

Table S1. List of 497 genes included in the targeted gene panel

ABCBI, ABCB7, ABCG2, ABCG5, ABCG8, ABL1, ABL2, ACD, ACTB, ACTN1, ADA, ADAMTS13, AIRE, AK1, AK2, AKT2, ALAS2, ALDOA, AMN, ANK1, ANKRD26, AP3B1, ARID1A, ARPC1B, ASXL1, ATG2B, ATM, ATR, ATRX, AXIN1, BCL11B, BCL2, BCL6, BCOR, BCORL1, BHLHE41, BIRC3, BLM, BPGM, BRAF, BRCA1, BRCA2, BRCC3, BRINP3, BRIP1, BTG1, BTK, BTLA, C3, C4BPA, C4BPB, CALN1, CALR, CARD11, CASP10, CBL, CBLB, CBLC, CCND1, CD200, CD247, CD27, CD36, CD3D, CD3E, CD40LG, CD46, CD58, CD59, CD79B, CDAN1, CDKN1B, CDKN2A, CDKN2B, CEBPA, CFB, CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, CHD1, CHD4, CHD9, CHMP2B, CLPB, CNOT3, COX4I2, CREBBP, CRLF2, CSF1R, CSF2RA, CSF3R, CTC1, CTCF, CTSC, CUBN, CUX1, CXCR4, CYB5R3, CYBA, CYBB, CYCS, DCLRE1C, DDX41, DGKE, DGKH, DHFR, DIS3, DKC1, DNMT1, DNMT3A, EBF1, ECT2L, EED, EGFR, EGLN1, EGLN2, EGLN3, EHMT1, ELANE, EP300, EPAS1, EPB41, EPB42, EPCAM, EPO, EPOR, ERCC4, ERG, ETNK1, ETV6, EZH2, F2R, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FANCM, FAS, FASLG, FAT1, FBXW7, FCGR1A, FCGR3B, FERMT3, FLI1, FLNA, FLT3, FOXP3, G6PC3, G6PD, GATA1, GATA2, GATA3, GCLC, GF11, GF11B, GIF, GINS1, GLRX5, GNAS, GNB1, GPIBA, GPIBB, GP9, GPI, GPRC5A, GPXI, GSKIP, GSN, GSR, GSS, HAX1, HBA1, HBA2, HBB, HBD, HCLS1, HFE, HIF1A, HIF1AN, HIF3A, HK1, HNRNPK, HOOK1, HOXA10, HOXA11, HRAS, HSPA9, HUWE1, ID3, IDH1, IDH2, IFNG, IFNGR1, IFNGR2, IKZF1, IKZF2, IKZF3, IL12RB1, IL2RB, IL2RG, IL3RA, IL7R, IRF1, ITGA2, ITGA2B, ITGB2, ITGB3, ITK, ITPKB, JAGN1, JAK1, JAK2, JAK3, JAKMIP2, JMJD1C, KDM5C, KDM6A, KDM7A, KIF23, KIT, KLF1, KMT2A, KMT2C, KMT2D, KRAS, LAMB4, LAMTOR2, LAPTM5, LCK, LEF1, LIG4, LMNA, LMO1, LMO2, LPIN2, LRP1B, LRRC4, LUC7L2, LYLI, LYST, MAD2L2,

