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Prognostic impact of t(16;21)(p11;q22) and t(16;21)(q24;q22) in pediatric AML: a retrospective study by the I-BFM Study Group

Short title: t(16;21) rearranged pediatric AML

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Key points

- t(16;21) translocations in AML comprise t(16;21)(p11;q22) (*FUS–ERG*) as well as t(16;21)(q24;q22) (*RUNX1–CBFA2T3*).
- Survival in pediatric AML with *FUS–ERG* is poor, whereas in *RUNX1–CBFA2T3* survival is similar to other core-binding factor leukemias.

Abstract

To study the prognostic relevance of rare genetic aberrations in AML, such as t(16;21), international collaboration is required. Two different types of t(16;21) translocations can be distinguished, t(16;21)(p11;q22) resulting in the *FUS–ERG* fusion gene and t(16;21)(q24;q22) resulting in *RUNX1–CBFA2T3*. We collected data on clinical and biological characteristics of 54 pediatric AML cases with t(16;21) rearrangements from 14 international collaborative study groups, participating in the international Berlin-Frankfurt-Münster (I-BFM) AML study group. The AML-BFM cohort diagnosed between 1997 and 2013 was used as a reference cohort. *RUNX1–CBFA2T3* (n=23) had significantly lower median WBC ($12.5*10^9$/l, p=0.03) compared to the reference cohort. *FUS–ERG* rearranged AML (n=31) had no predominant FAB type, whereas 76% of *RUNX1–CBFA2T3* had an M1/M2 FAB type (M1, M2), significantly different from the reference cohort (p=0.004). 4-year event free survival (EFS) of patients with *FUS–ERG* was 7% (SE=5%), significantly lower compared to the reference cohort (51%, SE=1%, p<0.001). 4-year EFS of *RUNX1–CBFA2T3* was 77% (SE=8%, p=0.06), significantly higher compared to the reference cohort. Cumulative incidence of relapse was 74% (SE=8%) in *FUS–ERG*, 0% (SE=0%) in *RUNX1–CBFA2T3,* compared to 32% (SE=1%) in the reference cohort (p<0.001). Multivariate analysis identified both *FUS–ERG* and *RUNX1–CBFA2T3* as independent risk factors with hazard ratios of 1.9 (p<0.0001) and 0.3 (p=0.025), respectively. These results describe two clinically relevant distinct subtypes of pediatric AML. Similarly to other core-binding factor AMLs, patients with RUNX1–CBFA2T3 rearranged AML may benefit from stratification in the standard risk treatment, whereas patients with FUS–ERG rearranged AML should be considered high-risk.

Introduction

Despite intensive chemotherapy, current outcome of pediatric acute myeloid leukemia (AML) has reached a plateau (1), with 5-year event free survival rates around 50-55% and 5-year overall survival rates reaching 70% (2-5). Apart from early clinical response, cytogenetic and molecular aberrations are the most reliable prognostic factors for survival (2, 3, 6). For future treatment stratification, identification of prognostic subgroups is important. Pediatric AML is a very heterogeneous disease, therefore the prevalence of specific genetic subgroups can be too low to allow individual study groups to evaluate prognostic relevance and requires international collaboration.

Over the past few years the I-BFM SG has described clinical and genetic characteristics of several rare pediatric AML subsets with the aim to provide clinicians with data for clinical decision making such as risk-group stratification (7-11). A pediatric AML group of interest is t(16;21), which, according to existing literature, is considered high-risk. This is mainly based on case reports or small series in adult patients (12-16). Two different t(16;21) translocations resulting in different fusion transcripts can be distinguished. These include t(16;21)(p11;q22), resulting in the *FUS–ERG* fusion(12); and t(16;21)(q24;q22), resulting in the *RUNX1–CBFA2T3* fusion(17).

To date, 63 patients with *FUS–ERG* rearranged AML have been described in patients from 1 to about 60 years of age(13), of which 19 were children(18). It has been reported that *FUS–ERG* AML presents with eosinophilia, micromegakaryocytes and hemophagocytosis, and outcome has been described to be poor. (13, 14, 19). *RUNX1–CBFA2T3* AML has been described in 24

patients, of which 5 were pediatric cases (18). This aberration is associated with the FAB M2 phenotype and eosinophilia (16, 20). In adults, *RUNX1–CBFA2T3* has been associated with treatment related AML, and is reported to have a poor outcome (20). However, this data is mainly based on adult cases, and the prognostic impact of these rearrangements in pediatric AML is unknown.

