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16th IHIW: International Histocompatibility Working Group in Hematopoietic Cell Transplantation

E. W. Petersdorf*, M. Malkki*, K. Hsu†, P. Bardy‡, A. Cesbron§, A. Dickinson¶, V. Dubois, K. Fleischhauer††, T. Kawase‡‡,§§, A. Madrigal¶¶, Y. Morishima‡‡,§§, B. Shaw***, S. Spellman†††, E. Spierings‡‡‡, M. Stern§§§, J.-M. Tiercy¶¶¶, A. Velardi**** & T. Gooley* on behalf of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation**

Summary

The International Histocompatibility Working Group is a collaborative international effort to understand the HLA and non-HLA genetics of the transplantation barrier. The Working Group is comprised of experts in the fields of histocompatibility and immunogenetics, hematopoietic cell transplantation and outcomes research. Data for 25 855 unrelated donor transplants were submitted in support of research studies for the 16th International Histocompatibility Workshop. Active investigation is in progress in seven key areas: the impact of HLA matching, role of race and ethnicity, identification of permissible HLA mismatches, haplotype-associated determinants, minor histocompatibility antigens, immune response genes and KIR genetics. New hypotheses for the 16th workshop were developed for immunogenetic studies in cord blood and haploidentical-related donor transplantation.

* Division of Clinical Research, Fred Hutchinson Cancer Research Center, Seattle, WA, USA, † Memorial Hospital, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, ‡ Red Cross Blood Transfusion Service, Adelaide, SA, Australia, § Laboratoire d'Histocompatibilité, EFS Pays de la Loire, Nantes, France, ¶ Haematological Sciences Institute of Cellular Medicine Newcastle University, Newcastle, UK, ** EFS Rhone Alpes-Laboratoire HLA, Lyon, France, †† Instituto Scientifico H. San Raffaele, Milan, Italy, ‡‡ Japan Marrow Donor Program, Tokyo, Japan, §§ Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan, ¶¶ Anthony Nolan Research Institute, London, UK, *** Royal Marsden Hospital and Anthony Nolan Research Institute, London, UK, ††† Center for International Blood and Marrow Transplant Research, Minneapolis, MN, USA, ‡‡‡ University Medical Center Utrecht, Utrecht, The Netherlands, §§§ Division of Hematology, Kantonsspital, Basel, Switzerland, ¶¶¶ LNRH/Transplantation Immunology Unit, University Hospital, Geneva, Switzerland and **** European Group for Blood and Marrow Transplantation, Perugia, Italy

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Correspondence: Effie W. Petersdorf, Division of Clinical Research, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave N, Seattle, WA 98109, USA. Tel: +12066675244; Fax: +12066675255; E-mail: epetersd@fhcrc.org

The IHWG in Hematopoietic Cell Transplantation: a unique collaboration of the HLA research and clinical transplant communities

The studies of the International Histocompatibility Working Group (IHWG) in Hematopoietic Cell Transplantation (HCT) were presented at the 16th International HLA and Immunogenetics Workshop in Liverpool in May, 2012. The projects within the Working Group represented a highly integrated group of studies drawing from a database of 25 963 transplants contributed by 170 participants representing 42 laboratories, 435 transplant centres, 10 transplant and donor registries and 18 countries (Fig. 1, Tables S1 and S2 in Supporting Information). The ethnic and racial diversity of the unrelated donor pairs continues to increase to meet the research goals of the working group (Fig. 2). How has the science changed since 1999? Hypotheses have increased sophistication, in part due to the larger and more ethnically diverse datafile, the development of novel laboratory methods and the elucidation of HLA and KIR by the research community. Since 1996, changes in the clinical practice of HCT have played an important role in shaping the specific research questions being addressed by the IHWG HCT Working Group. Notable developments by clinical transplant community include the development of reduced intensity conditioning regimens, expansion of transplantation to the treatment of non-malignant diseases, the growth of the size and composition of donor registries leading to increased racial and ethnic diversity of transplanted patients and donors, and increased use of cord blood as a stem cell source for both children and adults. The following sections describe the overall scientific goals of the IHWG HCT Working Group, the database and summaries of the studies presented at the 16th International HLA and Immunogenetics Workshop in Liverpool on 28–29 May 2012.

