

DUX4r, ZNF384r and PAX5-P80R mutated B-cell precursor acute lymphoblastic leukemia frequently undergo monocytic switch

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Table S1. S1a. List of antibodies used for diagnostic immunophenotyping.

Antibody	Fluorochrome	Clone	Catalogue number	Manufacturer
CD2	PE	39C1.5	A07744	Beckman Coulter
CD3	FITC	UCHT1	1F-202-T100	Exbio
CD4	PE-Cy7	MEM-241	T7-359-T100	Exbio
CD5	PerCP-Cy5.5	L17F12	T9-691-T100	Exbio
CD7	APC	124-1D1	1A-183-T100	Exbio
CD8	APC-Cy7	MEM-31	T4-207-T100	Exbio
CD10	PE	SS2/36	R084801	Dako
CD11b	PE	ICRF44	347557	BD Biosciences
CD11c	PerCP-Cy5.5	B-ly6	646784	BD Biosciences
CD13	PE	SJ1D1	A07762	Beckman Coulter
CD14	Pacific Blue	MEM-15	PB-293-T100	Exbio
CD15	FITC	MMA	332778	BD Biosciences
CD16	Alexa Fluor 700	3G8	A7-646-T100	Exbio
CD19	APC	LT19	1A-305-T100	Exbio
CD19	PE-Cy7	J3119	IM3628	Beckman Coulter
CD20	FITC	L27	345792	BD Biosciences
CD22	FITC	SJ10.1H11	IM0779U	Beckman Coulter
CD24	PE	ALB9	IM1428U	Beckman Coulter
CD27	PE	M-T271	560609	BD Biosciences
CD33	PE	D3HL60.251	A07775	Beckman Coulter
CD34	APC	581	IM2472	Beckman Coulter
CD38	PE	T16	IM1832U	Beckman Coulter
CD44	FITC	G44-26	555478	BD Biosciences
CD45	PerCP	2D1	PC-222-T100	Exbio
CD56	PE	NCAM16.2	345812	BD Biosciences
CD64	FITC	22	B49185	Beckman Coulter
CD65	FITC	88H7	B36299	Beckman Coulter
CD66c	FITC	KOR-SA3544	IM2039U	Beckman Coulter
CD71	Alexa Fluor 700	MEM-75	A7-235-T100	Exbio
CD79a	Alexa Fluor 647	ZL7-4	MCA1298A647	Exbio
CD99	PE	3B2/TA8	1P-658-T100	Exbio
CD117	PE	95C3	IM1360U	Beckman Coulter
CD371	APC	50C1	353606	BioLegend
IgM	FITC	G20-127	555782	BD Biosciences
HLA DR	PE	L243	1P-690-T100	Exbio
NG2	PE	7.1	B92429	Beckman Coulter
TSLPR	PE	1D3	322906	BioLegend
Kappa	PE	TB28-2	1P-674-T100	Exbio
Lambda	FITC	1-155-2	1F-673-T100	Exbio
CD3	APC	UCHT1	IM2467	Beckman Coulter
CD22	APC	S-HCL-1	333145	BD Biosciences
cyCD79a	PE	HM57	R715901	Dako
TdT	FITC	HT-6	F713950	Dako
Lysozyme	PE	LZ-2	GM-4133	Nordic Mubio
MPO	FITC	CLB-MPO-1	IM1874U	Beckman Coulter

S1b. Diagnostic antibody combinations. B1: FITC, B2: PE, B3: PerCP/PerCP-Cy5.5, B4: PE-Cy7, R1: APC/Alexa Fluor 647, R2: Alexa Fluor 700, R3: APC-Cy7, V1: Syto-41/Pacific Blue, V2: Pacific Orange.

B1	B2	B3	B4	R1	R2	R3	V1	V2
CD4	CD99	CD5	CD3	CD7	CD16+CD56	CD8	Syto-41	CD45
CD10	CD13	CD45	CD19					
CD10	CD33	CD45	CD19					
CD15	CD117	CD45	CD19	CD33	CD71		Syto-41	
CD20	CD10	CD45	CD19	CD34	CD38		Syto-41	
CD22	CD24	CD45	CD19	CD79a				
CD34	CD38	CD45	CD19					
CD44	CD27	CD34	CD19	CD10	CD38		CD20	CD45
CD64		CD45	CD19					
CD65	CD2	CD45	CD19					
CD66c	CD56	CD45	CD19					
CD19	NG2	CD45						
CD19	TSLPR	CD45						
CD33	VNN2	CD34	CD19	CD371			CD14	CD45
IgM	CD19	CD45						
Lambda	Kappa	CD20	CD19	CD5			CD45	
CD45	intra-Lysozyme	CD11c	CD34	CD33			CD14	
intra-TdT	CD7	CD45	CD3	intra-CD3				
CD7	CD11b	CD45	CD19	intra-CD22				
intra-MPO	intra-CD79a	CD45	CD19	intra-CD3				
intra-IgM	CD19	CD45						

Table S2. Association of switch with the most frequent recurrent genetic alterations within DUX4r subtype.

