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Adverse prognostic impact of complex karyotype (≥3 cytogenetic alterations) in adult T-cell acute lymphoblastic leukemia (T-ALL)

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ABSTRACT

The potential prognostic value of conventional karyotyping in adult T-cell acute lymphoblastic leukemia (T-ALL) remains an open question. We hypothesized that a modified cytogenetic classification, based on the number and type of cytogenetic abnormalities, would allow the identification of high-risk adult T-ALL patients. Complex

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karyotype defined by the presence of ≥3 cytogenetic alterations identified T-ALL patients with poor prognosis in this study. Karyotypes with ≥3 abnormalities accounted for 16 % (22/139) of all evaluable karyotypes, corresponding to the largest poor prognosis cytogenetic subgroup of T-ALL identified so far. Patients carrying karyotypes with ≥3 cytogenetic alterations showed a significantly inferior response to therapy, and a poor outcome in terms of event-free survival (EFS), overall survival (OS) and cumulative incidence of relapse (CIR), independently of other baseline characteristics and the end-induction minimal residual disease (MRD) level. Additional molecular analyses of patients carrying ≥3 cytogenetic alterations showed a unique molecular profile that could contribute to understand the underlying molecular mechanisms of resistance and to evaluate novel targeted therapies (*e.g.* IL7R directed) with potential impact on outcome of adult T-ALL patients.

1. Introduction

Genetic information in acute lymphoblastic leukemia (ALL) has long been obtained *via* conventional cytogenetics in clinical practice. Karyotyping performed at diagnosis in childhood and adult B-cell precursor ALL (BCP-ALL) patients has allowed to identify recurrent abnormalities with prognostic relevance [1–4]. This is particularly true for primary genetic events such as t(9;22)(q34;q11.2), t(4;11)(q21;q23), t(12;21)(p13;q22) or t(1;19)(q21;p13.3) [1–4], and numerical chromosomal changes like low hypodiploidy/near triploidy, high hyperdiploidy [1,3,5], and complex karyotypes (CK) [1–4]. In contrast, widely accepted classification based only conventional cytogenetics is still missing for T-ALL. Moreover, the cut-off set in ≥5 cytogenetic abnormalities to define CK in BCP-ALL, might not be appropriate for T-ALL, since in other leukemias such as acute myeloid leukemia (AML) and myelodysplastic syndromes, a different number of cytogenetic lesions is used for the definition of CK associated with adverse patient outcomes [6–8].

Few attempts have been made in T-ALL to assess the prognostic impact of conventional karyotyping, based on large cohorts of homogeneously treated patients. In a study from the Pediatric Oncology Group only a normal karyotype and t(10;14)(q24;q11) were associated with a better prognosis in childhood T-ALL [9]. In turn, CK defined according to the Moorman's criteria [1], was associated with a poorer overall survival (OS) in a large study (n = 356 adult cases) by the UKALL XII/ECOG Group [10]. Lastly, the Nordic study showed that only infrequent TCR rearrangements were associated with a poorer prognosis in pediatric T-ALL [11]. A major challenge in developing a cytogenetic classification with prognostic significance for T-ALL is the rarity of the disease. In addition, while many recurrent abnormalities are rather infrequent, others are better detected by genomic techniques, including some recurrent primary alterations in *TAL1*, *LMO2* and *TLX3* transcription factors, that have been associated with prognostic value in pediatric T-ALL patients [12].

Here we investigated the prognostic impact of cytogenetic data in a large series of 216 adult T-ALL patients treated within two consecutive minimal residual disease (MRD)-oriented trials from the Spanish PETHEMA (Programa Español de Tratamientos en Hematología) group. Our goal was to assess the potential usefulness of karyotypic data to identify high-risk adult T-ALL patients, and to further dissect the potential underlying molecular alterations.

2. Methods

2.1. Patient cohort

A total of 216 patients diagnosed with T-ALL according to the WHO criteria and treated within two consecutive MRD-oriented high-risk adults ALL protocols (ALL-HR-2003 [from 2003 to 2012, NCT00853008], [2011 to 2019, NCT01540812]) were analyzed. The immunological T-ALL subtypes were defined according to the European Group for the Immunological Characterization of Leukemias (EGIL) criteria [13], and the WHO [14] criteria was used to identify early T-cell precursor (ETP)-ALL, as previously described [15]. Treatment protocol schedules have been described elsewhere [16,17]. Briefly, in the ALL-HR-2003 trial the response to induction chemotherapy was evaluated by cytomorphology and flow cytometry. Good responders -<5 % blasts (cytologic CR), and MRD ≤ 0.1 %- proceeded to consolidation chemotherapy, and whenever a good MRD response was maintained (MRD ≤ 0.05 %) they followed maintenance chemotherapy treatment. Poor responders (> 5 % blasts and/or MRD ≥ 0.1 %) received intensification of induction treatment, followed by allogeneic-hematopoietic stem cell transplantation (Allo-HSCT). Poor responders after consolidation treatment (≥ 0.05 %) were also allocated to allo-HSCT. MRD assessment by flow cytometry was partially centralized in the ALL-HR-03 protocol. In the ALL-HR-11 trial the treatment allocation was exclusively based on fully centralized flow cytometry (MRD cut-off of ≤0.1 % for good responders after induction treatment and MRD cut-off of ≤0.01 % for good responders after consolidation treatment), using the EuroFlow guidelines, as previously described [18].

