# Bone marrow transplantation and haplotyping

openaccessgovernment.org/article/bone-marrow-transplantation-and-haplotyping/199058

Emily Warrender

October 1, 2025

## Brian Tait, chief scientific officer, Haplomic Technologies Pty Ltd, explores the clinical benefits of haplotyping in single-chromosome sequencing and unrelated donor bone marrow transplantation (HSCT)

The principal genes involved in transplant matching are the Human Leukocyte Antigen (HLA) genes, which are located on the short arm of chromosome 6 within the major histocompatibility complex (MHC). (which includes the HLA-A, -B, –C, –DR, -DQ and –DP genes). While HLA matching at individual alleles is a factor determining success in organ transplantation, there is little evidence that HLA haplotype matching improves graft survival.

## Organ transplantation and rejection

In the case of organ transplantation, the main concern is rejection of the transplant (graft) by the recipient (host), and this can be controlled well with modern immunosuppressive drugs. While it is possible to show with large cohorts that HLA matching has some impact on graft survival, the overall 1-year graft survival of nearly 90% in renal transplantation diminishes any interest clinicians may have in applying HLA haplotyping. Other forms of solid organ transplantation also have excellent outcomes.

## Haematopoietic stem cell transplantation (HSCT)

However, the situation with haematopoietic stem cell transplantation (HSCT), also referred to as bone marrow transplantation, is quite different. Prior to transplantation, leukemic patients are treated with drugs that, in addition to killing tumour cells, also kill healthy cells, which essentially depletes their immune system. In other patients, such as those with aplastic anaemia, the immune system has been severely compromised by the disease.

## Immune reconstitution in HSCT

Still, in both cases, the concept underlying HSCT is the reconstitution of the patient's immune system. Since we are transplanting immunocompetent cells into a patient with a compromised immune system, there are two rejection responses seen.

The first is the host versus graft (HVG) reaction, which occurs in solid organ transplantation, and can result in failure of engraftment of the transplanted cells when severe.

Secondly, a graft versus host (GVH) response is a condition whereby the transplanted cells attack the host. This is a serious complication which can result in damage to the skin, liver, and gut. In its most severe form, GVH disease can be fatal. The avoidance or minimisation of GVH is therefore of paramount importance.

## Importance of HLA matching in HSCT

The probability and the severity of GVH are positively associated with the number of recipient/donor HLA mismatches. Every effort is therefore made to HLA match the donor as closely as possible to the recipient.

An HLA identical sibling (where both individuals have inherited the same chromosome 6 from each parent) is the ideal HSCT donor, but unfortunately, this only occurs in approximately 30% of cases.

The haplotyping of the recipient can be expedited by single chromosome sequencing. Cases of apparent haplotype homozygosity in the parents can be resolved by single chromosome sequencing.

Approximately 70% of patients requiring an HSCT therefore require an unrelated donor. Due to the extensive polymorphisms of the HLA system, large numbers of potential unrelated donors are required to achieve an HLA match.

Patients, therefore, search the Australian Bone Marrow Donor Registry (ABMDR) or the numerous other registries worldwide. There are now in excess of 30 million HLA-typed donors worldwide [1]. HLA haplotypes are not determined in unrelated donors, firstly due to the expense of typing family members, and secondly because in many cases, family members are not available.

## Evidence supporting HLA haplotype matching Perth laboratory studies

Based on two lines of evidence, we know, however, that haplotype matching improves clinical outcome in HSCT. In the 1990s, the Royal Perth Hospital, under the direction of Roger Dawkins and later Frank Christiansen, developed a "block matching" approach to HLA matching.

Tay et al., in the two papers published in 1995 [2,3], first showed that each polymorphic block included haplotype-specific markers. Using DNA PCR amplification followed by electrophoresis and scanning with a laser, they were able to identify markers within each HLA β and Δ haplotype block. Those HSCT 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 conventional HLA genotyping. 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 was 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 permits allele-level matching.

However, this was addressed by the paper of Witt et al [4] and a follow-up analysis by the group [5]. Witt showed that HSCT patients who were matched at the β and Δ blocks had superior event-free survival than mismatched patients (63% vs 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 (5) studied 44 HSCT 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.