		Total (n=56)	Switch		p-Value Switch no versus yes
			No (n=11)	Yes (n=45)	
<i>ERG</i> deletion	No	16	4	12	0.46
	Yes	38	6	32	
	<i>No data</i>	2	1	1	
<i>IKZF1</i> deletion	No	35	8	27	0.17
	Yes	10	0	10	
	<i>No data</i>	11	3	8	
<i>CDKN2A</i> deletion	No	26	6	20	0.44
	Yes	19	2	17	
	<i>No data</i>	11	3	8	
<i>CDKN2B</i> deletion	No	26	6	20	0.44
	Yes	19	2	17	
	<i>No data</i>	11	3	8	
<i>PAX5</i> deletion	No	36	7	29	0.67
	Yes	9	1	8	
	<i>No data</i>	11	3	8	
<i>NRAS</i> mutation	No	36	9	27	0.25
	Yes	14	1	13	
	<i>No data</i>	6	1	5	
<i>KMT2D</i> mutation	No	39	9	30	0.42
	Yes	11	1	10	
	<i>No data</i>	6	1	5	

Table S3. Top ranking differentially expressed genes ($n = 50$) between switching and non-switching cases. Only protein-coding genes with absolute fold change ≥ 2.5 were considered. Genes with Cluster of Differentiation nomenclature IDs are highlighted.

ensembl_gene_id	gene	CD	log2Fold Change	padj	description
ENSG00000154188	ANGPT1		4.32	1.40E-60	angiopoietin 1
ENSG00000169083	AR		8.16	7.60E-57	androgen receptor
ENSG00000133687	TMTC1		4.3	1.10E-35	transmembrane O-mannosyltransferase targeting cadherins 1
ENSG00000171855	IFNB1		7.52	3.20E-35	interferon beta 1
ENSG00000203727	SAMD5		5.2	3.20E-35	sterile alpha motif domain containing 5
ENSG00000119698	PPP4R4		4.62	1.40E-33	protein phosphatase 4 regulatory subunit 4
ENSG00000119922	IFIT2		3.25	1.40E-33	interferon induced protein with tetratricopeptide repeats 2
ENSG00000145794	MEGF10		4.57	2.50E-32	multiple EGF like domains 10
ENSG00000178662	CSRNP3		7.07	3.70E-32	cysteine and serine rich nuclear protein 3
ENSG00000170421	KRT8		3.21	8.70E-28	keratin 8
ENSG00000088538	DOCK3		3.12	1.80E-25	dedicator of cytokinesis 3
ENSG00000144369	FAM171B		4.76	3.20E-25	family with sequence similarity 171 member B
ENSG00000145685	LHFPL2		2.71	3.40E-25	LHFPL tetraspan subfamily member 2
ENSG00000159674	SPON2		2.57	3.60E-25	spondin 2
ENSG00000182853	VMO1		2.79	9.70E-25	vitelline membrane outer layer 1 homolog
ENSG00000172322	CLEC12A	CD371	3.83	4.90E-24	C-type lectin domain family 12 member A
ENSG00000091129	NRCAM		3.49	6.30E-24	neuronal cell adhesion molecule
ENSG00000165509	MAGEC3		6.84	4.40E-23	MAGE family member C3
ENSG00000180767	CHST13		3.57	4.90E-23	carbohydrate sulfotransferase 13
ENSG00000205755	CRLF2		-4.09	5.60E-23	cytokine receptor like factor 2
ENSG00000256660	CLEC12B		4.06	8.90E-23	C-type lectin domain family 12 member B
ENSG00000169855	ROBO1		-3.7	1.30E-22	roundabout guidance receptor 1
ENSG00000010379	SLC6A13		3.21	1.30E-22	solute carrier family 6 member 13
ENSG00000134207	SYT6		6.83	1.30E-22	synaptotagmin 6
ENSG00000133101	CCNA1		3.96	1.80E-22	cyclin A1
ENSG00000129654	FOXJ1		4.03	2.60E-22	forkhead box J1
ENSG00000187783	TMEM72		-4.72	6.80E-22	transmembrane protein 72
ENSG00000144824	PHLDB2		-2.61	7.50E-22	pleckstrin homology like domain family B member 2
ENSG00000240403	KIR3DL2	CD158K	-4.01	1.70E-21	killer cell immunoglobulin like receptor, three Ig domains and long cytoplasmic tail 2
ENSG00000156738	MS4A1	CD20	-2.74	3.40E-21	membrane spanning 4-domains A1
ENSG00000132514	CLEC10A	CD301	3.67	7.90E-21	C-type lectin domain containing 10A
ENSG00000164023	SGMS2		3.18	8.20E-21	sphingomyelin synthase 2
ENSG00000100867	DHRS2		4.53	1.80E-20	dehydrogenase/reductase 2
ENSG00000157510	AFAP1L1		-3.1	9.40E-20	actin filament associated protein 1 like 1
ENSG00000011465	DCN		3.17	7.50E-19	decorin
ENSG00000158488	CD1E	CD1E	4.23	9.40E-19	CD1e molecule
ENSG00000137962	ARHGAP29		-3.38	3.20E-18	Rho GTPase activating protein 29
ENSG00000143850	PLEKHA6		2.56	3.20E-18	pleckstrin homology domain containing A6
ENSG00000182676	PPP1R27		3.24	3.30E-18	protein phosphatase 1 regulatory subunit 27
ENSG00000184507	NUTM1		-7.31	7.70E-18	NUT midline carcinoma family member 1
ENSG00000091181	IL5RA	CD125	3	7.70E-18	interleukin 5 receptor subunit alpha
ENSG00000183775	KCTD16		2.6	8.40E-18	potassium channel tetramerization domain containing 16
ENSG00000183421	RIPK4		-4.15	2.60E-17	receptor interacting serine/threonine kinase 4
ENSG00000198576	ARC		3.24	3.40E-17	activity regulated cytoskeleton associated protein
ENSG00000185028	LRRC14B		-2.67	4.40E-17	leucine rich repeat containing 14B
ENSG00000129595	EPB41L4A		2.51	5.90E-17	erythrocyte membrane protein band 4.1 like 4A
ENSG00000165186	PTCHD1		3.03	7.10E-17	patched domain containing 1
ENSG00000074590	NUAK1		3.3	7.90E-17	NUAK family kinase 1
ENSG00000147655	RSPO2		4.18	8.00E-17	R-spondin 2
ENSG00000178882	RFLNA		2.99	1.10E-16	refilin A

