Gene expression profiling identifies a subset of adult T-cell acute lymphoblastic leukemia with myeloid-like gene features and over-expression of miR-223

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ABSTRACT

Background
Until recently, few molecular aberrations were recognized in acute lymphoblastic leukemia of T-cell origin; novel lesions have recently been identified and a certain degree of overlap between acute myeloid leukemia and T-cell acute lymphoblastic leukemia has been suggested. To identify novel T-cell acute lymphoblastic leukemia entities, gene expression profiling was performed and clinico-biological features were studied.

Design and Methods
Sixty-nine untreated adults with T-cell acute lymphoblastic leukemia were evaluated by oligonucleotide arrays: unsupervised and supervised analyses were performed. The up-regulation of myeloid genes and miR-223 expression were validated by quantitative polymerase chain reaction analysis.

Results
Using unsupervised clustering, we identified five subgroups. Of these, one branch included seven patients whose gene expression profile resembled that of acute myeloid leukemia. These cases were characterized by over-expression of a large set of myeloid-related genes for surface antigens, transcription factors and granule proteins. Real-time quantitative polymerase chain reaction analysis confirmed over-expression of *MPO*, *CEBPA*, *CEBPB*, *GRN* and *IL8*. We, therefore, evaluated the expression levels of miR-223, involved in myeloid differentiation: these cases had significantly higher levels of miR-223 than had the other cases of T-cell acute lymphoblastic leukemia, with values comparable to those observed in acute myeloid leukemia. Finally, these patients appear to have an unfavorable clinical course.

Conclusions
Using gene profiling we identified a subset of adult T-cell acute lymphoblastic leukemia, accounting for 10% of the cases analyzed, which displays myeloid features. These cases were not recognized by standard approaches, underlining the importance of gene profiling in identifying novel acute leukemia subsets. The recognition of this subgroup may have clinical, prognostic and therapeutic implications.

Key words: T-ALL, molecular aberrations, acute myeloid leukemia.

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The online version of this article has a Supplementary Appendix.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of immature T cells, present in about 15% of children and 25% of adults.[1] While much is known on the biology of B-lineage acute lymphoblastic leukemia, until recently few molecular abnormalities were clearly recognized in T-ALL, with knowledge being mostly limited to aberrations involving the T-cell receptor.[2] The scenario is changing dramatically in this disease, since novel lesions are being ever more frequently recognized and will, most probably, allow therapeutic strategies to be tailored by targeting specific pathways. The most common aberrations include over-expression of *TLX1* and *TLX3* oncogenes;[3-5] both transcripts have prognostic significance, with *TLX1* being associated with a favorable outcome[6,7] and *TLX3* with a worse outcome,[8-10] although some exceptions have been reported.[11,12] The most frequent of the recognized mutations is *NOTCH1*,[13] found in roughly 50% of cases of T-ALL and generally associated with other aberrations, including *FBXW7* (detected in 8-16% of cases).[14,15] Other mutations include *JAK1*, *WT1* and *PTEN* reported to occur in 3-20%,[16-18] 8-13%[19] and 8%[20] of cases, respectively. Besides *NOTCH1* and *JAK1*, whose prognostic roles have, overall, been established,[16,21-24] the other mutations do not appear to affect outcome. Rearrangements include *SIL/TAL1*, *NUP214/ABL1*, *EML/ABL1*, *CALM/AF10* and *TAF_I-NUP214*;[25-29] finally, amplifications and deletions have been documented, the most frequent involving *MYB*, *CDKN2A* and *RB1*.[30-32]

The identification of these aberrations has led to the pinpointing of cellular functions that are specifically deregulated, such as cell cycle control, cell differentiation, proliferation and/or survival and self-renewal properties.[33] In this context, gene expression profiling (GEP) has previously proven useful in identifying signatures that are strictly associated with the over-expression of known oncogenes, with the stage of differentiation of the leukemic cell, with the recognition of novel subgroups, as well as with outcome.[34,35] Furthermore, GEP has been useful in showing that different genetic lesions may merge in the deregulation of the same pathways, a good example being the *HOXA* cluster genes.[28,29,36,37] A broad use of GEP has also enabled the identification of distinct subsets of patients and the recognition of similarities between different subsets and/or diseases. In acute myeloid leukemia (AML), Wouters *et al.* have recently described a subset of cases whose genomic profile is similar to that of patients with *CEBPA* mutations:[38] these patients are characterized by the over-expression of a set of T-lineage genes, *NOTCH1* mutations and hypermethylation of the *CEBPA* promoter, suggesting that *CEBPA* hypermethylation, together with *NOTCH1* mutations, may lead to a mixed T-lineage/myeloid lineage leukemia.

