Tohoku J. Exp. Med., 2023, **261**, 123-127

# Umbilical Cord Blood Transplantation for Myelodysplastic Syndromes with Donor-Specific Anti-HLA Antibodies against HLA-DP

**Yusuke Uchibori,[1] Koichi Onodera,[1] Yasushi Onishi,[1] Hiroka Komatsu,[1] Kenta Takenaka,[1] Yoshihiro Narumi,[1] Tatsuya Watanabe,[1] Hiroshi Nakamura,[1] Kazuki Sakurai,[1] Kazuki Hashimoto,[1] Kyoko Inokura,[1] Satoshi Ichikawa,[1] Noriko Fukuhara,[1] Hisayuki Yokoyama[1] and Hideo Harigae[1]**

[1]Department of Hematology, Tohoku University Hospital, Sendai, Miyagi, Japan

The presence of donor-specific anti-human leukocyte antigen (HLA) antibodies (DSAs) against anti-HLA-A, -B, -C, and -DRB1 in HLA-mismatched hematopoietic stem cell transplantation (HSCT) is associated with graft failure. DSAs against HLA-A, -B, -C, and -DRB1 with a mean fluorescence intensity (MFI) of greater than > 1,000 was shown to increase the risk of graft failure in single-unit umbilical cord blood transplantation (UCBT). Nevertheless, the impact of DSAs against HLA-DP or -DQ on transplantation outcomes is not fully understood. In this report, we present a case of UCBT in a patient with myelodysplastic syndrome who was positive for DSAs against HLA-DP with MFI of 1,263 before UCBT but successfully achieved neutrophil engraftment. If HLA-DP or -DQ is mismatched in UCBT, evaluating DSAs against HLA-DP or -DQ is crucial to avoid graft failure. However, the criteria for DSAs against HLA-A, -B, -C, and -DRB1 may not be directly applicable to those against HLA-DP or -DQ.

**Keywords:** donor-specific anti-HLA antibody; graft failure; HLA-DP; mean fluorescence intensity; umbilical cord blood transplantation
Tohoku J. Exp. Med., 2023 October, **261** (2), 123-127.
doi: 10.1620/tjem.2023.J063

## Introduction

Umbilical cord blood transplantation (UCBT) has been widely performed to treat patients for whom a human leukocyte antigen (HLA)-compatible stem cell donor is not available. Because of the less stringent HLA compatibility requirements in UCBT, HLA disparities often occur between recipient and donor.

Donor-specific anti-human leukocyte antigen antibodies (DSAs) have been associated with graft failure (GF) in HLA-mismatched hematopoietic stem cell transplantation (HSCT) (Choi et al. 2021). GF is an important complication that reduces the outcome of allogeneic HSCT and may occur more frequently in UCBT (Ozdemir and Civriz Bozdağ 2018; Fuji et al. 2020). A Japanese study reported that DSAs with a mean fluorescence intensity (MFI) ≥ 1,000 against HLA-A, -B, -C, and -DRB1 increase the risk of GF in single-unit UCBT (Fuji et al. 2020). Previous reports suggest that DSAs against HLA-DP and -DQ are also causative factors of GF (Ciurea et al. 2011; Jo et al. 2023); however, the applicability of criteria for DSAs against HLA-A, -B, -C, and -DRB1 to those against HLA-DP and -DQ has not been established.

## Case Presentation

A 65-year-old woman diagnosed with myelodysplastic syndromes (MDS)- refractory cytopenia with multilineage dysplasia (RCMD) with 46, XX, del(5)(q?) was referred to our hospital to be treated with lenalidomide (LEN). Despite 13 cycles of LEN monotherapy, the patient became transfusion dependent. Bone marrow aspiration (BMA) showed 14.0% of blasts, suggesting the progression of MDS; thus, azacitidine (AZA) was initiated. BMA after two courses of AZA revealed that the blast count had decreased to 1.8%,

Received May 6, 2023; revised and accepted July 27, 2023; J-STAGE Advance online publication August 10, 2023
Correspondence: Koichi Onodera, Department of Hematology, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8574, Japan.
e-mail: koichi.onodera.d6@tohoku.ac.jp

but the patient remained transfusion dependent. Because of the prolonged pancytopenia, AZA was discontinued and HSCT was proposed.