Table S4. Top ranking differentially expressed genes ($n = 50$) between switching and non-switching cases inside DUX4 rearranged cases. Only protein-coding genes with absolute fold change ≥ 2.5 were considered. Genes with Cluster of Differentiation nomenclature IDs are highlighted.

ensembl_gene_id	gene	CD	log2FoldChange	padj	description
ENSG00000169083	AR		9.91	6.60E-15	androgen receptor
ENSG00000133101	CCNA1		5.42	5.00E-13	cyclin A1
ENSG00000133104	SPART		2.87	2.50E-10	NA
ENSG00000179388	EGR3		5.24	1.00E-09	NA
ENSG00000245848	CEBPA		5.12	8.90E-09	NA
ENSG00000164283	ESM1		-6.7	2.80E-08	endothelial cell specific molecule 1
ENSG00000164023	SGMS2		4.28	4.70E-08	sphingomyelin synthase 2
ENSG00000159217	IGF2BP1		-6.64	8.00E-08	insulin like growth factor 2 mRNA binding protein 1
ENSG00000138798	EGF		4.6	8.30E-08	NA
ENSG00000159674	SPON2		3.57	8.30E-08	spondin 2
ENSG00000196460	RFX8		4.54	8.30E-08	NA
ENSG00000122025	FLT3	CD135	3.11	2.10E-07	NA
ENSG00000120738	EGR1		4.59	3.90E-07	NA
ENSG00000151650	VENTX		4	9.50E-07	NA
ENSG00000154188	ANGPT1		3.76	2.20E-06	angiopoietin 1
ENSG00000146147	MLIP		6.97	2.60E-06	NA
ENSG00000131724	IL13RA1	CD213a1	3.17	3.80E-06	NA
ENSG00000084110	HAL		2.68	4.90E-06	NA
ENSG00000112299	VNN1		4.54	5.00E-06	vanin 1
ENSG00000164171	ITGA2	CD49B	3.74	5.70E-06	NA
ENSG00000162711	NLRP3		2.72	6.60E-06	NA
ENSG00000203727	SAMD5		5.61	6.60E-06	sterile alpha motif domain containing 5
ENSG00000242265	PEG10		-5.56	8.20E-06	paternally expressed 10
ENSG00000101333	PLCB4		-3.92	1.10E-05	NA
ENSG00000141448	GATA6		4.31	1.10E-05	NA
ENSG00000134531	EMP1		3.93	1.90E-05	NA
ENSG00000171794	UTF1		4.99	2.10E-05	NA
ENSG00000171903	CYP4F11		5.72	2.60E-05	cytochrome P450 family 4 subfamily F member 11
ENSG00000147655	RSPO2		5.79	2.60E-05	R-spondin 2
ENSG00000152348	ATG10		-2.84	3.10E-05	NA
ENSG00000186998	EMID1		3.19	3.10E-05	NA
ENSG00000182601	HS3ST4		3.71	3.60E-05	NA
ENSG00000008197	TFAP2D		19.53	3.70E-05	NA
ENSG00000133216	EPHB2		3.63	3.70E-05	NA
ENSG00000152503	TRIM36		3.32	3.70E-05	NA
ENSG00000180549	FUT7		2.92	3.70E-05	NA
ENSG00000196805	SPRR2B		19.56	3.70E-05	NA
ENSG00000178662	CSRNP3		5.86	4.00E-05	cysteine and serine rich nuclear protein 3
ENSG00000183734	ASCL2		3.25	4.60E-05	NA
ENSG00000174123	TLR10	CD290	2.6	6.00E-05	NA
ENSG00000109339	MAPK10		3.25	6.70E-05	NA
ENSG00000105383	CD33	CD33	2.81	6.90E-05	NA
ENSG00000073464	CLCN4		2.63	7.30E-05	NA
ENSG00000122641	INHBA		3.36	7.30E-05	inhibin subunit beta A
ENSG00000130158	DOCK6		3.2	7.30E-05	NA
ENSG00000145632	PLK2		4.18	7.30E-05	NA
ENSG00000165757	JCAD		3.22	7.60E-05	NA
ENSG00000177989	ODF3B		3.23	7.80E-05	NA
ENSG00000110076	NRXN2		2.9	9.80E-05	NA
ENSG00000036565	SLC18A1		4.21	1.10E-04	solute carrier family 18 member A1