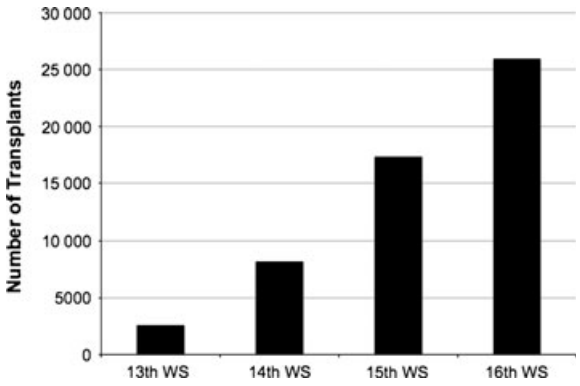

Figure 1. International Histocompatibility Working Group (IHWG) database as of 28 May 2012. The IHWG HCT Working Group database includes high-resolution HLA typing and clinical data for a total of 25 963 transplants (25 855 unrelated donor and 108 cord blood).

Research goals of the Working Group

The overall mission of the Working Group is to increase the availability and efficacy of HCT from alternative donors through an improved understanding of the genetic barrier. Today's landscape is very different from whence the 11th workshop studies were conducted. Over 20 million volunteer donors and 540 000 cord blood units are currently registered worldwide (www.bmdw.org) and provide patients a critical resource for identifying a suitable graft source for transplantation. HLA matching lowers morbidity and mortality of transplantation, but patients are still at risk of potentially life-threatening complications including acute and chronic graft-versus-host disease (GVHD). Furthermore, recurrence of disease after transplantation remains a significant cause of failure. Hence, the unmet needs today include an understanding of novel MHC and non-MHC genetic variation that could be responsible for risks after HLA-matched transplantation. At the same time, clinical experience has amply demonstrated the high-risk nature of HLA-mismatched unrelated donor transplantation, showcasing the need to understand the relative risks associated with mismatching at specific HLA loci and for specific combinations of mismatches.

The growth of cord blood and haploidentical-related donor transplantation as highly effective sources of stem cells now provide patients with a choice of graft sources. Although the risks associated with each of these modalities relative to each other are still coming into focus, the needs of patients are very clear: reduced morbidity and mortality in both the HLA-matched and HLA-mismatched settings.

The scientific goals of the IHWG HCT Working Group are severalfold: increase the sharing of donors and cord blood units internationally; refine the criteria for the selection of mismatched stem cell sources; lower transplant-associated risks through a more complete understanding of HLA and non-HLA variation and MHC haplotypes. The paradigm of the international HLA workshop serves the HCT Working Group well for four important reasons: collaboration, high standards of quality control, validation and translation of information to patient care. Collaboration within the framework of an international HLA workshop provides new avenues for exploring novel laboratory methods and analysis tools. Since transplant procedures differ from centre to centre and region to region, the effects related to different conditioning and GVHD prophylaxis regimens are important not only for adjusting for these effects in multivariate models, but open up new questions on the role of genetics in different transplant settings. Patient populations also differ with respect to disease and stage, and the specific HLA alleles and haplotypes. In this regard, an international HLA workshop is uniquely positioned to explore the clinical ramifications of HLA diversity. Finally, collaboration aids in the attainment of sufficiently large sample sizes that are required for adequate statistical power.

High standards for quality control have always been a benchmark of the international HLA workshops. For the studies in HCT, quality control not only applies to complete and precise characterization of the HLA system, but also complete, uniform definitions of clinical endpoints. Validation of study results in large independent populations is important prior to translation to clinical care. For the first time in its history, several studies are now poised to apply new information on the HLA and KIR systems to change clinical practice.

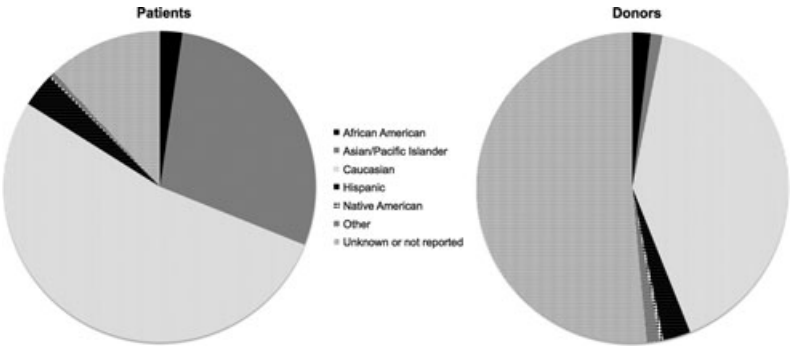

Figure 2. Self-defined ethnicity/race of the transplant patients and donors.

