Supplemental Data

Malignant Progression of Donor-engrafted Clonal Hematopoiesis in Sibling Recipients after Stem Cell Transplantation

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PART A: Supplemental Material and Methods

Next Generation Sequencing analysis of 21-gene myeloid panel

Targeted Next Generation Sequencing was performed using anchored multiplex PCR with a QiaSeq Targeted DNA custom panel (Qiagen, Hilden, Germany). The panel was designed and validated for analysis of 21 genes of clinical importance in myeloid malignancies, either diagnostic, prognostic or therapeutic.

Data was analysed by two bioinformatic pipelines using SeqNext (JSI medical systems, Ettenheim, Germany) and Genomics Workbench (Qiagen) software tools. Variants were classified according to Belgian Classification Guidelines.[1] Pathogenic, probably pathogenic and VUS variants above 2% VAF were reported. A sensitivity of 5% for substitution and small indel mutations (<25 bp) is guaranteed for all hotspot mutations and at least 99% of bases sequenced. The coding and splice site (intronic +/- 2 bp) sequences of the following genes, Ensembl transcripts and version numbers and exons were determined : ASXL1, ENST00000375687.4, exon, 13; CALR, ENST00000316448.5, exon, 9; CEBPA, ENST00000498907.2, exon, 1; CSF3R, ENST00000373103.1, exons, 14, 17; DNMT3A, ENST00000321117.5, exons, 8 to 23; EZH2, ENST00000320356.2, exons, 2 to 20; FLT3, ENST00000241453.7, exons, 13 to 15, 20; IDH1, ENST00000345146.2, exon, 4; IDH2, ENST00000330062.2, exon, 4; JAK2, ENST00000381652.3, exons, 12,14; KIT, ENST00000288135.5, exons, 8 to 11, 13, 17; MPL, ENST00000372470.3, exon, 10; NPM1, ENST00000296930.5, exon, 11; RUNX1, ENST00000300305.3, exons, 1 to 8; SETBP1, ENST00000282030.5, exon, 4; SF3B1, ENST00000335508.6, exons, 13 to 16; SRSF2(SFRS2), ENST00000392485.2, exon, 1; TET2, ENST00000380013.4, exons, 3 to 11; TP53, ENST00000269305.4, exons, 2 to 11; U2AF1, ENST00000291552.4, exons, 2, 6; WT1, ENST00000332351.3, exons, 6 to 9.

JAK2 V617F real time PCR

A quantitative JAK2 V617F real time PCR with a wild type blocking Locked Nucleid Acid (LNA) probe was performed according to Denys et al.,[2] with a reproducible sensitivity of 0.5%.

Shallow whole genome sequencing

Shallow depth WGS (sWGS) of tumor DNA was performed using an Illumina Hiseq3000 instrument (Illumina), starting from 200 ng input DNA. Tumor DNA was sheared to 200bp with a Covaris Ultrasonicator. Afterwards, library preparation was performed with the NEXTflex® Rapid DNA Sequencing Kit (BiooScientific), automated on a Hamilton MicroLab STAR Automated Liquid Handler (Hamilton). Libraries were pooled to equimolar concentrations and loaded on a Hiseq3000 instrument. The minimal number of reads per sample was set at 10 million (mean coverage of 0.4×). Data analysis and visualization was performed with the online analysis suite Vivar (http://cmgg.be/vivar/).[3] Each genome profile (line view and chromosome view) was manually checked for aberrations.

Estimated cumulative incidence

A cumulative incidence function for development of donor cell-derived hematological neoplasm (DCHN), giving the probability of a DCHN within a specific time frame after transplant, was estimated using R version 3.6.2,[4] cmprsk package version 2.2-10.[5] Death was considered a competing risk for the development of a DCHN.

Median follow-up time

Median follow-up time was calculated as the median time of follow-up for patients who remained event-free (no death, no DCHN).

PART B: Supplemental Results

Shallow Whole Genome Sequencing

Detailed results of shallow Whole Genome Sequencing is shown in Figure S1, Figure S2 and Figure S3. The VAF of the clone is 85% in both recipient and donor.

