stranded and the non-adherent strand was then removed by washing in specified buffer. This permitted the resultant single strand sequencing of both DR and DQ genes. In the succeeding 30 years there have been a multitude of papers from individual groups using sequencing for both HLA class I and class II matching in BMT. However, the International Workshops have been the catalyst for the biggest advances in our understanding of the importance of HLA matching in BMT. These large multicentre studies have allowed such questions as "how important are HLA-C and DP in ensuring good outcome?" and "are all sequence differences between donor and recipient important?" Some of the key findings from these workshops are discussed below. The 2012 IHIW held in Liverpool, UK served as the catalyst for the collection of genetic and outcome data on 25,855 unrelated BMT transplants.[32] The data was contributed by 42 laboratories, 435 transplant centres and 10 bone marrow donor registries in 18 countries. It became evident looking at the submitted data there had been a significant shift in focus during the previous 20 years. These changes included less intensive conditioning, growth in the percentage of patients with non-malignant diseases treated with BMT, increased size and number of donor registries which permitted the study of racial differences in BMT outcome and the use of cord blood as a stem cell source in both children and adults. The dramatic increase in numbers of patients submitted to the 16th Workshop, which had grown from approximately 2,500 at the 13th Workshop in 2001 to 8,000 at the 14th Workshop in 2005 and 17,000 at the 15th Workshop in 2009 to the nearly 29,000 reported in 2013 is a testament to the international co-operation which has always existed amongst the HLA community since the inception of workshops in 1964 and to the dedication of Effie Petersdorf and her colleagues at the Fred Hutchison Cancer Center in Seattle (named by his surgeon brother in honour of a famous US baseballer who died of lung cancer in 1964). In summary the data indicates that in order to optimise outcome for the recipient that allele sequencing at all class I loci (HLA- A-B and -C) and class II loci (HLA-DRB1-DRB3, - DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1) should be a match between donor and recipient.

Haplotype matching - how important is it?

As stated above, when searching International Bone Marrow registries, most transplant centres aim to find a donor who is allele matched at the class I loci and at the DR and DQ loci. Due to the relatively high degree of recombination between the DQ and DP loci, DP matching is considered a bonus. Allele matching at these loci do not, however, indicate that the patient

and donor are matched for two haplotypes. How important is haplotype matching?

While most transplant centres concentrated on establishing the effect of various HLA loci on clinical outcome in BMT, two laboratories explored the effect of matching for haplotypes in unrelated donor BMT. Roger Dawkins and co-workers in Perth, Australia investigated HLA microsatellites as markers of HLA ancestral haplotypes, so called because recombination occurs rarely within these blocks. They had previously shown that the HLA complex could be viewed as combinations of 5 blocks of DNA (α, (HLA-A), β (HLA B and C), ϒ (the complement region) Δ, (HLA-DR and DQ loci) and ε (HLA-DP loci). The group then developed and published a method for assigning haplotypes in BMT and examined the clinical significance.[33,34,35,36,37] Tay et al in the two papers published in 1995[34,35], firstly, showed that each polymorphic blot included haplotype specific markers. Using DNA amplification followed by electrophoresis and scanning with a laser they were able to identify markers within each HLA β and Δ haplotype block using this method of haplotype assignment. Those BMT recipients who were matched at both blocks had a 6-month survival rate of 54% which was superior to those matched by conventional typing methods. They further reported the use of this technique in selecting HLA matched siblings in the absence of HLA typing. Forty-six siblings from 10 families were genotyped by conventional typing methods, including C4 (complement C4) and Bf (properdin) typing. Forty-three siblings gave clear unambiguous results allowing the comparison of 22 compatible sibling pairs with 77 non compatible pairs using block matching. The comparative results yielded 100% concordance with 3 cases involving recombination, which were not detected by conventional typing, one of which was confirmed by block matching, the other two requiring further testing. One criticism that was levelled at these early studies is that matching levels were determined by serology and not sequencing which permitted allele level matching. However, this was addressed by the papers of Witt et al[36,37] and a follow up analysis by the group.[38] Witt showed that BMT patients who were matched at the β and Δ blocks had superior event free survival than mismatched patients (63% v 25%). Further analysis demonstrated that β block matching was correlated with sequence differences in exons 2 and 3 at the B locus, but less so with the C locus. Matching at the Δ block was strongly correlated with exon 2 sequences of DRB1.Kitcharoen and co-workers (38) studied 44 BMT recipients matched with donors of the Australian Bone Marrow Donor Registry. They correlated sequence matching at HLA-B, HLA-C, MIC-A and MIC-B and block matching with overall patient survival. Patients who were matched for HLA-B and HLA-C had statistically significantly improved survival when they were also β block matched. Further evidence for the importance of haplotyping in BMT was provided in three seminal papers by Effie Petersdorf's group from the Fred Hutchison Centre in Seattle.