Demographics of the clinical populations

In support of the 13th and 14th International HLA Workshops, the Working Group initially focused its efforts on the collection of HLA and clinical data for patients transplanted from unrelated donors for acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML) and myelodysplastic syndrome (MDS) using ablative conditioning, due to the relative homogeneity of transplant procedures used internationally for these disorders. Beginning with the 15th International HLA Workshop, the patient population was expanded to include all malignant and non-malignant diseases, and all transplant regimens. For the 16th International HLA and Immunogenetics Workshop, new hypotheses were developed for cord blood and haploidentical-related donor transplantation.

The studies presented at the 16th International HLA and Immunogenetics Workshop in Liverpool were supported by the IHWG HCT database of unrelated patient–donor pairs that included 10 937 HLA 10/10 matched, 6203 HLA 9/10 matched and 4636 8/10 matched (or less) pairs. This population reflected general clinical practice internationally. Transplantations were performed between 1983 and 2011, and the range of recipient age was 0.1–79 years. Patients received transplants for the treatment of acute myeloid or lymphoid leukaemia (52.1%), myeloproliferative disorders (20.0%; CML, polycythemia vera, myelofibrosis), MDS (13.4%), lymphoma (7.2%; Hodgkin and non-Hodgkin), plasma cell dyscrasias (1.2%; multiple myeloma and others) or non-malignancies (4.9%; aplastic anaemia, autoimmune disease, histiocytic disorders, inherited abnormality of platelets, inherited disorder of metabolism, inherited abnormalities erythrocyte differentiation, hemoglobinopathies, inherited abnormal of erythrocyte differentiation, severe combined immunodeficiency, chronic granulomatous diseases and other non-malignancies). Using standard definitions of disease severity, the distribution of disease stage in patients at the time of transplantation was low (34.3%), intermediate (24.1%), high (23.1%), non-malignant (4.9%), not applicable (0.8%) or undefined (12.8%). Transplant conditioning regimens included ablative with total body irradiation (TBI) (55.6%), ablative without TBI (20.3%), non-myeloablative (20.0%) or undefined (4.9%). GVHD prophylaxis regimens included use of a single agent (2.6%), multiple agents (66.1%), T-cell depleted (*ex vivo*, use of ATG/ALG and/or Campath in either conditioning regimen or GVHD prophylaxis regimen) (25.8%) or other/undefined (5.5%). The graft sources were 69% bone marrow, 30% peripheral blood stem cells and 1% undefined.

Data for 108 cord blood transplants were submitted by May 2012; data for a further 5003 cord blood transplants and for 1350 haploidentical-related donor transplants were committed from Working Group participants and are currently in progress.

Genetic and clinical data

An annual review of human subjects approval is undertaken to assure that the consenting process for the collection and analysis of de-identified transplant data is in place (Fig. 3). A certification and data sharing agreement is used for each new submission of data to the IHWG database; these agreements acknowledge appropriate research use of data based on informed consent and data sharing for research purposes. Proposals are reviewed by the Steering Committee which has the responsibility of assuring that the scientific goals of the Working Group are being met and that standard operating procedures and policies are updated.

HLA data

High resolution typing of HLA class I alleles were performed by sequence-based typing methods in 51% and by other methods (SSP, SSOP, RCA) in 32% of samples. The methods were not specified for 15% of samples. For class II alleles, sequence-based typing was employed for 44% of samples and other methods for the remainder of samples. The minimal criteria for acceptance of data are exon 2 and exon 3 for HLA-class I, exon 2 for HLA class II alleles, and the year in which the typing was performed; for future workshops, the Working Group will collect information on the version of the HLA nomenclature report used to define the allele. Data were collected for the following additional loci: DRB3, DRB4, DRB5 (15% of samples with appropriate DRB1 allele), DQA1 (42%), DPA1 (25%) and DPB1 (69%) of samples.

The distribution of pairs with 0, 1, 2 or more DNA-detectable mismatches is illustrated in Fig. 4. Of the 6203 single-locus HLA 9/10 transplants, 23% were mismatched at HLA-A, 10.4% at HLA-B, 41.9% at HLA-C, 6.2% at HLA-DRB1 and 18.3% at HLA-DQB1. Of the 15 027 pairs typed for HLA-DPB1, 82% were mismatched. Of the 4636 two-locus-mismatched HLA 8/10 transplants, the most common mismatch combinations were HLA-DRB1 and DQB1 (26%); HLA-B and C (21.6%) and HLA-A and C (15.6%). Of the 8/10 pairs, 58.8% were mismatched at HLA-C in addition to a second locus.

