Unique clinico-biological, genetic and prognostic features of adult early T-cell precursor acute lymphoblastic leukemia

Early T-cell precursor (ETP) acute lymphoblastic leukemia (ALL), was first identified within cases of childhood T-ALL based on its unique immunophenotypic and genetic features of limited (early) T-cell differentiation associated with (some) myeloid and stem cell features.[1] Thus ETP-ALL blasts express CD7, dim CD5 (<75% positive cells), in the absence of CD1a and CD8, and positivity for ≥1 myeloid/stem cell related markers (i.e., CD34, CD13 or CD33).[1,2] In turn, ETP-ALL frequently shows myeloid-associated gene alterations such as *FLT3, NRAS/KRAS, DNMT3A, IDH1* and *IDH2* mutations,[3,4] with lower frequencies of other T-ALL-associated mutations (e.g., *NOTCH1* and *CDKN2A/B* gene mutations).[5,6] The World Health Organization (WHO) 2016 classification of ALL included ETP-ALL for the first time, as a provisional entity,[7] but it failed to establish robust diagnostic criteria. Thus, after the first immunophenotypic characterization of ETP-ALL by Coustan-Smith *et al.*[1] the proposed criteria did not allow identification of all ETP-ALL cases as detected by gene expression profiling.[2] In addition, the "partial CD5 expression" criterion had a negative impact on the reproducibility of ETP-ALL diagnoses because of the lack of standardization of the method used for its assessment. Because of this, Zuubier *et al.* proposed refined immunophenotypic criteria by excluding CD5 expression while adding negativity for CD4.[2]

From the clinical point of view early studies based on limited numbers of pediatric patients indicated that ETP-ALL was associated with a very poor outcome.[1,8,9] More recent data, based on larger series of children treated with more intensive therapy, showed no significant differences in outcome for ETP-ALL *vs.* other T-ALL cases.[10] In contrast, limited data have been reported for adult ETP-ALL, with conflicting results.[11,12] In one study, adult ETP-ALL was associated with a worse prognosis following different frontline chemotherapy schedules.[11] The

GRAALL 2003 and 2005 trials showed that treatment intensification with allogeneic hematopoietic stem cell transplantation (HSCT) in ETP-ALL with early treatment resistance could improve survival.[12]

Here we retrospectively analyzed the frequency, clinico-biological and prognostic features of adult ETP-ALL *vs.* other T-ALL cases in a series of 185 adults with T-ALL treated within two consecutive minimal residual disease-oriented trials by the *Programa Español de Tratamientos en Hematología (PETHEMA):* ALL-HR-2003 (NCT00853008) and ALL-HR-11 (NCT01540812), the latter still ongoing).

All 185 patients were diagnosed with T-ALL using the WHO criteria. The treatment protocol schedules have been described previously elsewhere.[13,14] The patients' characteristics at diagnosis and at follow-up are summarized in Table 1. The immunological T-ALL subtype was defined according to the European Group for the Immunological Characterization of Leukemias (EGIL) criteria after centralized review of immunophenotypic reports. In turn, the criteria proposed by Zuurbier *et al.*[2] were used to define ETP-ALL. The combination of markers used (<5% CD1a, CD8 and CD4; >25% CD34 and/or CD33/CD13), resembles that proposed by Coustan-Smith *et al.*, while dim CD5 expression (<75% positive cells) was not considered. The inclusion of negativity for CD4 instead of dim CD5 expression as an immunophenotypic criterion, allows identification of most ETP-ALL cases identified by gene expression profiling (increased sensitivity of immunophenotyping to detect ETP-ALL cases).[2] Clinical data were obtained in accordance with the principles of the Declaration of Helsinki and Spanish legislation, after written informed consent had been provided by each patient. The study was approved by the Institutional Review Board of the Hospital Germans Trias i Pujol (Badalona, Spain).

Comparisons between ETP-ALL and other T-ALL cases were performed with the χ^2 test, Fisher exact test, and the median test, as appropriate. Event-free survival and overall survival curves were plotted using the Kaplan-Meier method and compared by the log-rank test. A secondary
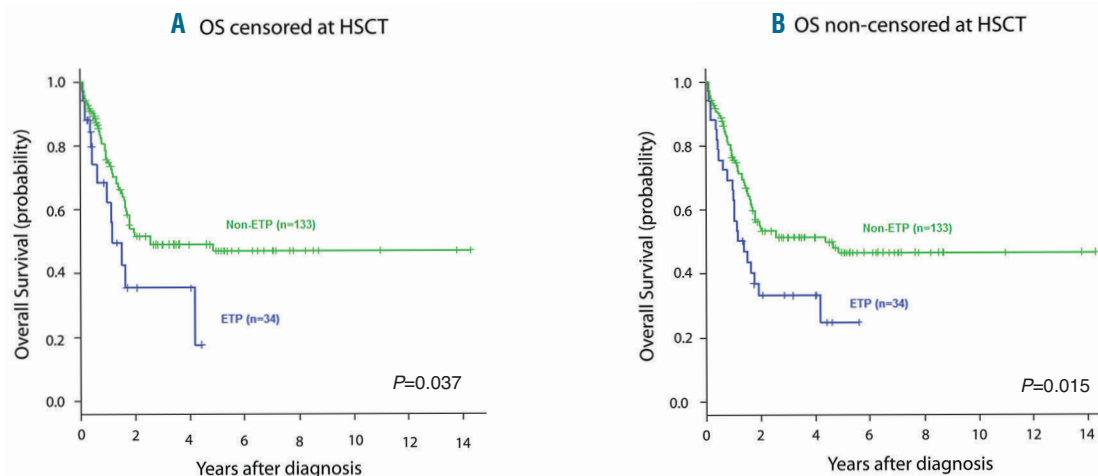