To get more insight in the relevance of these somatic aberrations, we conducted a collaborative retrospective international study, gathering data from 14 study groups participating in the I-BFM Study Group. The aim of this study was to describe the biological and clinical characteristics and outcome of pediatric patients with t(16;21) rearranged AML registered in I-BFM study group related data registries.

Patients and methods

Patients

To obtain the largest possible cohort of pediatric AML cases with t(16;21), aged 0 to 18 years of age, patient data was collected from 14 collaborative study groups and countries participating in the I-BFM Study Group (table S1). Patients diagnosed between 01/01/1995 and 01/01/2016 were included in the study. Patients were identified in the data registries of the study groups by reviewing karyotypes, FISH and/or PCR analyses. Both t(16;21) translocations can be detected by conventional karyotyping. AIEOP, BFM Austria, JCCG and BSPHO confirmed the translocation with either FISH or PCR as standard of care. In one case from NOPHO, the *FUS–ERG* fusion was detected through RNA sequencing.

For each case, a predefined set of data was collected and checked for consistency. This set of data included sex, age, date of diagnosis, white blood cell count (WBC), extramedullary disease, relation with prior treatment or cancer, FAB morphology, eosinophilia and other morphological characteristics, presence of erythrophagocytosis, karyotype, treatment protocol, including data on allogeneic hematopoietic stem cell transplant (HSCT), response to therapy, including data on minimal residual disease detection through flow cytometry (MRD) and events, including relapse, resistant disease, occurrence of secondary malignancy and death. Autologous HSCT was considered intensive chemotherapy.

Data of 1326 patients (excluding the t(16;21) cases) diagnosed between 1997 and 2013 were provided by the AML-BFM Study Group as a reference cohort. Patients with acute promyelocytic leukemia (APL) and Down syndrome were excluded. All patients in this cohort

were classified as either standard risk (SR) patients, comprising of inv(16) and t(8;21) or high risk (HR) patients comprising other cytogenetic subtypes.

RNA sequencing data of 1035 patients with de novo AML with a median age of 9.9 (range 0-29.6) from the COG AAML1031 trial (NCT01371981) were provided by COG for gene expression analysis(21, 22) and to identify the frequency of these aberrations. In this study, 93.9% of the patients were below 18 years of age.

Central cytogenetic review

All karyotypes were centrally reviewed by two independent expert cytogeneticists, W. Cuccuini and M. Pigazzi, following the International System for Human Cytogenetic Nomenclature (ISCN 2016). Patients with inconclusive karyotypes were screened by RT-PCR (supplemental methods). RNA was provided by the study groups.

Statistical analysis

Complete remission (CR) was defined as less than 5% blasts in the bone marrow, with regeneration of trilineage hematopoiesis and no leukemic cells in cerebrospinal fluid or elsewhere. If a patient did not obtain CR, treatment was considered a failure at day 0. Minimal residual disease (MRD) was measured by different study groups through flow cytometry after the first and second course of treatment. If more than 0.1% of the mononuclear cells (MNC) were leukemic cells, MRD was considered positive. OS was calculated from the day of diagnosis until the date of last follow up or death from any cause. EFS was measured from the day of

diagnosis to the date of the first event or the date of last follow up. Events considered in this analysis were resistant disease, relapse, occurrence of secondary malignancy and death.

Chi-square and Fisher exact tests were used to compare clinical characteristics. OS and event free survival (EFS) analysis were estimated according to Kaplan–Meier and compared with log-rank test. Cumulative incidence of relapse (CIR) was calculated according to Kalbfleisch and Prentice and compared with the Gray test(23). tMantel-Byar-test was used to compare groups with and without allogeneic hematopoietic stem cell transplantation (HSCT). The Cox proportional hazards model was used for multivariate analysis, considering age, WBC count at diagnosis, cytogenetic risk group (SR vs. HR) as covariables, and HSCT as time-dependent variable. Analyses were performed with SPSS Statistics version 21 and SAS 9.4. All tests were two-tailed, and a P-value of less than 0.05 was considered significant.

Gene expression analysis

Fusion transcripts from AML samples of patients included in the COG cohort were detected by RNA sequencing and validated by RT-PCR. Fractional counts were normalized to trimmed mean of m-values and counts per million mapped reads (CPM). The normalized counts were log2 transformed and filtered for genes with at least 1 CPM in 5% of samples. For hierarchical clustering the relative level of expression per gene in each sample was determined by mean centering the expression values, using the geometric mean. Pearson correlation coefficients were employed as a measure of dissimilarity with the ward.D2 linkage algorithm implemented in the R statistical programming environment (R v.3.4.0). Sample correlations were derived from the expression of the 2,412 differentially expressed genes, which are the union of those

identified in FUS–ERG or RUNX1–CBFA2T3 versus other AML. Differential expression analysis was completed using Limma v3.32.5 R package. Genes with absolute log2 fold-change > 1 and Benjamini-Hochberg adjusted p-values < 0.05 were retained.