We first scheduled transplantation with umbilical cord blood (UCB). Table 1 shows the HLAs of the patient and all candidate donors. Confirmation of the patient's anti-HLA antibodies revealed a wide range of anti-HLA antibodies with high MFI. Because she had DSAs with MFI of 11,842 against DRB1*15:02 of the initially selected UCB unit, it was judged as ineligible. We then considered performing haploidentical HSCT with one of her two sons. Since she had DSAs with high MFI against those of both sons (the eldest son: DSAs with MFI of 6,085 against A26:01, 11,091 against B40:01, and 12,475 against DRB1*04:03; the second son: DSAs with MFI of 17,697 against DRB1*09:01), they were also judged as ineligible. Therefore, we decided to perform UCBT with a second UCB unit. The patient did not have DSAs against HLA-A,

Table 1. Human leukocyte antigens (HLA) and patient's HLA antibodies.

| | HLA-A | | HLA-B | | HLA-C | | HLA-DRB1 | | HLA-DQA1 | | HLA-DQB1 | | HLA-DPA1 | | HLA-DPB1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | 02:07 | 33:03 | 46:01 | 58:01 | 01:02 | 03:02 | 08:03 | 13:02 | - | - | - | - | - | - | - | - |
| first-choice CB donor | 02:03 | 33:03 | 39:09 | 58:01 | 03:02 | 07:02 | 13:02 | **15:02** | - | - | - | - | - | - | - | - |
| eldest son | **26:01** | 33:03 | **40:01** | 58:01 | 03:02 | 03:03 | **04:03** | 13:02 | - | - | - | - | - | - | - | - |
| second son | 02:07 | 33:03 | 46:01 | 58:01 | 01:02 | 03:02 | **09:01** | 13:02 | - | - | - | - | - | - | - | - |
| second-choice CB donor | 02:15N | 33:03 | 46:01 | 58:01 | 01:02 | 03:02 | 08:03 | 13:02 | 01:02 | 01:03 | 06:01 | 06:09 | 01:03 | **02:02** | 02:01 | **05:01** |

