DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in IPD-IMGT/HLA at https://www.ebi.ac.uk/ipd/imgt/hla/, reference number HWS10062599.

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Identification of the novel *HLA-C*03:03:01:52N* allele, a splice-site variant at the boundary of intron1 and exon2

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*HLA-C*03:03:01:52N* differs from *HLA-C*03:03:01:01* by one nucleotide substitution at position gDNA 202 (A>G) in Intron1.

KEYWORDS
*HLA-C*03:03:01:52N*, new allele, splice site

HLA null alleles are characterized by the lack of a serologically detectable product. The failure to identify an HLA null allele as a non-expressed variant in the stem cell transplantation setting may result in an HLA mismatch.[1]

We report the sequence of a probable novel HLA-C null allele, *C*03:03:01:52N*, which differs from the common *C*03:03:01:01* by a splice site mutation at position gDNA 202 (A>G) at the boundary of intron1 and exon2 (Figure 1).

The novel allele was identified in a female patient suffering from acute myeloid leukemia during routine high resolution HLA typing using next generation sequencing (NGS).

Initial typing of genomic DNA extracted from peripheral blood was performed using NGSgo-AmpX, NGSgo-LibrX, and NGSgo-IndX kits (GenDx) on an Illumina MiSeqDx platform. Analyses of the resulting Fastq files with the corresponding NGSengine software Version 2.16.0 (GenDx) and implemented IPD-IMGT/HLA database version 3.38.0 identified the novel HLA-*C*03:03:01:52N* allele. Subsequent sequencing based typing of HLA C locus with Protrans S4 HLA C kit (Protrans GmbH) and allele assignment with SBTengine (GenDx) confirmed a splice site mutation at position gDNA 202 (A>G) at the boundary of intron1 and exon2 in the novel allele. This mutation leads to a change in the highly conserved ACCEPTOR SPLICE site (AG>GG) and alters the GT/AG mRNA processing rule applicable for almost all eukaryotic genes.[2,3]

In lymphocyte cytotoxicity test performed with HLA-Ready Plate ABC120 (inno-train Diagnostik GmbH), no reaction with Cw3 antibodies was detected. This fact explains the effect of splice site mutation.

```
gDNA                    80         90        100        110        120        130
C*03:03:01:01      GTGAGTG CGGGGTTGGG AGGGAATCGG CCTCTGCGGA GAGGAGCGAG GGGCCCGCCC
C*03:03:01:52N     ------- ---------- ---------- ---------- ---------- ----------

gDNA                   140        150        160        170        180        190
C*03:03:01:01   GGCGAGGGCG CAGGACCCGG GGAGCCGCGC AGGGAGGAGG GTCGGGCGGG TCTCAGCCCC
C*03:03:01:52N  ---------- ---------- ---------- ---------- ---------- ----------

gDNA                   200
C*03:03:01:01   TCCTCGCCCC CAG
C*03:03:01:52N  ---------- -G-
```

FIGURE 1 Alignment of sequence of intron1 of the novel *C*03:03:01:52N* compared to the sequence of *C*03:03:01:01* allele. Identity between sequences is indicated by dashes. Numbers above the sequences correspond to nucleotide positions

To confirm that the novel *HLA-C*03:03:01:52N* allele was present in the germline of the patient and not somatic mutation, confirmatory typing of genomic DNA extracted from buccal swabs was performed using NGSgo-MX11-3 (GenDx) and NGSengine software version 2.18.0 (GenDx) with IPD-IMGT/HLA database version 3.40.0.

The complete HLA type determined for this sample was *A*03:02:01*,*32:01:01*; *C*03:03:01:52N*,*05:01:01*; *B*15:01:01:01*,*44:02:01*; *DRB1*07:01:01:01*,*14:01P*; *DRB3*02:02P*, *DRB4*01:01:01:01*, *DQA1*01:04:01*,*02:01:01*; *DQB1*02:02:01:01*,*05:03:01*; *DPA1*01:03:01:04*,*02:01:01:02*; *DPB1*04:01:01*,*14:01:01:01*.

The sequence was submitted to GenBank under the accession number MW014810 and to the IPD-IMGT/HLA database[4] under the accession number HWS10061493. The name *C*03:03:01:52N* has been officially assigned by the World Health Organization (WHO) Nomenclature Committee for Factors of the HLA System in March 2021. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report, names would be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.[5]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Silvia Ulrich performed the analysis and interpretation of data, the submission to database, and wrote the manuscript. Lang Johanna performed the NGS sequencing. Bodem Oliver performed the Lymphocyte Cytotoxicity Test. All authors have read, corrected and approved the submitted manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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