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# Optimized outcome prediction of oncogenetic mutations in non-early T-cell precursor acute lymphoblastic leukemia

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## ABSTRACT

**Background:** Early biomarkers allowing effective treatment stratification in adult T-cell acute lymphoblastic leukemia (T-ALL) patients remain elusive.

**Materials and methods:** The mutation spectrum of 116 T-ALL adult patients enrolled in the Shanghai Institute of Hematology (SIH)-based hospital network or Multicenter Hematology-Oncology Protocols Evaluation System (M-HOPES) in China were studied by using RNA-sequencing or targeted next generation sequencing. A comprehensive survival analysis based on clinical characteristics, immunophenotype and oncogenetic classifier was performed.

**Results:** Early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) has higher mutation rates of N/K-RAS and lower mutation rates of FBXW7 compared to non-ETP ALL, but the survival probability of ETP-ALL patients is similar to that of non-ETP ALL patients. T-ALLs with a NOTCH1/FBXW7 (N/F) mutation in the absence of RAS or PTEN abnormalities (NFRP class I) show a more favorable outcome compared to T-ALLs with no N/F mutation and/or with the presence of RAS/PTEN alterations (NFRP class II). A survival analysis of T-ALL, taking into account both the ETP-ALL/non-ETP T-ALL groups and the NFRP oncogenetic classifier, demonstrates that, within the non-ETP T-ALL subtype, NFRP class II identifies a group with poor prognosis and significant decreases of both OS (14.8% versus 50.9%,  $P = 0.019$ ) and EFS (11.4% versus 42.4%,  $P = 0.001$ ). In contrast, no survival difference is observed within ETP-ALL between the NFRP class I or class II (OS: 37.9% versus 33%,  $P = 0.876$ ; EFS: 39.8% versus 33.7%,  $P = 0.969$ ).

**Conclusion:** In summary, the oncogenetic classifier based on the NFRP classes is particularly useful to improve the stratification of non-ETP ALL.

## 1. Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a malignant and heterogeneous disease caused by cells exhibiting developmental arrest at varying stages of differentiation. Immunophenotypic subtypes of T-ALL corresponding to T-cell maturation stages have long been proposed. Among them, early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) is a distinct form of leukemia compared to other T-ALL types and has been reported to represent 35–50% of adult T-ALL (Belver and Ferrando, 2016). ETP-ALL is characterized by reduced or absent expression of T-lymphoid markers (CD1a, CD5, CD8) and positivity for at least one hematopoietic stem cell and/or myeloid antigen (Coustan-Smith et al., 2009). Furthermore, the genomic profiles of pediatric ETP-ALL present more alterations in RAS signaling, JAK-STAT signaling, as well as in epigenetic regulation and less NOTCH1 mutations than non-ETP-ALL (Zhang et al., 2012; Liu et al., 2017). Lately, Bond et al. reported that the genotype of adult ETP-ALL is similar to the pediatric equivalent, although mutations in DNA methylation factor genes are more common (Bond et al., 2017).

Clinically, ETP-ALL was first reported to be associated with high risk of remission failure and inferior long-term outcomes in pediatric T-ALL (Coustan-Smith et al., 2009; Ma et al., 2012). However, subsequent larger series of pediatric cases treated with more intensive therapy showed no significant differences in the outcome between ETP-ALL and typical T-ALL cases. Meanwhile, several groups reported that survival of ETP-ALL was also comparable to typical T-ALL in adult (Bond et al., 2017; Neumann et al., 2012; Patrick et al., 2014). Data from the GRAALL suggested that poor outcome in ETP-ALL is exclusive to adult patients with HOXA-overexpression (Bond et al., 2016). More recently, Genesca et al. reported that adult ETP-ALL showed significantly poorer response to induction therapy and inferior overall survival than non-ETP-ALL in a series of 185 adult T-ALL patients (Genesca et al., 2020). Further studies would be required to finally conclude. Moreover, whether the presence of oncogenetic mutations is associated with the outcomes of ETP-ALL has never been investigated.

The NOTCH1 pathway plays a central role in T-cell lineage specification and proliferation (Gonzalez-Garcia et al., 2012). In contrast to the nearly 80% mutation rate of NOTCH1 signaling (mainly constitute with activating mutations in NOTCH1 or loss of function mutations in FBXW7) in typical T-ALL, ETP-ALL showed low frequency of NOTCH1 or FBXW7 mutation (Zhang et al., 2012). Prognosis relevance of NOTCH1/ FBXW7 (N/F) mutation has been widely explored in the whole cohort of T-ALL. While most agree that the presence of NOTCH1/FBXW7 mutation is associated with a favorable outcome (Asnafi et al., 2009; Van Vlierberghe et al., 2013), others suggest no significant effect (Baldus et al., 2009; Mansour et al., 2009). Later, N/K-RAS gene mutations or PTEN gene alterations were taken into account to provide a more refined oncogenetic classifier. Trinquand et al. demonstrated that the presence of N/F mutations in the absence of RAS or PTEN abnormalities predict good outcome in adult patients (Trinquand et al., 2013). However, this classifier has not been fully exploited in T-ALL in clinical. Up to now, few diagnostic features or molecular factors can accurately assign patients to a specific risk stage at the time of diagnosis.

In this study, we performed comprehensive clinical, genetic, and prognostic analyses of 116 adult T-ALL patients. By combining the analysis of the genetic alterations with the characterization of the immunophenotype status, we explored the impact of their possible association with the prognosis of adult T-ALL patients.

