Transmission of diffuse large B-cell lymphoma by an allogeneic stem-cell transplant

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doi:10.3324/haematol.2018.196907

Supplementary Information

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Supplementary Methods

Study oversight

Written consent was obtained for the collection and use of specimens for research purposes with ethical approval obtained locally; (London Research Ethics Committee (LREC) of the East London and the City Health authority (10/H0704/65 and 06/Q0605/69).

Immunohistochemistry and fluorescent *in situ* hybridization (FISH)

All biopsies were histologically reviewed to confirm the diagnosis by expert haemato-pathologists. Immunohistochemistry (IHC) was performed on 4 µm formalin fixed paraffin-embedded (FFPE) tissue sections using the DAKO Omnis staining machine with the following primary antibodies: anti-CD20 (RTU, clone L26, DAKO), anti-CD10 (RTU, clone 56C6; DAKO), anti-BCL6 (RTU, clone PG BP6; DAKO), anti-MUM1 (RTU, clone MUM1p; DAKO). Interphase FISH analysis for *MYC*, *BCL6*, and *BCL2* rearrangements and for the chromosome X and Y centromeres was performed on ~3 µm thick FFPE tissue sections according to standard validated protocols using the Cytocell *BCL2*, *BCL6* and *MYC* break-apart probe combinations, the Cytocell *IGH/MYC* Translocation dual fusion probe combination and the Cytocell dual labelled satellite X and Y probe set (Oxford Gene Technology, OGT; Cambridge, UK).

DNA extraction

DNA was extracted from formalin-fixed paraffin embedded (FFPE) biopsies using the Generead DNA FFPE Kit (QIAGEN) according to the manufacturer's instructions. For Immunoglobulin heavy-chain variable (IGHV) gene sequencing whole genome amplification (WGA) was performed using the GenomePlex Complete WGA Kit (Sigma Aldrich).

Immunoglobulin heavy-chain variable (IGHV) gene sequencing

The European BIOMED-2 protocol was followed to detect clonal IGHV sequences [1]. FR2 BIOMED primers and the JH consensus primer were used to PCR amplify tumor DNA and the amplified PCR products were separated using a 2% agarose gel. The clonal band was excised from the agarose gel and Sanger sequenced. Sequencing data was aligned to all known immunoglobulin genes using the IMGT/V-QUEST web-tool (www.imgt.org/IMGT_vquest/vquest) to identify the VH gene. The PCR and sequencing steps were then repeated as described above but with the specific VH family primer. The forward sequencing trace was analyzed using IMGT/V-QUEST to obtain the specific Diversity (D) and Joining (J) gene segments.

Targeted sequencing of the recipient and donor tumors.

Targeted sequencing of the donor and recipient tumors were performed using the Haloplex HS target enrichment system (Agilent Technologies) according to the manufacturer's instructions and using a custom DLBCL gene panel consisting of 158 genes recurrently mutated or implicated in lymphomagenesis (Supplementary Table 1). In brief, 50ng of genomic DNA was restriction enzyme digested before hybridization with the custom probe library for 16 hours and incorporation of molecular barcodes. Target DNA was captured and PCR amplified before multiplexing and sequencing on the Illumina Hiseq 2500 platform to generate 100bp paired end reads.

After de-multiplexing and de-barcoding, sequencing reads were processed using The Agilent Genomics NextGen Toolkit (AGeNT) (Agilent Technologies, http://www.genomics.agilent.com). Within the toolkit, the 'SurecallTrimmer' java application was first used to trim low quality bases from the ends, remove adaptor sequences and mask enzyme footprints. Filtered paired-end reads were then aligned to the reference genome hg19 using BOWTIE2 [2]. After alignment, the AGeNT "LocatIt" program was used to process the molecular barcode (MBC) information and remove MBC duplicates from the alignment SAM files. The output of this step was coordinate-sorted alignment BAM files, with duplicates merged and output of only the consensus read pair sequence per MBC. The VarScan2 tool [3] was used to examine the alignment pileup file to call variants against the reference genome based on high quality reads using a VAF cutoff of 10%. The strand bias filter was also applied. Identified variants were annotated using ANNOVAR [4] with nonsynonymous SNVs and coding indels further identified. Variants present in 1000 Genomes and NHLBI GO Exome Sequencing Project (ESP) with minor allele frequency (MAF) >1% were excluded. The remaining variants present in dbSNP138 and COSMIC v70 [5] were then marked. We compared identified variants with a larger series of samples sequenced on the same platform [6] to exclude sequencing artifacts defined as non-hotspot variants present in greater than three samples. Furthermore whole exome sequence (WES) data for the donor and recipient germline samples were available (Human All Exon v5 SureSelect XT kit (Agilent Technologies)), and variants present in the germline samples were removed, leading to a final set of high-confidence somatic calls. All non-synonymous somatic variants identified are listed in Supplementary Table 2.