MAGT1, MAP2K1, MAP2K2, MASTL, MBL2, MECOM, MED13, MEF2B, MEF2C, MEFV, MET, MLH1, MLLT10, MLLT3, MPL, MSH2, MSH4, MSH6, MTA1, MTAP, MTR, MTRR, MVK, MYB, MYC, MYD88, MYH9, MYSM1, NAF1, NBEAL2, NBN, NCF2, NCOR2, NF1, NHEJ1, NHP2, NLRP3, NOD2, NOP10, NOTCH1, NOTCH2, NPM1, NR3C1, NRAS, NT5C2, NT5C3A, NTRK3, NUP214, OS9, P2RY2, PALB2, PARN, PAX5, PBX1, PC, PCDH1B1, PDGFRA, PDGFRB, PDHA1, PDHX, PFKL, PFKM, PGK1, PGM3, PHF6, PICALM, PIEZO1, PIGA, PIK3CD, PIK3R1, PKLR, PML, PMS2, PNP, POT1, PRDM1, PRF1, PRKACG, PRPF40B, PTCH2, PTEN, PTK2B, PTPN11, PTPN2, PTPRC, PTPRD, PUS1, RAB27A, RAC1, RAC2, RAD21, RAD50, RAD51, RAD51C, RAF1, RAG1, RAG2, RB1, RBBP6, RBM8A, RELN, RFWD3, RHAG, RHOA, RIT1, RMRP, RNF168, RPL10, RPL11, RPL15, RPL23, RPL26, RPL27, RPL31, RPL35A, RPL36, RPL5, RPS10, RPS14, RPS15, RPS17, RPS19, RPS24, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS7, RTEL1, RUNX1, RUNX1T1, SAMD9L, SBDS, SBF2, SEC23B, SERPING1, SETBP1, SETD2, SF1, SF3A1, SF3B1, SH2B3, SH2D1A, SHOC2, SLC11A2, SLC19A2, SLC25A38, SLC2A1, SLC35C1, SLC37A4, SLC4A1, SLCO1B1, SLCO1B3, SLFN14, SLX4, SMARCD2, SMC1A, SMC3, SOS1, SPINK5, SPRED1, SPTA1, SPTB, SRC, SRCAP, SRP72, SRSF2, STAG1, STAG2, STAT3, STAT5B, STEAP3, STX11, STXBP2, SUZ12, SYNE1, TAL1, TAL2, TAZ, TBL1XR1, TBX1, TCF3, TCIRG1, TEC, TERC, TERF1, TERF2, TERF2IP, TERT, TET1, TET2, TET3, THBD, THPO, TINF2, TLX1, TLX3, TMPRSS6, TNFAIP3, TNFRSF13B, TNFRSF14, TNFRSF1A, TOX, TP53, TP11, TPMT, TRAF3, TRNT1, TSLP, TSR2, TUBB1, TYK2, U2AF1, U2AF2, UBE2T, UGT1A1, UGT1A7, UNC13B, UNC13D, UNC5D, USB1, USH2A, USP9X, VHL, VPS13B, VPS45, VWF, WAS, WDR1, WIPF1, WRAP53, WT1, XBPI, XIAP, XK, XRCC2, YARS2, ZAP70, ZFH4, ZNF197, ZRSR2, MRE11A, WHSC1, STON1, OBFC1

Table S2. The mutational profiles of 63 *de novo* acute lymphoblastic leukaemia patients

Patient ID	Diagnosis	Gene	Transcript number	NT alteration	AA alteration	Type	VAF (%)	
P2	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
		<i>RBI</i>	NM_000321.2	Partial gene deletion		CNA		
P3	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
		<i>CD200</i>	NM_001004196.3	Whole gene deletion		CNA		
		<i>BTLA</i>	NM_181780	Whole gene deletion		CNA		
		<i>BTG1</i>	NM_001731	c.295_325delinsTGAGG		p.Pro99Ter	TRUNC	8.1
P5	BLL	<i>KRAS</i>	NM_033360.2	c.183A>C		p.Gln61His	MISSENSE	10.5
		<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA		
P6	BLL	<i>CDKN2B</i>	NM_004936	Partial gene deletion		CNA		
		<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
		<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
P7	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
		<i>FANCA</i>	NM_000135.2	c.3720_3724del		p.Glu1240AspfsTer36	TRUNC	37.9
		<i>PTPN11</i>	NM_002834.4	c.1459G>A		p.Asp487Asn	MISSENSE	39.2
P8	BLL	<i>PTPN11</i>	NM_002834.4	c.182A>T		p.Asp61Val	MISSENSE	8.6
		<i>TP53</i>	NM_000546.5	c.536A>G		p.His179Arg	MISSENSE	84.3
P9	BLL	<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA		
		<i>CDKN2B</i>	NM_004936	Whole gene deletion		CNA		
		<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA		
		<i>PAX5</i>	NM_016734.2	c.169C>T		p.Gln57Ter	TRUNC	32.9
P10	BLL	Not detected						
P11	TLL	<i>IDH2</i>	NM_002168.3	c.419G>A		p.Arg140Gln	MISSENSE	45.1
		<i>NRAS</i>	NM_002524.4	c.34G>C		p.Gly12Arg	MISSENSE	45.3