## 2. Methods

### 2.1. Patients and samples

From Jan 2009 to Jun 2021, 116 T-ALL adult patients, aged from 15 to 69 years, were treated in the Shanghai Institute of Hematology (SIH)- based hospital network or Multicenter Hematology-Oncology Protocols Evaluation System (M-HOPES) in China. The diagnosis and classification of the T-ALL subtypes were based on the 2016 World Health Organization criteria (Arber et al., 2016). Patients were all enrolled in an SIH protocol [Chinese Clinical Trial Registry, number ChiCTR-ONRC- 14004968 (for treatment)], which was basically a modified VDLCp regimen. In brief, the induction therapy (weeks 1–4) consisted of vincristine 1.4 mg/m<sup>2</sup> on days 1, 8, 15, and 22; daunorubicin 60 mg/m<sup>2</sup> on days 1, 8, 15, and 22; Cyclophosphamide 750 mg/m<sup>2</sup> on day 1, 15; L- asparaginase 10 000 IU administered intravenously or intramuscularly on days 17 to 28 or PEG-asparaginase 2500 IU/m<sup>2</sup> on day 8 and day 22 (started from February 2014); prednisone 60 mg/m<sup>2</sup> administered orally in divided doses on days 1 to 28. Consolidation therapy consisted of four cycles of Hyper-CVAD alternating with four cycles of high-dose meth- otrexate (MTX) and cytarabine therapy, with intrathecal CNS prophylaxis monthly.

At least after two cycles of consolidation therapy, patients went on to receive allogeneic transplantation if an appropriate donor was available. Others went on to the rest consolidation treatment.

Patients who met the following criteria were encouraged to undergo allogeneic stem-cell transplantation (allo-SCT): (1) high WBC count ( $\geq 100 \times 10^9/L$ ); (2) complex karyotype (5 or more chromosomal abnormalities); (3) central nervous system involvement; (4) failure of remission (MRD  $\geq 0.01\%$ ) after the first induction treatment. A total of 27 patients received allo-SCT, while the others were unable to undergo allo-SCT mainly due to cost issues.

Bone marrow (BM) aspiration was conducted at diagnosis and mononuclear cells were enriched by density gradient centrifugation with Ficoll solution. Total RNA was extracted using AllPrep DNA/RNA/ Protein Mini Kit (Qiagen) or TRIzol reagent (Invitrogen).

### 2.2. Flow cytometry analysis

Fresh heparinized whole-bone marrow (BM) samples were processed on a standard NH<sub>4</sub>Cl whole-blood lysing technique for immunophenotyping at diagnosis and MRD monitoring during follow-up using standard methods as described previously (Weng et al., 2013). T-cell lineage and T-ALL sub-classifications were assessed by immunophenotyping according to the National Comprehensive Cancer Network (NCCN) guidelines version 1.2020. ETP-ALL was defined as Coustan-Smith et al: reduced/absent expression of CD1a, CD5, and CD8, and positivity for at least one of the following antigens: CD34, CD117, HLA-DR, CD13, CD33, CD11b, or CD65 (Coustan-Smith et al., 2009).

Minimal residual disease (MRD) in bone marrow was evaluated by using flow cytometry to detect a cluster of events with an aberrant antigen pattern that resembled that of leukemic cells at diagnosis. MRD negative was defined as  $< 0.01\%$  residual leukemia cells at the end of induction course. Since 2004 and onwards, MRD was assessed by multicolor flow cytometry

with a sensitivity of  $10^{-4}$  in remission bone marrow samples for patients with T-ALL as described previously (Weng et al., 2013). MRD was not available for 3 patients, who all died before the end of the induction treatment.

The study was approved by the institution review board of Ruijin Hospital. All patients or guardians provided informed consent for sample collection and research in accord with the Declaration of Helsinki.

### 2.3. RNA-Seq data analysis

The gene mutations of 54 patients were mainly analyzed using RNA-sequencing and confirmed by whole exome sequencing (Chen et al., 2018). Reads from fastq files were aligned using STAR 2.5.2b software for UCSC hg19 reference genome. The aligned reads were counted using HTSeq framework (version 0.9.1). RPKM (reads per kilobase million) values were obtained by dividing the RPM (reads per million) values by a cumulated length of exons in kilobases and log-transformed by computing  $\log_2(1 + \text{RPKM})$ .

Analysis of mutational profiles by mutation calling from RNA-seq data has been reported previously (Chen et al., 2018).

### 2.4. Molecular characterization

We used the Sanger sequencing and targeted next generation sequencing to detect genes mutations in 72 additional patients. Sequencing of NOTCH1 was performed as described previously (Chen et al., 2012). Sanger sequencing of FBXW7 was carried out following PCR amplification of the FBXW7 WD40 domains (exons 8–12), previously reported to be the most frequently mutated regions.

The NOTCH1/FBXW7/RAS/PTEN classifier defined two molecular groups, respectively NFRP class I, for the presence of N/F mutation but no RAS/PTEN mutation and NFRP class II for all the other cases.

The transcripts of STIL-TAL1, SET-NUP214, and PICALM-MLLT10 fusion genes were analyzed by RNA-seq and confirmed using reverse transcription polymerase chain reaction (RT-PCR). The transcripts of STIL-TAL1 fusion gene were detected as previously described (Chen et al., 2012). The transcripts of PICALM-MLLT10 and SET-NUP214 were detected according to Meijerink and Macintyre (Van Vlierberghe et al., 2008; Asnafi et al., 2003), respectively.