KIR data

The KIR genetic region has emerged as a significant regulator of innate immune function and contributor to the success of hematopoietic stem cell transplants. Variation at the level of gene content as well as significant allelic polymorphism have stimulated investigations of donor KIR genotype on transplant outcome, identifying specific KIR genes in the donor as beneficial for protection from leukaemic relapse and survival. As our understanding of how KIR and HLA molecular interaction dictates natural killer (NK) cell

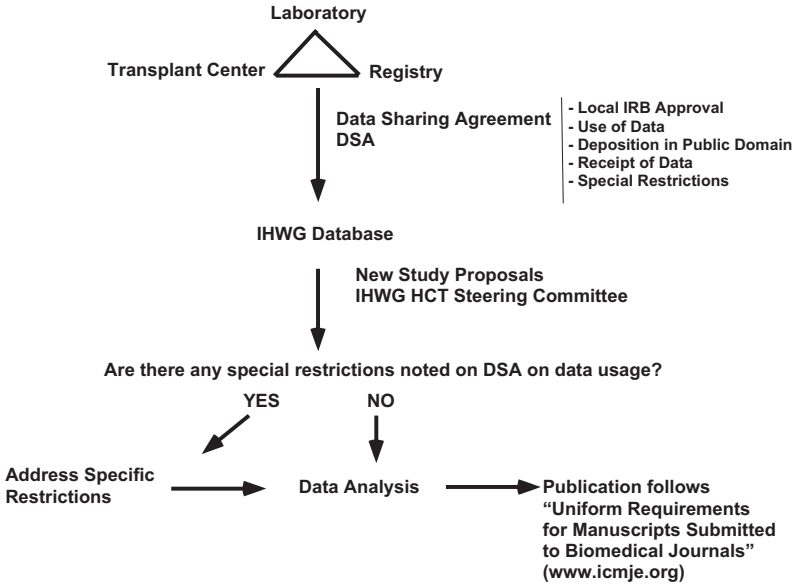

Figure 3. Schema for data collection, study design, analysis and reporting. The data sharing agreement (DSA) certifies that the participant has received approval for the use of research samples by their local IRB; that the data may be included in IHWG HCT Working Group studies; whether de-identified data may be made available for public access; whether the participant is interested in receiving the genotyping data performed by the IHWG Coordinating Laboratory on the participant's samples, and whether there are specific restrictions for the use of the data. Approval for the conduct of research involving human subjects for IHWG HCT studies is reviewed annually by the institutional review board of the Fred Hutchinson Cancer Research Center, Seattle. Specific restrictions on the use of data are reviewed by research study committees from participating institutions and registries including the CIBMTR, EBMT and JMDP.

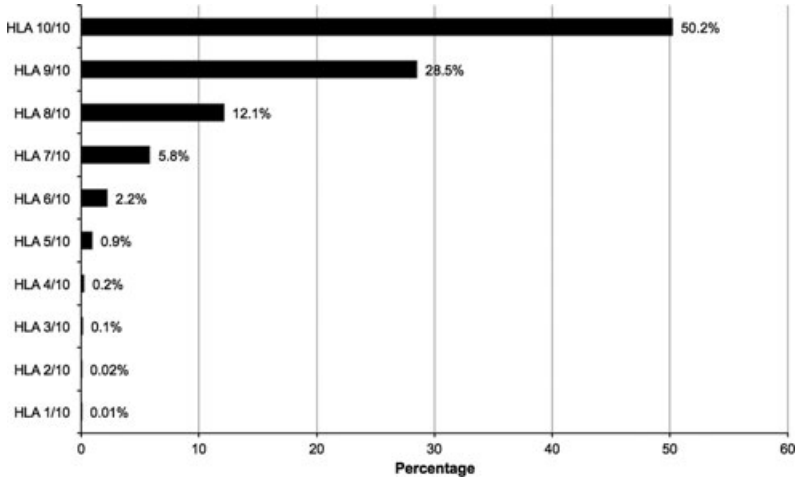

Figure 4. HLA match grades of unrelated patient-donor pairs. The HLA match grade is defined for 10 HLA determinants from high resolution typing of HLA-A, C, B, DRB1, DQB1 loci in 21 777 unrelated donor transplant pairs.

function, research questions are now focusing on how KIR/HLA genotype combinations in the donor and recipient impact transplant outcome. At the Victoria workshop in 2002, the Working Group first established its intent to examine the role of KIR ligands and KIR/HLA interaction on transplant outcome. Since that time, 17 laboratories in addition to the Center for International Blood and Marrow Transplant Research (CIBMTR) and Japan Marrow Donor Program (JMDP) have contributed genotyping and clinical data on 2816 pairs, resulting in three published studies examining KIR ligands, donor KIR genotype and donor–patient KIR/HLA genotype combinations (Hsu *et al.*, 2006; Venstrom *et al.*, 2010, 2012).

