8. Kiyoi H, Yanada M, Ozekia K. Clinical significance of FLT3 in leukemia. *Int J Hematol*. 2005;82:85-92.
9. Yanada M, Matsuo K, Suzuki T, Kiyoi H, Naoe T. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations for acute myeloid leukemia: a meta-analysis. *Leukemia*. 2005;19:1345-1349.
10. Frohling S, Schlenk RF, Breitruck J, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood*. 2002;100:4372-4380.
11. Murphy KM, Levis M, Hafez MJ, et al. Detection of FLT3 internal tandem duplication and D835 mutations by a multiplex polymerase chain reaction and capillary electrophoresis assay. *J Mol Diagn*. 2003;5:96-102.
12. Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/ Eastern Cooperative Oncology Group Study. *Blood*. 2000;96: 4075-4083.
13. Karp JE, Blackford A, Smith BD, et al. Clinical activity of sequential flavopiridol, cytosine arabinoside, and mitoxantrone for adults with newly diagnosed, poor-risk acute myelogenous leukemia. *Leuk Res*. 2010;34:877-882.
14. Vaughan WP, Karp JE, Burke PJ. Two-cycle timed-sequential chemotherapy for adult acute nonlymphocytic leukemia. *Blood*. 1984;64:975-980.
15. Castaigne S, Chevret S, Archimbaud E, et al. Randomized comparison of double induction and timed-sequential induction to a "3 + 7" induction in adults with AML: long-term analysis of the Acute Leukemia French Association (ALFA) 9000 study. *Blood*. 2004;104:2467-2474.
16. McCune JS, Batchelder A, Deeg HJ, et al. Cyclophosphamide following targeted oral busulfan as conditioning for hematopoietic cell transplantation: pharmacokinetics, liver toxicity, and mortality. *Biol Blood Marrow Transplant*. 2007; 13:853-862.
17. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14:641-650.
18. Luznik L, Fuchs EJ. High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation. *Immunol Res*. 2010;47:65-77.
19. Gale RE, Hills R, Kottaridis PD, et al. No evidence that FLT3 status should be considered as an indicator for transplantation in acute myeloid leukemia (AML): an analysis of 1135 patients, excluding acute promyelocytic leukemia, from the UK MRC AML10 and 12 trials. *Blood*. 2005;106:3658-3665.
20. Meshinchi S, Arceci RJ, Sanders JE, et al. Role of allogeneic stem cell transplantation in FLT3/ITD-positive AML. *Blood*. 2006;108:400-401.
21. Bornhauser M, Illmer T, Schaich M, et al. Improved outcome after stem-cell transplantation in FLT3/ITD-positive AML. *Blood*. 2007;109:2264-2265.
22. Fernandez HF, Sun Z, Yao X, et al. Anthracycline dose intensification in acute myeloid leukemia. *N Engl J Med*. 2009;361:1249-1259.
23. Santos FP, Jones D, Qiao W, et al. Prognostic value of FLT3 mutations among different cytogenetic subgroups in acute myeloid leukemia. *Cancer*. 2010.
24. Gaidzik V, Dohner K. Prognostic implications of gene mutations in acute myeloid leukemia with normal cytogenetics. *Semin Oncol*. 2008;35:346-355.
25. Mead AJ, Linch DC, Hills RK, et al. FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. *Blood*. 2007;110:1262-1270.

Predictions in the Face of Clinical Reality: *HistoCheck* versus High-Risk HLA Allele Mismatch Combinations Responsible for Severe Acute Graft-versus-Host Disease

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HLA polymorphism remains a major hurdle for hematopoietic stem cell transplantation (HSCT). In 2004, Elsner et al. proposed the *HistoCheck* Web-based tool to estimate the allogeneic potential between HLA-mismatched stem cell donor/recipient pairs expressed as a sequence similarity matching (SSM). SSM is based on the structure of HLA molecules and the functional similarity of amino acids. According to this algorithm, a high SSM score represents high dissimilarity between MHC molecules, resulting in a potentially more