Another area of research that is rapidly evolving is the identification of microRNA (miR), small RNA molecules whose main role is negative regulation of mRNA function at the post-transcriptional level.[39] Among these, miR-223 has been shown to regulate myeloid differentiation[40,41] and, more in particular, granulocytic differentiation; in fact, its expression regulates a microcircuit involving both *CEBPA* and *NFIA*, ultimately inducing differentiation.

In this study, we used GEP to evaluate 69 newly diagnosed cases of adult T-ALL and identified a subgroup of cases, representing 10% of our cohort, characterized by the over-expression of a large set of myeloid genes. Detailed molecular characterization, including molecular analysis and miR quantification, was carried out in these samples.

Design and Methods

Patients

Fifty-two adult patients with a diagnosis of T-ALL were evaluated by GEP within the first phase of the Microarray Innovations in Leukemia (MILE) project[42] and 17 adult T-ALL patients were subsequently included in the second phase of the same study. Patients were enrolled in the *Gruppo Italiano Malattie Ematologiche dell'Adulto* (GIMEMA) protocols 2000 and 0904, and were evaluated prior to any chemotherapy. Of the total of 69 patients, 52 were males and 17 were females; their median age was 31 years (range, 15-55 years). Immunophenotypic analysis, according to the EGIL classification,[43] revealed that 3 cases were pro-T, 13 pre-T, 32 cortical and 4 mature T-ALL; in the remaining cases, the immunophenotype was not further classified. Molecular screening analysis was performed according to the reverse transcriptase polymerase chain reaction (RT-PCR) multiplex protocol described by Elia *et al.*, which includes screening for *ALL1* rearrangements, *SIL/TAL1* and *NUP98/RAP1GDS1*, *BCR/ABL* p210 and p190, and, more recently, *TAF_I-NUP214*.[44]

Molecular biology studies showed the presence of a fusion transcript in 19 patients. The detailed characteristics of the patients are summarized in Table 1. In selected cases, molecular analysis was also performed to evaluate the presence of rearrangements detected in AML, namely *PML/RARA*, *AML1/ETO*, *MLL* self-fusion, *CBFB/MYH11*, *DEK/CAN*, *FLT3-ITD*, and *BCR/ABL* p210 and p190.[45]

This study was approved by our Institutional Review Board. All patients gave their informed consent to collection of blood or bone marrow and to the biological analyses included in the present study, in agreement with the Declaration of Helsinki.

RNA extraction

Leukemic cells were obtained from either bone marrow or peripheral blood of patients with newly diagnosed ALL: the percentage of leukemic cells was at least 80% in all cases, in order to minimize contamination with non-leukemic cells.

Total RNA was extracted either by using Trizol reagent (Life Technologies, Grand Island, NY, USA) and further purified with the SV total isolation system (Promega, Madison, WI, USA), according to the manufacturer's instructions with minor modifications, or by using the RNeasy mini kit (Qiagen, Hilden, Germany).

For miR-223 quantification, total RNA was extracted using Trizol reagent followed by isopropanol precipitation. No further purification was performed in order to avoid loss of small RNA molecules. The quality of total RNA was checked by agarose gel electrophoresis and RNA concentration was determined by measuring the absorbance at 260 nm; for all samples, the 260/280 ratio was greater than 1.8, as required for microarray analysis.

Gene expression profiling and statistical analysis

HGU133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA, USA) were used for GEP. First strand cDNA was synthesized from 5 μg total RNA using T7-$(dT)_{24}$ primers and reverse transcribed with the Roche Applied Science Microarray CDNA Synthesis kit (Mannheim, Germany); after the second strand cDNA synthesis, the product was used in an *in vitro* transcription reaction (Roche Applied Science Microarray RNA Target Synthesis (T7) kit) to gen-

erate biotinylated complementary RNA (cRNA). Eleven micrograms of fragmented cRNA were hybridized on microarrays for 16 h and the gene chips were subsequently washed, stained and scanned.

