Mutational and transcriptomic profiling of acute leukemia of ambiguous lineage reveals obscure but clinically important lineage bias

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Supplementary Methods

Samples

One patient (case 348) has collection of sequential samples (diagnosis, remission and relapse. We sequenced all his samples and used his remission sample as germline control). We unfortunately do not have matched germline samples for the addtional 14 patients. The sequenced ALAL samples were obtained from cryopreserved diagnostic bone marrow aspirate specimens. These samples were taken when a potential patient was referred for a bone marrow biopsy after the appropriate consents were given. The samples were diluted with equal samples of Hank's balanced salt solution, layered onto Ficoll, centrifuged and aspirated into a sterile tube. Samples were then counted, washed and resuspended to obtain concentration of 10 million cells per ml. The speciments were then aliquoted into cryovials and gradually cooled to -80 degrees Celsius before being transferred into liquid nitrogen. DNA and RNA were extracted from the liquid nitrogen samples and used for sequencing.

Exome sequencing analysis

We used a highly stringent bioinformatics pipeline that we developed previously extensively to remove germline SNP (1-8). The mutation list was filtered with dbSNP131, 1,000 genome, The Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org/), Esp5400 [NHLBI Exome Sequencing Project (ESP) Exome Variant] exome database (http://evs.gs.washington.edu/EVS/), UCSC repeat filters and our in-house manually curated SNP database with the following criteria (10, 11):

For dbSNP database: Latest versions of dbSNP database (dbSNP137, dbSNP 138 etc.) were not utilized as they are contaminated with some well-characterized somatic oncogenic mutations: e.g., *NRAS* G12D (rs121913237), *IDH2* R140Q (rs121913502) [see below Table (10)]. For SNPs present in dbSNP131 but also marked with CLINSIG=pathogenic in ClinVAR database, each of them were manually inspected to determine whether those SNPs were associated with leukemia/cancer, before they were filtered from our list.

Presence of well appreciated somatic cancer/leukemic drivers in dbSNP and exome sequencing database of ExAC and Esp5400:

		1000 genome	dbSNP131	dbSNP137	dbSNP138	dbSNP141	ExAC frequency	ESP5400 frequency
DNMT3A	R882H	NA	NA	rs147001633	rs147001633	rs147001633	0.0005449	0.000744
DNMT3A	R882C	NA	NA	NA	rs377577594	rs377577594	0.0003551	0.000279
DNMT3A	S714C	NA	NA	NA	rs367909007	rs367909007	0.00004119	0.000093
IDH2	R140Q	NA	NA	rs121913502	rs121913502	rs121913502	0.00009884	NA
IDH2	R172K	NA	NA	rs121913503	rs121913503	rs121913503	NA	NA
IDH1	R132H	NA	NA	rs121913500	rs121913500	rs121913500	NA	NA
FLT3	D835Y	NA	NA	rs121913488	rs121913488	NA	NA	NA
U2AF1	S34F	NA	NA	NA	rs371769427	rs371769427	0.00004213	0.000093
KRAS	G12D	NA	NA	rs121913529	rs121913529	NA	0.00001976	NA
KRAS	G13C	NA	NA	rs121913535	rs121913535	NA	NA	NA
KRAS	G13D	NA	NA	rs112445441	rs112445441	NA	NA	NA
NRAS	Q61K	NA	NA	rs121913254	rs121913254	NA	NA	NA
NRAS	G12D	NA	NA	rs121913237	rs121913237	NA	0.000008237	NA

NA: not detected in the database.

We filtered the mutation list with 1,000 genome, ExAC, and Esp5400 etc. Because some important leukemia drivers [such as *DNMT3A* R882H (0.0005) or *IDH2* R140: *DNMT3A* R882 (ExAC: *DNMT3A*, Arg882His= 0.0005449; Arg882Cys=0.0003551; Arg882Pro=0.00005779; Arg882Leu = 0.000008256) or *IDH2* R140 (Arg140Gln = 0.00009884)] were also present at very low frequency in the blood samples of normal elderly people (9-11) and annotated as SNP in some database, we manually checked and curated the list (before and after filtering) to make sure that those well appreciated AML drivers had not been removed.