Data contributed to the KIR–HLA studies of the Working Group are comprised of high-resolution HLA genotyping of the donor and the patient, and donor KIR genotyping, where KIR genotype may be submit-

ted at gene content or allele-level resolution. The relevant HLA loci include HLA-A, B and C, as these molecules are known ligands for the KIR receptors. Criteria for acceptable HLA genotype data are as per the HLA component of the Working Group.

The KIR genetic region contains up to 15 genes and pseudogenes, whose gene/alleles can be identified in a number of ways, including PCR-SSP, PCR-SSOP, SBT and mass spectrometry-based (MALDI) methods. Acceptable genotyping for submission must include typing for the known activating (2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DS1) and inhibitory KIR genes (2DL1, 2DL2, 2DL3, 2DL5, 3DL1), in addition to the framework genes (3DL3, 2DL4, 3DL2). Pseudogene typing is not mandatory, but is accepted. Laboratories wishing to contribute KIR genotyping to the Working Group must first complete a quality-control step to verify accuracy of their KIR typing methods. Evidence of completion of KIR genotyping of either a CEPH DNA panel (provided by the Working Group) or the UCLA KIR reference panel is needed before KIR genotyping data can be accepted for inclusion in Working Group research projects.

Clinical data

Transplant and donor registries continued to play a critical role in the collection of clinical data. For the Liverpool workshop studies, 89.5% of the clinical data was contributed by the Australian Bone Marrow Donor Registry (ABMDR), the Anthony Nolan (AN), the CIBMTR, the European Group for Bone Marrow Transplantation (EBMT), the JMDP and the Société Française de Greffe de Moelle et Therapie Cellulaire (SFGM) registries. Data from registries and from transplant centres followed conventional definitions of disease stage: high [any relapse of acute or chronic leukaemia, lymphoma, myeloma, MDS more advanced than refractory anaemia (RA) and refractory anaemia with ringed sideroblasts (RARS)]; intermediate [acute leukaemia in second or higher complete remission (CR); CML in second or higher chronic phase (CP); lymphoma in second or higher CR], and low (acute leukaemia in first CR; CML in first CP; MDS RA/RARS; multiple myeloma in remission). Relapse was defined according to hematologic/molecular evidence.

Specific topics of the IHWG HCT Working Group

Clinical significance of HLA matching in unrelated donor and cord blood transplantation

Extensive clinical experience demonstrates that complete and precise HLA matching of potential-unrelated donors is associated with improved clinical outcome compared with HLA mismatching. When HLA-matched donors are not available, the use of donors with selected HLA mismatches is feasible. When an unrelated donor search yields more than 1 otherwise equivalent HLA-mismatched donor, the criteria with which to select the least risky mismatch are unknown. The IHWG studies test the hypotheses that risks after unrelated donor transplantation are shaped by the total number of HLA mismatches, the locus or loci that are mismatched, the resolution of the mismatch combination (low-resolution antigen-equivalent versus high-resolution allele) and the specific amino acid mismatch at a given residue. To meet these research objectives, studies defined the transplant populations by the number of HLA mismatches (10/10, 9/10, 8/10, etc.) and the mismatched locus (loci). Multivariate models adjusted for the non-genetic variables that influenced the clinical endpoint of interest. For the 16th workshop, data for 10 938 HLA 10/10 matched, 6203 single-locus (9/10) matched, and 4636 multilocus mismatched pairs were evaluated. Mortality increased as the total number of HLA mismatches increased. The absolute value of the odds of acute GVHD and the hazards of relapse and mortality differed for each locus. For HLA-C mismatches, there was a suggestion that mortality depended on the number of mismatched residues; furthermore, not all mismatched residues contributed equally to risks. A comprehensive report of these results will follow. Future studies will investigate the role of HLA-DRB3, DRB4, DRB5, DQA1, MICA, MICB, HLA-E and HLA-G in unrelated donor transplantation using workshop genotyping protocols and quality-control panels. Analysis of residues on an expanded data set of ethnically diverse transplant pairs is desirable to increase the diversity of amino acid residue mismatch combinations. Molecular modelling approaches for identifying permissible HLA mismatches remains an area of interest (Yanover *et al.*, 2011).