Results

Clinical features

A total of 55 patients with t(16;21) were identified, 32 patients with *FUS–ERG* rearranged AML and 23 patients with *RUNX1–CBFA2T3* rearranged AML. After central review of the karyotypes, there was one patient who did not meet our criteria, as only 1 out of 20 cells analyzed displayed a t(16;21)(p11;q22). It was not possible to confirm this fusion by RT-PCR and therefore we excluded this patient from further analysis. The total cohort thus consisted of 54 patients with t(16;21), 31 with *FUS–ERG* rearranged AML and 23 with *RUNX1–CBFA2T3* rearranged AML. In the COG AAML1031 cohort, 5 *FUS–ERG* and 4 *RUNX1–CBFA2T3* cases were indentified, hence the frequency of *FUS-ERG* was 0.5% and 0.3% for RUNX1-CBFA2T3, as compared to 0.3% and 0.1% in the BFM reference cohort (karyotype only), respectively. Clinical characteristics were compared to the AML-BFM SG reference cohort.

The patient characteristics of the t(16;21) subgroups are described in table 1. No significant differences in sex and median age could be found when we compared patients in the *FUS–ERG* or *RUNX1–CBFA2T3* groups to the reference cohort. No patients with *FUS–ERG* rearranged AML were less than 2 years of age and neither *FUS–ERG* nor *RUNX1–CBFA2T3* rearrangements were found in infants below 1 year of age (figure S1). The median WBC of *FUS–ERG* ($14.0*10^9$/l) was not significantly different compared to the reference cohort ($19.4*10^9$/l, p=0.66), whereas the WBC of *RUNX1–CBFA2T3* ($12.5*10^9$/l) was significantly lower (p=0.030).

Patients with *FUS–ERG* had no predominant French–American–British (FAB) type, whereas 76% of those with *RUNX1–CBFA2T3* had a M1/M2 FAB type, compared with 42.1% in the

reference cohort (P=0.004). There was one patient with *FUS–ERG* with Auer rods. No other specific morphological features were reported.

Cytogenetics

At initial diagnosis, 9 out of 31 (29.0%) patients with *FUS–ERG* had t(16;21)(p11;q22) as a sole aberration. Ten out of 31 (32.3%) had a complex karyotype, defined as at least 3 chromosomal aberrations. In 3 cases, the t(16;21)(p11;q22) translocation was not detected by conventional karyotyping but by PCR, FISH or RNAseq. Recurrent additional cytogenetic aberrations were trisomy 8 (n=6, 19.3%) and trisomy 10 (n=4, 12.9%).

Complete karyotype data was available for 19 patients with *RUNX1–CBFA2T3*. In 3 (15.8%) of these patients, t(16;21)(q24;q22) was the sole abnormality. Five patients (26.3%) had a complex karyotype. Recurrent additional cytogenetic aberrations were trisomy 8 (n=8, 42.1%) and deletion of the Y chromosome (n=3, 15.7%). In 3 patients, cytogenetic analysis failed, but the *RUNX1–CBFA2T3* translocation was detected by FISH or PCR. In one patient, the t(16;21)(q24;q22) translocation was not detected by conventional karyotyping, but was detected by PCR. A detailed list of the karyotypes is provided in table S1.

Secondary AML

A total of seven patients had secondary AML, two presenting with FUS–ERG rearrangements and five with RUNX1–CBFA2T3. Those with *FUS–ERG* rearranged AML, presented with AML with myelodysplastic features and received chemotherapy prior to HSCT.

One patient relapsed and died of disease, the second patient is still in remission after 7 years of follow up.

Of the 5 patients diagnosed with *RUNX1–CBFA2T3*, two had Ewing sarcoma as primary malignancy. Four patients had been diagnosed with MDS prior to AML development, one of whom also had previous Ewing sarcoma. The median time to development of AML after MDS diagnosis was 6.7 months (range 4-28 months). None of the 4 patients with MDS was transplanted prior to AML diagnosis. In one patient, a 21q22 rearrangement was detected by FISH at time of MDS diagnosis. All patients received chemotherapy after being diagnosed with AML and 3 patients underwent HSCT in first CR.