We used the UCSC repeat filters (genomicSuperDups, microsatellite_hits, interupted_repeats, repeat_masker, simple_tandem_repeats) to remove all of the SNVs site located in the repeat and low complexity region.

Concerning the recent discovery that some of the early leukemia mutations (e.g., *DNMT3A*, *IDH2* etc.) often persisted in remission sample (1, 9-14), we also performed analysis of each sample of case 384 (diagnosis, remission and relapse) separately to examine whether any such mutation exist.

Supplementary Table 1. Summary of ALAL patients of this study.

Case	Diagnosis	Age	Karyotype	Immunophenotype
774	AUL With t(v;11q23.3)	17	46, XY, t(11;19)(q23;p13.3)	MPO-, CD117+ CD33-, partial CD13+, partial CD11b+, CD36-, CD64-, CD14-, Cytoplasmic CD3- CD7+ CD2- CD5- CD4- CD8- CD1a- CD56- CD19- CD10- Tdt- CD123-.
1542	AUL With t(v;11q23.3)	53	44, XX, der(4)t(4;11)(p16;q23), add(5)(q11.2), -7, -8, der(11;18)t(11;18)(p11.2;p11.2) t(4;11)(p16;q23), add13(p13), del(17)(p13), -18, -21, -22	MPO-, Partial DIM CD33+, CD13+, CD117+, CD11b-, CD36-, CD14-, CD34+, HLA-DR+, GA -, CD41-, CD61-, Cytoplasmic CD3-, CD4-, CD7-, CD8-, CD19-, CD79a-, CD10-, CD22-, CD123-, CD56-, Tdt-.
1190	AUL	61	46, XX	MPO-, CD33+, CD13+, CD117+, CD11b-, CD36-, CD14-, CD34+, HLA-DR+, GA-, Cytoplasmic CD3-, CD1a-, CD19-, CD79a-, CD10-, CD123-, CD56-, Tdt-
1373	AUL	64	47, XX +10	CD1a-, CD2-, CD3-, Cytoplasmic CD3-, CD4-, CD8-, CD5+, CD7+, Partial CD34+, TdT-, CD56-, CD11b-, CD14-, CD64-.
1169	AUL	65	40-42, XY, -5, add(7)(q11.2), -12, -13, add15(p11.2), -16, -17,-20	CD34+, CD117+, MPO-, CD3-, CD79a-, CD19-, CD10-, CD11b-, CD14-, CD4-, CD56-.
1568	AUL	78	45, XY, -2, add(3)(q12), add(4)(q25), i(5)(p10), -6, del(7)(q22), +8, -11, add13(q34), -16, -17, -20	Dim CD45+, MPO-, CD13+, CD33-, CD117+, Partial CD34+, CD36-, CD64-, CD14-, CD11b-, cCD3-, CD7-, CD19-, CD10-, CD79a-, CD41-, CD61-, GA-, CD123-, CD4-, CD56-.
5694	AUL	54	45, X -Y	CD7+, Partial CD33+, Partial CD117+, Partial HLA-DR+, CD13-, CD11b-, CD4+, CD123dim+, CD71dim+, CD38-, MPO-, CD19-, Cytoplasmic CD3-, CD79a-, CD56-, CD64-, CD14-, Tdt-, CD45dim+.