### 2.5. Statistical analysis

Comparisons of categorical variables were determined by Pearson's Chi-square test or Fisher's exact test. The Mann-Whitney  $U$  test was used for median comparisons. Fisher exact P-test was used to compare the CR and relapse rate. Survival was measured from the date of diagnosis of T-ALL to the date of death from any cause or to the date of last contact. OS was defined as from the diagnosis of the disease to death or alive at last follow-up (censored); EFS was from disease diagnosis to treatment failure, including relapse, death, or alive in CR at last follow-up (censored). OS and EFS were estimated by Kaplan – Meier method and compared by log-rank test for stratifications of the risk groups. The last follow-up was carried out on September 30th 2021. Multivariate analyses were performed using the Cox regression model. A two-sided  $P$

< 0.05 was regarded as statistically significant. All statistical procedures were performed with the SPSS Version 20.0 statistical software package.

### 3. Result

#### 3.1. Characteristics of adult acute lymphoblastic leukemia

A total of 116 adult T-ALL patients enrolled in our center, among them, including 40 patients classified as ETP-ALL, which represent 34% of all T-ALL patients. The clinical characteristics were compared between patients with ETP-ALL and those with non-ETP ALL (Table 1). Notably, we found that ETP-ALLs in our group were older (median age, 42 versus 27;  $P = 0.001$ ) and showed lower WBC counts at diagnosis (median, 6.6 versus 27.9;  $P = 0.002$ ) (Table 1). A higher frequency of age over 35 years (57.5% versus 30.3%,  $P = 0.004$ ) was consistently observed in ETP-ALL.

The CR rate in patients with ETP-ALL was significantly lower than that of non-ETP-ALL patients (60% versus 84.2%,  $P = 0.004$ ) and fewer patients tend to have negative MRD (blasts < 0.01%) after induction treatment (23.7% versus 53.3%;  $P = 0.003$ ) (Table 1). A total of 27 patients went to allo-SCT after achieving remission, including 9 (22.5%) patients with ETP-ALL and 18 (23.7%) patients with non-ETP ALL, respectively.

More specifically, among the 79 patients (respectively 26 ETP-ALL and 53 non-ETP ALL) whose MRD data was available on day14, 77% (20/26) of the ETP-ALLs patients showed poor early response (MRD > 10%), which is much higher compared to patients with non-ETP ALLs (34% (n = 18/53),  $P < 0.001$ ). Moreover, only one (3.8%) patient of the ETP-ALL subgroup showed MRD < 0.01% at day14, while 19 (35.8%) of the non-ETP-ALL showed negative MRD ( $P < 0.001$ ). Taken together, these clinical observations suggest that ETP-ALL patients tend to show worse treatment response than patients with non-ETP T-ALLs.

#### 3.2. Distinct molecular and genetic features of adult ETP-ALL and non-ETP ALL

We performed RNA-sequencing (RNA-seq) of 54 patients (14 ETP-ALLs and 40 non-ETP-ALLs) for which high-quality diagnostic RNA samples were available. There was no difference in the clinical characteristics between those with RNA-seq or without RNA-seq (Table S1).

The mutational spectrum within these 54 patients was analyzed (Fig. 1). Consistent with previous reports, alterations in RAS signaling (54.3% versus 20%,  $P = 0.006$ ) and genes related to DNA methylation (42.9% versus 7.5%,  $P = 0.006$ ) were more commonly seen in the ETP-ALL subgroup. Notably, a significantly higher rate of mutations in DNMT3A was observed in ETP-ALL patients compared to non-ETP ALLs (35.7% versus 5%,  $P = 0.01$ ). On the contrary, gene alterations commonly reported in typical T-ALL such as FBXW7 were less frequently seen in ETP-ALL patients than in non-ETP ALL, although this difference did not reach statistical significance (21.4% versus 45%,  $P = 0.119$ ). Unlike in pediatric ETP-ALLs, alterations in JAK-STAT signaling (50% versus 47.5%,  $P = 0.872$ ), PRC2 complex (35.7% versus 22.5%,  $P = 0.497$ ) and cell cycle (21.4% versus 17.5%,  $P = 0.708$ ) showed similar frequencies in patients with ETP compared to non-ETP ALL. Overall, we further confirmed that, although there are genes commonly altered in both adult and pediatric ETP-ALLs, the adult ETP-ALL subset has its own distinct deregulated molecular pathways, implicating different pathophysiological processes and therefore different outcome impact.

Differences in the somatic mutations of candidate genes and gene rearrangements between ETP-ALLs and non-ETP-ALLs were also explored (Table 1). Notably, ETP-ALLs were associated with a lower rate of FBXW7 mutations and a higher rate of N/K-RAS mutations compared to non-ETP ALLs (15% versus 30.3%,  $P = 0.071$ ; 30% versus 9.2%,  $P = 0.004$ ). While there is a trend toward high rate of SET-NUP214 rearrangements in ETP-ALL ( $P = 0.091$ ), no STIL-TAL1 rearrangement were found in ETP-ALL ( $P = 0.004$ ) (Table 1). We did not observe any significant difference in the NOTCH1 mutation frequencies between these two groups.

### 3.3. Patient outcome according to ETP-ALL and non-ETP ALL subtypes

The ETP-ALL subtype in pediatric patients had been almost unanimously reported to correlate with higher rates of corticosteroid resistance, early bone marrow chemotherapy resistance and poor long-term survivals compared to non-ETP (Coustan-Smith et al., 2009; Malard and Mohty, 2020), whereas the clinical impact of the ETP phenotype has been more variable in adult patients (Bond et al., 2017; Van Vlierberghe et al., 2013).

Therefore, we performed clinical and survival comparisons of ETP- ALLs and non-ETP ALLs in our cohort.