Figure S1. Flow chart showing the number of patients.

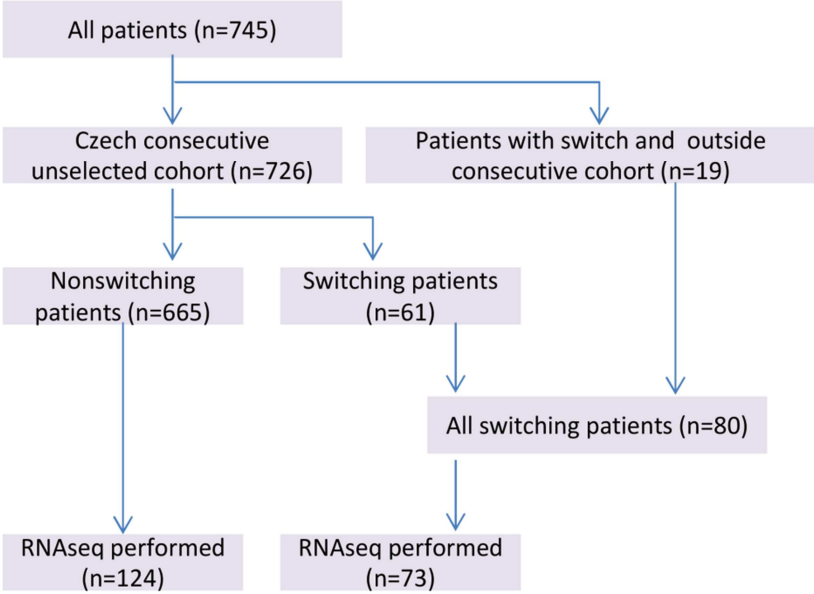


Figure S2. Monocytic switch in a typical patient with the DUX4r subtype, d+8 PB sample. Changes in expression of the precursor, B lineage and monocytic markers and side scatter (SSC) are depicted.

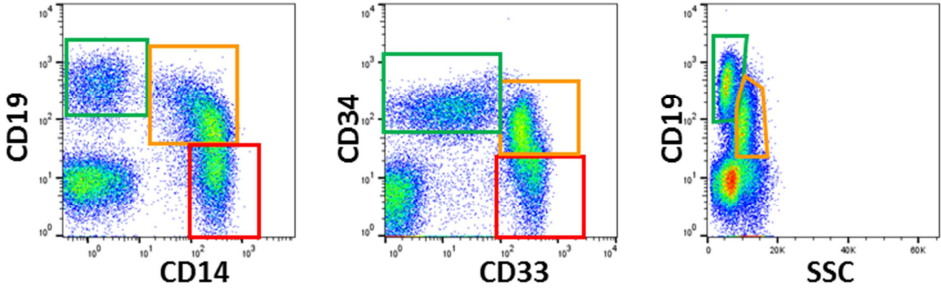
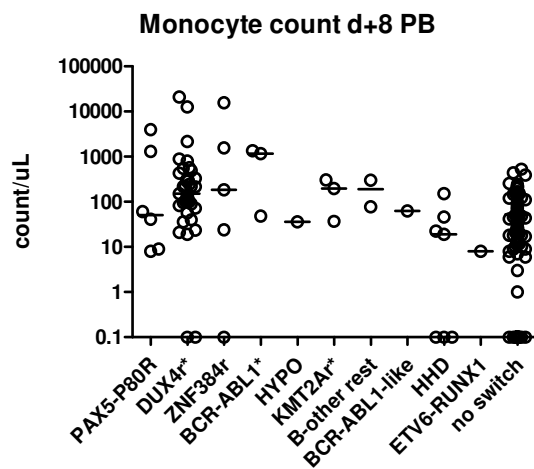
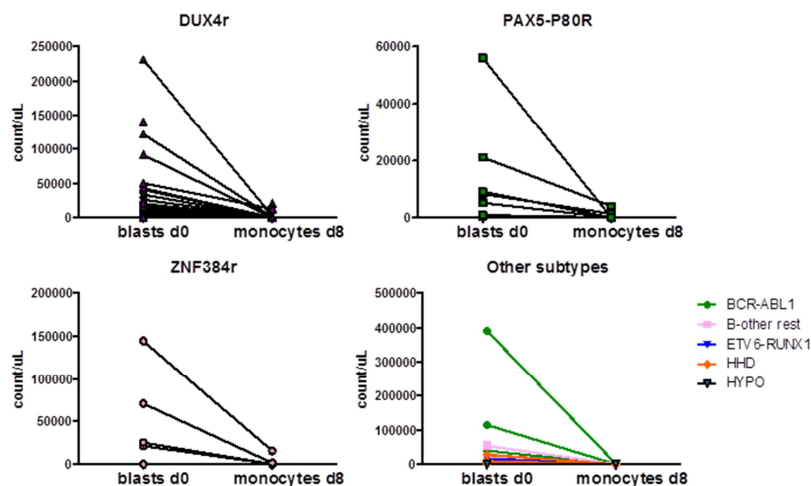