| | Anti-HLA antibody | Antigen Site | MFI |
|---|---|---|---|
| Patient | Class I Ab positive | A25:01, A26:03, A26:02, **A26:01**, A23:01, A66:01, A24:02, A01:01 | 9438, 9051, 8415, **6085**, 5085, 5012, 4137, 4106 |
| | | A01:02, A24:03, A11:02, A11:01, A43:01 | 3788, 1889, 1833, 1480, 1022 |
| | | B15:12, B67:01, B40:03, B49:01, B50:02, B15:20, B50:01, B15:27 | 15730, 15129, 14887, 14677, 14306, 14044, 13655, 13491 |
| | | B15:18, B56:01, B40:05, B15:07, B45:01, B40:04, B48:02, B15:01 | 13041, 13000, 12909, 12776, 12372, 12209, 12179, 12054 |
| | | B40:06, B40:02, B41:02, **B40:01**, B56:03, B15:06, B55:02, B15:04 | 11882, 11436, 11126, **11091**, 10871, 10258, 10140, 9872 |
| | | B15:03, B52:01, B27:04, B39:13, B27:08, B42:01, B55:04, B41:01 | 9710, 9580, 9532, 9460, 9214, 9205, 9165, 9094 |
| | | B44:02, B42:02, B55:01, B44:03, B27:06, B27:05, B39:05, B07:14 | 8971, 8764, 8371, 8347, 8331, 8253, 8121, 7596 |
| | | B15:24, B07:02, B35:02, B39:04, B15:10, B39:02, B15:21, B82:01 | 7586, 7436, 7252, 7023, 6963, 6876, 6687, 6514 |
| | | B39:06, B48:01, B39:01, B35:12, B38:02, B51:01, B13:02, B35:08 | 6390, 6323, 6238, 6237, 6196, 5869, 5830, 5487 |
| | | B54:01, B35:03, B78:01, B38:01, B51:02, B35:01, B81:01, B47:01 | 5417, 5365, 4736, 4526, 4477, 4446, 4422, 4169 |
| | | B15:02, B13:01, B73:01, B18:01, B53:01, B15:11, B59:01, B14:01 | 4114, 3697, 3180, 2874, 2120, 1929, 1918, 1570 |
| | | B15:13, B37:01, B08:01 | 1561, 1422, 1268 |
| | Class II Ab positive | DRB1*09:02, **DRB1*09:01**, DRB4*01:03, DRB4*01:01, DRB1*04:01 | 19131, **17697**, 16485, 15144, 14621 |
| | | DRB1*07:01, DRB1*04:04, DRB1*04:02, **DRB1*04:03**, DRB1*15:01 | 14201, 14126, 13016, **12475**, 12211 |
| | | DRB1*04:06, **DRB1*15:02**, DRB5*02:02, DRB5*01:02, DRB1*04:07 | 12051, **11842**, 11689, 11675, 11499 |
| | | DRB5*01:01, DRB1*04:05, DRB1*15:03, DRB1*16:02, DRB1*16:01 | 11449, 11062, 10502, 10489, 10099 |
| | | DRB1*10:01, DRB1*04:11, DRB1*01:01, DRB1*04:10, DRB1*01:02 | 8969, 8550, 8157, 7904, 6839 |
| | | DRB1*01:03, DRB1*12:02, DRB1*12:01, DRB3*02:02, DRB3*02:01 | 5984, 3230, 2920, 2470, 2310 |
| | | DRB1*14:01, DRB1*14:54, DRB1*14:04, DRB3*01:01, DRB1*14:05 | 1880, 1700, 1654, 1330, 1162 |
| | | DQB1*03:01+DQA1*06:01, DQB1*03:01+DQA1*05:03 | 15701, 14964 |
| | | DQB1*03:02+DQA1*03:02, DQB1*03:19+DQA1*05:05 | 14473, 14038 |
| | | DQB1*03:03+DQA1*03:02, DQB1*03:19+DQA1*02:01 | 13022, 12031 |
| | | DQB1*03:02+DQA1*03:01, DQB1*02:01+DQA1*05:01 | 11850, 9784 |
| | | DQB1*04:01+DQA1*03:03, DQB1*03:01+DQA1*02:01 | 9655, 9427 |
| | | DQB1*03:03+DQA1*02:01, DQB1*03:02+DQA1*02:01 | 9351, 8689 |
| | | DQB1*03:03+DQA1*03:01, DQB1*03:01+DQA1*03:01 | 8157, 7229 |
| | | DQB1*04:02+DQA1*02:01, DQB1*04:01+DQA1*02:01 | 5983, 5038 |
| | | DQB1*04:02+DQA1*04:01, DQB1*05:01+DQA1*01:01 | 4555, 4100 |
| | | DQB1*02:01+DQA1*03:01, DQB1*05:02+DQA1*01:02 | 4053, 2986 |
| | | DQB1*02:01+DQA1*04:01, DQB1*05:03+DQA1*01:01 | 2831, 2646 |
| | | DQB1*06:03+DQA1*01:03, DQB1*02:01+DQA1*02:01 | 2186, 1699 |
| | | DQB1*02:02+DQA1*02:01 | 1431 |
| | | DPB1*01:01+DPA1*02:01, **DPB1*05:01+DPA1*02:02** | 2288, **1263** |

Numbers in bold are indicated in the Case Presentation.

-B, -C, and -DRB1 of the second candidate; however, considering the wide range of anti-HLA antibodies including HLA-DP and -DQ, these HLAs were additionally investigated in the second UCB unit (Table 1). The patient had DSAs with MFI of 1,263 against DPB1*05:01 and DPA1*02:02 of the second UCB unit. Although the MFI was over 1,000, we decided to perform UCBT with the second selected UCB unit due to a lack of other suitable UCB units. The patient underwent UCBT (total nucleated cell dose: 2.76 × $10^7$/kg, CD34 cell dose: 0.68 × $10^5$/kg) with fludarabine [22 mg/$m^2$ (dose reduction because of decreased renal function) day −7 to −2], busulfan (3.2 mg/kg day −7 to −4), and melphalan (40 mg/$m^2$ day −3 to −2) as conditioning and tacrolimus and mycophenolate mofetil as graft-versus-host disease (GVHD) prophylaxis. On day 9, she developed a high-grade fever, skin rash, weight gain (from 41.6 kg to 46.4 kg), and renal dysfunction (eGFR 27 mL/min/1.73 $m^2$; Cre 1.52 mg/dL) indicative of pre-engraftment immune reaction (PIR) and were given total six doses of hydrocortisone (100 mg) from day 10 to 13. BMA performed due to delayed hematological recovery revealed numerous activated macrophages, along with elevated serum ferritin (6,947 ng/mL), suggesting the development of hemophagocytic syndrome (HPS). Administration of methylprednisolone (mPSL) (1 mg/kg on day 14 to 15) followed by dexamethasone palmitate (10 mg on day 16 to 17 and tapered) resulted in good hematological recovery with improvement in PIR and HPS, and neutrophil engraftment was achieved on day 20. Shortly thereafter, the patient developed gastrointestinal acute GVHD, and mPSL (1 mg/kg) was administered again from day 26 (Fig. 1). The administration of mPSL was effective and BMA on day 28 revealed complete donor chimerism with 0.6% of blasts, suggesting hematological remission. Platelet engraftment was achieved on day 48.

