CHARACTERIZATION OF A *DE NOVO* RECIPIENT-SPECIFIC ANTI-HLA ANTIBODY FOLLOWING A SECOND HAPLOIDENTICAL TRANSPLANT

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INTRODUCTION

The occurrence of **recipient-specific anti-HLA antibodies (RSA)** in hematopoietic cell transplantation (HCT) has been scarcely reported. Herein, we describe a rare *de novo* RSA emerging after a second haploidentical HCT

CASE STUDY

A 1-year-old patient with acute megakaryoblastic leukemia underwent haploidentical HCT with the father in 2020 and relapsed three years later. Therefore, a second haploidentical HCT with the mother was carried out in June 2023 **(Figure 1)**. The patient achieved hematologic recovery on day +15 and had complete donor chimerism, using STR and NGS, on day +29. Several Luminex Single Antigen (LSA) tests were performed, and no DSA was detected on days +41, +49, +57, +69, +86, +108. On day +132, the patient experienced chronic graft-versus-host disease (cGVHD). A new LSA test was performed on day +155 and, surprisingly, an **anti-DQ5 RSA was detected (MFI=24335) (Figure 2).** A new sample was requested to confirm the RSA reactivity, showing similar results (MFI=16991). In eplet analysis, the RSA was justified by eplets 52PQ/55R/77R. We performed an Adsorption/Elution test using a cell expressing DQ5 to rule out the RSA as a false-positive reaction. Remarkably, **the eluate result showed that DQ5 was recovered, thereby validating the RSA reactivity (Figure 3).** Next, we examined a serum sample from the mother and conducted a new LSA test to assess whether the donor had been previously sensitized to DQ5**. No RSAs (MFI<100) were detected in the mother's serum (Figure 4)**. Thus, we hypothesized that the RSA was associated with a de novo response. Another LSA test was performed using an anti-IgM secondary antibody, and reactivity against eplets 52PQ/55R/77R was also observed. This finding suggests that the emerging RSA could be related to a primary humoral response. The anti-DQ5 RSA was also detected on day +227 (MFI=10105). Indeed, the patient has presented **100% donor cells in STR and NGS chimerism assays (Figure 5)** on days +108, +174, +213, +241, +280, +310, +346, and +372. Currently (day +372), the patient is in complete remission without evidence of disease relapse.

Although it is tempting to speculate whether this de novo RSA was associated with a **humoral graft-versus-host alloreactivity**, leading to concomitant cGVHD and graft-versus-leukemia effect, previous evidence (Delbos, 2016; Umino, 2023) supports such hypothesis

RESULTS

Sample Date	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DRB3/4/5*	HLA-DQB1*	HLA-DQA1*	HLA-DPB1*	HLA-DPA1*	COMPATIBILITY
Recipient* (18.05.23)	01:01	15:01	03:03	01:03	AUSENTE	05:01	01:01	02:01P	01:03	
*Swab Sample	02:01	35:02	04:01	11:01	B3*02:02	03:01P	05:05	04:02P	01:03	
Mother (09.05.23)	02:01	35:02	04:01	11:01	B3*02:02	03:01	05:05	04:02	01:03	Haploidentical
	02:01	49:01	07:01	04:11	B4*01:03	03:02	03:01	27:01	02:01	
Father (26.03.20)	01:01	15:01	03:03	01:03	AUSENTE	05:01	01:01	02:01P	01:03	Haploidentical
	31:01	40:01	03:04	04:04	B4*01:03	03:02P	03:01	04:01	01:03	

Figure 1. High-resolution HLA typing from the patient and the haploidentical donors

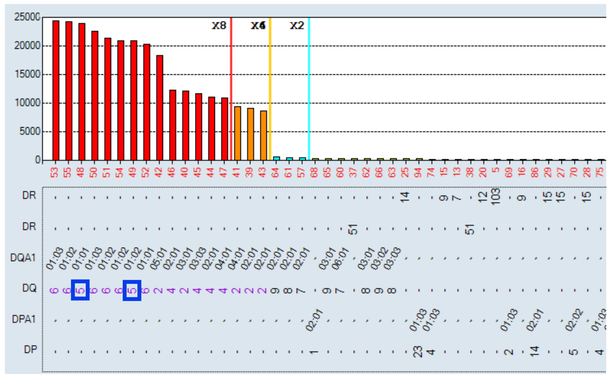

Figure 2. Anti-DQ5 RSA detected on day +155.