In a specific analysis of the effect of ethnicity on the importance of HLA-C mismatched KIR ligand mismatches in GVHD, Japanese patients had a higher risk of mortality than did non-Japanese patients (Kawase *et al.*, 2011). This analysis will be extended to test the hypothesis that risks of HLA-A, B, DRB1 and DQB1 mismatches depend on the diversity of HLA genotypes of the patient and donor, by examining mismatches that are shared among Japanese and non-Japanese transplant recipients, as well as those mismatches that are unique to the two populations.

Impact of donor–recipient race and ethnicity on risk of acute GVHD, leukaemia relapse and survival in HLA-matched unrelated donor transplantation

These studies seek to define the role of donor–recipient ethnicity on transplant outcome (Morishima *et al.*, 2009). The results have major significance in the effort to identify the genes that are responsible for acute GVHD across transplant populations. The IHWG study led by Prof. Yasuo Morishima included 4335 HLA-matched pairs of which 1734 transplants were performed in Japan

and the remainder in non-Japanese transplant centres. Analyses were restricted to patients receiving transplants for the treatment of AML, ALL and CML. Compared with Japanese patients, patients of all other ethnic backgrounds had higher risks of clinically severe acute GVHD and mortality. Among Japanese patients with standard risk leukaemia, there was a trend for a lower risk of relapse independent of disease diagnosis or grade of acute GVHD (a publication detailing this work will follow). Future studies will investigate the impact of ethnicity on risk of chronic GVHD, and define high-risk HLA haplotypes associated with GVHD.

Role of HLA-DP

The HLA-DP studies were designed to test the hypothesis that permissive HLA-DP mismatches can be defined by T-cell epitopes (TCEs) encoded by polymorphisms within HLA-DPB1 exon 2 and that the effect of TCE-(non)permissive mismatches is synergistic with that from HLA-A, B, C, DRB1, DQB1 mismatches. Based on a definition of TCEs defined by cross-reactivity of alloreactive T-cell clones isolated from a patient who experienced graft failure (Zino *et al.*, 2004), an algorithm for TCE matching was applied to the analysis of 5428 HLA 10/10-matched and 3111 single-locus-mismatched HLA 9/10 pairs led by Drs. Katharina Fleischhauer and Bronwen Shaw (Fleischhauer *et al.*, 2012). Compared with patients transplanted from HLA 10/10-matched donors with a TCE-permissive HLA-DPB1 mismatch, patients with a HLA 12/12-matched donor had a significantly higher risk of relapse and a suggestively lower risk of grades III–IV acute GVHD, suggesting the lack of a beneficial graft-versus-leukaemia (GVL) response. However, patients transplanted from HLA 10/10-matched but TCE-non-permissive HLA-DPB1 mismatched donors had significantly increased mortality, non-relapse mortality and grades III–IV acute GVHD, demonstrating the high-risk nature of the TCE-non-permissive HLA-DPB1 mismatches. Single-locus-mismatched HLA 9/10 transplantation from TCE-non-permissive donors was associated with the poorest survival when compared with HLA 12/12, HLA 10/10 TCE-permissive or non-permissive, and HLA 9/10 with a TCE-permissive HLA-DPB1 mismatch. In contrast, transplants from HLA 9/10 donors with a TCE-permissive HLA-DPB1 mismatch were not associated with significantly different risks of mortality compared with those from HLA 10/10 donors with a TCE-non-permissive HLA-DPB1 mismatch. These results demonstrate that permissivity of HLA-DPB1 mismatches can be defined by alloreactive TCEs, both in the HLA 10/10 and in the HLA 9/10 setting. In these transplants, a TCE-non-permissive HLA-DPB1 mismatch leads to substantially increased risks that can be avoided with prospective donor–recipient typing for HLA-DPB1 alleles and avoidance of TCE-non-permissive HLA-DPB1 mismatches (Fleischhauer *et al.*, 2012). An IMGT/HLA Database web-based tool has been developed by Dr. Steven Marsh to aid in the classification of recipient–donor HLA-DPB1 TCE groups and is available at www.ebi.ac.uk/imgt/hla/dpb.html. Future studies will focus on the role of TCE-defined mismatches in different clinical populations defined by the GVHD prophylaxis regimen (T-cell replete versus T-cell deleted), the graft source (marrow versus peripheral blood stem cells), the patient's disease diagnoses and in transplants with two HLA mismatches (HLA 8/10) or more.