Gene expression data were analyzed using dChip software (*www.dchip.org*, Dana-Farber Cancer Institute, Boston, MA, USA), which employs an invariant set normalization method in which the array with median overall intensity is chosen as the baseline for normalization. Model-based expressions were computed for each array and probe set using the *perfect match/mismatch* model.[46]

Unsupervised clustering was performed as described by Eisen *et al.*[47] and the distance between two genes was computed as 1 minus the correlation between the standardized expression values across samples. Non-specific filtering criteria for unsupervised clustering were defined as follows: gene expression level was required to be higher than 200 in more than 5% of the samples and the ratio of standard deviation to the mean expression across all samples was required to be between 0.5 and 1000.

Analysis of variance (ANOVA), with *P* values of 0.0001 or less, was performed to compare the three most homogeneous subgroups of T-ALL patients identified by unsupervised analysis, i.e. those with *HOXA* over-expression, patients with *SIL/TAL1* and patients with a 'myeloid-like' profile. In each group, genes with a mean expression value of 300 or more and a fold change difference of 1.5 or more compared to any other group were retained. Finally, principal component analysis was performed using the top 100 genes (ordered by their *P* values) from the ANOVA analysis.

Real-time quantitative polymerase chain reaction

cDNA was generated from 1 μg of total RNA using the Advantage RT-for-PCR Kit (Clontech, CA, USA). Real-time quantitative polymerase chain reaction (Q-PCR) analysis was performed using the ABI PRISM 7300 Sequence Detection System and the SYBR green dye method (Applied Biosystems, CA, USA) was applied as previously described.[48] For each sample, *GAPDH* C_T values were used for normalization purposes. For each gene, expression levels were computed as the difference (ΔC_T) between the target gene C_T and *GAPDH* C_T. The $\Delta\Delta C_T$ method was then applied,[49] using a $CD2^+$ healthy control for calibration purposes. A $CD2^+$ healthy control was chosen since our main goal was to highlight differences between "myeloid-like" T-ALL and the remaining cases of ALL; furthermore, there was no variability among the $CD2^+$ samples from healthy donors. The following primers were used for the evaluation of *GAPDH*: forward, 5'-CCACCCATGGCAAATTCC-3'; reverse, 5'-GATGGGATTTC-CATTGATGACA-3'. The primers for *CEBPA*, *CEBPB*, *MPO*, *GRN* and *IL8* were as follows: CEBPA forward, 5'-AAGAAGTCGGTGGACAAGAACAG-3', reverse, 5'-GCGGT-CATTGTCACTGGTCA-3'; CEBPB forward, 5'-GCC-CTCGCAGGTCAAGAG-3', reverse 5'-TGCGCACGGCGAT-GT-3'; MPO forward 5'-GTGGCATTGACCCCATCCT-3', reverse 5'-GATCTCATCCACTGCAATTTGGT-3'; GRN forward 5'-CAGAGTAAGTGCCTCTCCAAGGA-3', reverse 5'-CTCAC-CTCCATGTCACATTTCAC-3'; IL8 forward 5'-CAATGCGC-CAACACAGAAAT-3', reverse 5'-TCTCCACAACCCTCTG-CACC-3'.

Box plots and a Student's t-test, used to compare "myeloid-like" cases with T-ALL or AML, were generated using the statistical tools available at *http://www.physics.csbsju.edu/stats/t-test_bulk_form.html*. Correlations between gene expression and Q-PCR results were calculated using Pearson's coefficient. miR-223 was quantified by the standard curve method, as previously described.[50] Standard curves were obtained by Q-PCR amplification of serial dilutions of synthetic DNA templates. Blanks and reverse transcription minus controls were included. The following primers were used: RT primer: 5'ATTCCGGTAGTAACGT TGCGGGGTATTTG3'; forward primer: 5'T+GT+CA+ GTTTGTCAAA3'; reverse primer: 5'ATTCCGGTAG-TAACGTTGC3' ("+" indicates the LNA modified bases). U6 small nuclear RNA was measured with the same method and used for normalization (i.e. the amount for miR-223 measured for each sample was divided by the one obtained for U6 in the same sample). U6 reverse primer: 5'GCTTCGGCAGCACATATACT3'; forward primer: 5'GCTTCGGCAGCACATATACT 3'.