Despite the high rates of poor early bone marrow response observed in ETP-ALL, there were similar outcomes between ETP-ALL and non-ETP ALL patients considering both 3-year OS (35% versus 40.6%,  $P = 0.224$ ) and EFS (36.8% versus 33.8%,  $P = 0.623$ ) (Fig. 2). No difference was observed in the ratio of patients who underwent allo-SCT between ETP- ALLs and non-ETP ALLs (22.5% versus 23.7%,  $P = 0.886$ ).

Overall, these results suggest that, although there is a noticeable trend for ETP-ALL to be poor responders to the early treatment, the ETP- ALL phenotype does not necessarily associate with poor long-term outcome in adult patients.

### Collaborative prognostic impact of oncogenetic classifier and immunophenotype in adult T-ALLs

Beyond ETP-ALL, other recurrent mutations carry prognostic significance. Among them, the most common ones occur in the NOTCH1/ FBXW7 pathway (60% in adult patients), and associated with a favorable prognosis in most studies. A risk classification based on the presence or absence of NOTCH1/FBXW7, PTEN, or N/K-RAS mutations has been proposed (see details in Methods).

In our cohort, 66 (56.9%) patients with N/F mutations and RAS/ PTEN (R/P) germline were classified as NFRP class I, whereas 50 (43.1%) with N/F germline and R/P mutations or N/F and R/P mutations were classified as NFRP class II (Table 2). We observed no statistical difference between these two groups regarding gender, age and WBC count (Table 2). In particular, due to the lower rate of FBXW7 mutations and higher rate of RAS mutations in the ETP-ALL subtype (Fig. 1), we observed that ETP-ALL presented a significantly lower proportion of the NFRP class I compared to NFRP class II (22.7% versus 50%,  $P = 0.002$ ).

This observation led us to make the hypothesis that, by combining the immunophenotype subgroups with this oncogenetic classifier, we could more accurately stratify T-ALLs.

We initially explored the prognostic impact of this oncogenetic classifier in our group. Consistent with previous reports, the NFRP class I was associated with a longer overall survival compared to the NFRP class II (46.9% vs. 28.1%,  $P = 0.061$ ), as well as a significantly improved EFS at 3 years (40.7% versus 28.6%,  $P = 0.025$ ) (Fig. 3A-B).

We then subdivided the NFRP classes into ETP-ALL and non-ETP ALL subgroups and compared the outcomes. We found that, within non-ETP ALL group, the NFRP class II predicted a highly aggressive outcome, with regard to both OS (14.8% versus 50.9%,  $P = 0.019$ ) and EFS (11.4% versus 42.4%,  $P = 0.001$ ) (Fig. 4A-B). In contrast, no difference in OS or EFS between the two NFRP classes was observed within the ETP-ALL subgroup (37.9% versus 33%,  $P = 0.876$  for OS; 39.8% versus 33.7%,  $P = 0.969$  for EFS) (Supplementary Fig. 1A-B). Specifically, among the 25 patients with non-ETP ALL together with NFRP class II, 12 patients (16/25, 64%) relapsed, while only 14 non-ETP ALL patients with NFRP class I relapsed (14/51, 27.5%) ( $P = 0.002$ ), suggesting that NFRP class II can serve as a more precise predictor of relapse in non-ETP ALL.

After subdividing the non-ETP ALLs with NFRP status, multivariate cox regression analysis including other prior-treatment factors was performed. Among them, NFRP classes remained as the most significant prognostic covariate when considering EFS (HR: 2.885; 95% CI, 1.474–5.649;  $P = 0.002$ ) when adjusting to age (using the 35-year cut-off) and WBC count (using the  $100 \times 10^9 /L$  cutoff).

Prognostic importance of MRD has been proved by numerous studies in both childhood and adult ALL, and this is also true in our group. Based on a multivariate Cox regression analysis including all patients, MRD positivity after induction was identified as the most significant independent factor predicting a decreased EFS, while the NFRP classifier was not significantly associated with EFS (Table S2). A multivariate Cox regression analysis restricted to the non-ETP subgroup, demonstrated that the MRD as well as the NFRP classifier could serve as independent parameters predicting EFS (Table S3). These results also support the ability of this oncogenetic classifier in predicting the outcome of non-ETPs.

Altogether, when focusing on the non-ETP ALL subgroup, the NFRP oncogenetic classifier was the most significantly factor associated with survival among those available at pre-treatment stages.

#### 4. Discussion

In this study, we first explored the clinical features and prognostic significance of ETP-ALL in our series of patients. Compared to patients with non-ETP ALL, those with ETP-ALL showed lower rates of CR and negative MRD ( $P = 0.004$  and  $P = 0.003$ , Table 1), consistent with the poor induction response of ETP-ALLs reported by Genesca (Genesca et al., 2020). However, no significant differences were observed in overall survival or event-free survival between ETP-ALL and non-ETP ALL patients and long-term outcomes in our group, which is in line with the observations from other cohorts of patients treated with the GRAALL protocols (Bond et al., 2017).

