Identification of HLA-B*14:02 Somatic Mutation in Patient Evaluated for Hematopoietic Stem Cell Transplantation

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BACKGROUND

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for patients with hematological malignancies.

In HSCT setting, the degree of donor and recipient Human Leukocyte Antigen (HLA) matching influences graft-versus-host disease, the risk of graft rejection, and overall survival.

Therefore, precise HLA typing is essential for identifying the donor-recipient HLA matching and optimal donor selection.

CASE HISTORY

A 30-year-old male with aplastic anemia was evaluated for an allogenic hematopoietic stem cell transplantation.

The patient's initial HLA genotyping performed on peripheral blood using next generation sequencing (NGS) shows HLA-B*14:85N null allele (**Figure 1**).

The confirmatory typing done on a separately collected peripheral blood sample by intermediate resolution rSSO confirms HLA-B locus typing at antigen level as HLA-B*14:FHXFC (**Figure 2**).

The patient's sibling was evaluated as a potential donor. The sibling appears HLA haploidentical except HLA-B locus. His sibling carries HLA-B*14:02 (**Figure 3**).

The HLA-B*14:02:01:01 and HLA-B*14:85N alleles differ at exon-1, codon 18.1. The B*14:02 allele encodes CGA (Arginine) whereas B*14:85N contains TGA stop codon (**Figure 4**).

The patient's buccal swab and the peripheral blood samples obtained from his parents were HLA typed using NGS. The buccal swab sample reveals HLA-B*14:02. His father carries HLA-B*14:02 allele as well. (**Figure 5** and **Figure 6**).

Therefore, the patient's final typing was reported as HLA-B*14:02. Patient and his family HLA typing results are shown in Table 1.

LABORATORY FINDINGS

Figure 1. Patient's peripheral blood sample initial HLA typing by NGS



Figure 2. Patient's confirmatory typing performed by rSSO

Locus	DNA TYPING RESULT		NMDP Code	
	Allele	Allele		
A*	02	03	02:FHVJU	03:FHVJW
B*	14	51	14:FHXFC	51:DWMJU
C*	08	16	08:FKPKK	16:FJFMM
DRB1*	08	13	08:EWDNN	13:FHYUF

FHXFC - 14:02/14:16/14:17/14:20/14:22/14:24/14:29/14:31/14:38/14:39/14:45/14:51/14:58/14:61/14:64/14:66/14:67/14:68/14:69N/14:74/14:75/14:76N/14:84/14:85N/14:88/14:89/14:91/14:92/14:94/14:98/14:100N/14:101N/14:106/14:107/14:109/14:110/14:114/14:115/14:116/14:119/14:122/14:123/14:125/14:126/14:127/14:131N/14:132/14:135/14:136

Figure 3. Sibling's peripheral blood sample HLA typing by NGS



Figure 4. HLA-B*14:02 vs B*14:85N sequence comparison

Figure 4 A. HLA Sequence Alignment: full protein

```
AA Pos.              -21        -11        -1         10         20         30         40         50         60         70
B*14:02:01:01        MLVM APRTV.....LLLLS AALALIETWA GSHSMRYFYT AVSRPGRGEP RFIS.....VGYVDD TQFVRFDSDA ASPREEPRAP WIEQEGPEYW DRNTQICRTN
B*14:85N             ---- --X

AA Pos.                        80         90        100        110        120        130                   140        150        160        170
B*14:02:01:01        TQ.......TDRESLRN LRGYY.NQSEA GSHTLQWMYG CDVGPDGRLL RGYNQFAYD.G KDYIALNEDL SSWTA.........ADTAA ....QITQRMEAA REAEQLRAYL EGTCVEWLRR
B*14:85N

AA Pos.              180        190        200        210        220        230        240        250        260        270
B*14:02:01:01        HLENGKETLQ RA.DPPKTHVT HHPISDHEAT LRCWALGFYP AEITLTWQRD GEDQTQDTEL VETRPAGDRT FQMWAAVVVP SGEEQRYTCH VQHEGLPKPL
B*14:85N

AA Pos.              280        290        300        310        320        330
B*14:02:01:01        TLRWEPSSQS TVPIVGIVAG LAVLAV.VVI G AVVAAVMCRR KSSGGKGGSY SQAASSDSAQ GSDVSLTA
B*14:85N
```