2.2. Karyotyping

Bone marrow (BM) and/or peripheral blood (PB) samples were processed at the institution-of-origin laboratories using standard cytogenetic methods, and karyotypes were reported according to the International System for Human Cytogenetic Nomenclature [19]. Cytogenetic data were compiled and centrally analyzed. Karyotypes with ≥15 metaphases without abnormalities were classified as normal karyotypes (NK), whereas cases with <15 normal metaphases or with no growth of leukemic cells were considered to be non-evaluable (NE). Any additional genetic data coming from FISH analysis, was not considered in the genetic classification. Monosomal karyotype (MK) was defined according to Breems et al. definition [20], and the number of karyotypic abnormalities was assessed according to the criteria of Chu et al. [21]. Conventional CK included cases with ≥5 unrelated chromosomal abnormalities [1] and hyperdiploidy was defined as >46 chromosomes without structural abnormalities.

2.3. Next generation sequencing (NGS)

T-ALL cases with available DNA obtained at diagnosis (n = 30) from the PETHEMA cohort, were analyzed by target deep sequencing (TDS) using a NGS panel (NGSp) designed at the Agilent SureDesing platform (Agilent Technologies, Santa Clara, CA), from which 5 DNA corresponded to patients with ≥3 CK and 25 to patients with <3 alterations. The genes included in the panel are listed in Supplemental Table 1. To increase genomic data of cases with ≥3 CK, we took advantage of the genomic data generated at the MLL Munich Leukemia Laboratory (Munich, Germany) and we included 38 T-ALL cases from the MLL cohort from which whole genome sequencing (WGS) data was available. Main patient characteristics are shown in Supplemental Table 2. We select adult patients in the same age range as the Spanish cohort [15;60]. We distributed them in cases with ≥3 CK or <3 alterations, similarly as we did for the PETHEMA cohort, taking only into account cytogenetic data. Briefly, WGS libraries were prepared with the TruSeq PCR free library prep kit. Hundred-fifty base pair (bp) paired-end sequences were generated on a NovaSeq 6000 or HiSeqX instrument (Illumina, San Diego, CA) with 100x coverage. NGSp libraries were prepared with the

SureSelect XT HS (Agilent technologies) chemist. Pooled libraries were sequenced 2 × 75bp paired-end sequences on a Miseq instrument (Illumina) at 300X mean depth. Fastq files from WGS and TDS were aligned to the hg19 reference genome. The Illumina tumor/unmatched normal workflow was used for the WGS variant calling. Here, a mixture of genomic DNA from multiple anonymous donors (n = 6) was used as normal controls. TDS variant calling was performed using Samtools4 (version 1.10, http://www.htslib.org/) and VarScan2 (version 2.4.0, https://dkoboldt.github.io/varscan/). Exonic and splice-site variants obtained by WGS and TDS were annotated using ANNOVAR (version 2018-04-16, http://annovar.openbioinformatics.org/en/latest/). Polymorphisms described in population databases (1000Genomes, ExAC, gnomAD and Exome Variant Server) with a calculated mean population frequency > 0.1 % were excluded from further analyses. Annotated variants were selected after filtering out calls according to the following criteria: coverage <30X, <8 alternative reads or variant allelic fraction (VAF) <5 %. Finally, selected variants were annotated using DBNSFP *in silico* predictors (SIFT, Polyphen2, LRT, MutationTaster, MutationAssessor, FATHMM, PROVEAN, VEST3, MetaSVM and MetaLR), excluding benign predicted variants (>5 predictor scores considering a variant as benign).

2.4. Outcome variables and statistical analysis

Overall survival (OS) was measured from the time of diagnosis to the time of death or last follow-up. Event-free survival (EFS) was calculated from diagnosis to last follow-up, considering failure, relapse or death by any cause as events. Cumulative incidence of relapse (CIR) was measured from first CR date to relapse or last follow-up, being non-relapse mortality the competing event. Main demographic, clinical, laboratory and genetic characteristics were described. Comparisons between groups were performed by the chi-square test or Fisher's exact test (categorical variables) and the median test (numerical variables). EFS and OS curves were plotted by the Kaplan-Meier method and were compared by the log-rank test. Estimates of CIR were calculated using cumulative incidence functions and compared using Gray's test. Multivariable analyses were performed using the Cox proportional hazards model for OS and EFS, and the Fine and Gray model for CIR. The significance level was set at P < .05. All statistical analyses were carried out with the SPSS package v24.0 and the R 3.6.3 software.