Role of HLA-DR15 in transplant outcome

These studies tested the hypothesis that DR15 is involved in alloimmune reactions after unrelated donor transplantation (Stern *et al.*, 2006). The study population consisted of 19 143 HLA 10/10 and 9/10 matched pairs defined by presence or absence of HLA-DR15. A subset analysis of DRB1*15:01 versus DRB1*15:02 was also performed. Among HLA-matched and single-locus-mismatched transplants, the risks of grades III–IV acute GVHD and mortality were not statistically significantly different between DR15-positive and DR15-negative patients. There was suggestive evidence for differences in risk of relapse based on HLA match grade and ethnicity, suggesting possible mechanistic pathways involved in relapse. A publication detailing this work will follow.

Mapping novel MHC determinants

GVHD and other risks after HLA-matched unrelated donor transplantation may depend on untyped genetic variation within the MHC. This hypothesis was tested in a discovery-validation study of 4205 patients transplanted in US centres for AML, CML, MDS or ALL from HLA 10/10 matched unrelated donors (Petersdorf *et al.*, 2012). Two variants were identified as risk markers for GVHD and disease-free survival. Based on these findings, a new Working Group study of SNPs will determine the frequency of donor–recipient matching in ethnically diverse transplant populations.

MHC haplotypes

The clinical significance of specific HLA haplotypes will be investigated in unrelated donor, haploidentical-related donor and cord blood transplantation. Haplotypes will be characterized for HLA-E, F, G, MICA and MICB in addition to HLA-A, C, B, DRB, DQA1, DQB1, DPB1. Risks associated with the presence of specific haplotypes will be defined.

Cytokine polymorphisms

The importance of genetic variation in 13 cytokine genes, including IL1B, TNF, IL10, TGFB1, HSPE, has been extensively evaluated by participants from France (Table S2 in Supporting Information). Of these, TNF

was also recently identified by Bettens *et al.* (2012) as a risk marker for acute GVHD and mortality. The IHWG data set serves as an invaluable population for the validation of cytokine gene polymorphisms of interest. In collaboration with Dr. Jean-Marie Tiercy, the IHWG study will elucidate the SNP content of TNFd microsatellite alleles in a side-by-side evaluation of samples typed for the microsatellite alleles described by Bettens *et al.* (2012) with SNPs residing with the TNF block. Upon completion of the pilot study, formal analysis of TNF block haplotypes will be undertaken to test the hypothesis that genetic variation within the TNF block influences clinical outcome after transplantation. The study population includes all unrelated donor transplants (without restriction to HLA match status) haploidentical-related and cord blood transplants. Testing of TNF block variants will involve workshop quality-control panels prior to large-scale testing of patients, donors and cord blood units. Validation of other reported polymorphisms in cytokine genes remains an important goal for the Working Group (Malkki *et al.*, 2007; Chien *et al.*, 2012).

Minor histocompatibility antigens

Synergistic effects of mismatching for minor and major histocompatibility antigens may explain why there is heterogeneity of risks among transplanted patients, particularly when female donors are used for male recipients. To test this hypothesis, Dr. E Spierings *et al.* have analysed 1332 HLA 12/12 and 3329 HLA 11/12 DPB1 mismatched pairs. Multivariate analysis reveals a synergistic effect of HLA-DP mismatching with HY-mismatching for grades III –IV acute GVHD (publication to follow). Future analyses will re-evaluate the significance of HLA-DPB1 mismatches by way of TCE-defined epitopes (Fleischhauer *et al.*, 2012) and inclusion of the HLA restriction molecule.

Cord blood transplantation

The addition of cord blood transplants to the study population represents a natural extension of the IHWG studies to define the genetics of the transplantation barrier. The role of HLA matching in clinical outcome will evaluate HLA-A, B and DRB1 as the initial six determinants, where HLA-A and B are defined by low-resolution antigen-equivalent level testing, and DRB1 is defined at the allele level. Subsequently, the role for HLA-C and for typing at high resolution for all HLA loci will be evaluated. Analysis of MHC SNPs and haplotypes, and cytokine polymorphisms as outlined for unrelated donor transplants, are planned for future studies.

Role of non-inherited maternal antigens

Recent validation of the protective effect of matching for non-inherited maternal antigens (NIMA) in cord blood transplantation (Rocha *et al.*, 2012; van Rood *et al.*, 2012) provides impetus for the analysis of NIMAs in haploidentical-related and cord blood transplantation in the IHWG HCT Working Group. As described above, the haploidentical-related donor and cord blood transplant populations are newly recruited. They will be analysed for the presence of NIMA-matching and the total number of HLA mismatches in multivariate models that control for non-genetic variables affecting the clinical endpoints. The inclusion of patients, donors and cord blood units representing individuals of diverse ethnic and racial backgrounds is a main theme of the IHWG studies to increase representation of diverse HLA mismatches and haplotypes.