The first publication[39] described the microsatellites specific for HLA haplotypes which could be used for providing haplotype matching in BMT. The second publication described a method for separating haplotypes using genomic DNA from individuals of known HLA genotype.[40] Probes specific for the two HLA-B alleles were adhered to a glass

surface and hybridized with the genomic DNA. After washing off excess DNA, the two captured DNA fragments were placed in separate tubes and PCR primers were used to amplify the HLA-A and DRB1 genes. Using this method of physical separation, Petersdorf was able to study the clinical effect of HLA haplotype matching in 246 unrelated donor BMT.[41] Of the 246 recipients who were HLA-A, B, C, DRB1 and DQB1 matched with their donors, 191 (78%) were haplotype matched with their donors while 55 (22%) were mismatched. There was a strong association of HLA haplotype mismatching and increased levels of acute GVHD Grades 3 and 4 which was observed after a few days, peaked at day 20 and at 100 days the incidence of acute GVHD was 35% greater in those haplotype mismatched (60%v 25%).

These results clearly demonstrate the value of block matching in collaboration with sequence-based matching, and it is surprising that these techniques have not been incorporated into routine testing in HLA matching laboratories for unrelated donor transplants.

The ability to sequence individual chromosomes[42] would, firstly, replace block matching techniques and secondly, would indicate which non-HLA differences contribute to haplotype differences measured by block matching and importantly which sequence differences influence survival in BMT. This advance would have significance to the $41x10^6$ donors who are listed on the registries world-wide, the majority of which, HLA haplotypes have not been determined.

The improved outcome seen in HLA haplotyped matched unrelated donor transplants suggest there are other genes within the MHC, currently not part of the matching algorithm, which influence BMT outcome. Haplomic Technologies in partnership with SCHOTT Minifab in Melbourne have developed a chip which enables single metaphase cells to be trapped, lysed and individual chromosomes collected for sequencing. The use of this technology will enable the assignment of unambiguous HLA haplotypes in the absence of family studies and haplotype matching in unrelated donor BMT. This technology will also permit the evaluation of the role of other MHC genes in determining outcome.

Conclusions

There are many factors which have improved outcome in bone marrow transplant recipients since its introduction into routine practice in the 1970s. One of the major factors, particularly in the unrelated transplant situation, has been the improvements in HLA matching techniques, which have progressed from serology, to matching at the sequence level, made possible by the introduction of sequencing methods into the science of tissue matching. Sequencing of HLA alleles has had a twofold effect. Firstly, it has improved outcome for many patients and secondly has unearthed a level of polymorphism of which was not predicted amongst HLA aficionados. The science of matching therefore has moved from matching for a handful of serological specificities for class I and relying on the mixed lymphocyte reaction as a surrogate marker for class II matching, to sequencing and matching for potentially thousands of alleles at both HLA class I and II.

An additional matching barrier remains. Despite the fact that convincing data exists, which suggests that matching for HLA haplotypes with unrelated donors improves outcome, in

terms of a reduction in GVHD, compared with recipients whose donors are matched at individual HLA class I and class II loci, donors are chosen regardless of genetic phase. Haplotype matching is rarely investigated in bone marrow registry sourced donors.

Family studies, which are difficult logistically in registry donors, or individual chromosome sequencing are two options for assessing haplotype matching in unrelated donor bone marrow situations. The latter option is currently being actively pursued. In addition to improving clinical outcome, individual chromosome sequencing has the potential to unearth other loci which may be clinically important in producing the better outcome.

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