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deleterious impact on stem cell transplant outcomes. We investigated the potential of SSM to predict high-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease (aGVHD grades III and IV) published by Kawase et al., by comparing SSM in low- and high-risk combinations. SSM was calculated for allele mismatch combinations using the *HistoCheck* tool available on the Web (www.histocheck.org). We compared ranges and means of SSM among high-risk (15 combinations observed in 722 donor/recipient pairs) versus low-risk allele combinations (94 combinations in 3490 pairs). Simulation scenarios were created where the recipient's HLA allele was involved in multiple allele mismatch combinations with at least 1 high-risk and 1 low-risk mismatch combination. SSM values were then compared. The mean SSM for high- versus low-risk combinations were 2.39 and 2.90 at A, 1.06 and 2.53 at B, 16.60 and 14.99 at C, 4.02 and 3.81 at DRB1, and 7.47 and 6.94 at DPB1 loci, respectively. In simulation scenarios, no predictable SSM association with high- or low-risk combinations could be distinguished. No DQB1 combinations met the statistical criteria for our study. In conclusion, our analysis demonstrates that mean SSM scores were not significantly different, and SSM distributions were overlapping among high- and low-risk allele combinations within loci HLA-A, B, C, DRB1, and DPB1. This analysis does not support selecting donors for HSCT recipients based on low *HistoCheck* SSM scores.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many hematologic and nonhematologic disorders. The steady expansion of unrelated stem cell donor registries has facilitated finding a matched donor for many transplant candidates, particularly those with common human leukocyte antigens (HLA) alleles and haplotypes. However, the extensive polymorphism of HLA and the remarkable disparity in the distribution of alleles and haplotypes among individuals of different ethnic and racial backgrounds remain a major hurdle for access of many patients to HSCT. A number of studies have shown that donor/recipient matching for alleles at HLA-A, -B, -C, -DRB1, and -DQB1 loci lowers the risk of clinically severe acute graft-versus-host disease (aGVHD) [1-3]. Recently, HLA-DPB1 allele mismatches were also significantly associated with an increased incidence of GVHD [4-6]. When only HLA mismatched donors are available for a given patient, the challenge becomes determining which mismatch has a less deleterious impact on clinical outcomes. Bray and colleagues [7], in a comprehensive commentary, described the National Marrow Donor Program (NMDP) guidelines for unrelated HSC donor selection including the impact of mismatches at different loci on HSCT clinical outcomes. In 2004, Elsner and colleagues [8] proposed the *HistoCheck* Web-based tool to estimate the allogenicity of mismatches with a sequence similarity matching (SSM) concept. In this concept, an SSM score (ie, allogenicity index) is generated by rating the amino acid (AA) differences between HLA allelic products based on the position within the HLA molecule and the functional similarity of AA within proteins [9]. A high SSM score (also referred to as Dissimilarity Score [DSS]) represents high dissimilarity between HLA alleles resulting in a potentially greater deleterious impact on clinical outcomes. However, this algorithm has been challenged by 2 single-center analyses that could not associate higher SSM scores with aGVHD (in 26 patients) or in vitro T cell reactivity (in 74 patients) [10,11]. In the present study, we investigated the potential of SSM scores to predict high-risk HLA allele mismatch combinations responsible for severe aGVHD (grades III and IV) observed in a large cohort (5120 consecutive patients) of HSCT donor/recipient pairs. These allele combinations were observed in HSC transplants facilitated by the Japan Marrow Donor Program (JMDP) and published by Kawase et al. [12]. This investigation was conducted by comparing SSM scores in high-risk and low-risk allele combinations at HLA-A, -B, -C, -DRB1, and -DPB1 loci. No high-risk allele combinations at DQB1 locus met the predetermined level of statistical significance ($P < .005$) and thus SSM predictions were not evaluated in this study.

METHODS

Identification of High- and Low-Risk HLA Allele Mismatch Combinations

Significant high-risk HLA allele mismatch combinations were identified by retrospective analysis of 5210 consecutive registered patients who underwent transplantation through the JMDP. Patient characteristics, HLA matching and typing methods, and transplant procedures are described elsewhere [12]. Briefly, 15 mismatch allele combinations were identified as high-risk allele mismatch combinations (4 at HLA-A, 1 at HLA-B, 6 at HLA-C, 1 at HLA-DRB1, and 2 at HLA-DPB1 loci). Only 1-allele mismatched pairs in the same HLA locus were considered, and