Figure S3. Intermediate B-monocytoid cells and monocytes in patients with monocytic switch. **S3a.** Absolute monocyte count by morphology in switching patients. Separately are shown data from randomly selected 122 patients without switch. Subtypes are marked with asterisk if significantly different from non switching patient values by Mann-Whitney test. HYPO, hypodiploid; HHD, hyperdiploid. **S3b.** Dynamics of diagnostic blast count in PB compared to d+8 monocyte count in PB in patients with monocytic switch. HYPO, hypodiploid; HHD, hyperdiploid. **S3c.** The maximum proportion of intermediate B-monocytoid cells defined as $CD19^{pos}CD14^{pos}$ at time-points d0 (BM, PB), d+8 (PB), d+15 (BM, PB), are d+33 (BM) are shown. Each dot represents the maximum value of a single patient. HYPO, hypodiploid; HHD, hyperdiploid; B-other unknown, B-other without performed RNA-seq.

S3a.



S3b.



S3c.

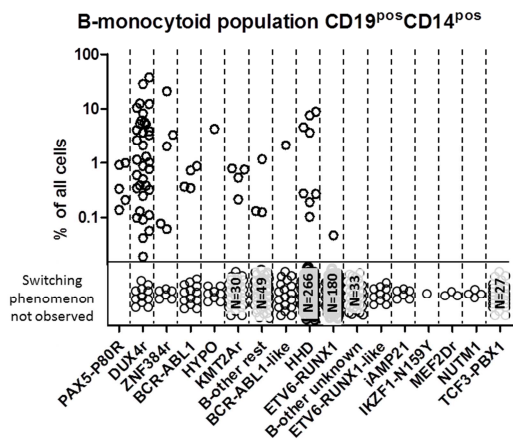


Figure S4. Unsupervised Hierarchical Clustering Analysis of all cases ($n = 197$) based on most variable genes (only genes with SD higher than 0.4 times maximum SD, $n = 271$).

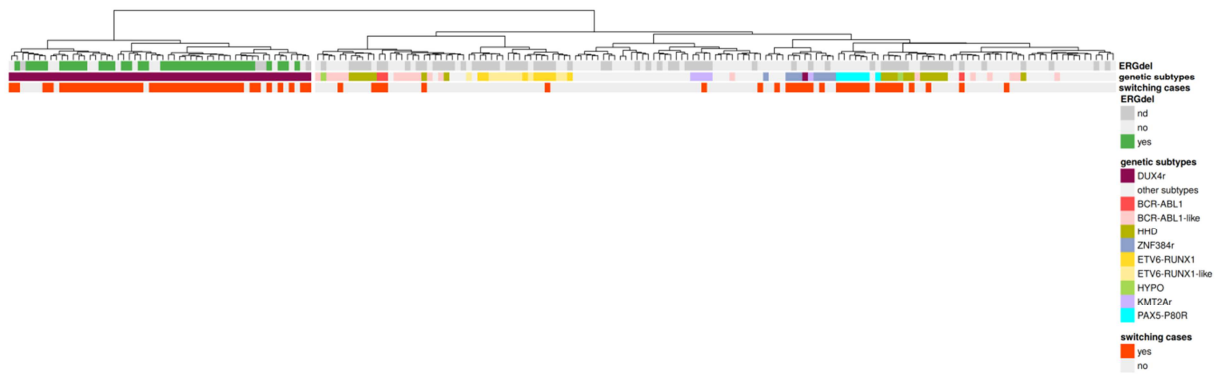


Figure S5. Unsupervised Hierarchical Clustering of DUX4r cases ($n = 55$) based on most variable genes (only genes with SD higher than 0.4 times maximum SD).