3. Results

From a total of 216 treated adults, 207 were included in the study (100 from the ALL-HR-2003 and 107 from the ALL-HR-11 trials). Lack of karyotype (n = 5), lack of follow-up (n = 2), protocol violation (n = 1) and T-cell lymphoma (n = 1) were the reasons of exclusion. Detailed patient characteristics at diagnosis and at follow-up are described in Table 1. Overall, 74/207 (36 %) showed a normal karyotype (NK) and 68/207 (33 %) had non-evaluable (NE) cytogenetic results. Patients with abnormal karyotypes (65/139, 47 %) were grouped as follows: 1) structural abnormalities (SA) only, (38/139 [29 %]); 2) structural + numerical abnormalities (SNA) (21/139 [15 %]; and 3) hyperdiploidy only (6/139 [4 %]). In addition, karyotypes were classified according to classical CK (≥5 abnormalities, 10/139 [7 %]) and MK (10/139 [7 %]). Chromosome 14 was the most frequently lost chromosome (n = 3) and no cases only showing chromosomal losses were detected in our cohort. The percentages of NK, NE karyotypes, and CK, were similar to those reported by others [10]. Previously reported primary cytogenetic alterations involving T-ALL-associated transcription factors such as *TLX1/TLX3* and *LMO* [22–25] were also identified at very low frequencies (Table 1). Of note, patients carrying the *TLX1/TLX3* and *LMO2* alterations belonged to the SA category and the genetic event (rearrangement) was mostly found as unique abnormality or it was associated with another single alteration. In turn, 7/11 (64 %) of cases with del(6q) were found as a single or as a partner alteration. In parallel, karyotypes were also classified according to the number of chromosomal alterations, independently of their nature (Supplemental Table 3).

Table 1
Demographic, clinical, laboratory and genetic features of adult T-ALL patients included in this study.

	Patient distribution
Clinical and laboratory features	
Age (yrs)*	32 [15; 60]
Female/male ratio	54/153
Lymph node enlargement	109/183 (60 %)
Splenomegaly	72/201 (36 %)
Hepatomegaly	47/198 (24 %)
Mediastinal mass	96/205 (47 %)
WBC count ($x10^9$/L)*	40.8 [0.6; 842]
CNS involvement	20/198 (10 %)
Immunophenotype	
Unclassified	19 (9 %)
ETP-ALL	35 (17 %)
Pre-T	35 (17 %)
Cortical	79 (38 %)
Mature	39 (19 %)
T-ALL genetic hallmarks	
TLX1	3/207 (1.5 %)
TLX3	1/207 (0.5 %)
TAL1	4/207 (2 %)
LMO2	2/207 (1 %)
KMT2A rearranged	3/207 (2 %)
TCR rearranged	1/207 (0.5 %)
del(6q)	11/207 (5 %)
CDKN2A/B gene deletion	31/53 (58 %)
TCRG gene deletion	24/31 (77 %)
Treatment	
Chemotherapy	97/139 (70 %)
Allo-HSCT	42/139 (30 %)
Response to treatment	
Slow response at day +14	90/186 (48 %)
Nº of induction cycles to CR:	
One induction	156/187 (83 %)
Two inductions	31/187 (17 %)
CR	187/207 (90 %)
MRD day +35 < 0.1 %	125/162 (77 %)
MRD day +35 < 0.01 %	90/154 (58 %)

Results expressed as number of cases (percentage) or * as a median [range]. Specific alterations detected included: *TLX1,* 3 cases with t(10;14); *TLX3* 1 case with t(5;7)(q35;q21); *TAL1* 1 case with t(1;14)(p32;q21) and t(1;12)(p32;q13), and 2 cases with del(1)(p32); *LMO2,* 2 cases with t(11;14)(p13;q11).
MRD values were considered only for those patients that reached CR. Yrs: years; WBC: white blood cells; CNS: central nervous system; ETP-ALL: early T-cell precursor acute lymphoblastic leukemia; CR: complete remission; MRD: minimal residual disease; d+14: fourteen days after starting induction treatment; d+35: thirty-five days after starting induction treatment; Allo-HSCT: allogeneic hematopoietic stem cell transplantation.