348	MPAL M/T, NOS	33	46, XY, add(4)(q21), add(9)(p22), del(20)(q1?)	Cytoplasmic CD3+, MPO+, CD7+, CD13+, CD33+, CD34+, CD56+, HLA-DR+, Tdt+, CD4-, CD8-, CD10-, CD20-, CD117-.
281	MPAL M/B, NOS	44	46, XX[20]	CD19+, CD79a+, Partial CD33+, CD34+, CD11b+, MPO+, Partial Tdt+, CD3- and CD117-.
683	MPAL M/B, NOS	36	46, XX, t(13;14)(q14;q24)	MPO+, Partial CD3+, CD33-, CD11b-, CD117-, CD34+, CD19+, CD10-, CD79a+, Partial TDT+, CD3-, CD7-.
1251	MPAL with t(9;22)(q34.1;q11.2)	61	46, XY, t(9;22)(q34;q11.2)	One population expressing CD34+, CD19+, CD10+, CD79a+, Tdt+, cCD3- and another expressing CD34+, MPO+ CD19+ (partial), CD10-, CD79a-, cCD3-.
1408	MPAL with t(9;22)(q34.1;q11.2)	47	45, XY, -7, t(9;22)	CD3-, Cytoplasmic CD3-, CD7+, CD19+, CD10+, CD79a+, TdT+, CD13+, CD33+, CD117+, CD11b-, CD34+ and MPO+.
1034	MPAL with t(9;22)(q34.1;q11.2)	22	46, XY, t(9;22)(q34;q11.2)	MPO+, Partial CD33+, CD13-, CD36-, CD64-, CD14-, Partial CD19+, CD10+, CD79a+, TdT+, CD34+, CD117-, CD3-, CD7-.
1030	MPAL with t(9;22)(q34.1;q11.2)	32	46, XY, add(1)(q21), der(9)t(1;9) (q25;q34), der(22)t(9;22)(q34;q11.2)	MPO+, CD33+, CD13-, CD19+ CD79a+, TdT+, CD34+, CD3-
5684	MPAL with t(9;22)(q34.1;q11.2)	64	46, XX, der9 inv9 t(9;22)	CD3-, CD7+, CD19+, CD10+, CD79a+, TdT+, CD13+, CD33+, CD117+, CD11b-, CD34+ and MPO+.

Age: age at diagnosis; v, variable chromosome; MPO, Myeloperoxidase, a protein synthesized during myeloid differentiation.

Supplementary Table 2. ALAL patients: DNA repair and chromosome stability gene mutations.

Gene information were cited from Genecards and Wikipedia.

Patient ID	Mutations
1408	*BRCA2* (stop-gain, S1001*), involved in repair of damaged DNA(15). VAF=0.51.
1373	*ERCC6* (frameshift, K476Rfs*11). Coding for Cockayne syndrome B (CSB) protein, which is important in transcription-coupled excision DNA repair (16). VAF=0.42.
1568	*MDC1* (Inframe deletion of 40 amino acids, S1570_E1610del), Mediator of DNA Damage Checkpoint protein 1(17-20). VAF=0.23. *TP53* (V143M, and deletion of 17/17p), involved in DNA repair. V143M is a deleterious common mutation according to TP53 mutation database [http://p53.iarc.fr/, number of occurrence in somatic dataset (number of tumors reported to carry this mutation) = 35]. Mutation in the same position have been recorded in 50 cancer samples in COSMIC database and 39 cancer samples in cbioportal TCGA pan cancer database (21). VAF=0.25.
683	*PRKDC* (frameshift, I1085Sfs*17), also known as DNA-PKcs is a kinase that acts as a molecular sensor for DNA damage. Involved in DNA non-homologous end joining required for double-strand break (DSB) repair and VDJ recombination (22-24). VAF=0.15.
774	*RAD21* (K70E), involved in the repair of DNA double-strand breaks, as well as in chromatid cohesion during mitosis (25, 26). VAF=0.13.
1542	*FANCD2* (frameshift, Y103Lfs*77), required for maintenance of chromosomal stability. Promotes accurate and efficient pairing of homologs during meiosis. Involved in the repair of DNA double-strand breaks. FANCD2 mutant mice have a significantly increased incidence of cancer. Humans with a FANCD2 deficiency have increased risk of AML (27-33). VAF=0.69