Figure S1. Detailed result of sWGS of chromosome-20 on peripheral blood DNA of case-1 recipient (upper graph) and case-1 donor (lower graph)

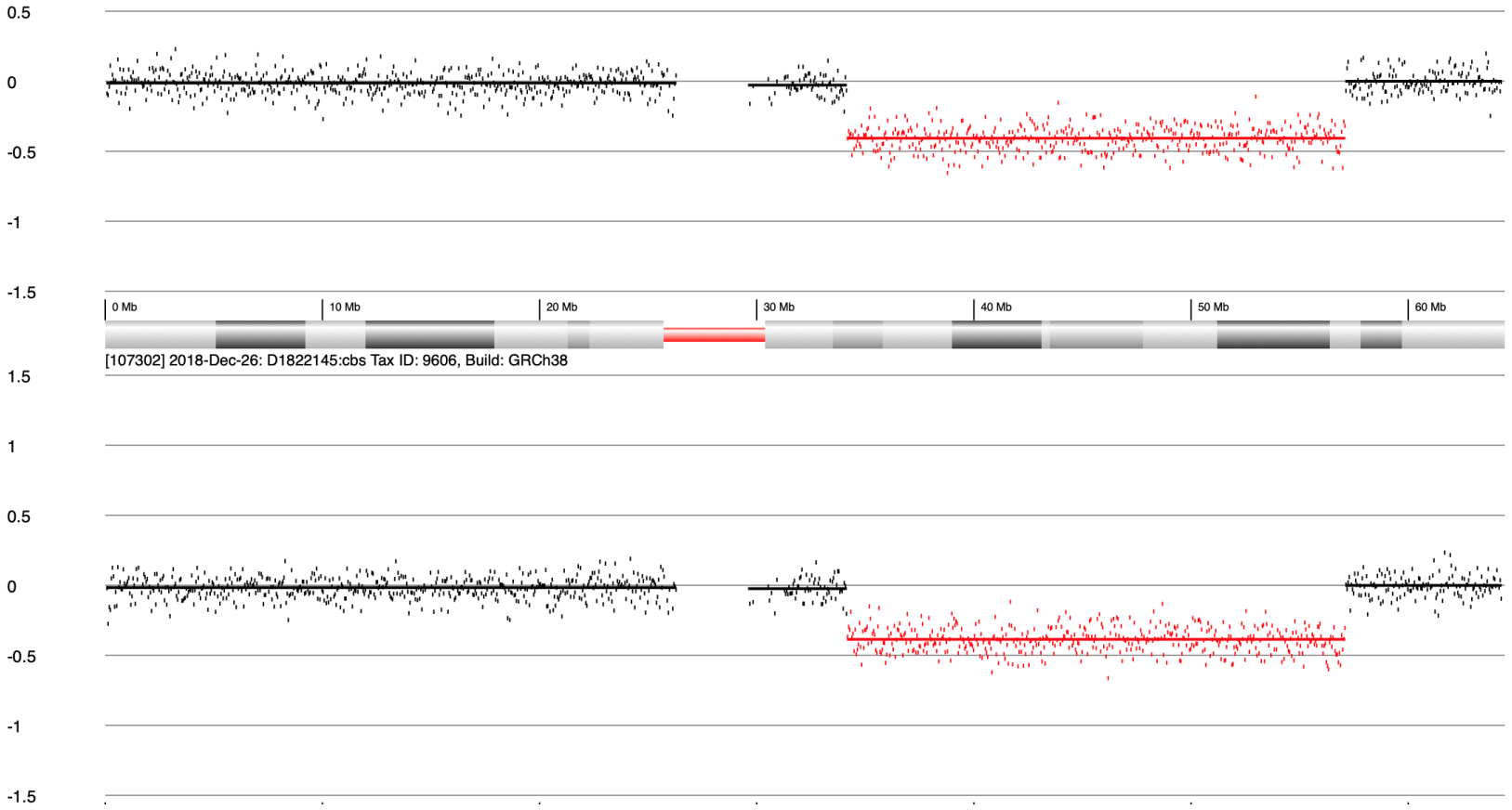

Figure S2. Detailed result of beginning of deletion of sWGS of chromosome-20 on peripheral blood DNA of case-1 recipient (upper graph) and case-1 donor (lower graph)

Figure S3. Detailed result of end of deletion of sWGS of chromosome-20 on peripheral blood DNA of case-1 recipient (upper graph) and case-1 donor (lower graph)

Estimated cumulative incidence

The estimated cumulative incidence function for development of a DCHN is reported in Figure S4. Table S1 gives estimated cumulative incidences at times post-transplant of DCHN occurrence.