Based on the above main cytogenetic subgroups, we investigated the potential clinical impact of this cytogenetic classification on patient outcome (OS, EFS and CIR). Overall, patients with NE karyotype had a very similar outcome to cases with NK; in contrast, the outcome of NE and NK cases was clearly better than that of patients with an abnormal karyotype in terms of EFS, but not of OS and CIR (Supplemental Fig. 1). These findings suggest the absence of poor prognosis karyotypes among NE cases. Despite this, cases with NE karyotypes were excluded from further prognostic analyses. In such analyses, specific cytogenetic subgroups such as MK and CK (≥5 cytogenetic abnormalities) were associated with poor OS (3y prob [CI 95 %] of 15 % [0 %; 40 %] for MK *vs* 51 % [41 %; 61 %] for non-MK cases, p = 0·012; and of 13 % [0 %; 36 %] for ≥5 CK *vs* 51 % [41 %; 61 %] for other cases with <5 chromosomal alterations, p = 0·012), EFS (1y prob [CI 95 %] of 40 % [10 % ; 70 %] for MK *vs* 64 % [55 %; 73 %] for non-MK, p=<0·001; and of 40 % [10 %; 70 %] for ≥5 CK *vs* 64 % [55 %; 73 %] for those with <5 cytogenetic alterations, p = 0·004), and CIR (1y prob [CI 95 %] of 88 % [6 %; 99 %] for MK *vs* 43 % [33 %; 53 %] for non-MK, p = 0·001; and of 56 % [16 %; 83 %] for ≥5 CK *vs* 45 % [34 %; 55 %] for those with <5 chromosomal

changes, p = 0·013), as previously reported by others [3,10]. Patients with MK and CK (≥5 abnormalities) were both included in the large SNA cytogenetic group (15 % T-ALL cases) and retained their adverse clinical outcome when compared to non-SNA patients (Supplemental Fig. 2). In contrast, hyperdiploidy and SA did not show an adverse impact on T-ALL patient outcome (data not shown). Further investigation of the prognostic impact of the number of chromosomal alterations independently of their nature, showed that the presence of ≥3 cytogenetic abnormalities (≥3 CK) in our adult T-ALL patients, already conferred a poorer prognosis in terms of OS, EFS and CIR, similar to that of cases showing a classical CK with ≥5 cytogenetic alterations (Fig. 1 and Supplemental Table 3). Of note, karyotypes with ≥3 abnormalities accounted for 16 % of all evaluable karyotypes (22/139), which would represent the largest poor prognosis cytogenetic subgroup identified so far in T-ALL. All cases (n = 4) in which chromosomal rearrangements or deletion involving the *TAL1* transcription factor were detected, were included in this category, in addition to patients with MK and CK (≥5 abnormalities) (Supplemental Table 4). Other variables than the new ≥3 CK cytogenetic subgroup, the MK, ≥5 CK and SNA cytogenetic category, that showed a significant impact on patient outcome, included treatment protocol, age, white blood cell (WBC) count, and ETP-ALL (Table 2). Multivariate analysis confirmed the independent adverse prognostic value of the presence of ≥3 cytogenetic abnormalities at diagnosis, for all the three prognostic end-points evaluated (Table 2). Importantly, karyotypes with ≥3 abnormalities retained their adverse prognostic impact also when MRD data obtained at the end of induction therapy (day+35) were included in the multivariate analyses (Table 3). Of note, in our cohort having ≥3 CK was the only risk factor, together with age, with an independent predictive value to identify at diagnosis patients that will relapse (Table 2). In turn, the ≥3 CK together with the WBC count at diagnosis and MRD status at day+35, were the only independent predictors for OS and EFS (Tables 2 and 3). Other variables such as the ETP immunophenotype did not prove to be independent predictors of patient outcome in the multivariate analysis. No relationship was found between ≥3 CK and other patient characteristics such as age, WBC count, CNS involvement and ETP-ALL.

In order to analyze the underlying reasons for the poor prognosis of ≥3 CK in adult T-ALL, we investigate the potential association of ≥3 CK with response to therapy. Despite the similar initial response to therapy (day+14) between ≥3 CK and other T-ALL cases (p = 0·167), ≥3 CK patients showed a significantly lower end-induction CR rate (77 % *vs.* 94 % for the remaining T-ALL, p = 0·032). Of note, 2/5 ≥ 3 CK patients who did not reach CR at the end of induction therapy died due to toxicity whereas the other 3/5 did not continue the protocol because of refractory disease. Among those patients that achieved CR, a similar number of induction cycles were administered for ≥3 CK *vs* other T-ALL cases. Consistent with the lower CR rate observed for ≥3 CK patients, lack of end-induction MRD response (>0.01 %) was more frequently observed among patients with ≥3 CK than the remaining patients (65 % *vs.* 40 %, p = 0·06). However, no differences in the frequencies of chemotherapy or allo-HSCT allocation at the end of consolidation therapy were observed on comparison of patients within ≥3 CK with the remaining.