Figure 1. Impact of allogeneic hematopoietic stem cell transplantation on overall survival of patients with or without early T-cell precursor acute lymphoblastic leukemia. (A) Overall survival of adults with early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) *vs.* other non-ETP T-ALL adult patients censored at transplantation; the survival probability, with 95% confidence interval (95% CI) at 4 years was 36% (95% CI: 13-59%) for the ETP-ALL group and 49% (95% CI: 39-59%) for the other non-ETP patients. (B) Overall survival of ETP and non-ETP T-ALL patients not censored at transplantation. The survival probability at 4 years was 33% (95% CI: 16-50%) for ETP-ALL patients *vs.* 51% (95% CI: 42-60%) for the non-ETP T-ALL group.

Table 1. Clinico-biological and prognostic characteristics together with response to treatment of early T-cell precursor acute lymphoblastic leukemia vs. other types of T-cell acute lymphoblastic leukemia.

	Wholes series (n=185)	ETP-ALL (n=34)	Other non-ETP T-ALL (n=133)	P
Age (years)*	33.5 (12)	39 (12)	33 (12)	**0.01**
Female/male ratio	49/136	13/21	30/103	0.06
Lymph node enlargement	65/163 (40%)	23/29 (79%)	66/118 (56%)	0.02
Splenomegaly	61/178 (34%)	8/31 (26%)	49/130 (38%)	0.21
Hepatomegaly	40/175 (23%)	7/31 (23%)	30/127 (24%)	0.90
Mediastinal mass	86/182 (47%)	15 (44%)	65/131 (50%)	0.57
WBC count (x10^9/L)*	94.3 (121.0)	72.2 (155.1)	96.6 (111.7)	0.004
CNS involvement	18/178 (10%)	6/33 (18%)	12/130 (9%)	0.21
Complex karyotype**	9/141 (6%)	3/22 (14%)	6/104 (6%)	0.19
Del (6q)	10/140 (7%)	1/21 (5%)	9/104 (9%)	0.47
CDKN2A/B gene deletion	31/53 (58%)	1/11 (9%)	29/40 (73%)	**<0.001**
TCRG gene deletion	24/31 (77%)	3/8 (37%)	19/20 (95%)	**0.003**
Slow response at day +14	82/172 (48%)	27/33 (82%)	46/123 (37%)	**<0.001**
1 induction cycle to CR	153/167 (92%)	19/26 (73%)	118/125 (94%)	**0.003**
2 induction cycles to CR	14/167 (8%)	7/26 (27%)	7/125 (6%)	
CR	167/185 (90%)	26 (77%)	125/133 (94%)	**0.005**
MRD day +35 <0.1%	106/141 (75%)	7/20 (35%)	90/110 (82%)	**<0.001**
MRD day +35 <0.01%	74/132 (44%)	3/20 (15%)	65/104 (63%)	**<0.001**
Chemotherapy	86/121 (71%)	6/20 (30%)	72/91 (79%)	**<0.001**
Allogeneic HSCT	35/121 (29%)	14/20 (70%)	19/91 (21%)	

Results expressed as number of cases (percentage) or *as a mean (standard deviation). **More than five cytogenetic abnormalities. Minimal residual disease values were considered only for those patients who reached complete remission. Statistically significant values are shown in bold. ETP: early T-cell precursor; ALL: acute lymphoblastic leukemia; WBC: white blood cell; CNS: central nervous system; CR: complete remission; MRD: minimal residual disease; HSCT: hematopoietic stem cell transplantation.

analysis of overall survival was performed, with transplanted patients censored at the time of allogeneic HSCT. Cumulative incidence of relapse and non-relapse-related mortality were analyzed as competing events with the Gray test. A Cox proportional hazard regression model was used to identify predictive factors for event-free and overall survival, whereas multivariate analysis for cumulative incidence of relapse was performed with the Fine and Gray model. Statistical significance was set at *P*-values <0.05.