Table 1. Main biological features of the 69 adult T-ALL cases evaluated by oligonucleotide arrays within the MILE study.

	MILE phase I	MILE phase II
N. of patients	52	17
Immunophenotype		
Pro-T	2	1
Pre-T	8	5
Cortical	22	10
Mature	4	–
Not further classified	16	1
Molecular biology		
Negative	38	12
SIL/TAL	6	2
BCR/ABL1	2	–
SET/NUP214	2	2
NUP214/ABL1	1	1
MLL rearrangement*	2	–
CALM/AF10	1	

*One MLL/ENL rearrangement, one unknown partner.

Results

Unsupervised clustering revealed the presence of several subgroups

Non-specific filtering selected 3539 probe sets differentially expressed among the first 52 T-ALL samples analyzed (Figure 1A). This analysis identified five subgroups: (i) a subgroup including cases positive for *JAK1* mutations; a detailed description of this subgroup has been previously reported;[16] (ii) a subcluster including cases characterized by high expression of the *LYL* oncogene ($P<0.0001$); (iii) a subset including cases expressing high levels of several *HOXA* genes: it is noteworthy that this cluster included patients who harbored *MLL* rearrangements, *CALM/AF10* rearrangements and the *TAF_I-NUP214* fusion transcript; (iv) a cluster including patients with *SIL/TAL1*; and (v) the last cluster of five cases, representing roughly 10% of the whole cohort, characterized by high expression of a large set of myeloid genes, such as genes codifying for myeloid antigens (*CD11b*, *CD66c* *CD24*, *CD14*, *CD163* and *CD114*), transcription factors (*MNDA*, *CEBPA*, *CEBPB*, *CEBPD*, *KLF4*, *KLF5*, *MAFB* and *MXD1*) and granule proteins (*MPO*, *LYZ*, *CTSB*, CTSD, *CTSG*, *CTSS*, *DEFA1*, *DEFA4*, *ELA2*, *MMP8*, *AZU1*, *ECP*, and *GRN*). These cases also showed high levels of *IL8* expression, while *LCK* was specifically down-modulated. Of note, unsupervised clustering analysis including 19 AML cases branched the above-described T-ALL cases with the AML cases, confirming

the close similarity between the two subsets of patients (Figure 1B). This signature was not sustained by residual contaminating cells, since the mean percentage of leukemic cells in this group was 87%.

In this report, we have focused on the last subgroup: for simplicity, these cases will be defined throughout the text as "myeloid-like" cases.

Analysis of variance highlighted a specific signature in "myeloid-like" cases

ANOVA was used to compare "myeloid-like" cases with patients with *SIL/TAL1* and patients with high levels of expression of *HOXA* cluster genes. We decided to perform comparisons considering only these three subclusters since they were the best characterized and most homogeneous, and also comprised a large set of cases. Hierarchical clustering of the samples highlighted a distinct signature for the "myeloid-like" cases (Figure 2A). Remarkably, principal component analysis of the top 100 genes yielded similar results: in fact, "myeloid-like" patients were distinctly separated from the other two subgroups (Figure 2B). Overall, functional annotation analysis of gene ontology biological processes, performed using DAVID software (*http://david.abcc.ncifcrf.gov*), highlighted a strong enrichment for genes involved in defense responses, as well as inflammatory responses to stress and responses to injury (BH adjusted $P=1.1\times10^{-10}$, 1.8×10^{-8}, 6.5×10^{-5}, respectively) in the "myeloid-like" subgroup. In addition, several members of the *MAPK* cascade (*MAP2K1*, *MAP2K3*, *MAP3K2*, *MAP3K3*, *MAPK14*, *MAPKAPK2*, *RAF1*, *TGFB1*, *MKNK2*) were over-represented (*Online Supplementary Table S1*).

Confirmation of results on an extended cohort of patients

To confirm the results obtained, and in particular the presence of the "myeloid-like" subgroup, GEP analysis was broadened to a total of 69 patients, comprising cases from both the first and second phase of the MILE study: partition of samples into a training-set and a test-set was not feasible because of the relatively small number of samples in the second phase of the MILE study.