ETP-ALL is characterized by pluripotency and shared features with both hematopoietic stem cells and myeloid progenitor cells. Although the prognostic impact of the ETP-ALL phenotype alone is still controversial, a poor response to the induction treatment has been consistently reported (Coustan-Smith et al., 2009; Malard and Mohty, 2020). In an adult cohort of patients

with T-ALL/lymphoblastic lymphoma treated at the MD Anderson Cancer Center, 17% had the ETP subtype. In their study, the ETP-ALL/lymphoblastic lymphoma (LBL) patients were found to have significantly lower complete remission rates (73% versus 91%,  $P = 0.03$ ) and poorer median overall survival (20 months versus not reached,  $P = 0.008$ ) in comparison with other T-ALL/T-LBL patients (Jain et al., 2016). Genesca et al., also reported poorer outcome for ETP-ALL patients due to poor response to the induction therapy (Genesca et al., 2020). Accordingly, efforts to improve the depth of remission might be highly beneficial for these patients. Inhibitors of the BCL2 family, including venetoclax and navitoclax, appear to be promising agents against ETP-ALL. Venetoclax has been reported to be clinically active in patients with relapsed/refractory ETP-ALL (Numan et al., 2018; Richard-Carpentier et al., 2020). In a phase I study (NCT03181126) including patients with relapsed/refractory ALL and lymphoblastic lymphoma, the anti-tumor activity of venetoclax in combination with navitoclax and chemotherapy showed improved response rates in ETP-ALL (N = 8/12, 66.7%) (Pullarkat et al., 2021). Notably, adults with ETP-ALL treated using the GRAALL protocols were also reported to show high levels of post-induction MRD (71.4% of patients had MRD level  $\geq 10^{-4}$ ), although no inferior long-term outcome compared to the non-ETP-ALL group was observed ( $P = 0.33$  for 5-year OS;  $P = 0.17$  for 5-year EFS) (Bond et al., 2017). It is also worth noting that patients in the above mentioned study received an intensified treatment when there was evidence of early treatment resistance. Moreover, approximately 49% of ETP-ALL patients received allogeneic stem cell transplant, suggesting an important role of allogeneic SCT transplant in improving the outcome of ETP- ALL patients.

Beyond ETP-ALL, recurrent mutations that carry prognostic significance in T-ALL were also assessed. RNA-sequencing was performed to explore mutational landscape in our series of 54 adult T-ALLs.

In agreement with previous studies, we found a significantly higher rate of mutations in DNMT3A in ETP-ALL patients while common gene alterations in typical T-ALL such as FBXW7 were less likely seen in ETP- ALL patients. Unlike in pediatric ETP-ALLs, alterations in JAK-STAT signaling and the PRC2 complex were similar between patients with ETP and non-ETP. We further confirmed that, although there are genes commonly altered in adult and pediatric ETP-ALLs, the adult ETP-ALL subset has its own distinct deregulated molecular pathways, implicating different pathophysiological processes and thus different outcome impact. Moreover, the high somatic mutational frequency in signaling pathways such as Ras or JAK-STAT suggests that inhibitors targeting these pathways may provide new strategies to improve out- comes (Inaba et al., 2017). Among these genetic alterations in T-ALL, the most common alterations occur in the NOTCH1/FBXW7 pathway (60% of adult patients), and genetic alterations in this pathway have long been proposed as a prognosis classifier in previous studies. The good-risk group (significantly superior OS and inferior cumulative incidence of relapse) harbored mutations in the NOTCH1/FBXW7 pathway with no associated mutations in PTEN or N/K-RAS (NFRP class I); all other (NFRP class II) patients were classified as poor risk with OS and cumulative incidence of relapse (Trinquand et al., 2013). In the present study, our results support that the NFRP class II is associated with significantly inferior EFS. We also noticed that NFRP class II was associated with a lower percent of non-ETP ALL. The difference in the distribution of this oncogenetic classifier between ETP-ALLs and non-ETP ALLs led us to address the question of prognostic impact of these pre- treatment factors in combination in order to more accurately identify high risk T-ALLs.

We found that the NFRP class II was associated with a negative outcome exclusively in adults with non-ETP ALL, despite similar rates of post-induction MRD observed in non-ETP-ALL between NFRP class I and class II.

Therefore, our results suggest that the NFRP oncogenetic classifier can be used to separate non-ETP ALL patients into groups who may respond well to standard therapy and those who may benefit from more intensive or targeted therapy at diagnosis. Targeting the NOTCH1 signaling pathway may be a promising option in the management of adult non-ETP ALL patients, since mutational rates are rather high in this group. Novel inhibitors of NOTCH1 pathway such as OMP-52 M51 (Agnusdei et al., 2020), BMS-906024 (Knoechel et al., 2015) showed encouraging anti-tumor efficacy in both T-ALL xenograft models and clinical trials. Endeavors to increase the depth of remission might also be highly profitable for non-ETP ALL patients. Clinical trials evaluating inhibitors targeting JAK-STAT (Delgado-Martin et al., 2017) mTOR, (Daver et al., 2015), BCL2 (Peirs et al., 2014) are emerging as novel approaches to strengthen remission in T-ALL. Moreover, immunotherapies involving endogenous T cells are proposed as a new strategy to treat T cell ALL. Ongoing clinical trials, which include CD7-based CAR T-cell, CD1a-based CAR T-cell and other approaches, are also promising therapies to alleviate the adverse outcome of adult T-ALL (Safarzadeh Kozani et al., 2021). Since CD38 expression was stably expressed in both baseline and relapsed samples, the CD38-targeting antibody daratumumab (DARA) is another attractive option to induce profound remission of T-ALL (Naik et al., 2019; Ofran et al., 2020). These newer treatment options may result in improved survival outcomes for these patients.

Nevertheless, we have to mention that the overall survival of the patients of our cohort is lower than the 60% or even higher rate of long-term OS of patients from other published studies. Unfortunately, a large proportion of the patients of our cohort did not have access to allo-SCT, the high cost of allo-SCT being too much of a burden for the average family, and being only partially covered by the national health system. Hence, the rate of allo-SCT in our group (<25%) was much lower than that in other groups (~50%). Since allo-SCT is known to greatly improve survival of patients, this lower rate of access to allo-SCT is likely a major factor contributing to the poor overall survival of the patients from our cohort. Indeed, according to a recently published article by Ganzel (Ganzel et al., 2020), patients who received only chemotherapy as a consolidation treatment have a much higher risk of early relapse compared to those who received allo-SCT after achieving CR (early relapse rates of 60.8% versus 13.2%), and these relapsed patients duly have a much lower probability of overall survival. This explains why our long-term survival is lower than that of other studies.

