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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data generated during and/or analyzed during the current study are available from thecorresponding author on reasonable request.

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Characterization of the novel *HLA-B*44:324:02* allele by sequencing-based typing

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*HLA-B*44:324:02* differs from *HLA-B*44:324:01* by one nucleotide substitution in codon 99 in exon 3.

KEYWORDS
HLA, *HLA-B*44:324:02*, novel allele, sequencing-based typing

We report here a novel *HLA-B*44* allele, now named *HLA-B*44:324:02* that carries one nucleotide substitution in exon 3 when compared to the *HLA-B*44:324:01* allele, identified in a volunteer bone marrow donor. The HLA typing was performed using Next Generation Sequencing (AllType NGS, One Lambda, Canoga Park, CA) on the Ion S5 system platform (ThermoFisher Scientific, Waltham, MA),[1] from exons 1 to 7. The reads were analyzed using the TypeStream Visual Software version 3.0 (One Lambda). This donor was found to have a new *HLA-B*44* allele and was consequently typed *A*24:02*, *29:02*; *B*07:02:01*, *44:324:02*; *C*07:02*, *16:01*; *DRB1*07:01*, *15:01*; *DRB4*01:01*; *DRB5*01:01*; *DQA1*01:02*, *02:01*; *DQB1*02:02*, *06:02*; *DPA1*01:03*, *02:01*; *DPB1*04:01*, *11:01*. Using the IPD-IMGT/HLA Database,[2] nucleotide sequence alignment with HLA-B alleles shows that this new allele has one nucleotide change from *B*44:324:01* in codon 99 in exon 3 where T → C, not resulting in a coding change (TAT → TAC, Figure 1). This nucleotide change was confirmed using other NGS reagents provided by GenDX NGSgo-MX6-1 (Utrecht, Netherlands) run on the Illumina MiSeq system (San Diego, CA) and analyzed with the NGSEngine software (GenDX, version 2.26). We were very confident in the phasing as the sample displayed a mean read length of 313 base pairs over all the loci, the mismatched C base was attributed 198 times to the new *HLA-B*44* allele and can be only attributed to this