		<i>KMT2D</i>	NM_003482.3	c.11779_11793delinsAGGCAA	p.Gln3927_Leu3931delinsArgGln	INFRAME	26.9
		<i>BCOR</i>	NM_001123383.1	c.3163A>T	p.Lys1055Ter	TRUNC	93.7
P12	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
P13	BLL	Not detected					
P14	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>SETD2</i>	NM_014159.6	Partial gene deletion		CNA	
		<i>SETD2</i>	NM_014159.6	c.7509_7519delinsGGCGAA	p.Asp2504AlafsTer14	TRUNC	10.9
P15	BLL	<i>SETD2</i>	NM_014159.6	c.607_608del	p.Ser203IlefsTer33	TRUNC	8
P16	BLL	Not detected					
P17	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>RBI</i>	NM_000321.2	Partial gene deletion		CNA	
P18	BLL	<i>IKZF1</i>	NM_006060.4	Whole gene deletion		CNA	
P19	BLL	<i>TP53</i>	NM_000546.5	c.818G>A	p.Arg273His	MISSENSE	21.1
		<i>TP53</i>	NM_000546.5	c.846dup	p.Arg283AlafsTer23	TRUNC	8.3
P22	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>PAX5</i>	NM_016734.2	Partial gene deletion		CNA	
		<i>SETD2</i>	NM_014159.6	c.6626_6642delinsCAGGTCG	p.Leu2209Serfs*36	TRUNC	15.7
		<i>SETD2</i>	NM_014159.6	c.7575_7582del	p.Asn2526GlyfsTer6	TRUNC	6.9
P23	BLL	Not detected					
P24	BLL	Not detected					
P25	TLL	Not detected					
P26	TLL	<i>IKZF1</i>	NM_006060.4	c.908_909insT	p.Glu304ArgfsTer185	TRUNC	5.6
		<i>SUZ12</i>	NM_015355.3	c.856C>T	p.Arg286Ter	TRUNC	32.1
P28	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>ETV6</i>	NM_001987	Partial gene deletion		CNA	
P31	BLL	<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA	
		<i>CDKN2B</i>	NM_004936	Partial gene deletion		CNA	

		<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
P32	BLL	<i>RBI</i>	NM_000321.2	c.1775_1776insGA	p.Asn593IlefsTer19	TRUNC	66.7
		<i>RBI</i>	NM_000321.2	Whole gene deletion		CNA	
P34	BLL	Not detected					
P36	MPAL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>PAX5</i>	NM_016734.2	Partial gene deletion		CNA	
P37	BLL	<i>IKZF1</i>	NM_006060.4	c.1062_1069delinsACCTCCCC	p.Ala355Profs*134	TRUNC	12.8
		<i>SRCAP</i>	NM_006662.2	c.4316_4317insG	p.Ile1441HisfsTer407	TRUNC	6.3
P38	BLL	<i>TP53</i>	NM_000546.5	Whole gene deletion		CNA	
P40	BLL	<i>SETD2</i>	NM_014159.6	c.5463_5467delinsCCACCC	p.Pro1822Hisfs*10	TRUNC	11.9
P41	BLL	<i>KMT2D</i>	NM_003482.3	c.12661C>T	p.Gln4221Ter	TRUNC	31
		<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA	
		<i>CDKN2B</i>	NM_004936	Whole gene deletion		CNA	
		<i>NR3C1</i>	NM_001018077.1	c.1275_1278dup	p.Glu427Ter	TRUNC	6.2
		<i>MED13</i>	NM_005121.2	c.4075dup	p.Met1359AsnfsTer21	TRUNC	32.1
P42	BLL	<i>TP53</i>	NM_000546.5	c.846dup	p.Arg283AlafsTer23	TRUNC	82.6
P43	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>NBEAL2</i>	NM_015175.2	Partial gene deletion		CNA	
		<i>NBEAL2</i>	NM_015175.2	c.3700del	p.Ala1234GlnfsTer4	TRUNC	
P44	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
P45	BLL	<i>PIK3R1</i>	NM_181523.2	c.1425+1G>C		SPLICE	15.8
		<i>CTCF</i>	NM_006565.3	c.1207+2T>C		SPLICE	28
P46	TLL	<i>RPL5</i>	NM_000969.3	c.357T>A	p.Tyr119Ter	TRUNC	28.8
		<i>DNMT3A</i>	NM_022552.4	c.2645G>A	p.Arg882His	MISSENSE	39.5
		<i>DNM2</i>	NM_001005360.2	c.1136_1137insAGGTGAGC	p.Phe379LeufsTer38	TRUNC	10.4
P47	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
P48	BLL	<i>SETD2</i>	NM_014159.6	c.5137_5138insC	p.Asp1713AlafsTer15	TRUNC	32.4
		<i>SETD2</i>	NM_014159.6	c.2837_2865delinsTGGG	p.Arg946MetTer28	TRUNC	6.1
		<i>IKZF1</i>	NM_006060.4	Whole gene deletion		CNA	