Full immunophenotypic data according to the EGIL criteria were available for 167/185 T-ALL patients; 34/167 (20%) T-ALL cases met criteria for ETP-ALL as defined by both Zuurbier *et al*. and the WHO.[7] This frequency is similar to that observed in previously published cohorts of adults with T-ALL[11,12] in which the phenotypic criteria applied in early pediatric studies to define ETP-ALL were used.[1] Significant differences were observed when comparing ETP-ALL and non-ETP-ALL cases at diagnosis (Table 1). Patients with ETP traits were older [mean ± standard deviation (SD): 39±12 years *vs.* 33±12 years, *P*=0.01], more frequently had lymph node enlargement (79% *vs.* 56%, *P*=0.02), had lower white blood cell counts (mean±SD: 72.2±155.1x10^9/L *vs.* 96.6±111.7 x10^9/L, *P*=0.004), and showed a slightly different female to male ratio (13/21 *vs.* 30/103, *P*=0.06), similarly to what has been shown for the GRAALL cohort.[12] In addition, our ETP-ALL cases frequently showed absence of *CDKN2A/B* (*P*<0.001) and *TCRG* (*P*=0.003) gene deletions. These results are in line with those previously reported in the literature[6,14,16] and support the use of both

genetic markers for the diagnosis of ETP-ALL. However, some discordance between immunophenotypic and genetic results was observed, which might be due to the limited sensitivity of current immunophenotypic criteria, highlighting the need for more precise and robust criteria and standardized methods for the diagnosis of ETP-ALL.

No significant differences in complete response, overall survival, event-free survival and cumulative incidence of relapse rates were observed between ETP-ALL patients enrolled in the two treatment protocols (*data not shown*). This allowed a combined evaluation of the data from both PETHEMA trials. ETP-ALL patients showed significantly poorer response to induction therapy compared to other T-ALL patients (Table 1). Thus, ETP-ALL patients had poorer bone marrow response (<10% blasts) on day +14 compared to other T-ALL cases (18% *vs.* 63%, *P*<0.001); half of ETP-ALL cases required second induction treatment (*vs.* 10% of the remaining T-ALL cases, *P*<0.001) and 23% of them did not attain a complete response (*vs.* only 6% of other T-ALL cases, *P*=0.005). Minimal residual disease data were available for 132/167 patients who reached a complete resposne after one or two induction cycles. Two-thirds (65%) of ETP-ALL cases showed minimal residual disease levels ≥0.1% (*vs.* 18% of non-ETP-ALL cases, *P*<0.001), the percentage increasing to 85% (*vs.* 37%, *P*<0.001) at a cut-off level of 0.01%. Consequently, as indicated per protocol, more ETP-ALL patients underwent allogeneic HSCT (70% *vs.* 21%, *P*<0.001). Despite treatment intensification with allogeneic HSCT, ETP-ALL patients did not attain a significant improvement in the probability of overall sur-

vival (Figure 1A, B). In fact, differences in overall and event-free survival between ETP-ALL and other non-ETP T-ALL patients were mostly due to the greater rate of failure to induction therapy, with a minimal contribution of the higher (non-statistically significant) cumulative incidence of relapse observed among ETP-ALL patients *vs.* other T-ALL cases (*Online Supplementary Figure S1*). However, it should be noted that the overall survival rate of adult T-ALL patients from the PETHEMA cohort who underwent allogeneic HSCT was lower than that observed in other studies.[12,13] Thus, the improvement of non-transplant-related mortality in transplanted patients might have a positive impact on survival of ETP-ALL patients. Multivariate analyses showed that ETP-ALL was an independent adverse predictor for both overall survival and event-free survival [hazard ratio of 2.14 (95% confidence interval: 1.29- 3.53), *P*=0.003 and 1.91 (1.18-3.11), *P*=0.009, respectively] together with a high white blood cell count (*Online Supplementary Table S1*).

In summary, here we confirmed the previously reported frequency and clinico-biological features of adult ETP-ALL and showed that adult ETP-ALL patients should be considered as a group with a worse prognosis. The poorer outcome of ETP-ALL patients in our study was due to a lower response to induction therapy and not to an increased relapse rate, which highlights the need to investigate the potential utility of alternative induction therapy for these patients. The use of acute myeloid leukemia-oriented chemotherapy, together with intensification of chemotherapy regimens, has been effective in clearing disease in pediatric ETP-ALL cases refractory to initial induction chemotherapy.[17] A similar strategy could be applied to adult ETP-ALL patients. Based on these findings, and given the poor response attained in adults with the conventional induction regimen, it makes sense to explore the potential benefit of the FLAG-IDA regimen (idarubicin; fludarabine; ARA-C and granulocyte colony-stimulating factor), a more myeloid-oriented chemotherapy schedule, as front-line induction treatment together with allogeneic HSCT after standard consolidation. In addition, new therapies targeting specific genes altered in ETP-ALL patients, such as FLT3 inhibitors, have emerged as novel alternative treatment strategies. However, the potential benefit of this latter treatment is restricted to ETP-ALL patients carrying *FLT3* mutations in the tyrosine kinase domain (1/9, ETP-ALL cases in our cohort; *data not shown*). Similarly, other potential targeted therapies (e.g. Jak2 inhibitors or Bcl-2 antagonists) may also provide a benefit, but require further investigation.

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