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AA Codon                        95                  100                 105                 110                 115
B*44:324:02        GG TCT CAC ATC ATC CAG AGG ATG TAC GGC TGC GAC GTG GGG CCG GAC GGG CGC CTC CTC CGC GGG TAT GAC CAG
B*44:324:01        -- --- --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                       120                 125                 130                 135                 140
B*44:324:02        GAC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAC GAG GAC CTG AGC TCC TGG ACC GCG GCG GAC ACC GCG GCT
B*44:324:01        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                       145                 150                 155                 160                 165
B*44:324:02        CAG ATC ACC CAG CGC AAG TGG GAG GCG GCC CGT GTG GCG GAG CAG CTG AGA GCC TAC CTG GAG GGC CTG TGC GTG
B*44:324:01        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                       170                 175                 180
B*44:324:02        GAG TCG CTC CGC AGA TAC CTG GAG AAC GGG AAG GAG ACG CTG CAG CGC GCG G
B*44:324:01        --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
```

FIGURE 1 Alignment of the sequence of exon 3 of *HLA-B*44:324:02* with the sequence of *HLA-B*44:324:01*. Dashes indicate nucleotide identity with the *HLA-B*44:324:01* allele. Numbers above the sequence indicate codon position.

allele because it was possible to discriminate from the associated *HLA-B*07:02:01:01* allele by virtue of seven variant positions each distant by less than 100 base pairs. HLA typing by Luminex reverse sequence-specific oligonucleotide (SSO) was performed (One Lambda Labtype XR, Canoga Park, CA).[3] With this assay (lot 007, catalog RSSOX1B_007_04), the most likely HLA-typing of the donor was *B*07:EENNR*, *44:EGAFV* (most likely allele *B*07:02*, *44:03*, respectively) without any bead modification. Indeed the IPD-IMGT/HLA Database 3.51.0 release describes many alleles displaying a TAC in codon 99 (as *HLA-B*44:03*) and few other HLA-B alleles displaying an ACC sequence in codon 283 (as *HLA-B*44:324:01*), explaining why the manufacturer did not include probes targeting this codon. The coding nucleotide sequence of the new allele has been submitted to the GenBank database (Accession No. OQ378320) and to the IPD-IMGT/HLA Database (Submission No. HWS10065195). The name *B*44:324:02* has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in February 2023. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report,[4] names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

AUTHOR CONTRIBUTIONS

Marine Cargou and Jonathan Visentin contributed to the design of the study. Marine Cargou and Jonathan Visentin participated in the writing of the paper. Marine Cargou, Vincent Elsermans, Isabelle Top, Gwendaline Guidicelli and Jonathan Visentin participated in the performance of the research. Marine Cargou, Vincent Elsermans, Isabelle Top, Gwendaline Guidicelli and Jonathan Visentin participated in data analysis. Vincent Elsermans, Isabelle Top and Gwendaline Guidicelli were involved in critical revision of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The sequence is freely available in the IPD-IMGT/HLA Database.

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The novel *HLA-B*44:369* allele characterised by two different sequencing-based typing techniques

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The novel allele *HLA-B*44:369* differs from *HLA-B*44:02:01:01* by one non-synonymous nucleotide substitution in exon 3.

KEYWORDS
HLA, *HLA-B*44*, novel allele, sequencing-based typing

We hereby report the novel allele *HLA-B*44:369* identified in a volunteer stem cell donor.

The complete HLA typing of the individual in which the novel allele was identified was found to be: *HLA-A*02:01:01, 32:01:01*; *-B*44:369, 55:01:01*; *-C*03:03:01, 05:01:01*; *-DRB1*04:08:01, 13:01:01*; *-DRB3*02:02:01*; *-DRB4*01:03:01*; *-DQA1*01:03:01, 03:03:01*; *-DQB1*03:01:01G, 06:03:01G*; *-DPA1*01:03:01*; *-DPB1*04:01:01*.

Genomic DNA was extracted from self-collected saliva (Oragene DNA kit) with an automated method using Genomic STARlet pipetting robot (Hamilton Company, Bonaduz, Switzerland) with NucleoSpin® Blood L Vacuum kit (Macherey Nagel GmBH & Co. KG, Dueren, Germany) according to the suppliers' recommendations.

The HLA typing was first performed using ALLType NGS 11 loci kit (One Lambda, Canoga Park, CA, USA) on a Miniseq platform (Illumina, San Diego, SA, USA). The fastq files were analysed thanks to dedicated software, that is, TypeStream Visual version 2.1.0 (One Lambda). The metrics were excellent for all loci and especially for the HLA-B locus with a minimal depth of 667X considering all exons. The third exon bearing the nucleotide substitution had minimal depth of 928X and an average depth of 1202X with a good allelic balance.

To confirm the novel allele, a single-allele specific sanger sequencing was then performed using Protrans S4 HLA-B kit (Protrans, Hockenheim, Germany) on an ABI 3130 XL (Applied BioSystems, Waltham, Massachusetts, MA, USA). The generated files were analysed using SeqScanner, version 2.0 (Applied BioSystems) to assess the quality of raw data, then SeqPilot version 5.2.0 (JSI medical systems GmBH, Ettenheim, Germany) for the sequence interpretation.

Using both techniques, the novel allele presents a single non-synonymous nucleotide substitution in exon 3 compared to its closer match *HLA-B*44:02:01:01*. The nucleotide change occurs at position 595 of the CDS sequence where guanine (G) is replaced by thymine (T) compared to the reference allele *HLA-B*44:02:01:01* (Figure 1). This results in a codon change from GGG to TGG, therefore to an amino acid change at residue 175 where Glycine (G) is replaced by Tryptophan (W).

The 175G of the *HLA-B*44:02* molecule is not known to be an eplet in the HLA epitope registry.[1] The analysis