## Gamma block and complement gene polymorphisms

However, David Sayer, who worked in the Perth laboratory and who founded and was the CEO of Conexio Genomics, has developed a kit which utilises complement gene polymorphisms, which are located in the HLA class 3 region (gamma block), to define haplotypes better, but this is not routinely used in HSCT.

Sayer showed [6] that in 23 SNPs, there was no difference between those mismatched and those matched or with the degree of mismatch. The endpoints measured were acute GVHD, chronic GVHD, and mortality. C4A and C4B are the most polymorphic genes in the gamma block. An exception was the association between 1–3 mismatches at the composite of SNPs C13193/T14952/ T19588 with the development of acute GVHD (P = 0.012) and with grades III-IV of this disease (P = 0.004).

Maskalan et al [7] looked at gamma matching using SSP primers, which covered the 23 SNPs found in the gamma block. This group showed that, in a cohort of 51 patients, measuring 23 SNPs across the gamma block using the OLERUP SSP AB, SWEDEN, there was a borderline association between haplotype matching and acute GVHD (p<0.041). Surprisingly, 70.59% of patients were haplotype mismatched. All donor/recipient pairs were HLA-A, B, C, DRB1, DQB1 allele matched (10/10).

## Seattle studies on haplotype matching

The second strand of evidence comes from Effie Petersdorf and her co-workers, Fred Hutchison Cancer Research Centre, Seattle, who showed some years ago that patients HLA haplotype matched with their donors at the HLA-B, class 3 region and class 2 region had superior clinical outcome to those donor-recipient pairs who were allele matched at these loci.

They utilised a different technique from the Perth laboratory [8]. Probes specific to the two HLA-B alleles were adhered to a glass surface and hybridised with the genomic DNA. After washing off excess genomic DNA, the two captured DNA fragments were placed in separate tubes, and PCR primers were used to amplify the HLA-A and DRB1genes. This method relies on having DNA strands of 2mb, so that A and DRB1 are removed together, and prior knowledge of the individual's HLA genotype.

Using this method, Petersdorf and her co-workers [9] were able to show that of 246 unrelated donor transplant pairs who were HLA-A-, B-, C-, DRB1, and DQB1 allele matched, 22% (55) were 1 or 2 haplotypes mismatched with their donors. The difference in acute GVHD was approximately 40% between the matched and mismatched groups (matched = 20%, mismatched = 60%, $p < 0.0001$). These data suggest there are important genes within the MHC that, unless they are matched, produce GVHD.

Despite this evidence that indicates haplotype matching is clinically efficacious, no test has been developed for routine use to determine HLA haplotypes in HSCT involving unrelated donors.

## Haplomic Technologies and single chromosome sequencing

Haplomic Technologies' approach of isolating and lysing metaphase cells and using fluorescent probes to identify chromosome 6 and hence provide a source of DNA for amplification and haploid sequencing of HLA genes could provide a platform for such routine use.

The potential market for a routine assay is all laboratories worldwide that provide HLA genotyping services to National Bone Marrow Donor Registries. From a marketing point of view, contact with the various registries should be part of the overall strategy since the registries, to a large extent, dictate HLA genotyping requirements for inclusion in their database. This, therefore, is the first target market for HT's single chromosome technology.

### References

1. https://wmda.info/
2. Tay, GK.et al,1995 Bone Marrow Transplantation 15(3) pp381-385.
3. Tay GK et al 1995 Exp. Hematol.23: pp1655-1660.
4. Witt, C et al 2000. Human Immunology, 61(2), pp.85-91.
5. Kitcharoen K et al 2006.Human Immunology; 67(3); pp.238-246.

6. Getz J et al. Transfusion and Cell Therapy, 42 (3), pp. 221-229
7. Maskalan M et al 2020. Human Immunology 81;pp 12-17.
8. Guo Z et al 2006. Proc. of the Nat. Acad.of Sciences, 103(18), pp.6964-6969
9. Petersdorf E et al. PLoS Medicine; 4(1); p. e8

Primary Contributor

Brian Tait
Haplomic Technologies Pty Ltd