Compared to BCP-ALL, where molecular abnormalities are commonly used for risk assessment and adaptation of the treatment, in T-ALL only a few molecular lesions or pre-treatment factors can be exploited for risk stratification (Matlawska-Wasowska et al., 2016; Roy and Raghavan, 2021). Our results offer a better understanding of the genetic lesions in adult T-ALL and highlight the value of oncogenetic alterations to improve the risk assessment in non-ETP ALLs. The identification of this new prognostically relevant diagnostic marker could be instrumental in refining risk-adapted treatment. A therapeutic strategy combining conventional chemotherapy with pathway inhibitors or immunotherapies may be considered as an option of choice for patients with high risk oncogenetic mutations, especially those with non-ETP ALLs.

## 5. What's new

T-cell acute lymphoblastic leukemia (T-ALL) is associated with poor outcome in adult patients. Early biomarkers allowing effective treatment stratification in adult T-ALL patients are still needed. In the present study, we not only confirmed the prognostic impact of an oncogenetic classifier based on the mutation status of NOTCH1, FBXW7, RAS and PTEN (NFRP classifier) but also demonstrate, by a comprehensive survival analysis combining the ETP-ALL/non-ETP-ALL phenotype and the oncogenetic classifier, that the NFRP classes can more accurately separate patients with high risk of relapse in the non-ETP ALL subgroup. We therefore propose a more accurate stratification model to predict prognosis in adult T-ALL.

### Data availability

The datasets have been deposited at The National Omics Data Encyclopedia (NODE) (<https://www.biosino.org/node>) under accession no. OEP000760 or the following URL: <https://www.biosino.org/node/project/detail/OEP000760>.

### Ethics statement

All patients or guardians provided informed consent for sample collection and research in accord with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of Shanghai Jiao-tong University School of Medicine affiliated Ruijin Hospital (200807).