		<i>CD200</i>	NM_001004196.3	Whole gene deletion		CNA	
		<i>BTLA</i>	NM_181780	Whole gene deletion		CNA	
P49	BLL	Not detected					
P50	BLL	Not detected					
P51	BLL	Not detected					
P52	TLL	<i>SUZ12</i>	NM_015355.3	c.1150_1151del	p.Leu385ProfsTer10	TRUNC	42.1
		<i>KMT2C</i>	NM_170606.2	c.2104_2105dup	p.Glu704MetfsTer15	TRUNC	45.1
		<i>ETV6</i>	NM_001987	Whole gene deletion		CNA	
P53	BLL	<i>TBL1XR1</i>	NM_024665.5	c.297_298insTT	p.Arg100LeufsTer43	TRUNC	15.8
		<i>SETD2</i>	NM_014159.6	c.4187dup	p.Asn1396LysfsTer2	TRUNC	49.2
		<i>NRAS</i>	NM_002524.4	c.35G>A	p.Gly12Asp	MISSENSE	18.2
		<i>KMT2D</i>	NM_003482.3	c.11674C>T	p.Gln3892Ter	TRUNC	46.5
		<i>ARID1A</i>	NM_006015.4	c.1882_1883insAAAAG	p.Met628LysfsTer3	TRUNC	8.9
P54	BLL	<i>CDKN2A</i>	NM_000077	Partial deletion		CNA	
		<i>CDKN2B</i>	NM_004936	Partial deletion		CNA	
		<i>NRAS</i>	NM_002524.4	c.38G>T	p.Gly13Val	MISSENSE	35.5
		<i>KMT2D</i>	NM_003482.3	c.12844C>T	p.Arg4282Ter	TRUNC	41.2
P55	BLL	<i>SETD2</i>	NM_014159.6	c.6118C>T	p.Arg2040Ter	TRUNC	5.2
		<i>KMT2D</i>	NM_003482.3	c.8053C>T	p.Arg2685Ter	TRUNC	16.8
		<i>KMT2D</i>	NM_003482.3	c.7228C>T	p.Arg2410Ter	TRUNC	6.6
		<i>KMT2D</i>	NM_003482.3	c.6343_6346del	p.Ser2115ArgfsTer28	TRUNC	9
		<i>KMT2D</i>	NM_003482.3	c.2817_2818insAGGC	p.Ser940ArgfsTer30	TRUNC	5.4
		<i>IKZF1</i>	NM_006060.4	Partial deletion		CNA	
		<i>FLT3</i>	NM_004119.2	c.2503G>T	p.Asp835Tyr	MISSENSE	37.7
P56	BLL	<i>CTCF</i>	NM_006565.3	c.1176C>G	p.Tyr392Ter	TRUNC	6.3
		<i>PHF3</i>	NM_001290259.1	c.2728A>T	p.Lys910Ter	TRUNC	16.3
P57	BLL	Not detected					
P59	BLL	<i>KMT2D</i>	NM_003482.3	c.5064_5065insGCCCCCA	p.Arg1689AlafsTer4	TRUNC	15.7