	ERCC8 (P53T), Excision Repair 8, involved in DNA repair pathway. VAF=0.51.
1190	*TDG* (Inframe insertion, I137_P141dup), G/T Mismatch-Specific Thymine DNA Glycosylase, involved in DNA base excision repair (34, 35). VAF=0.06 *PARP1* (K324Q), involved in DNA base excision repair pathway (36-40). VAF=0.45.
1169	*TP53* (E286K, and deletion of 17/17p), involved in DNA repair. E286K is a common deleterious mutation according to TP53 mutation database [http://p53.iarc.fr/, number of occurrence in somatic dataset (number of tumors reported to carry this mutation) = 96]. Hotspot mutation, mutation in the same position have been recorded in 113 cancer samples in COSMIC database and 101 cancer samples in cbioportal TCGA pan cancer database(21). VAF=0.2. *NEK1* (stop-gain, Y168*), involved in DNA damage sensing/repair pathway (41-43). VAF=0.26.
5694	*CHEK1* (D47N), checkpoint Kinase 1, It is required for checkpoint mediated cell cycle arrest in response to DNA damage (44, 45).VAF=0.14.
1251	*E2F7* (Q506L), involved in DNA damage response: up-regulated by P53 following genotoxic stress, and acts as a downstream effector of TP53-dependent repression of target genes involved in DNA replication (46-48). VAF=0.47. SPC24 (R5C), acts as a component of the essential kinetochore-associated NDC80 complex, which is required for chromosome segregation and spindle checkpoint activity (49). VAF=0.5.
1030	*RAD51AP1* (A104D), may participate in a common DNA damage response pathway associated with the activation of homologous recombination and double-strand break repair (50-54). VAF=0.06.
1034	*RPA2* (A100V), Replication Protein A2, involved in DNA replication, repair, recombination and telomere maintenance (55-57). VAF=0.19.

Supplementary Table 3. The mutation list of ALAL patients.

(see excel file "Mutation list of ALAL.xlsx")

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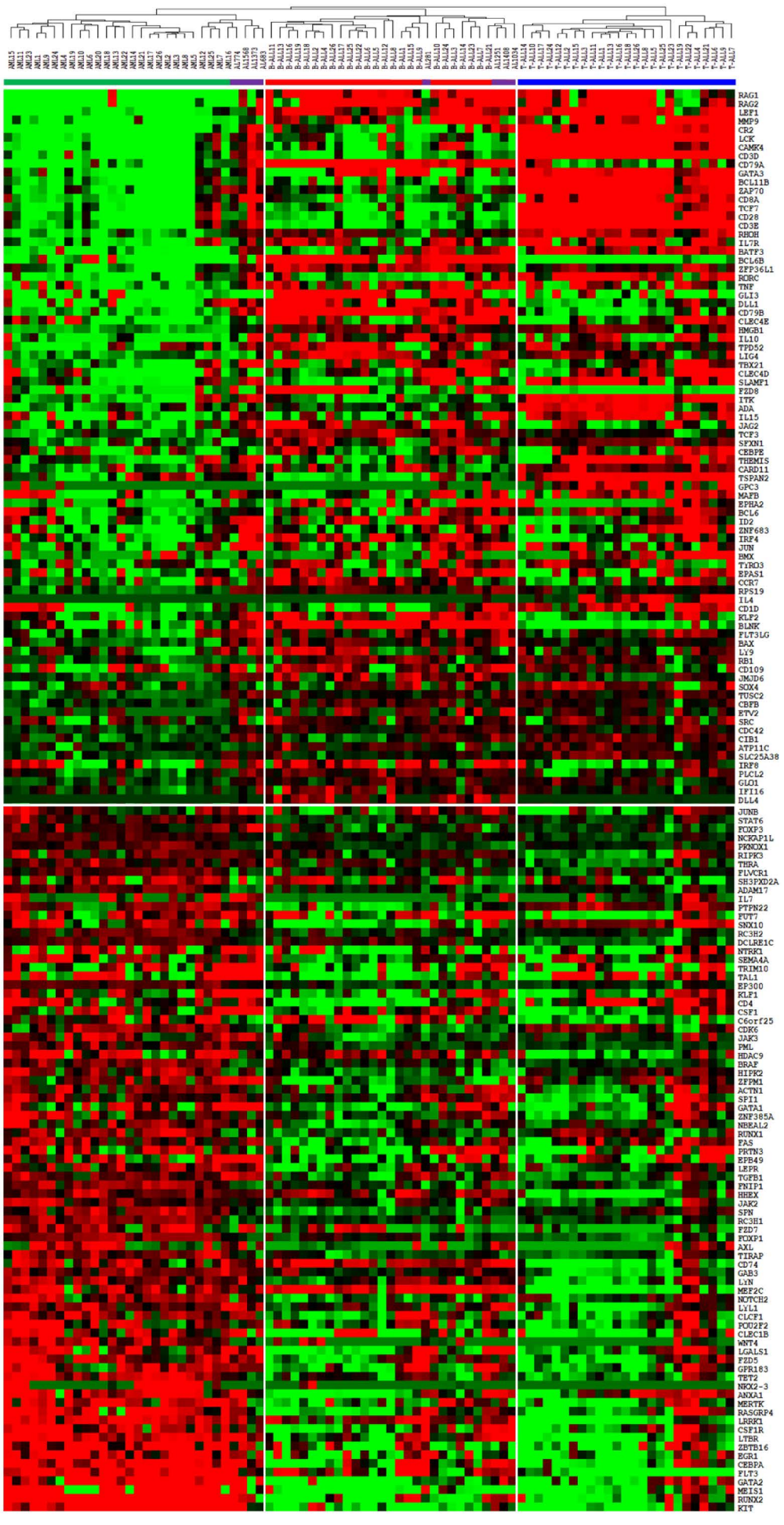
Supplementary Figure Legends