This analysis led to the identification of another two patients for a total of 7/69 patients (10%) with a comparable profile, corroborating the reproducibility of the results described as well as the incidence of this specific pattern of expression.

Qualitative real-time polymerase chain reaction confirmation of the high level of expression of myeloid genes

Q-PCR for *CEBPA*, *CEBPB*, *MPO*, *GRN* and *IL8* was performed on 7 "myeloid-like" samples, 27 T-ALL cases, 23 AML patients and 3 $CD2^+$ samples from healthy donors. This analysis confirmed the results obtained by oligonucleotide arrays: expression levels were similar in "myeloid-like" T-ALL and AML, whereas significant differences were found between "myeloid-like" T-ALL and the remaining T-ALL ($P<0.0001$ for *CEBPA*, *CEBPB* and *MPO*, $P=0.0016$ and $P=0.0078$ for *GRN* and *IL8*, respectively) (Figure 3).

Pearson's correlation coefficients between gene expression and Q-PCR were fairly high, being 0.72, 0.89, 0.74, 0.74, 0.77 for *CEBPA*, *CEBPB*, *MPO*, *GRN* and *IL8*, respectively.

miR-223 is more highly expressed in "myeloid–like" cases

Since miR-223 regulates myeloid differentiation, we hypothesized that patients with "myeloid-like" features could have altered expression of miR-223: an analysis to test this hypothesis was carried out on five "myeloid-like" cases (RNA was not available for the remaining two patients), 12 T-ALL patients, 8 AML patients and 3 $CD2^+$ samples from healthy donors. The results indicated that patients with the "myeloid-like" profile had levels of expression of miR-223 comparable to those of patients with AML and remarkably higher than those of patients with other T-ALL ("myeloid-like" cases *versus* AML cases, $P=0.85$, "myeloid-like" cases *versus* T-ALL cases, $P=0.0005$) (Figure 4).

Clinico-biological characteristics of the "myeloid-like" cluster

The median age of the patients with "myeloid-like" fea-

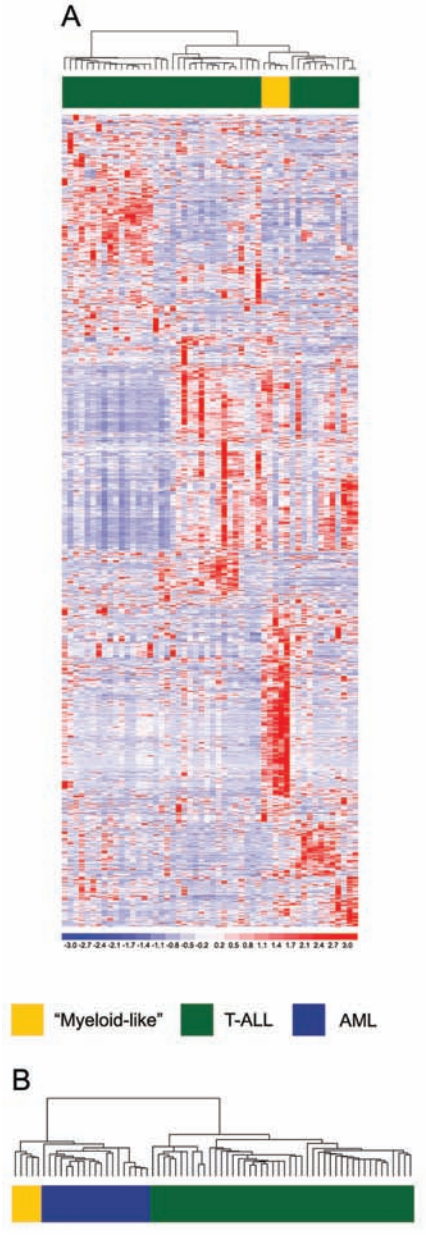

Figure 1. (**A**) Unsupervised hierarchical clustering of 52 cases of adult T-ALL. Each row represents a probe set, each column represents a sample. Bottom, a color scale indicates the relative levels of expression: dark blue, lowest levels of expression; red, highest levels of expression. (**B**) Unsupervised hierarchical clustering including AML patients.

