### 84\_\_\_\_WILEY\_HLA

The complete HLA typing of the donor was *HLA-A\*24:02:01*, *32:01:01*; -*C\*07:02:01*, *07:04:01*; -*B\*07:02:01*, *15:09:01*; -*DRB1\*11:03:01*, *15:01:01/15:204*; -*DQB1\*03:338N*, *06:02:01*.

The novel *HLA-DQB1\*06:467* (GenBank OQ087130, IPD-IMGT/HLA HWS10064733) allele was identified in a Caucasian individual from Orenburg, Russia. The most similar allele is *HLA-DQB1\*06:04:01:01*, from which *HLA-DQB1\*06:467* differs at nucleotide 754 in exon 4, where exchange from C to T (codon 220, CGT to TGT) results in a coding change (Arginine to Cysteine).<sup>3</sup> The complete HLA typing of the donor was *HLA-A\*01:01:01*, *03:01:01*; *-C\*04:01:01*; *-B\*35:01:01*, *35:03:01*; *-DRB1\*01:01:01/01:* 01:35/01:01:41/01:100, 13:02:01; *-DQB1\*05:01:01*, 06:467.

The *HLA-B\*44:481:02* and *HLA-DQB1\*03:338N* alleles were confirmed using a sequence-based typing (SBT) method with AlleleSEQR HLA Kit (GenDx, Utrecht, Netherlands), exons 2–4 of the HLA-B, exons 2 and 3 of the HLA-DQB1 genes were amplified by polymerase chain reaction (PCR) and sequenced on an ABI 3500xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Sequences were analyzed with SBTEngine software (GenDx, Utrecht, Netherlands).

#### **AUTHOR CONTRIBUTIONS**

**Maria Loginova:** Registering novel alleles, writing a manuscript. **Daria Smirnova:** Primary HLA-typing. **Igor Paramonov:** Registering novel allele, scientific edition of the manuscript.

### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

Data is available from the IPD-IMGT/HLA Database.

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# Characterization of the novel *HLA-B\*51:370* allele by sequencing-based typing

Marine Cargou<sup>1</sup> | Vincent Elsermans<sup>2</sup> | Isabelle Top<sup>2</sup> | Mamy Ralazamahaleo<sup>1</sup> | Jonathan Visentin<sup>1,3</sup>

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<sup>2</sup>CHU de Lille, Institut d'Immunologie-HLA, Lille, France
<sup>3</sup>Univ. Bordeaux, CNRS, ImmunoConcEpT, UMR 5164, Bordeaux, France

#### Correspondence

Marine Cargou, CHU de Bordeaux, Laboratoire d'Immunologie et Immunogénétique, Hôpital Pellegrin, Place Amélie Raba Léon, 33076 Bordeaux Cedex, France. Email: marine.cargou@chu-bordeaux.fr *HLA-B\*51:370* differs from *HLA-B\*51:01:01:04* by one nucleotide substitution in codon 276 in exon 5.

K E Y W O R D S HLA, *HLA-B\*51:370*, novel allele, sequencing-based typing

We report here a novel *HLA-B\*51* allele, now named *HLA-B\*51:370* that carries one nucleotide substitution in

exon 5 when compared with the *HLA-B\*51:01:01:04* allele, identified in a volunteer bone marrow donor. The

CARGOU ET AL.				1LA _	WILEY *
				Immune Response Genetics	
AA Codon		100	105	110	115
DRB1*01:02:01:01	TT GAG CCT AAG GT	G ACT GTG TAT CCT TC.	A AAG ACC CAG CCC CTG	CAG CAC CAC AAC CTC	CTG GTC TGC TCT GTG
DRB1*01:140					
AA Codon	120	125	130	135	140
DRB1*01:02:01:01	AGT GGT TTC TAT CC.	A GGC AGC ATT GAA GT	C AGG TGG TTC CGG AAC	GGC CAG GAA GAG AAG	GCT GGG GTG GTG TCC
DRB1*01:140					
AA Codon	145	150	155	160	165
DRB1*01:02:01:01	ACA GGC CTG ATC CA	G AAT GGA GAT TGG AC	C TTC CAG ACC CTG GTG	ATG CTG GAA ACA GTT	CCT CGG AGT GGA GAG
DRB1*01:140	G				
AA Codon	170	175	180	185	
DRB1*01:02:01:01	GTT TAC ACC TGC CA	A GTG GAG CAC CCA AG	T GTG ACG AGC CCT CTC	ACA GTG GAA TGG A	
DRB1*01:140					

**FIGURE 1** Alignment of the sequence of exon 5 of *HLA-B\*51:370* with the sequence of *HLA-B\*51:01:01:04*. Dashes indicate nucleotide identity with the *HLA-B\*51:01:01:04* allele. Numbers above the sequence indicate codon position.

