LOW-LEVEL DONOR-SPECIFIC HLA ANTIBODIES DO NOT IMPACT ENGRAFTMENT AFTER PEDIATRIC HEMATOPOIETIC CELL TRANSPLANTATION: A CASE SERIES

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AIM

It is currently unknown whether **low-level donor-specific HLA antibodies (DSA)** affect engraftment in pediatric patients undergoing hematopoietic cell transplantation (HCT). Thus, we report a case series assessing low-level DSAs before and after transplant in a reference pediatric HCT service.

METHODS

Low-level DSAs were defined as those that are positive (MFI>300) in the Luminex Single Antigen Bead (SAB) assay but negative in Flow Cytometry Crossmatch (FCXM). DSA evaluation was conducted with One Lambda's SAB assay. FCXM was performed using the Go-Halifaster protocol. Desensitization strategies were tailored to the patient's disease and clinical status. SAB testing was repeated on day zero (graft infusion) and day +2 to monitor potential DSA rebound. Chimerism analysis was performed using the STR method

RESULTS

From 2022 to 2025, six pediatric patients were closely monitored for low-level DSAs. Details on the patient's diagnoses, desensitization, pre- and post-HCT MFI values, and HCT outcomes are provided in **Fig. 1A**. In brief, **patients #1 to #4** had low-level DSAs. After desensitization, all DSAs were below 1000 MFI on day zero, and no DSA rebounds were observed on day +2. All four patients achieved hematologic recovery, with complete chimerism on day +28.

Patient #5, transplanted for Wiskott-Aldrich syndrome, had an anti-DR7 DSA (MFI=3820), which was explained by 98E eplet. This DSA displayed an overlapping low-level pan-DR reactivity **(Fig. 1B)**, and FCXM was clearly negative. Despite this unusual DSA pattern, which is probably a false-positive reaction, the HCT team desensitized the patient due to the underlying disease, and the patient fully engrafted as expected.

Patient #6 had a 10/10 matched unrelated donor with DP3 and DP14 mismatches, classified as nonpermissive in the host-versus-graft direction. We noted a 56ED eplet pattern below 300 MFI, with DP3 and DP14 clustering with DP6, DP9, DP17, and DP20 **(Fig. 1C)**. Due to the high risk of rejection of the nonpermissive DPB1 mismatch, we monitored for 56ED DSA rebound, and no increase in DP3/DP14 reactivity was observed. Patient #6 had hematologic recovery, with complete chimerism documented on day +28.

Figure 1A) Patient, donor and DSA characteristics and HCT outcomes

Patient number	Diagnosis	Donor type	Desensitization protocol	Number of DSAs	DSA specificity	MFI before desenstitization	MFI after desensitization	MFI on day zero	MFI on day +2	Neutrophil recovery	Platelet recovery	Chimerism on day +28	Last Chimerism	Survival Status	Last Follow-Up
1	ALL	Haploidentical	MD Anderson#	1	DQ7 (DQA1*05:05/ DQB1*03:01)	2807	408	942	672	Day +16	Day +21	100% donor	100% donor	Deceased with sustained engraftment due to hepatic GVHD	Day +332
2	SCD	Haploidentical	Vanderbilt†	1	B*35:01	2322	359	521	736	Day +16	Day +23	100% donor	100% donor	Alive	Day +748
3	SAA	Haploidentical	MD Anderson#	2	DRB1*09:01	1831	400	925	783	Day +14	Day +19	100% donor	95.5% donor	Alive	Day +194
					DQ9 (DQA1*03:02/ DQB1*03:03)	2598	571	658	785						
4	SCD	Haploidentical	Vanderbilt†	1	B*07:02	1300	478	761	31	Day +18	Day +20	100% donor	97% donor	Alive	Day +793
5	WAS	Haploidentical	Three session of plasmapheresis	1	DRB1*07:01	3820	859	NT	NT	Day +18	Day +20	100% donor	100% donor	Alive	Day +121
6	SAA secondary to PNH	10/10 MUD with 2 DPB1 mismatches	One session of plasmapheresis for PTR due to strong class I HLA antibodies	2	DPB1*03:01	NT	NT	270	329	Day +19	Day +21	100% donor	100% donor	Alive	Day +258
					DPB1*14:01	NT	NT	170	150						

Abbreviations: ALL, Acute lymphoblastic leukemia; SCD, Sickle cell disease; SAA, Severe aplastic anemia; WAS, Wiskott-Aldrich syndrome; PNH, Paroxysmal nocturnal hemoglobinuria; MUD, matched unrelated donor; PTR, Platelet transfusion refractoriness; DSA, donor-specific anti-HLA antibodies; MFI, mean fluorescence intensity; NT, not tested; GVHD, graft-versus-host disease.

#**MD Anderson protocol:** Alternate day plasma exchange, followed by 1 dose of 375 mg/m2 rituximab (Rituxan) and 1 dose of intravenous immunoglobulin (IVIG) 1 g/kg and donor buffy coat.