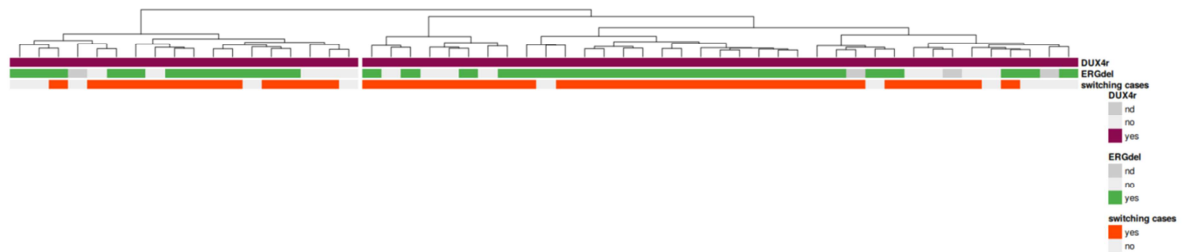


Figure S6. Correlation of FC and PCR MRD on d+8 and w+12 in *DUX4r*, *PAX5-P80R* and *ZNF384r*. Spearman's rank correlation coefficient indicated if p value < 0.05.

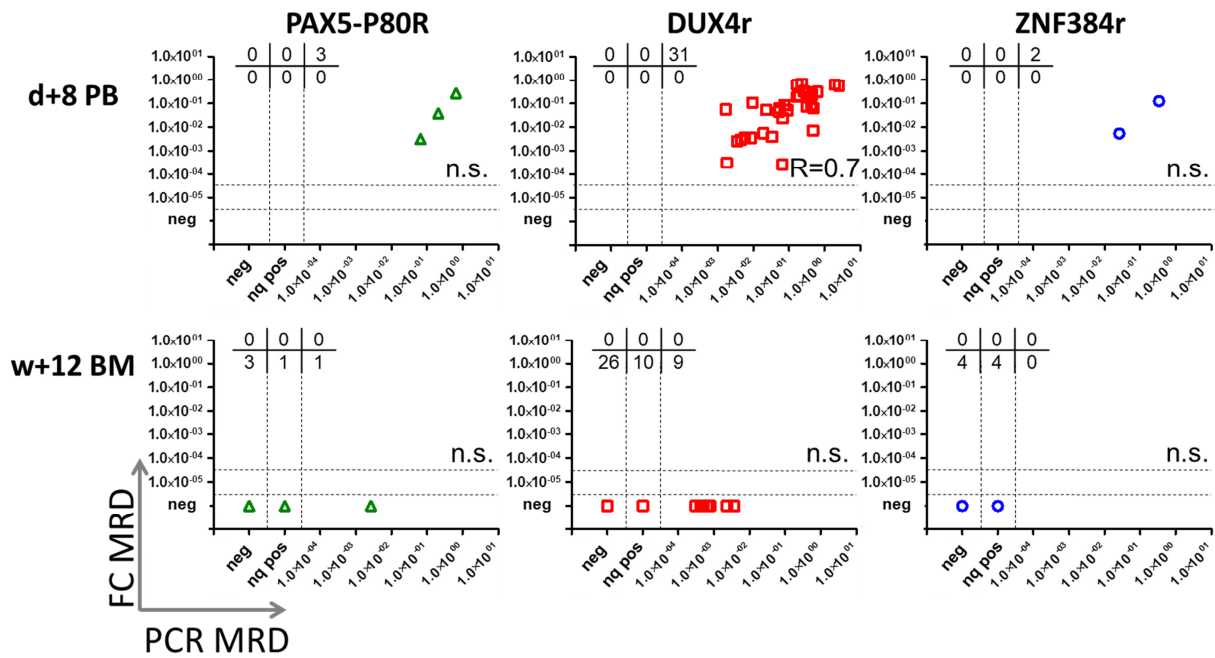


Figure S7. Correlation of FC and PCR MRD in *DUX4r*^{neg}*PAX5-P80R*^{neg}*ZNF384r*^{neg} cases with monocytic switch in different time points. Spearman's rank correlation coefficient indicated if p value < 0.05.