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),<sup>1</sup> from exons 1 to 7. The reads were analyzed using the TypeStream Visual Software version 2.1 (One Lambda). This donor was found to have a new B\*51 allele and was consequently typed A\*02:01, 11:01; B\*07:02, 51:370; C\*07:02, 15:02; DRB1\*13:01, 15:01; DRB3\*01:01; DRB5\*01:01; DQA1\*01:02, 01:03; DQB1\*06:02, 06:03; DPA1\*01:03, 01:03; DPB1\*02:01, 04:01. Using the IPD-IMGT/HLA Database,<sup>2</sup> nucleotide sequence alignment with HLA-B alleles shows that this new allele has one nucleotide change from B\*51:01:01:04 in codon 276 in exon 5 where  $C \rightarrow A$ , resulting in a coding change (CCA  $\rightarrow$  CAA, Proline  $\rightarrow$  Glutamine, 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 310 base pairs over all the loci, the mismatched A base was attributed 475 times to the new HLA-B\*51 allele and can be only attributed to this allele because it was possible to discriminate from the associated HLA-B\*07:02:01:01 allele by virtue of 15 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).<sup>3</sup> With this assay (lot 007, catalog RSSOX1B\_007\_02), the most likely HLA-typing of the donor was B\*07:DWCSZ, 51:DWCSY (most likely allele B\*07:02, 51:01 respectively) without any bead modification. Indeed the IPD-IMGT/HLA Database 3.50.0 release describe no other HLA-B alleles displaying a CAA sequence in codon 276, explaining why the manufacturer did not include probes targeting this codon. The analysis of the localization of this amino-acid and its antibody accessibility

with the pHLA3D database<sup>4</sup> indicated that this amino-acid is located out of the peptide binding groove while its surface accessibility is unclear. Therefore, its clinical significance is unclear. The coding nucleotide sequence of the new allele has been submitted to the GenBank database (Accession No. OP807953) and to the IPD-IMGT/HLA Database (Submission No. HWS10064321). The name B\*51:370 has been officially assigned by the WHO Nomenclature Committee for Factors of the HLA System in November 2022. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report,<sup>5</sup> 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**

1.11

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, Mamy Ralazamahaleo, and Jonathan Visentin participated in the performance of the research. Marine Cargou, Vincent Elsermans, Isabelle Top, Mamy Ralazamahaleo, and Jonathan Visentin participated in data analysis. Vincent Elsermans, Isabelle Top, and Mamy Ralazamahaleo 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.

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## The novel *HLA-B\*56:91* allele characterised by three different sequencing-based typing techniques

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Adèle Dhuyser, HLA and Histocompatibility Laboratory, CHRU de Nancy, Vandoeuvre les Nancy, France. Email: a.dhuyser@chru-nancy.fr The novel allele *HLA-B*\*56:91 differs from *HLA-B*\*56:33 by one non-synonymous nucleotide substitution in exon 2.

#### K E Y W O R D S

HLA, HLA-B\*56, nanotype sequencing, novel allele, sequencing-based typing

We hereby report a novel *HLA-B*\*56 allele, now named, *HLA-B*\*56:91, which was identified in a voluntary stem cell donor.

The complete HLA typing was as followed: was as followed: *HLA-A\*11:01:01, 24:02:01; -B\*15:01:01G, 56:91;* -*C\*01:02:01; -DRB1\*01:01:01, 13:02:01; -DRB3\*03:01:01;* -*DQA1\*01:01:01, 01:02:01; -DQB1\*05:01:01G, 06:04:01;* -*DPA1\*01:03:01, 02:01:01; -DPB1\*02:01:02, 04:01:01.* 

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

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). Fastq files were analysed using TypeStream Visual version 2.0 (One Lambda). Data metrics were excellent for all loci, however the analysis retrieved an unexpected combination of HLA-B alleles: HLA-B\*15:552, 15:new.