tures (three females, four males) was 25 years (range, 23-48 years); their median white blood cell count was 79.3×10^9/L (range, 15.9-351$\times10^9$/L). Immunophenotypic analysis highlighted the presence of CD5 and CD8 in all cases but one and did not detect a specific maturation block: in fact two cases had a pre-T stage, four had a cortical stage, and one had a mature stage leukemia. MPO was negative in all cases but one, while CD13 and CD33 proved positive in two patients. Molecular biology analysis for both lymphoid and myeloid recurrent fusion genes was negative in all patients but one who harbored a *BCR/ABL* rearrangement.

Figure 2. (A) **Hierarchical clustering of the cases with *HOXA* genes+, *SIL/TAL1* and myeloid-like profiles: 1317 probests, selected by ANOVA analysis, are displayed. A distinct signature is observed for the myeloid-like cases.** (B) **Principal component analysis performed on the same set of samples. Principal component analysis was performed using the top 100 genes (according to their *P* values); results show a clear distinction among the various T-ALL subsets.**

Although this subset of patients is relatively small to draw definitive conclusions, the clinical course and outcome was poor: in fact, of the seven patients, two died during induction chemotherapy, two were refractory to induction chemotherapy and only two achieved complete remissions, with one relapsing shortly after. Response to induction therapy is not known for one patient.

The clinico-biological characteristics of these seven patients are summarized in Table 2.

Discussion

In this study, performed in the context of the MILE project, we used oligonucleotide arrays to profile 69 adult patients with a diagnosis of T-ALL at the onset of the disease. Unsupervised analysis of these cases identified several clusters, some of which are of particular interest.

The *JAK1* cluster grouped cases harboring a mutation of the *JAK1* gene; this mutation, recently identified almost exclusively in adult patients with T-ALL by our group, has been associated with a specific gene signature, constitutive activation of STAT1 transcriptional activity, IL-3 cytokine-independent cell growth and, from a clinical standpoint, an inferior outcome.[16]

A second cluster included cases characterized by the over-expression of several *HOXA* genes and comprised patients with *MLL* rearrangements, the *CALM/AF10* rearrangement and the *TAF_I-NUP214* fusion transcript, strengthening the notion that all the above mentioned alterations lead to activation of the *HOXA* genes.[28,29,36,37]

A third cluster of interest included cases harboring *SIL/TAL1*. Intriguingly, this cluster included three cases whose molecular analysis was initially negative for the *SIL/TAL1* rearrangement; however, the use of primers that target an extended portion of *SIL* and *TAL1*, revealed the presence of the rearrangement.[51] Overall, the set of data obtained by performing an unsupervised analysis corroborate the knowledge that GEP can identify, at least in some cases, molecular lesions more promptly than molecular biology itself, supporting the fact that GEP should be used more broadly in the diagnostic setting.

However, the most interesting outcome of this analysis was the identification of a set of patients, accounting for 10% of the cohort of adult T-ALL patients analyzed, who displayed "myeloid-like" gene expression features. These patients showed over-expression of a large set of genes that are typical of the myeloid lineage, such as antigens, transcription factors and granule proteins. These findings suggest that the leukemic cells of these cases, while exhibiting T-lineage features at the phenotypic level, maintain an active myeloid transcription program. This result was not impaired by a possible contamination of residual myeloid cells, since all samples contained at least 80% leukemic cells and the percentage of leukemic cells did not differ from that of the other cases analyzed.

Furthermore, it is important to mention that none of the routine diagnostic approaches, such as morphology, flow cytometry and molecular analysis, revealed any peculiar feature that could suggest an AML, indicating a discrepancy between genomic and phenotypic features. Moreover, flow cytometry did not reveal a specific differentiation stage: in fact, two cases were pre-T, four were cortical and one was a mature T-ALL, thus suggesting that the signature observed is not correlated to any maturation stage.

Remarkably, unsupervised clustering including a set of AML branched "myeloid-like" cases together with AML cases.