HLA peptides

These newly proposed studies by Dr. Eric Spierings will assess whether GVHD and GVL are related to the number of recipient-derived HLA peptides presented by donor-shared recipient HLA molecules. The studies are designed to specifically address the relative risks of peptide mismatching with respect to gender mismatching, whether the intensity of the conditioning regimen can influence the peptide-mismatch effects, and whether the impact of mismatching at HLA-C or HLA-DP is influenced by peptide-mismatch effects. The study population includes HLA 12/12 and 11/12 matched pairs.

KIR

Initial studies examining the role of NK cells focused on KIR ligand incompatibility, a condition fulfilled only in the HLA-mismatched setting and requiring only knowledge of the HLA genotypes of the transplant donor and patient. The Working Group has evaluated KIR ligands in both HLA-matched and mismatched transplant pairs, concluding that KIR ligand incompatibility between the HLA-mismatched donor and patient as a marker of NK alloreactivity is not a necessary requirement for protection from leukaemic relapse, but that homozygosity for HLA-B and C epitopes predictive of 'missing KIR ligand' present even in HLA-matched transplant pairs could also confer a protective effect (Hsu *et al.*, 2006). Evaluation of donor KIR genotypes revealed that the presence of activating KIR3DS1 in the donor genotype is associated with lower GVHD, lower transplant-related mortality and higher overall survival (Venstrom *et al.*, 2010). Most recently, translating *in vitro* findings of the role of KIR2DS1 and its HLA-C2 ligand in NK education, Venstrom *et al.* (2012) have shown that patients with AML experience lower post-transplant relapse if their unrelated stem cell donors have KIR2DS1, but only if the donors are not homozygous for HLA-C2 (Venstrom *et al.*, 2012).

The immediate priority of the Working Group is to expand the KIR–HLA transplant data set so that the

multiple distinct mechanisms of donor NK alloreactivity can be evaluated in parallel. This will help to establish the relative strength of each mechanism to the spectrum of donor NK alloreactivity, as assessed by outcomes of relapse, infection, GVHD and survival. In turn, the hierarchy can then be used in grading suitability of stem cell donors based on their KIR and HLA genotypes. A study in progress is examining combinations of KIR and HLA alleles on transplant outcome for patients with acute leukaemia. Planned studies in the KIR–HLA will extend research inquiries to include non-myeloablative transplants, transplants utilizing alternative stem cell donor sources (umbilical cord allografts, haploidentical donors) and patients with lymphoma.

The future

The IHWG HCT Working Group has effectively applied the international HLA workshop paradigm as a model for research studies in transplantation. Collaboration among immunogenetics and transplant clinicians has been an essential feature of the Working Group at many levels. Novel methods include the use of SNPs and haplotypes to identify new variants that are clinically relevant. Collaboration is essential to increase the sample size required for adequate statistical power to address the significance of amino acid residues and SNPs. Differences in the transplant regimens used from centre to centre mandate collaboration to identify homogeneous patient populations to minimize the confounding effects of these variables, and to test the hypothesis that the effect of genetic variation is the same under different transplant conditions.

Central to the success of international workshop studies is a high standard for quality control of the data. For transplantation, this includes not only the genetic data, but also clinical data which uses standardized definitions and unified report forms by registries. The addition of new hypotheses in cord blood and haploidentical-related donor transplantation put the traditional workshop values to the test: complete and precise characterization of the HLA system along with high-quality clinical data will be fundamental to the success of these studies in the future.

The IHWG database has served as an independent cohort for the validation of observations made by individual laboratories and transplant centres. The studies planned for permissible HLA mismatches, cytokines, minor histocompatibility antigens represent the next generation of testable hypotheses that will benefit from a large, ethnically diverse set of data.

Finally, the research goals of the IHWG are all centred on the global mission to increase the efficacy and availability of alternative donor transplantation as treatment for hematologic disorders. To that end, the translation of research findings to prospective donor selection, particularly the concepts of permissive mismatches, SNP matching and KIR typing, are a new and exciting concept for international HLA workshops to come.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 The members of the International Histocompatibility Working Group in Hematopoietic Cell Transplantation.

Table S2 The number of transplant pairs in the IHWG Hematopoietic Cell Transplantation database as of 28 May 2012.