### *CRedit authorship contribution statement*

Li-Jun Peng: Formal analysis, Investigation, Methodology, Software, Writing – original draft. Si-si Wang: Formal analysis, Methodology, Software, Writing – original draft. Shan-Shan Guo: Methodology, Investigation. Jiao-Jiao Zhang: Data curation. Yuan-Fang Liu: Investigation. Sophie Rousseaux: Supervision. Saadi Khochbin: Writing – review & editing. Bing Chen: Data curation, Supervision. Jin Wang: Conceptualization, Funding acquisition. Jian-Qing Mi: Writing – review & editing, Conceptualization, Funding acquisition.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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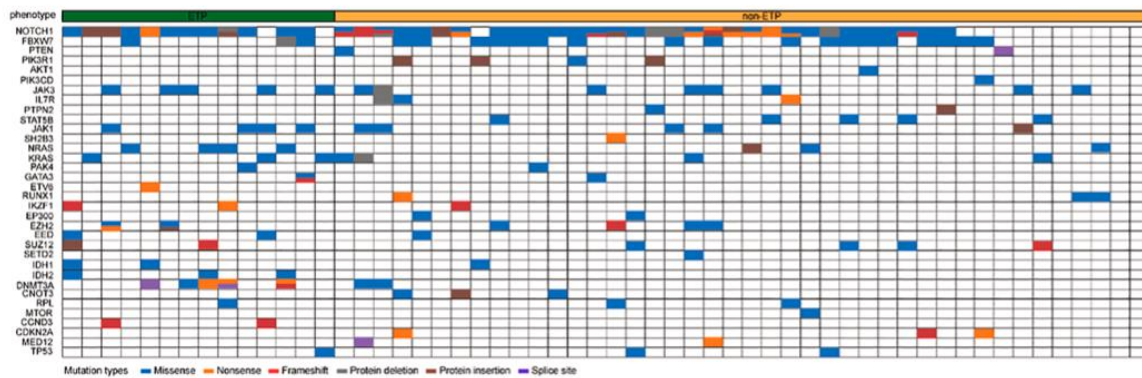
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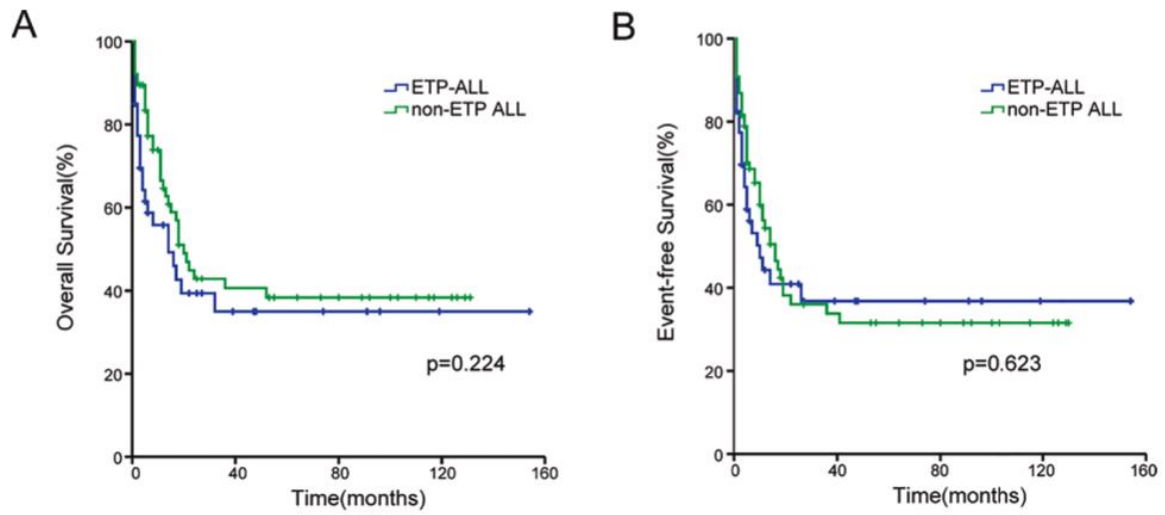
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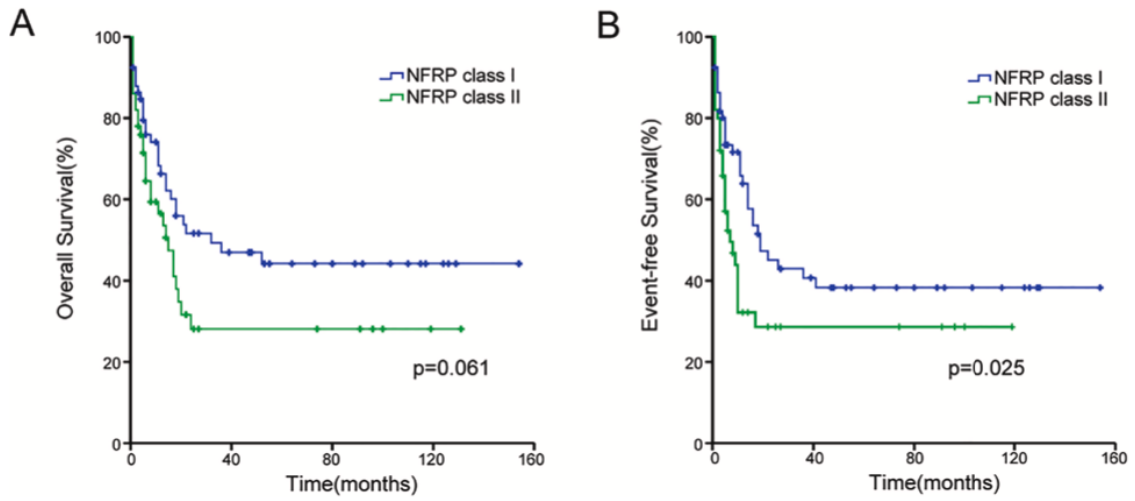
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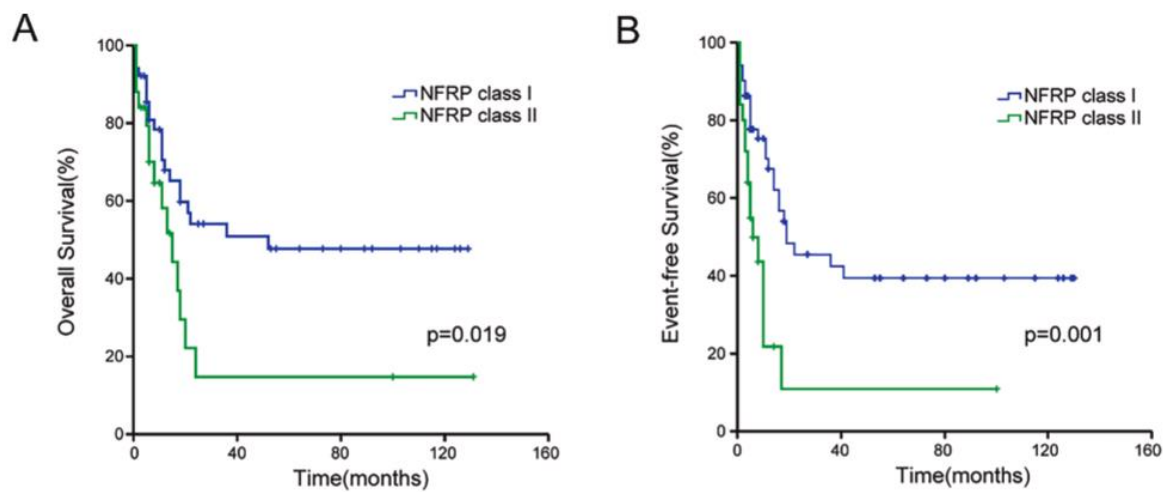
**Fig. 1.** Details of mutations found in adult T-ALL according to immunophenotype.