In order to gain further biological insights, we first confirmed our GEP results by Q-PCR focusing on typical myeloid transcripts. Among the genes that were more highly expressed we identified, and confirmed by Q-PCR, *CEBPA*. We, therefore, assumed that, given their "myeloid" features and the fact that *CEBPA* is a regulator of miR-223, the latter could be differentially expressed: this appears to be the case, with miR-223 being specifically up-regulated in "myeloid-like" cases. It must be underlined that the miR-223 up-regulation was substantial, being at least 1 log more highly expressed than in other cases of T-ALL. To the best of our knowledge, this is the first report associating miR-223 to ALL, while a role in patients' stratification has been reported in chronic lymphocytic leukemia.[50,52] Overall, these results suggest that in "myeloid-like" T-ALL miR-223 deregulation may play a pivotal role in determining biological behavior. Since *NFIA* is part of the miR-223 microcircuit, we also determined its mRNA levels in these patients, but we failed to detect a down-modulation of this gene (*data not shown*). There are two possible explanations for this: (i) we evaluated primary leukemic T cells, whereas this circuit was described in myeloid cell lines treated with retinoic acid;[40] (ii) we did not evaluate the protein expression of NFIA because of lack of material.

Although the number of patients is relatively small to draw definitive conclusions or obtain meaningful statistics, from a clinical standpoint these patients seem to have a poor response to treatment and an overall unfavorable outcome. Intriguingly, these cases were also characterized by high expression levels of *IL8*, a gene previously associated with refractoriness to induction chemotherapy.[35] Given these observations and the biological features, it is tempting to speculate that patients with T-ALL with a "myeloid-like" profile could benefit from a different chemotherapeutic approach, such as regimens used for the management of AML. Furthermore, the evidence of the involvement of several members of the MAPK signaling pathway, more frequently activated in AML, suggests a potential role for MAPK inhibitors in such cases.[53]

Coustan-Smith *et al.*[54] recently described a subset of pediatric T-ALL, accounting for 12% of cases, which retained multilineage differentiation potential and

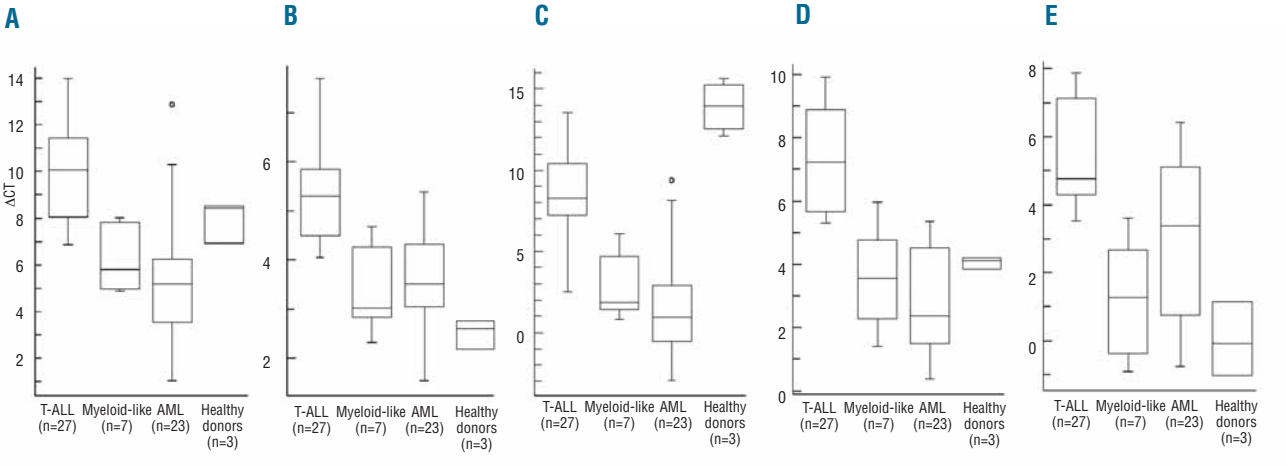

Figure 3. Q-PCR results for 27 T-ALL samples, 7 "myeloid-like" *cases*, 23 AML samples and 3 $CD2^+$ samples obtained from healthy donors. Gene expression values are expressed by ΔΔCT values: all values are normalized to the values of a $CD2^+$ sample and are expressed in log-scale. Box plots define the median values, 25% to 75% of values around the median, and the range of values. (A) Q-PCR results for ***CEBPA***. (B) Q-PCR results for ***CEBPB***. (C) Q-PCR results for ***MPO***. (D) Q-PCR results for ***GRN***. (E) Q-PCR results for ***IL8***.