Supplementary Figure 1. A. Cluster analysis of RNA sequencing results of 8 ALAL samples, clustered together with RNA sequencing data of AML (randomly selected, 25 cases from the TCGA AML cohort), pediatric B-ALL and T-ALL (randomly selected, 25 cases of B-ALL and 25 cases of T-ALL from EGAS00001001858)(58). The cluster analysis was performed using 367 myeloid/lymphoid expressing signature genes. B. Hierarchical tree showing the Cluster analysis result of (A). The ALAL samples were highlighted with blue color rectangle boxes.

Supplementary Figure 2. A. Overall survival analysis of ALAL patient based on disease subtype. B. Overall survival analysis of ALAL patient based on the age (>60 vs <60) of patients. P values were calculated by Log-rank test.

Supplementary Figure 1

A



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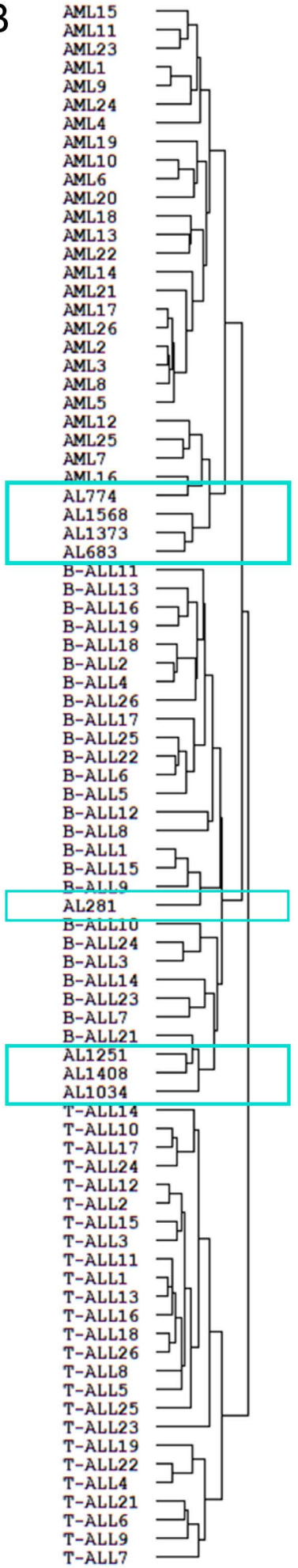

Supplementary Figure 2