**Fig. 2.** Overall survival (OS) (A) and Event-free survival (EFS) (B) of ETP-ALL and non-ETP ALL. 4



**Fig. 3.** Overall survival (OS) and Event-free survival (EFS) according to NFRP classes.



**Fig. 4.** OS and EFS according to oncogenetic classifier within non-ETP ALL subgroup.

| Characteristics                      | Total               | ETP              | non-ETP            | P value |
|--------------------------------------|---------------------|------------------|--------------------|---------|
| <b>Total</b>                         | 116                 | 40               | 76                 |         |
| <b>Gender, male</b>                  | 79(68.1%)           | 22 (55%)         | 57 (75%)           | 0.028   |
| <b>Age, years, Median(Range)</b>     | 28.5(15–69)         | 42(15–69)        | 27(15–60)          | 0.001   |
| <b>Laboratory data</b>               |                     |                  |                    |         |
| WBC, $\times 10^9/L$ , Median(Range) | 15.95 (0.29–454.48) | 6.6 (0.4–179.11) | 27.9 (0.29–454.48) | 0.002   |
| Hb, g/L, Median (Range)              | 112.5(47–180)       | 101(47–160)      | 117.5(51–180)      | 0.075   |
| PLT, $\times 10^9/L$ , Median(Range) | 78(5–470)           | 81(9–376)        | 72(5–470)          | 0.248   |
| <b>Karyotype</b>                     |                     |                  |                    |         |
| Normal karyotype                     | 57(49.1%)           | 22(55%)          | 35 (46.1%)         | 0.204   |
| Abnormal karyotype                   | 45(38.8%)           | 12 (30%)         | 33 (43.4%)         |         |
| NA                                   | 14(13.2%)           | 6(15%)           | 8(10.5%)           |         |
| <b>Achieved remission No. (%)</b>    | 88(75.9%)           | 24(60%)          | 64(84.2%)          | 0.004   |
| <b>MRD-Induction</b>                 |                     |                  |                    |         |
| $\geq 10^{-4}$                       | 64(55.2%)           | 29(76.3%)        | 35(46.7%)          | 0.003   |
| $< 10^{-4}$                          | 49(42.2%)           | 9(23.7%)         | 40(53.3%)          |         |
| <b>Allo-SCT (%)</b>                  | 27(23.3%)           | 9(22.5%)         | 18(23.7%)          | 0.886   |
| <b>Relapse, No. (%)</b>              | 30/88(34.1%)        | 6/24 (25%)       | 24/64 (37.5%)      | 0.271   |
| <b>Oncogenetics</b>                  |                     |                  |                    |         |
| NOTCH1                               | 76(65.5%)           | 23 (57.5%)       | 53 (69.7%)         | 0.188   |
| FBXW7                                | 29(25%)             | 6 (15%)          | 23 (30.3%)         | 0.071   |
| N/K-RAS                              | 20(17.2%)           | 12 (30%)         | 7 (9.2%)           | 0.004   |
| PTEN                                 | 7(6%)               | 2 (5%)           | 5 (6.6%)           | 1       |
| IL7R                                 | 8(6.9%)             | 2 (5%)           | 6 (7.9%)           | 0.713   |
| PHF6                                 | 24(20.7%)           | 6 (15%)          | 18 (23.7%)         | 0.272   |
| SET-NUP214                           | 10(8.6%)            | 6 (15%)          | 4 (5.3%)           | 0.091   |
| PICALM-AF10                          | 4(3.4%)             | 1 (2.5%)         | 3 (3.9%)           | 1       |
| STIL-TAL1                            | 13(11.2%)           | 0 (0%)           | 13 (17.1%)         | 0.004   |

**Table 1**

Clinical and genomic comparison of ETP-ALL and non-ETP ALL patients.

Precursors; CR, complete remission; MRD, Minimal Residual Disease; Allo-SCT, allogeneic stem-cell transplantation; NA, not available. *P*-value were determined by using Pearson's Chi-square or Fisher's exact test for categorical variables and Mann-Whitney *U* test for median comparisons.

| Characteristics                 | No. | NFRP          |               | P value |
|---------------------------------|-----|---------------|---------------|---------|
|                                 |     | class I(%)    | class II(%)   |         |
| <b>Total</b>                    | 116 | 66 (56.9%)    | 50 (43.1%)    | /       |
| <b>Gender, male/female</b>      |     |               |               |         |
| male                            | 79  | 44 (66.7%)    | 35 (70%)      | 0.703   |
| female                          | 37  | 22 (33.3%)    | 15 (30%)      |         |
| <b>Age (years)</b>              |     |               |               |         |
| ≤35                             | 70  | 44 (66.7%)    | 26 (52%)      | 0.11    |
| >35                             | 46  | 22 (23.3%)    | 24 (48%)      |         |
| <b>WBC, ×10<sup>9</sup>/L</b>   |     |               |               |         |
| ≤100                            | 95  | 53 (80.3%)    | 39 (78%)      | 0.406   |
| >100                            | 21  | 10 (19.7%)    | 11 (22%)      |         |
| <b>Immunophenotype</b>          |     |               |               |         |
| ETP                             | 40  | 15 (22.7%)    | 25 (50%)      | 0.002   |
| non-ETP                         | 76  | 51 (77.3%)    | 25 (50%)      |         |
| <b>Karyotype</b>                |     |               |               |         |
| Normal karyotype                | 57  | 31 (50.8%)    | 26 (63.4%)    | 0.209   |
| Abnormal karyotype              | 45  | 30 (49.2%)    | 15 (36.6%)    |         |
| NA                              | 14  | /             | /             | /       |
| <b>CR</b>                       | 88  | 49 (74.2%)    | 39 (78%)      | 0.640   |
| <b>Post-induction MRD/total</b> |     |               |               |         |
| ≥10 <sup>-4</sup>               | 64  | 32/64 (50%)   | 32/49 (65.3%) | 0.104   |
| <10 <sup>-4</sup>               | 49  | 32/64 (50%)   | 17/49 (34.7%) |         |
| <b>Relapse/total</b>            | 30  | 14/48 (29.2%) | 16/39 (41%)   | 0.247   |

**Table 2**

Clinical characteristics of 116 adult T-ALL patients according to oncogenetic classifiers.

NFRP class I: N/F mutations and RAS/PTEN (R/P) germ line (GL); NFRP class II: N/F GL and R/P GL mutations or N/F and R/P mutations; WBC, White Blood Cells counts; Hb, hemoglobin; PLT, platelet; ETP, Early T cell Precursors; CR, complete remission; MRD, Minimal Residual Disease; Allo-SCT, allogeneic stem- cell transplantation; NA, not available. *P*-value were determined by using Pearson's Chi-square or Fisher's exact test for categorical variables and Mann- Whitney *U* test for median comparisons.