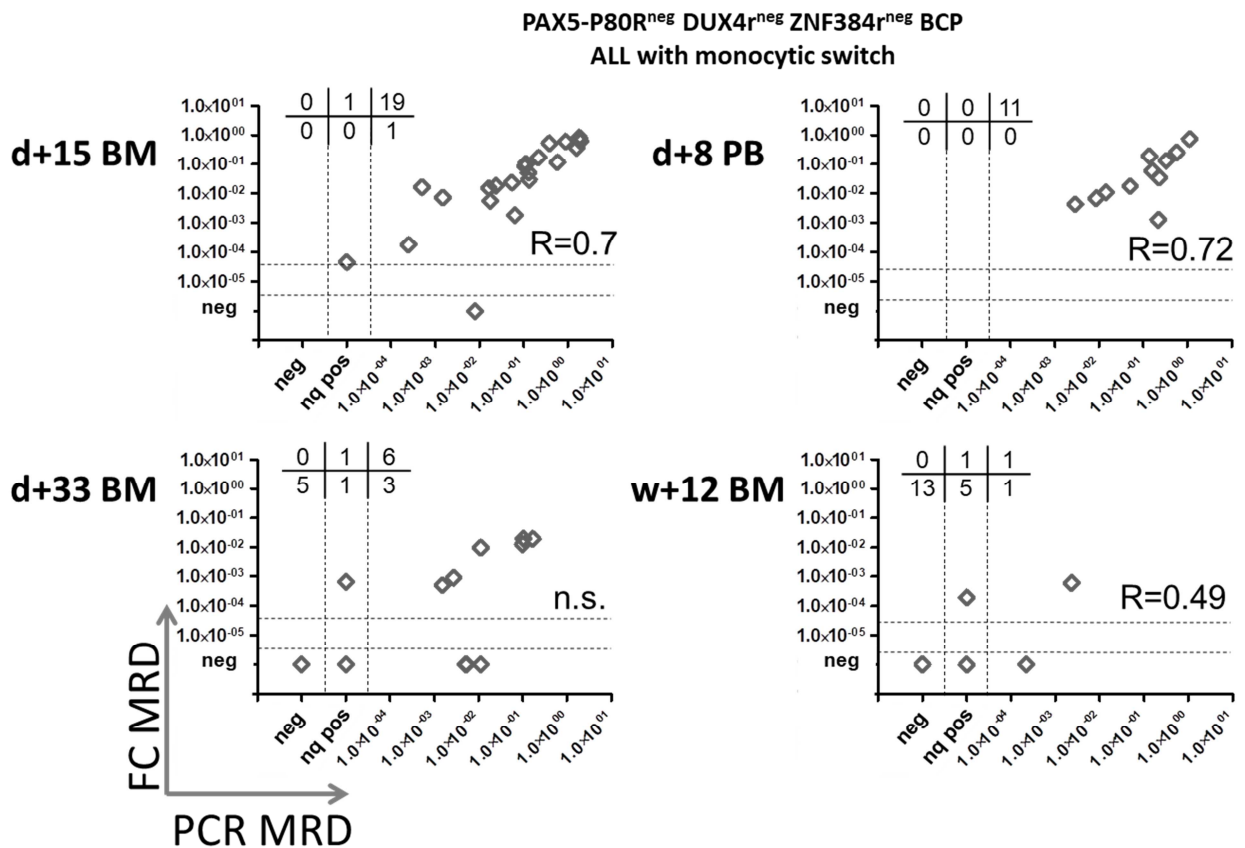
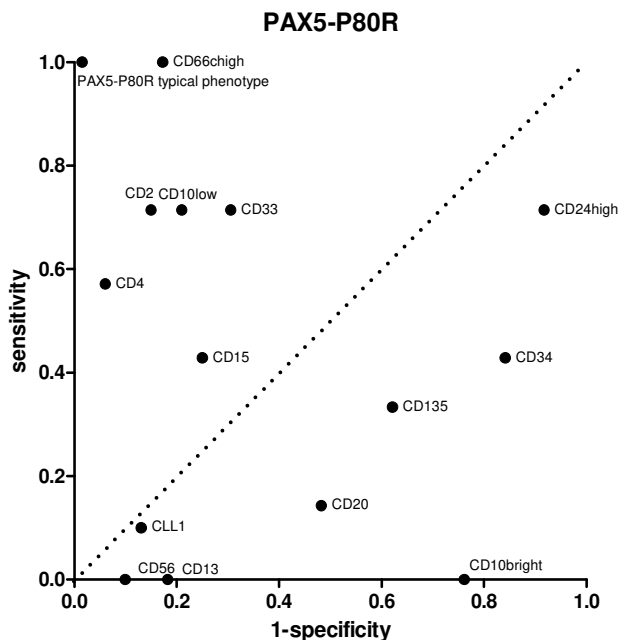
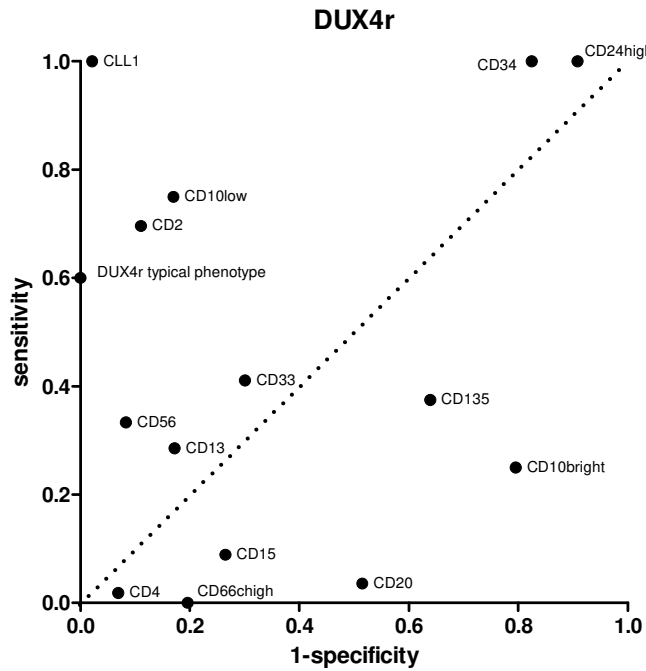