## Discussion

We present a case of UCBT in a patient with MDS who had a wide range of anti-HLA antibodies. Although the patient developed relatively severe PIR, HPS, and GVHD and had DSAs against HLA-DP with an MFI > 1,000, she successfully achieved neutrophil engraftment.

Several studies have reported the association of DSAs with GF and their adverse impact on transplantation outcomes. DSAs with MFI ≥ 2,000 in unrelated HSCT (Spellman et al. 2010) and MFI ≥ 1,000 in single-unit UCBT (Fuji et al. 2020) increased the risk of GF. Whereas the impact of DSAs after haplo-HSCT with posttransplant cyclophosphamide is controversial (Ciurea et al. 2015; Bramanti et al. 2019; Lima et al. 2021), the presence of DSAs with MFI ≥ 5,000 is significantly associated with worse neutrophil recovery in haplo-HSCT using GVHD prophylaxis with steroid (Yoshihara et al. 2012). However, most of these reports focused on the effect of DSAs against HLA-A, -B, -C, and -DRB1 and the studies that analyzed the impact of DSAs against HLA-DP and -DQ on the transplantation outcomes are limited.

Few studies have found an association between DSAs against HLA-DP or -DQ with GF. In a study involving 175 patients who underwent single-unit UCBT, patients with

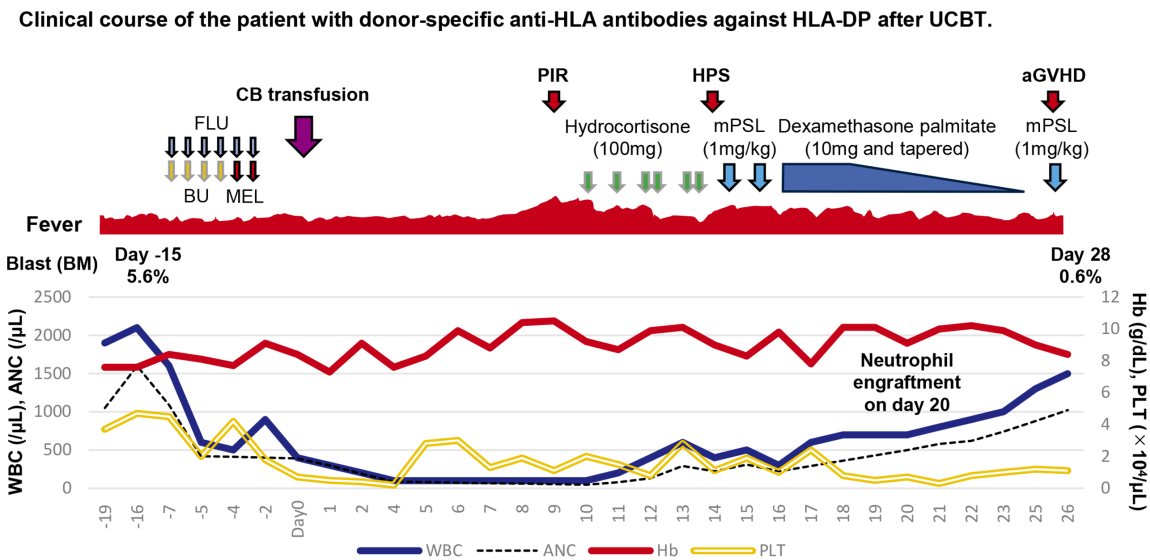


Fig. 1. Clinical course of the patient.
The patient underwent umbilical cord blood transplantation (UCBT) with fludarabine, busulfan, and melphalan as conditioning and tacrolimus and mycophenolate mofetil as graft-versus-host disease (GVHD) prophylaxis. She developed pre-engraftment immune reaction (PIR), hemophagocytic syndrome (HPS), and acute GVHD. The treatment with corticosteroids resulted in good clinical response. WBC, white blood cell; Hb, hemoglobin; PLT, platelet; ANC, absolute neutrophil count; BM, bone marrow; CB, cord blood; FLU, fludarabine; BU, busulfan; MEL, melphalan; mPSL, methylprednisolone.