		<i>CREBBP</i>	NM_004380.2	c.3982+2_3982+13delTAAGTTTCGGGA		SPLICE	9.8
		<i>CDKN2B</i>	NM_004936	Whole gene deletion		CNA	
		<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA	
		<i>CDKN2A</i>	NM_000077	c.262G>T	p.Glu88Ter	TRUNC	80.5
P60	BLL	Not detected					
P62	BLL	Not detected					
P63	BLL	<i>IKZF1</i>	NM_006060.4	Partial gene deletion		CNA	
		<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA	
		<i>PAX5</i>	NM_016734.2	Whole gene deletion		CNA	
P64	TLL	<i>PHF6</i>	NM_001015877.1	c.297T>A	p.Cys99Ter	TRUNC	90.9
		<i>FBXW7</i>	NM_033632.3	c.1394G>T	p.Arg465Leu	MISSENSE	43.5
P65	BLL	<i>IKZF1</i>	NM_006060.4	c.1509C>G	p.Tyr503Ter	TRUNC	15.6
P67	TLL	<i>RBI</i>	NM_000321.2	Whole gene deletion		CNA	
		<i>PTEN</i>	NM_000314.4	c.698_701delinsGGGGGAGA	p.Arg234GlyfsTer10	TRUNC	49.6
		<i>PTEN</i>	NM_000314.4	c.734_740del	p.Gln245HisfsTer9	TRUNC	15.1
		<i>MED12</i>	NM_005120.2	c.205-2A>G		SPLICE	88.5
		<i>BRAF</i>	NM_004333.4	c.1780G>A	p.Asp594Asn	MISSENSE	45.1
P68	BLL	<i>PAX5</i>	NM_016734.2	Partial gene deletion		CNA	
		<i>EBF1</i>	NM_024007.3	c.1708_1714dupCCCACCT	p.Cys572SerfsTer28	TRUNC	29.5
		<i>CD200</i>	NM_001004196.3	Whole gene deletion		CNA	
		<i>BTLA</i>	NM_181780	Whole gene deletion		CNA	
P71	BLL	<i>TP53</i>	NM_000546.5	c.518T>C	p.Val173Ala	MISSENSE	45.1
		<i>SETD2</i>	NM_014159.6	c.7531_7532insG	p.Lys2511ArgfsTer9	TRUNC	20.6
P72	BLL	Not detected					
P73	BLL	<i>RBI</i>	NM_000321.2	c.1848dupA	p.Gly617ArgfsTer36	TRUNC	57.4
		<i>RBI</i>	NM_000321.2	Partial gene deletion		CNA	
P75	TLL	<i>WT1</i>	NM_024426.4	c.1372C>T	p.Arg458Ter	TRUNC	40
		<i>TP53</i>	NM_000546.5	c.560-27_560delinsTAGA	p.Gly187Asp	MISSENSE	23.7
		<i>TP53</i>	NM_000546.5	c.844C>T	p.Arg282Trp	MISSENSE	42.7