†**Vanderbilt protocol:** Alternate-day, single-volume plasmapheresis with IVIg (100 mg/kg), tacrolimus (1 mg, IV per day) and mycophenolate mofetil (1 g, twice daily).

Figure 1B) DSA pattern of patient #5 (98E eplet reactivity overlapping with pan-DR pattern)

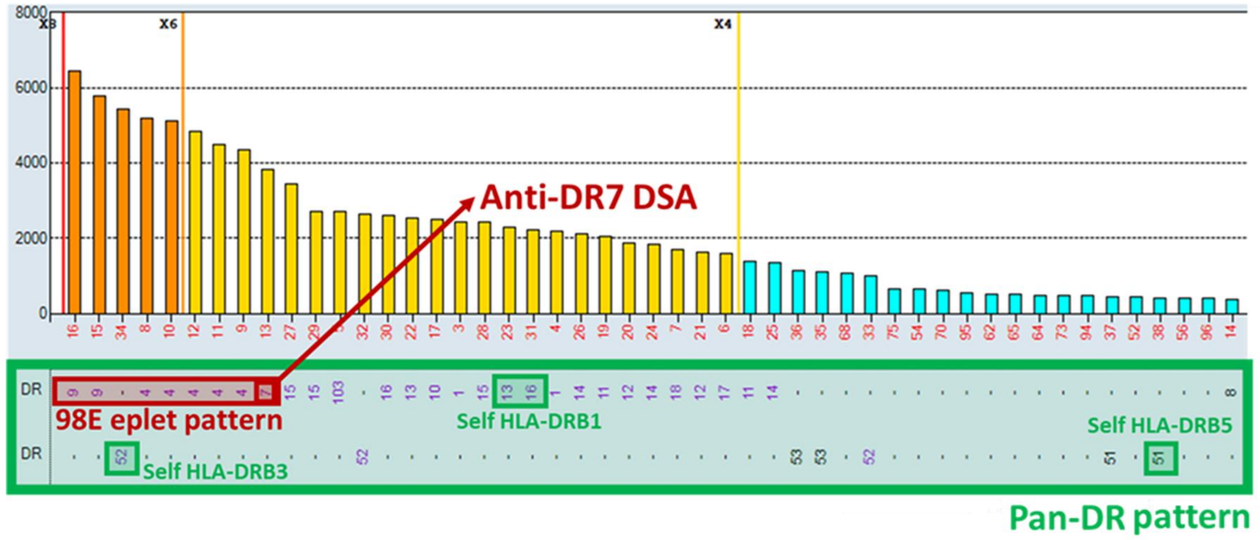
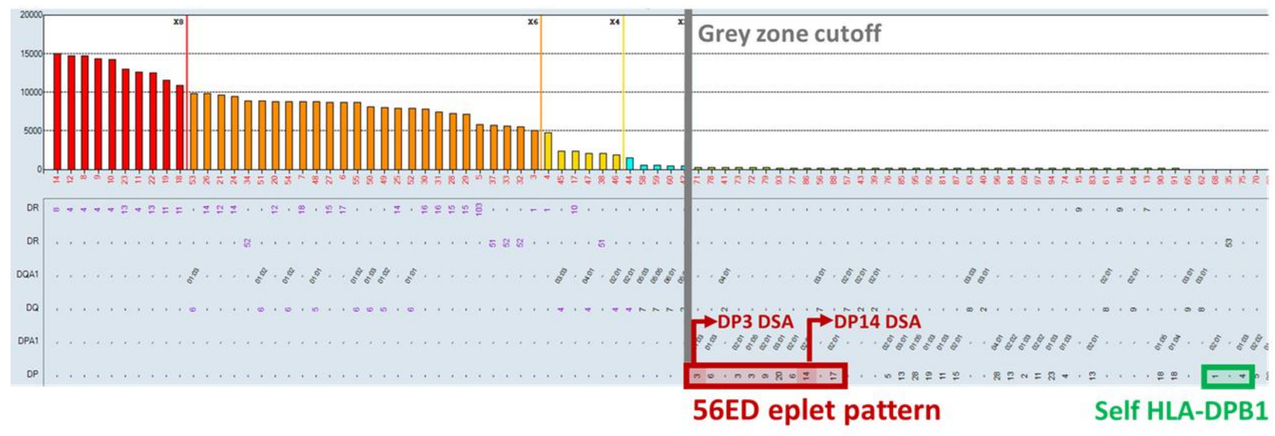


Figure 1C) DSA pattern of patient #6 (β56ED eplet reactivity just below the grey zone cutoff)



CONCLUSIONS

- **Our case series suggests that low-level DSAs, promptly desensitized, with negative FCXM and without DSA rebound post-HCT, are permissive for engraftment after HCT.**
- **Independent validation of our findings in the pediatric HCT setting is warranted.**

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All authors declare that they have no conflict of interest.