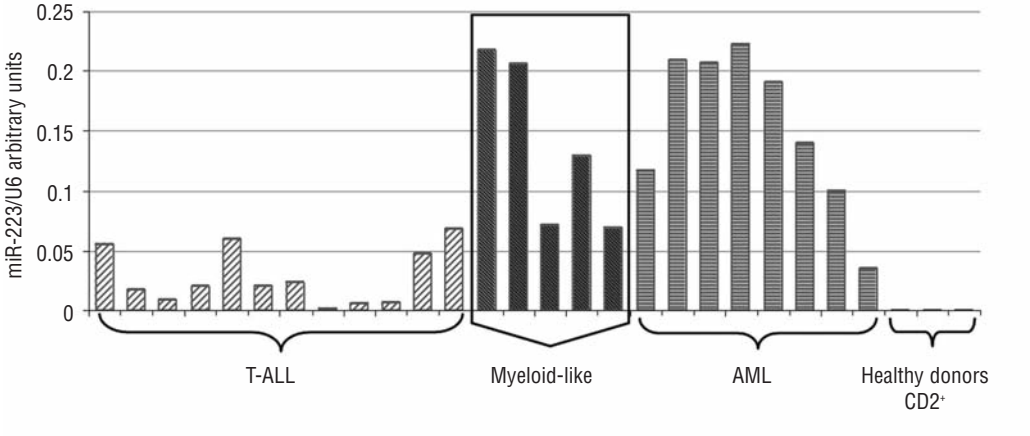

Figure 4. Q-PCR results for miR-223 in 12 T-ALL, cases, 5 "myeloid-like", cases, 8 AML cases and $CD2^+$ cells from three healthy donors.

Table 2. Clinico-biological characteristics of the "myeloid-like" cases.

UPN	Age	Gender	Leukemic cells (%)	Immunophenotype	Molecular biology	Response to induction therapy	Follow-up
ROM_00050	25	F	98	Cortical	Negative	Complete remission	Relapse
ROM_00129	25	M	84	Cortical MPO:20%, CD13:14%, CD33:11%	*BCR/ABL*	Not available	Lost to follow-up
ROM_00196	25	F	80	Pre-T MPO:0%, CD13:0%, CD33:5%	Negative	Refractory	Death
ROM_00208	23	M	80	Mature MPO, CD13, CD33:0%	Negative	Refractory	Death
ROM_00217	31	F	84	Cortical MPO, CD13, CD33:0%	Negative	Death in induction	-
ROM_00373	48	M	92	Pre-T MPO:0%, CD13:90%, CD33:40%	Negative	Death in induction	-
ROM_00348	38	M	95	Cortical MPO, CD13, CD33:0%	Negative	Complete remission	Continuous complete remission

responded poorly to chemotherapy: these cases had an early T-cell phenotype and co-expressed at least one myeloid marker. Our cases appear to resemble these pediatric cases in the light of: (i) an active myeloid and/or stem cell program coupled, in some cases, to co-expression of myeloid antigens; (ii) the poor clinical outcome; and (iii) similar incidence. Nevertheless, at the immunophenotypic level our cases expressed CD5, CD8 and CD1a. It is intriguing to speculate that these patients might be the adult counterpart of the small subset of children described by Coustan-Smith *et al.*,[54] who did express CD5 and lacked myeloid antigens.

In summary, we have described a subset of adult T-ALL cases whose genomic profile, but not phenotypic features, resembles that of AML patients, again underlining the role of GEP in the identification of novel subgroups. This subset accounts for about 10% of adult T-ALL cases and appears to be associated with an unfavorable outcome, leading to the suggestion that a different treatment approach may be beneficial in these patients if identified early.

Authorship and Disclosures

SC and MM performed research and wrote the manuscript. ST, GZ, LE, AF, PG and GS performed research, IB provided expertise on *CEBPA*. AV, CF and AC recruited patients, AP performed the statistical analysis. AG and RF designed the research and critically revised the manuscript.

The authors reported no potential conflicts of interest.

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