Figure 5. Father's peripheral blood sample HLA typing by NGS

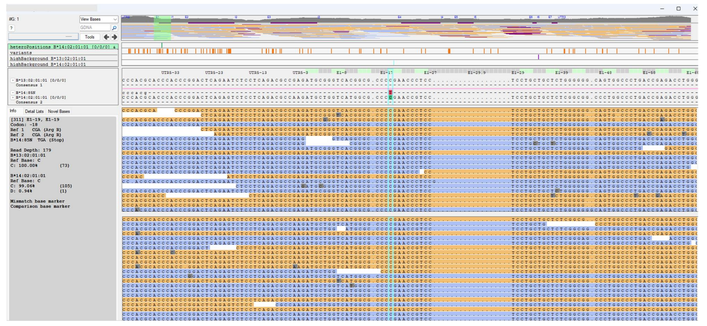


Figure 6. Patient's buccal swab sample confirmatory typing by NGS

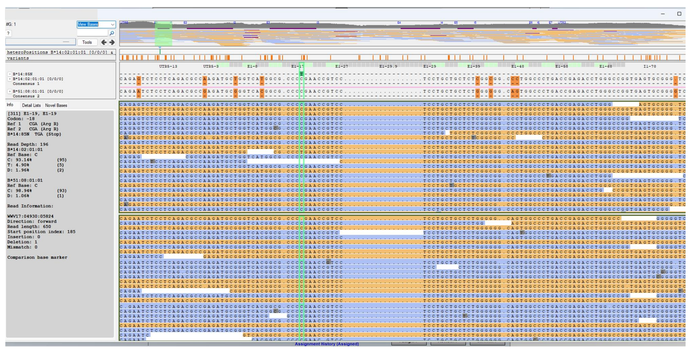


Figure 4B. HLA Sequence Alignment: nucleotide Exon-1, Exon -2, Exon-3

```
AA Codon                    -20        -15        -10         -5
B*14:02:01:01        ATG CTG GTC ATG GCG CCC CGA ACC GTC CTC CTG CTG CTC TCG GCG GCC CTG GCC CTG ACC GAG ACC TGG GCC G
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -

AA Codon                     5         10         15         20         25
B*14:02:01:01        GC TCC CAC TCC ATG AGG TAT TTC TAC ACC GCC GTG TCC CGG CCC GGC CGC GGG GAG CCC CGC TTC ATC TCA GTG
B*14:85N             -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                    30         35         40         45         50
B*14:02:01:01        GGC TAC GTG GAC GAC ACG CAG TTC GTG AGG TTC GAC AGC GAC GCC GCG AGT CCG AGA GAG GAG CCG CGG GCG CCG
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                    55         60         65         70         75
B*14:02:01:01        TGG ATA GAG CAG GAG GGG CCG GAA TAT TGG GAC CGG AAC ACA CAG ATC TGC AAG ACC AAC ACA CAG ACT GAC CGA
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                    80         85         90         95         100
B*14:02:01:01        GAG AGC CTG CGG AAC CTG CGC GGC TAC TAC AAC CAG AGC GAG GCC G|GG TCT CAC ACC CTC CAG TGG ATG TAT GGC
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -|-- --- --- --- --- --- --- --- ---

AA Codon                   105        110        115        120        125
B*14:02:01:01        TGC GAC GTG GGG CCG GAC GGG CGC CTC CTC CGC GGG TAT AAC CAG TTC GCC TAC GAC GGC AAG GAT TAC ATC GCC
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   130        135        140        145        150
B*14:02:01:01        CTG AAC GAG GAC CTG AGC TCC TGG ACC GCG GCG GAC ACC GCG GCT CAG ATC ACC CAG CGC AAG TGG GAG GCG GCC
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   155        160        165        170        175
B*14:02:01:01        CGT GAG GCG GAG CAG CTG AGA GCC TAC CTG GAG GGC ACG TGC GTG GAG TGG CTC CGC AGA CAC CTG GAG AAC GGG
B*14:85N             --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

AA Codon                   180
B*14:02:01:01        AAG GAG ACG CTG CAG CGC GCG G
B*14:85N             --- --- --- --- --- --- --- -
```

Table 1. Patient & Family HLA typing

	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*	HLA-DPB1*
Patient (peripheral blood)	03:01 02:01	**14:85N** 51:08	08:02 16:02	13:02 08:04	06:09 03:01	02:01 02:01
Sibling (peripheral blood)	03:01 02:05	**14:02** 51:01	08:02 16:02	13:02 04:03	06:09 03:02	02:01 04:01
Father (peripheral blood)	03:01 03:01	**14:02** 13:02	08:02 16:04	13:02 03:01	06:09 02:01	02:01 03:01
Patient (buccal swab)	03:01 02:01	**14:02** 51:08	08:02 16:02	13:02 08:04	06:09 03:01	02:01 02:01

CONCLUSIONS

HLA-B*14:02 is one of the high-risk alleles associated with clonal evolution in patients with immune-mediated aplastic anemia (AA).

In AA patients, hematopoietic stem cells often acquire a mutation or structural alternation in HLA genes to escape HLA-mediated immune surveillance and destruction.

The incorrect identification of HLA typing can delay the donor search process and result in selection of an incompatible donor.

In addition, an understanding disease associated high-risk HLA alleles may be important for risk assessment at the time of transplantation.

REFERENCES

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