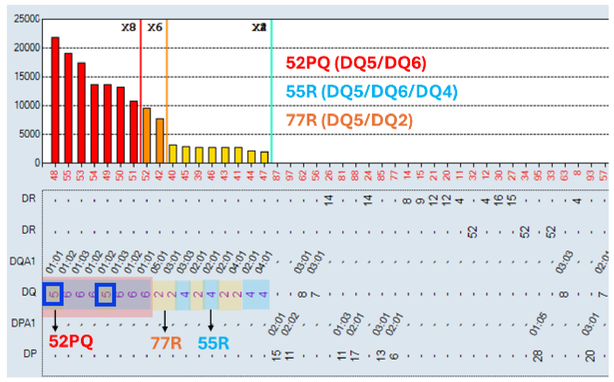

Figure 3. Eluate reactivity after adsorption with a cell expressing HLA-DQ5.

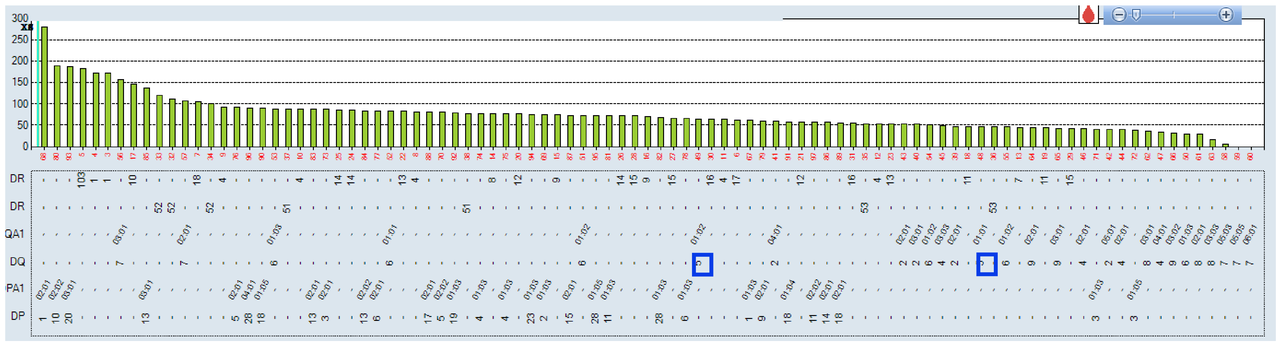

Figure 4. LSA test with the mother's serum. No Anti-DQ5 RSA (MFI<100) was detected.

Days after BMT	Results
29	Full Chimerism – 100% Donor
84	Full Chimerism – 100% Donor
108	Full Chimerism – 100% Donor
174	Full Chimerism – 100% Donor
213	Full Chimerism – 100% Donor
241	Full Chimerism – 100% Donor
280	Full Chimerism – 100% Donor
310	Full Chimerism – 100% Donor
346	Full Chimerism – 100% Donor
372	Full Chimerism – 100% Donor

Figure 5. Sustained full donor chimerism in STR and NGS assays.

CONCLUSIONS

- **In summary, we described an unusual case of *de novo* RSA after a second haploidentical hematopoietic cell transplantation**
- **Further studies are warranted to clarify the clinical impact of RSA on HCT outcomes and its role in humoral graft-versus-host alloreactivity**

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All authors declare that they have no conflict of interest.