Figure S8. Immunophenotype of genetic subtypes. Expressions are positive if present on $\geq 10\%$ blasts, dim expressions as being positive on $\leq 50\%$ of blasts. $CD10^{bright}$ is defined as $CD10$ overexpression on $\geq 20\%$ of blasts; $CD10^{low}$ as being positive ($\geq 10\%$ of blasts) but not fulfilling definition for $CD10^{high}$; $CD24^{high}$ as expressed on $\geq 60\%$ of blasts; $CD66c^{high}$ as expressed on $\geq 75\%$ of blasts. Typical phenotype of DUX4r is defined as $CD10^{low} CD20^{neg} CD34^{pos} CD2^{pos} CD371^{pos}$ (sensitivity 60%, specificity 100%), typical phenotype of ZNF384r as $CD10^{neg/low} CD13^{pos} CD66c^{neg} CD34^{pos} CD135^{pos} CD24^{low}$ (sensitivity 50%, specificity 99.7%) and PAX5-P80R as $CD10^{neg/low} CD66c^{high} CD2^{dim/neg} CD4^{neg} CD34^{pos} CD33^{neg/dim}$ or $CD10^{neg/low} CD66c^{high} CD2^{pos} CD4^{pos} CD34^{neg} CD33^{pos}$ (sensitivity 100%, specificity 98.5%).



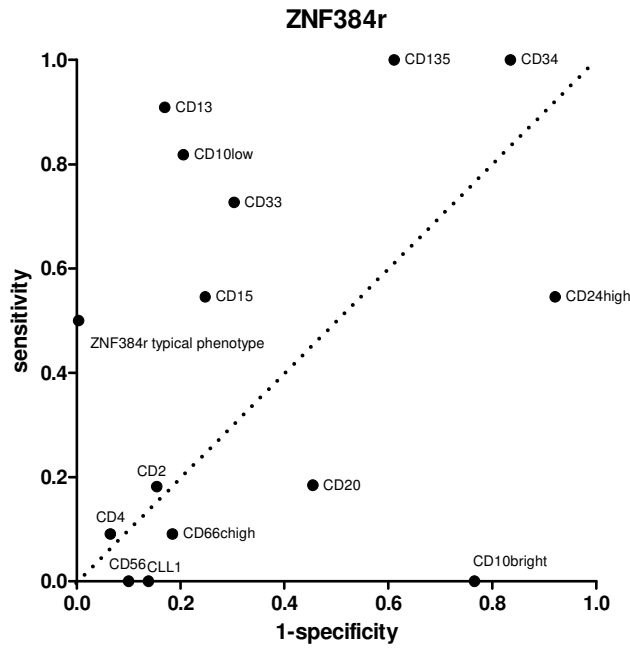
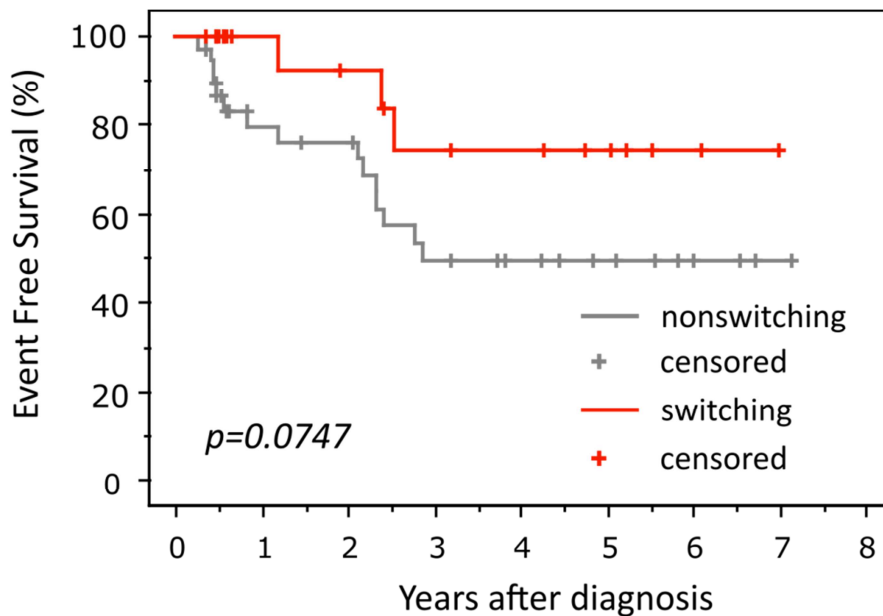


Figure S9. Comparison of EFS in patients with ($n=18$) or without ($n=23$) monocytic switch belonging to slow early response (SER) or high risk (HR) prognostic group using PCR MRD data at day 33 and week 12. 5-year EFS of patients with monocytic switch was $75 \pm 13\%$, 5-year EFS of patients without monocytic switch was $50 \pm 9.5\%$.



Number at risk						censored
nonswitching	38	21	10	4	0	23
switching	21	11	7	2	0	18