In order to better understand the molecular mechanisms underlying the poorer outcome of ≥3 CK patients, molecular studies by NGS were performed. T-ALL cases with available DNA obtained at diagnosis (n = 30) from the PETHEMA cohort, were analyzed by TDS. To increase genomic data of cases with ≥ 3 CK, we included additional 38 T-ALL cases from the MLL cohort from which WGS data was available. Selection criteria of the T-ALL cases from the MLL cohort included in the study and homogenization and standardization of NGS data analysis is described in material and methods section. Overall, we observed that the mutational profile identified in the T-ALL patients analyzed was similar to those previously reported by others (Fig. 2) [26,27]. Interestingly,

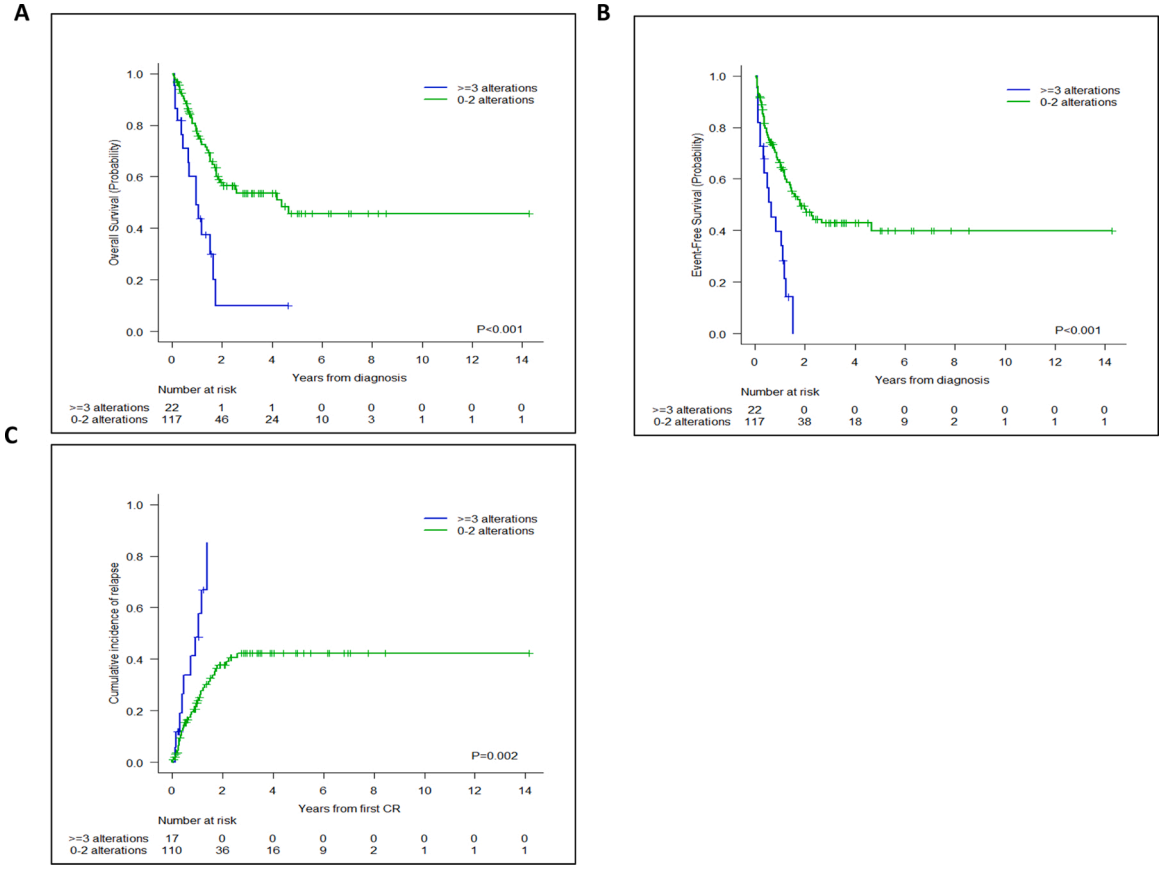

Fig. 1. Impact of karyotypes with ≥3 *vs* 0-2 cytogenetic alterations for OS (A), EFS (B) and CIR (C) at 3-year probability in the PETHEMA cohort.

Table 2
Univariate and multivariate analysis of prognostic factors for OS, EFS and CIR in adult T-ALL patients.