Figure S4. cumulative incidence as a function of time since transplant

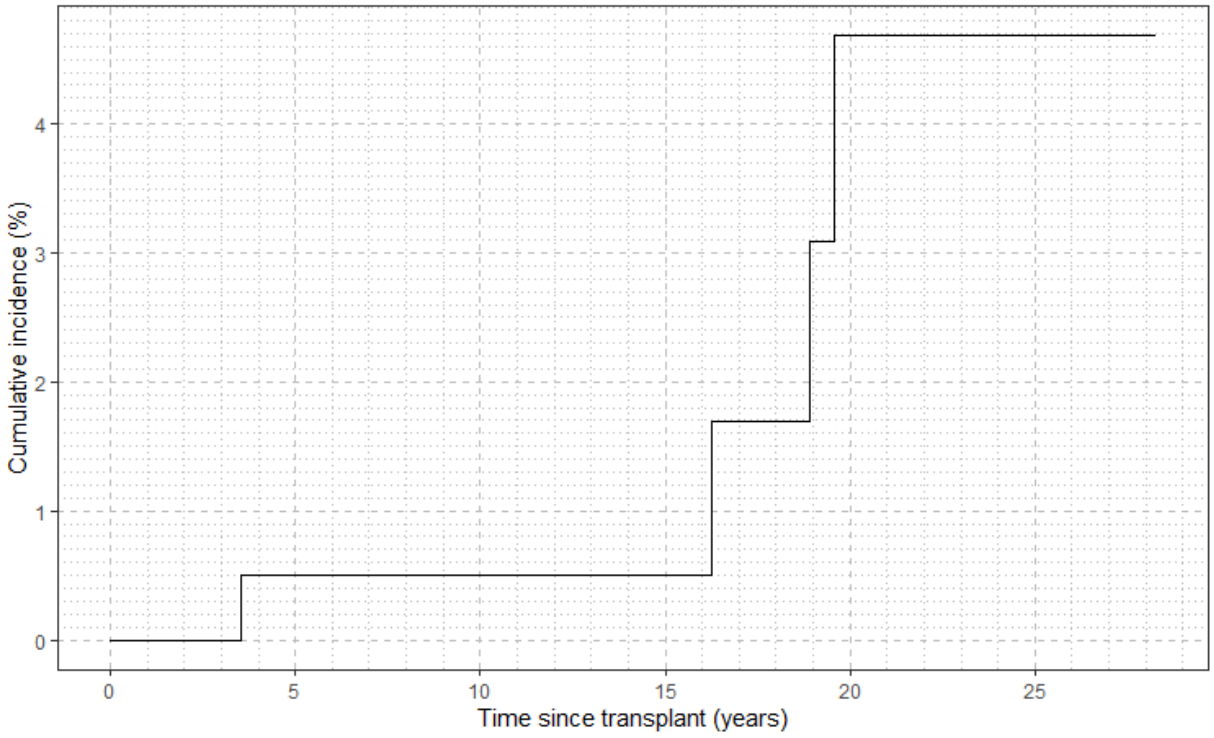

Table S1. Cumulative incidence estimates at occurence times of DCHN

Time since transplant (years)	Cumulative incidence (%)
3.548	0.507
16.246	1.688
18.916	3.090
19.562	4.683

Median follow-up time

Median follow-up for those remaining event-free was 7.97 years.

PART C: Supplemental References

1. Froyen G, Mercier M Le, Lierman E, et al. Standardization of somatic variant classifications in solid and haematological tumours by a two-level approach of biological and clinical classes: An initiative of the belgian compermed expert panel. *Cancers (Basel)*. 2019;11(12).

2. Denys B, El Housni H, Nollet F, Verhasselt B, Philippé J. A real-time polymerase chain reaction assay for rapid, sensitive, and specific quantification of the JAK2V617F mutation using a locked nucleic acid-modified oligonucleotide. *J Mol Diagnostics*. 2010;12(4):512-519.

3. Sante T, Vergult S, Volders PJ, et al. ViVar: A comprehensive platform for the analysis and visualization of structural genomic variation. *PLoS One*. 2014;9(12):1-12.

4. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.

5. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat*. 1988;16(3):1141-1154.