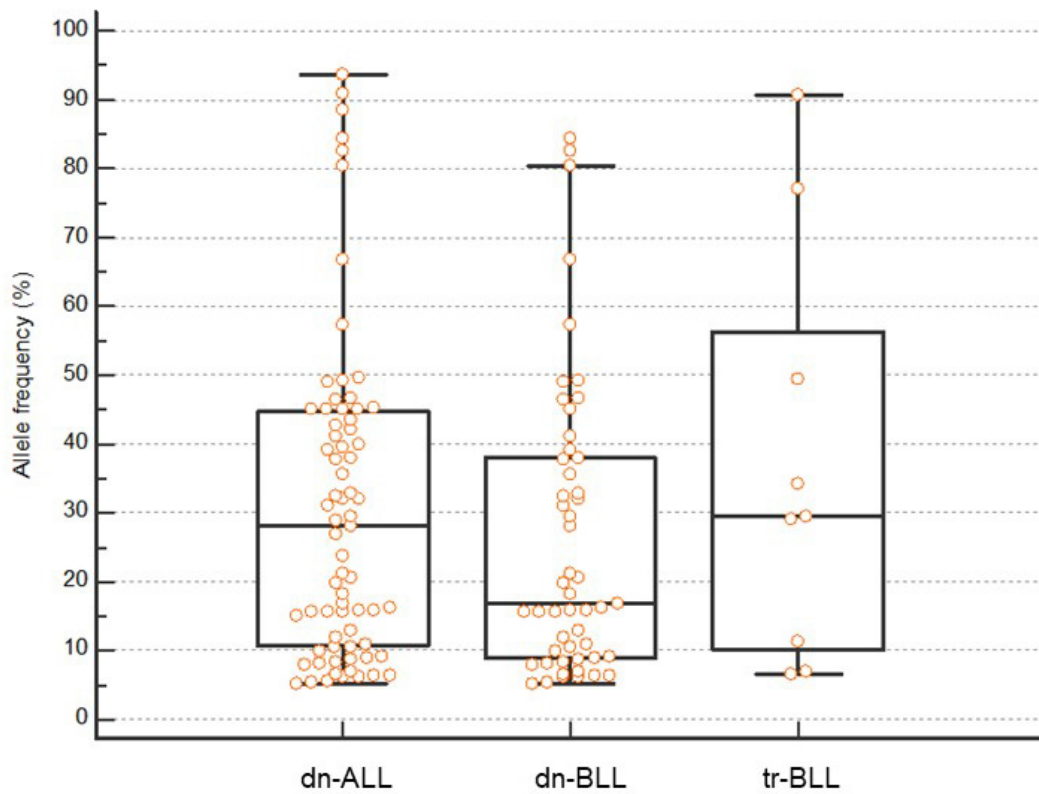
P76	BLL	<i>DNMT3A</i>	NM_022552.4	c.2644C>A	p.Arg882Ser	MISSENSE	46.7
		<i>CDKN2B</i>	NM_004936	Whole gene deletion		CNA	
		<i>CDKN2A</i>	NM_000077	Whole gene deletion		CNA	
P83	BLL	<i>IKZF1</i>	NM_006060.4	Whole gene deletion		CNA	
		<i>CDKN2A</i>	NM_000077	c.199_200insCTCG	p.Gly67AlafsTer54	TRUNC	19.8
P89	BLL	Not detected					
P155	BLL	Not detected					

Table S3. Characteristics and treatment outcomes of the tr-ALL patients (*n*=9)

Patient characteristics	Median (IQR) or number (%)
Duration from prior malignancy diagnosis to tr-ALL diagnosis, yrs (range)	6.4 (5.5-11.7)
Type of prior malignancy	
Solid cancer	8 (89)
Hematologic malignancy	1 (11)
Prior malignancy	
More than one prior diagnosis	2 (22)
Breast cancer	2 (22)
Stomach cancer	2 (22)
Osteosarcoma	2 (22)
Rectal cancer	1 (11)
Hepatocellular carcinoma	1 (11)
Ovarian cancer	1 (11)
Acute promyelocytic leukaemia	1 (11)
Thyroid cancer	1 (11)
Type of prior therapy	
Chemotherapy +/- Operation	7 (78)
Chemotherapy + Radiation +/- Operation	1 (11)
TACE and RFA	1 (11)
Chemotherapeutic agents used for prior malignancy	
Anthracyclines	6 (86)
Alkylating agents	5 (71)
Antimicrotubules (Taxanes)	3 (17)
Antimetabolites	2 (29)
Camptothecin analogues	1 (14)
Retinoids	1 (14)
Original disease status at tr-ALL diagnosis	
NED or CR	8 (89)
Residual or progression	1 (11)

ALL induction regimen	
Hyper-CVAD +/- TKIs	7 (78)
GRAALL	1 (11)
VPD	1 (11)
CR1 achievement after ALL induction	8 (89)
Allogenic hematopoietic stem cell transplantation	4 (44)
Matched sibling donor	2 (22)
Haploidentical donor	2 (22)

1 **Figure S1.** The variant allele frequencies (VAFs) of single-nucleotide variants from 63 *de*
2 *novo* acute lymphoblastic leukaemia (dn-ALL), 54 *de novo* B-lymphoblastic leukaemia (dn-
3 BLL), and 8 therapy-related BLL (tr-BLL) patients. The median VAFs of each group are 28.0%
4 (IQR, 10.6-44.7), 16.80% (IQR, 8.93-37.85), and 29.40% (IQR, 10.13-56.4), respectively.



6 **Figure S2. Survival outcome of *de novo* acute lymphoblastic leukemia (dn-ALL) and therapy-related ALL (tr-ALL).** The differences in
7 median (A) overall survival and (B) progression-free survival between dn-ALL and tr-ALL patients.