	N (positive/ evaluable)	OS HR (95 %CI)	P value	N (positive/ evaluable)	EFS HR (95 %CI)	P value	N (positive/ evaluable)	CIR HR (95 %CI)	P value
Univariate analysis									
ALL-HR2003 protocol	100/207	1.62 (1.05;2.5)	0.028	100/207	1.43 (0.97;2.09)	0.069	89/187	1.18 (0.73;1.89)	0.500
Age*	207	1.01 (0.99;1.02)	0.456	207	0.99 (0.98;1.01)	0.897	/87	0.97 (0.95;0.99)	0.022
WBC count (x10^9/ L)*	207	1.002 (1.000;1.004)	0.028	207	1.002 (1.000;1.003)	0.032	187	1.001 (0.999;1.003)	0.480
CNS involvement	20/198	1.16 (0.56;2.41)	0.681	20/198	1.24 (0.66;2.3)	0.498	16/178	1.08 (0.47;2.48)	0.850
ETP-ALL	35/188	1.83 (1.11;3.01)	0.018	35/188	1.67 (1.05;2.66)	0.029	27/170	1.27 (0.69;2.33)	0.440
CK (≥3 alterations)	22/139	3.04 (1.67;5.51)	<0.001	22/139	2.96 (1.68;5.2)	<0.001	17/127	2.76 (1.41;5.4)	0.003
CK (≥5 alterations)	10/139	2.52 (1.19;5.31)	0.016	10/139	2.72 (1.34;5.54)	0.006	9/127	2.57 (1.05;6.3)	0.038
MK	10/139	2.53(1.19;5.35)	0.015	10/139	3.23 (1.64;6.35)	0.001	8/127	3.67 (1.73;7.77)	<0.001
SNA	21/139	2.30 (1.27;4.19)	0.006	21/139	3.01 (1.75;5.17)	<0.001	17/127	3.23 (1.81;5.75)	<0.001
SA	38/139	1.23 (0.72;2.09)	0.441	38/139	1.14 (0.69;1.82)	0.595	35/127	0.95 (0.50;1.79)	0.860
Multivariate analysis									
ALL-HR2003 protocol	–	–	NS (0.75)	–	–	NS (0.29)	–	NA	NA
Age*	–	NA	NA	–	NA	NA	–	0.97 (0.94;0.99)	0.032
WBC count (x10^9/ L)*	–	1.003 (1.001;1.005)	0.001	–	1.004 (1.002;1.005)	<0.001	–	NA	NA
ETP-ALL	–	–	NS (0.33)	–	–	NS (0.33)	–	–	–
CK (≥3 alterations)	–	3.37 (1.76;6.46)	<0.001	–	3.43 (1.86;6.31)	<0.001	–	2.91 (1.48;5.5)	0.002

* Age and WBC were considered as continuous variables. HR: Hazard ratio; CI: confidence interval; OS: overall survival; EFS: event-free survival; CIR: cumulative incidence of relapse; WBC: white blood cell; ETP-ALL: early T-cell precursor ALL; CNS: central nervous system; CK: complex karyotype; SNA: structural + numerical abnormalities; SA: only structural alterations.

Table 3
Univariate and multivariate analysis of prognostic factors for OS and CIR in adult T-ALL patients including end-induction MRD level.

	N (positive/evaluable)	OS HR (95 %CI)	P value	N (positive/evaluable)	CIR HR (95 %CI)	P value
Univariate analysis						
ALL-HR2003 protocol	89/187	1.72 (1.05;2.82)	0.031	89/187	1.18 (0.73;1.89)	0.500
Age*	187	0.99 (0.98;1.02)	0.760	187	0.97 (0.95;0.99)	0.022
WBC count (x10^9/L)*	187	1.002 (1.000;1.004)	0.054	187	1.001 (0.999;1.003)	0.480
CNS involvement	16/178	0.98 (0.39;2.43)	0.960	16/178	1.08 (0.47;2.47)	0.850
ETP-ALL	27/170	1.47 (0.79;2.70)	0.219	27/170	1.27 (0.69;2.33)	0.440
CK (≥3 alterations)	17/127	2.64 (1.30;5.36)	0.007	17/127	2.76 (1.41;5.38)	0.003
CK (≥5 alterations)	9/127	2.81 (1.26;6.27)	0.012	9/127	2.57 (1.05;6.30)	0.038
MK	8/127	2.53 (1.07;5.96)	0.034	8/127	3.67 (1.73;7.78)	<0.001
SNA	17/127	2.07 (1.03;4.16)	0.040	17/127	3.23 (1.81;5.75)	<0.001
SA	35/127	1.32 (0.74;2.37)	0.348	35/127	0.95 (0.50;1.79)	0.860
MRD day +35 (≥0.1 %)	37/162	1.94 (1.11;3.37)	0.019	37/162	1.36 (0.76;2.44)	0.300
MRD day +35 (≥0.01 %)	64/154	2.30 (1.30;4.08)	0.004	64/154	1.20 (0.71;2.05)	0.490
Multivariate analysis						
ALL-HR2003 protocol	–	–	NS (0.316)	–	NA	NA
Age*	–	NA	NA	–	0.97 (0.94;0.99)	0.032
WBC count (x10^9/L)*	–	1.005 (1.002;1.009)	0.001	–	NA	NA
ETP-ALL	–	–	NS (0.807)	–	–	–
CK (≥3 alterations)	–	4.17 (1.79;9.69)	0.001	–	2.91 (1.48;5.70)	0.002
MRD day +35 (≥0.01 %)	.	2.27 (1.12;4.60)	0.023	–	NA	NA

* Age and WBC were considered as continuous variables. HR: Hazard ratio; CI: confidence interval; OS: overall survival; CIR: cumulative incidence of relapse; WBC: white blood cell; ETP-ALL: early T-cell precursor ALL; CNS: central nervous system; CK: complex karyotype; SNA: structural + numerical abnormalities; SA: only structural alterations; MRD: minimal residual disease.