DSAs against HLA-C, -DP, -DQ, and -DRB3/4/5 had lower engraftment rates than those without such DSAs (Yamamoto et al. 2014). More specifically, a retrospective study analyzing 567 single-unit UCBT recipients demonstrated that DSAs against HLA-DP or -DQ were associated with a significantly lower neutrophil engraftment rate, resulting in an increased risk of bacterial infection and decreased survival (Jo et al. 2023). Nevertheless, no consensus was obtained among these studies on the MFI threshold of DSAs against HLA-DP and -DQ which potentially causes GF. Our patient underwent UCBT with a UCB unit which had DSAs with MFI of 1,263 against HLA-DP and successfully achieved sustained neutrophil engraftment. Our case suggests that DSAs against HLA-DP need a higher MFI to induce GF in single-unit UCBT than DSAs against HLA-A, -B, -C, and -DRB1. The observation reported by Jo et al. (2023) that the MFI of DSA against HLA loci other than HLA-DP or -DQ is relatively lower for HLA-DP or -DQ supports our hypothesis; however, this notion that the MFI threshold for DSAs against HLA-DP may be higher for DSAs against HLA-A, -B, -C, and -DRB1 is speculative. The specific threshold of DSAs against HLA-DP and -DQ which can lead to GF needs to be confirmed in larger prospective studies.

Moreover, little attention has been given to the relationship between DSAs against HLA-DP and -DQ and transplantation outcomes other than GF, such as PIR, HPS, and GVHD. Given the relatively severe PIR, HPS, and GVHD in our case, DSAs against HLA-DP and -DQ may be associated with the development of PIR, HPS, and GVHD; however, there are no reports suggesting potential associations between DSA against HLA-DP and the development of PIR, HPS, and GVHD. Given that several studies have reported an association between HLA-DP mismatch and increased risk of acute GVHD in unrelated HSCT (Morishima et al. 2018; Petersdorf et al. 2020; Zou et al. 2022), this may be due to the HLA-DP mismatch itself.

In conclusion, the present case suggests that the criteria for DSAs against HLA-A, -B, -C, and -DRB1 in UCBT may not be directly applicable to those against HLA-DP. Furthermore, the MFI threshold for DSAs against HLA-DP could be higher than that for DSAs against HLA-A, -B, -C, and -DRB1. To clarify the MFI threshold for DSAs against HLA-DP and the actual impact on long-term outcomes in single-unit UCBT, further studies are required.

## Author Contributions

Article drafting or critical revision for important intellectual content: Y.U. and K.O. Final approval of the submitted version: Y.U., K.O., Y.O., H.K., K.T., Y.N., T.W., H.N., K.S., K.H., K.I., S.I., N.F., H.Y., and H.H.

## Conflict of Interest

The authors declare no conflict of interest.

## References


Bramanti, S., Calafiore, V., Longhi, E., Mariotti, J., Crespiatico, L., Sarina, B., De Philippis, C., Nocco, A., Santoro, A. & Castagna, L. (2019) Donor-specific anti-HLA antibodies in haploidentical stem cell transplantation with post-transplantation cyclophosphamide: risk of graft failure, poor graft function, and impact on outcomes. *Biol. Blood Marrow Transplant.*, **25**, 1395-1406.

Choi, A.Y., Manook, M., Olaso, D., Ezekian, B., Park, J., Freischlag, K., Jackson, A., Knechtle, S. & Kwun, J. (2021) Emerging new approaches in desensitization: targeted therapies for HLA sensitization. *Front. Immunol.*, **12**, 694763.

Ciurea, S.O., Thall, P.F., Milton, D.R., Barnes, T.H., Kongtim, P., Carmazzi, Y., López, A.A., Yap, D.Y., Popat, U., Rondon, G., Lichtiger, B., Aung, F., Afshar-Kharghan, V., Ma, Q., Fernández-Viña, M., et al. (2015) Complement-binding donor-specific anti-HLA antibodies and risk of primary graft failure in hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant.*, **21**, 1392-1398.

Ciurea, S.O., Thall, P.F., Wang, X., Wang, S.A., Hu, Y., Cano, P., Aung, F., Rondon, G., Molldrem, J.J., Korbling, M., Shpall, E.J., de Lima, M., Champlin, R.E. & Fernandez-Vina, M. (2011) Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation. *Blood*, **118**, 5957-5964.