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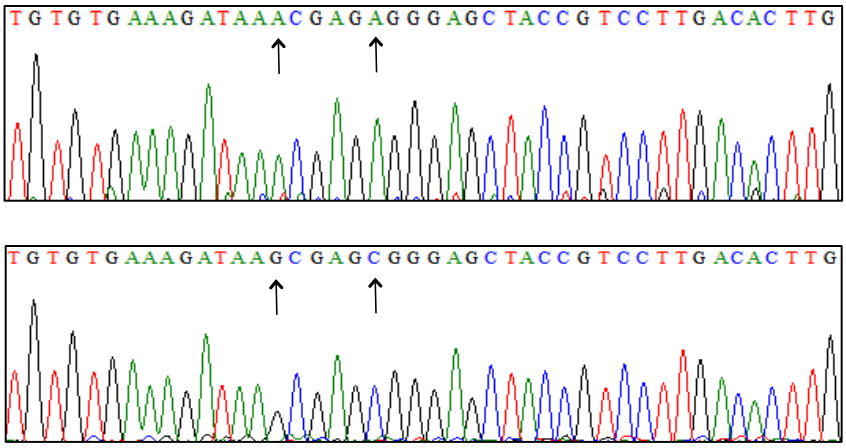
Supplementary Figure 1: *IGHV* analysis of the donor and recipient tumors

An identical VDJ rearrangement was detected in both the donor and recipient tumors and a portion of the sequenced CDR3 region is demonstrated (discordant somatic hypermutation changes are denoted by the black arrows).



Supplementary Table 1: Haloplex HS targeted gene panel used for targeted sequencing

ACTB	ETS1	LYST	TCF3
APC	ETV1	MALT1	TET2
ARHGEF1	ETV6	MAP2K1	TLR2
ARID1A	EZH2	MCL1	TMEM30A
ARID1B	FAS	MEF2B	TMSB4X
ATM	FAT2	MEF2C	TNFAIP3
ATP10A	FAT4	MPEG1	TNFRSF14
ATP13A4	FBXO11	MTOR	TP53
ATP6AP1	FBXW7	MYC	TRAF3
ATP6AP2	FOXO1	MYD88	UBE2A
ATP6V0A1	FOXP1	NFKBIA	VMA21
ATP6V0C	GNA12	NFKBIE	XPO1
ATP6V0D1	GNA13	NFKBIZ	ZFHX3
ATP6V1A	GNAI2	NOTCH1	ZMYM3
ATP6V1B2	HDAC7	NOTCH2	
ATP6V1F	HIST1H1B	NOTCH3	
ATRX	HIST1H1C	NOTCH4	
B2M	HIST1H1D	P2RY8	
BCL10	HIST1H1E	PAX5	
BCL2	HIST1H2AC	PCLO	
BCL6	HIST1H2AE	PIK3C2G	
BCL7A	HIST1H2AG	PIK3CD	
BCOR	HIST1H2AL	PIK3R1	
BCR	HIST1H2AM	PIM1	
BRD4	HIST1H2BC	PLCB1	
BTG1	HIST1H2BD	POU2AF1	
BTG2	HIST1H2BG	POU2F2	
CARD11	HIST1H2BJ	PRDM1	
CCND3	HIST1H2BK	PRKCB	
CD22	HIST1H2BO	PTEN	
CD274	HIST1H3B	PTPN6	
CD36	HIST1H3C	RB1	
CD58	HIST1H3G	REL	
CD70	HIST1H3H	RHOH	
CD74	HVCN1	RRAGC	
CD79A	ID3	S1PR2	
CD79B	IKZF3	SETD2	
CD83	IRF4	SGK1	
CDKN2A	IRF8	SLC22A16	
CDKN2B	ITPKB	SMARCA4	
CIITA	KDM2B	SMARCB1	
CREBBP	KLHL14	SOCS1	
CXCR4	KLHL6	SPEN	
DTX1	KMT2A	STAT3	
DUSP2	KMT2C	STAT6	
EEF1A1	KMT2D	SYK	
EP300	LRRN3	TAF1	
EPHA7	LYN	TBL1XR1	

Supplementary Table 2: List of all non-synonymous mutations in the donor and recipient tumors

SHARED VARIANTS

			Donor		Recipient	
Gene	**Position**	**aa _change**	**Total reads**	**VAF (%)**	**Total reads**	**VAF (%)**
HIST1H1D	6:26234759	p.A135T	120	34.17	540	25.37%
CDKN2A	9:21971186	p.R58X	40	60%	833	20.41%
DTX1	12:113496211	Y72N	107	48.60%	355	29.58%
CD70	19:6590146	p.W55X	209	78.47%	768	22.14%
MEF2B	19:19260052	p.N81H	34	73.53%	372	32.26%
BCL6	3:187447773	p.R140fs	16	31.25%	44	29.55%

DONOR SPECIFIC VARIANTS

Gene	**Position**	**aa _change**	**Total reads**	**VAF (%)**
HIST1H1E	6:26156854	p.R79H	272	23.53%
PIM1	6:37139124	p.V246E	46	23.91%
PIM1	6:37139143	p.C252W	46	23.91%
CCND3	6:41909273	p.V39I	99	25.25%
CARD11	7:2979559	p.D230N	41	24.39%
B2M	15:45003747	p.M1I	42	16.67%

RECIPIENT SPECIFIC VARIANTS

Gene	**Position**	**aa _change**	**Total reads**	**VAF (%)**
CD70	6590875	p.Q47X	1396	33.02%
SMARCA4	11144146	p.R1243W	2885	32.27%
MYC	128750684	p.P74infs	532	29.70%