three distinct genetic signatures were identified manually, when comparing ≥3 CK *vs.* <3 CK patient groups. Thus, there were genes that were not differentially mutated in ≥3 CK *vs* other T-ALL cases. These included mutations in the *NOTCH1/FBXW7* signaling pathway (53 % for ≥3 CK *vs.* 67 % for other T-ALL patients; p = 0·31) and in *PHF6* (35 % for ≥3 CK *vs.* 22 % for other T-ALL cases; p = 0·26) gene. A subset of genes that was found to be non-mutated in ≥3 CK patients while mutated in other T-ALL cases, such as genes involved in the *JAK1/3/AKT1* signaling pathway (0 % for ≥3 CK *vs.* 25 % for other T-ALL cases, p = 0·02) and genes related to DNA methylation, *e.g.* the

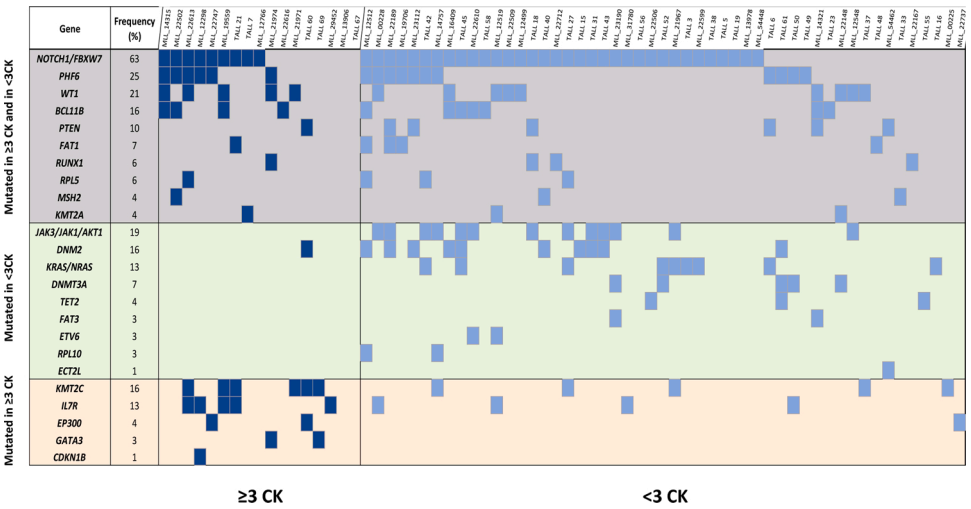

Fig. 2. Mutational spectrum of ≥3 CK and <3 CK adult T-ALL patients. Genomic data from the PETHEMA (n = 30) and MLL (n = 38) cohorts are shown together. Global mutational frequency for each gene is shown on the left. Genes are grouped according the three signatures identified and ordered by frequency. Each colored square indicates a positive mutation for the indicated gene. Blue dark squares identify patients with ≥3 abnormalities. Light blue squares identify patients <3 CK.

DNMT3A (0 % for ≥3 CK *vs.* 10 % among other T-ALL cases: p = 0·18) and *TET2* (0 % for ≥3 CK *vs.* 6 % for other T-ALL patients; p = 0·31) gene. These genes have been previously associated with an immature T-ALL immunophenotype [28]. Finally, a third group of genes that were mutated at greater frequencies in ≥3 CK patients *vs* other T-ALL cases, including the *KMT2C* (35 % *vs* 10 %, respectively; p = 0·01), *IL7R* (29 % *vs* 8 %, respectively; p = 0·02) and *GATA3* (12 % *vs* 0 %, respectively; p = 0·01) genes.

4. Discussion

Conventional cytogenetics is still a widely used technique in the diagnostic of ALL for the fast and robust identification of Ph^+ ALL or *KMT2A/AFF1* patients with important therapeutic consequences. However, with the irruption of high-throughput genomic techniques, the usefulness of conventional cytogenetics has been questioned, particularly for T-ALL patients, due to the low number of cases with informative abnormalities (30 %) and the lack of a widely-accepted standard cytogenetic classification. This, together with the low prevalence of patients with T- *vs* BCP-ALL, has translated into a very limited information on the prognostic value of karyotyping, particularly among adult T-ALL.