Fuji, S., Oshima, K., Ohashi, K., Sawa, M., Saito, T., Eto, T., Tanaka, M., Onizuka, M., Nakamae, H., Shiratori, S., Ozawa, Y., Hidaka, M., Nagamura-Inoue, T., Tanaka, H., Fukuda, T., et al. (2020) Impact of pretransplant donor-specific anti-HLA antibodies on cord blood transplantation on behalf of the Transplant Complications Working Group of Japan Society for Hematopoietic Cell Transplantation. *Bone Marrow Transplant.*, **55**, 722-728.

Jo, T., Arai, Y., Hatanaka, K., Ishii, H., Ono, A., Matsuyama, N., Mori, J., Koh, Y., Azuma, F. & Kimura, T. (2023) Adverse effect of donor-specific anti-human leukocyte antigen (HLA) antibodies directed at HLA-DP/-DQ on engraftment in cord blood transplantation. *Cytotherapy*, **25**, 407-414.

Lima, A.C.M., Bonfim, C., Getz, J., Dornelles, L.N., do Amaral, G.B., Petterle, R.R., Loth, G., Nabhan, S.K., Pereira, N.F. & Pasquini, R. (2021) The impact of donor-specific anti-human leukocyte antigen antibodies in salvage haploidentical hematopoietic cell transplantation with posttransplant cyclophosphamide in patients with nonmalignant disorders. *HLA*, **97**, 493-504.

Morishima, S., Shiina, T., Suzuki, S., Ogawa, S., Sato-Otsubo, A., Kashiwase, K., Azuma, F., Yabe, T., Satake, M., Kato, S., Kodera, Y., Sasazuki, T. & Morishima, Y.; Japan Marrow Donor Program (2018) Evolutionary basis of HLA-DPB1 alleles affects acute GVHD in unrelated donor stem cell transplantation. *Blood*, **131**, 808-817.

Ozdemir, Z.N. & Civriz Bozdağ, S. (2018) Graft failure after allogeneic hematopoietic stem cell transplantation. *Transfus. Apher. Sci.*, **57**, 163-167.

Petersdorf, E.W., Bengtsson, M., De Santis, D., Dubois, V., Fleischhauer, K., Gooley, T., Horowitz, M., Madrigal, J.A., Malkki, M., McKallor, C., Morishima, Y., Oudshoorn, M., Spellman, S.R., Villard, J., Stevenson, P., et al. (2020) Role of HLA-DP expression in graft-versus-host disease after unrelated donor transplantation. *J. Clin. Oncol.*, **38**, 2712-2718.

Spellman, S., Bray, R., Rosen-Bronson, S., Haagenson, M., Klein, J., Flesch, S., Vierra-Green, C. & Anasetti, C. (2010) The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*, **115**, 2704-2708.

Yamamoto, H., Uchida, N., Matsuno, N., Ota, H., Kageyama, K., Wada, S., Kaji, D., Nishida, A., Ishiwata, K., Takagi, S., Tsuji,

M., Asano-Mori, Y., Yamamoto, G., Izutsu, K., Masuoka, K., et al. (2014) Anti-HLA antibodies other than against HLA-A, -B, -DRB1 adversely affect engraftment and nonrelapse mortality in HLA-mismatched single cord blood transplantation: possible implications of unrecognized donor-specific antibodies. *Biol. Blood Marrow Transplant.*, **20**, 1634-1640.

Yoshihara, S., Maruya, E., Taniguchi, K., Kaida, K., Kato, R., Inoue, T., Fujioka, T., Tamaki, H., Ikegame, K., Okada, M., Soma, T., Hayashi, K., Fujii, N., Onuma, T., Kusunoki, Y., et al. (2012) Risk and prevention of graft failure in patients with preexisting donor-specific HLA antibodies undergoing unmanipulated haploidentical SCT. *Bone Marrow Transplant.*, **47**, 508-515.

Zou, J., Kongtim, P., Oran, B., Kosmoliaptsis, V., Carmazzi, Y., Ma, J., Li, L., Rondon, G., Srour, S., Copley, H.C., Partlow, D., Ciurea, S.O., Greenbaum, U., Ma, Q., Shpall, E.J., et al. (2022) Refined HLA-DPB1 mismatch with molecular algorithms predicts outcomes in hematopoietic stem cell transplantation. *Haematologica*, **107**, 844-856.