The prognostic impact of conventional cytogenetics was assessed in a large cohort of adult T-ALL patients treated with two sequential trails from the PETHEMA group. Our results show for the first time, that karyotype is a strong independent prognostic marker that can be obtained already at diagnosis to predict outcome of adult-TALL patients. Specifically, the presence of ≥3 cytogenetic alterations was the most informative adverse prognostic factor for OS, EFS and CIR when T-ALL karyotypes were classified according to the number of cytogenetic alterations, using a well standardized method [21]. Karyotypes with ≥3 abnormalities accounted for 16 % of all evaluable karyotypes, thereby representing the largest poor prognosis cytogenetic subgroup identified so far in T-ALL. However, it should be pointed out that the classification of adult T-ALL patients from this series according to previously defined primary cytogenetic events (gene rearrangements), was not possible due to the low number of cases in each of these subgroups (*i.e.* t(10;14); t (5;7)(q35;q21); t(1;14)(p32;q21); t(1;12)(p32;q13); del(1)(p32) or t (11;14)(p13;q11)). Underrepresentation of the primary genetic events in our cohort might be due to differences in the prevalence of these alterations in adult *vs.* childhood T-ALL, to the locally performed conventional cytogenetics and to the lower sensitivity of cytogenetics *vs,* molecular techniques, among other reasons.

Despite the above limitations, our results show that compared to BCP-ALL, a lower genetic complexity is required in adult T-ALL patients to define adverse complex karyotypes. Thus, complex karyotype with ≥3 alterations is a marker of inferior outcome in terms of OS, EFS and CIR, independently of other prognostic features such as age, WBC count at diagnosis and the MRD status after induction therapy. The adverse prognostic impact of ≥3 CK together with age were the only independent predictors for an impending relapse after starting therapy, ruling out the predictive value of MRD to identify patients with early relapse. This is probably due to the fact that while MRD could identify those patients at lower (MRD negative) *vs* high (MRD positive) risk of relapse, ≥3 CK discriminated among the MRD positive patients those at risk of earlier recurrence of the disease. These results are of clinical relevance because in contrast to MRD results, karyotypic data are already available at diagnosis, which facilitates early risk stratification of the disease for early risk-adapted treatment (intensification) decisions.

In order to better understand the mechanisms underlying the poorer prognosis of patients with ≥3 CK we investigated the reasons for their dismal outcome and the associated gene mutational profiles. Patients with ≥3 CK showed a significantly higher rate of refractoriness to induction therapy used in the two PETHEMA protocols, together with a poorer clearance of blast cells, as reflected by lower rates of both CR and MRD negativity after induction therapy. Treatment intensification by allo-HSCT was not more prevalent in patients with ≥3 CK, possibly due to premature exclusion of these patients due to refractoriness or early relapse. Treatment resistance and tumor genetics have both been recurrently associated with specific gene mutational profiles in ALL [29, 30]. Of note, genomic analysis performed combining NGS data revealed the presence of unique mutational profiles among ≥3 CK cases *vs* other T-ALL patients. These profiles consisted in a predominance of mutations in the *KMT2C, GATA3* and *IL7R* genes, and absence of mutations involving genes of the *JAK1/3/AKT1* signaling pathway and genes related to DNA methylation, such as the *DNMT3A* and *TET2*. Such mutational profile might contribute to explain the poorer outcome for ≥3 CK patients. As an example, activating mutations in *IL7R*, confer glucocorticoid resistance and poor clinical outcome in childhood T-ALL [31]. Thus, IL7R works as biomarker of reduced steroid response, which could contribute to explain, at least in part, the increased resistance to treatment and the poorer blast clearance observed in patients with high genetic complexity (≥3 CK). In this sense, a recent report from the GRAALL cooperative group showed that patients with IL7Rp mutated T-ALL were slow-responders, with a high level of MRD in day-8 bone marrow, compared to unmutated cases, although no correlation was observed with response to prednisone [32].

In summary, here we propose a definition for CK in adult T-ALL based on the presence of ≥3 cytogenetic alterations, with important implications for improved risk stratification at diagnosis. In addition, we have shown that patients with ≥3 CK carry unique gene mutational profiles, that might benefit in the future from novel targeted therapies directed against the mutated genes (*e.g.* IL7R). Further studies in large series of adult T-ALL patients treated with similar and novel therapies are necessary to confirm our results.

Contributors

E. Genescà, designed the study and wrote the manuscript. M. Morgades performed statistical analysis. C. González-Gil and F. Fuster performed NGSp sequencing and analysis. M.M. performed WGS sequencing and analysis. E. Genescà and F. Solé, performed cytogenetic central review and karyotype analysis. E. Genescà, M. Morgades, I. Granada, F. Solé and J.M. Ribera analyzed data. P. Montesinos, P. Barba, C. Gil, R. Coll, M.J. Moreno, D. Martínez-Carballeira, I. García-Cadenas, S. Vives, J. Ribera, J.González-Campos, M. Díaz-Beya, S. Mercadal, M.T. Artola, A. Cladera, M. Tormo, A. Bermúdez, F. Vall-llovera, P. Martínez, M.L. Amigo, S. Monsalvo, A. Novo, M. Cervera, A. García, J. Cervera and JM Hernández-Rivas provided clinical data and C. Haferlach, M. Meggendorfer and T. Haferlach, F.Solé, A. Orfao and JM Ribera reviewed the manuscript. All authors have read and approved the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2021.106612.

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