Treatment and outcome

All patients in this cohort were treated with curative intent. Complete remission (CR) was achieved in 87.1% of the patients with *FUS–ERG* and 82.6% of those with *RUNX1–CBFA2T3*. Two patients with *RUNX1–CBFA2T3* AML suffered from early death before reaching CR.

In total, 23 patients had data available on MRD measured by flow cytometry, 12 with *FUS–ERG* and 11 with *RUNX1–CBFA2T3* patients (table S2). In the *FUS–ERG* group, 5 out of 12 were MRD negative after the first course of chemotherapy (MRD1), and an additional 2 were MRD negative after the second course of treatment (MRD2). No difference in the incidence of relapse could be observed between the MRD positive and negative patients, as 10 out of 12 patients experienced a relapse. One of the patients that did not suffer from relapse was MRD2 positive, but had a short follow up time of only 2 months, the other patient was MRD1 negative

and received an HSCT in CR1. In the *RUNX1–CBFA2T3* group, 8/10 patients were MRD negative after the first course of chemotherapy. After the second course, all patients were MRD negative and none relapsed.

Four-year EFS, OS and cumulative incidence of relapse (CIR) for the reference cohort were 51% (SE=1%), 68% (SE=1%) and 32% (SE=1%), respectively. The SR group in the reference cohort had a 4-year EFS, OS and CIR of 74% (SE=3%), 88% (SE=2%) and 19% (SE=2%), respectively. For the HR group, EFS was 45% (SE=2%), OS 62% (SE=2%) and CIR 36% (SE=2%).

Median follow up for survivors in the t(16;21) cohort was 1.6 years for those with *FUS–ERG* and 5.0 years for *RUNX1–CBFA2T3*. Patients with *FUS–ERG* had a 4-year EFS of 7% (SE=5%, p<0.0001), an OS of 21% (SE=8%, p<0.0001) and a CIR of 74% (SE=8%, p<0.0001). The median time to relapse was 10.2 months. Almost all relapses occurred early within the first year after start of treatment (18/21).

For *RUNX1–CBFA2T3*, 4-year EFS was 77% (SE=9%, p=0.06), OS was 81% (SE=8%, p=0.34) and CIR was 0%. As EFS rates of the SR patients 74% (SE=3%), Thus, the patients with *RUNX1–CBFA2T3* had a similar outcome as BFM SR patients (figure 1).

A total of 30 patients underwent an allogeneic HSCT: 22/31 (71.0%) patients with *FUS–ERG,* and 8/23 (34.8%) with *RUNX1–CBFA2T3*. Of the 22 *FUS–ERG* patients who received an HSCT, 14 (42.2%) received the HSCT in CR1. The 4-year EFS for transplanted patients with *FUS–ERG* was 15% (SE=15%) compared with 0% (SE=0%) for patients receiving chemotherapy only (p=0.50).

Multivariate analysis of EFS and OS revealed that *FUS–ERG* was an independent predictor of poor outcome for both EFS and OS (hazard ratio [HR] 2.9, p<0.0001 and 2.61, p<0.0001, respectively), whereas *RUNX1–CBFA2T3* was a predictor of favorable outcome for EFS but not OS (HR 0.33, p=0.02 and 0.42, p=0.14, respectively). The hazard ratios for *RUNX1–CBFA2T3* were comparable to the SR group of the reference cohort, with HR for EFS of 0.36 (p<0.001) and OS of 0.25 (p<0.001). In addition, WBC>$100*10^9$/l was an independent predictor of poor outcome for both EFS and OS (HR 1.4, p=0.0005 and 1.27, p=0.046, respectively) (table 2). All other covariates, including HSCT, were not significantly associated with outcome.

Gene expression profiling

Unsupervised hierarchical clustering revealed that *RUNX1–CBFA2T3* and *FUS–ERG* cluster separately (figure 2). *FUS–ERG* also clusters separately from other cytogenetic subgroups, like KMT2A-rearrangements, t(8;21)(q22;q22) and inv(16)(p13;q22), whereas *RUNX1–CBFA2T3* cases cluster in close proximity to t(8;21)(q22;q22) cases. Comparing gene expression of *FUS–ERG* to the remainder of the pediatric AML cohort revealed 1314 differentially expressed genes with an adjusted p-value of less than 0.05. Among these, 428 genes were up-regulated. In hematopoiesis, *ERG* is known to upregulate *GATA2* and *RUNX1*, however, there was no differential expression of these genes in *FUS–ERG* rearranged AML(24). The top 100 most differentially expressed genes of *FUS–ERG* are provided in table S3.

Comparing *RUNX1–CBFA2T3* gene expression to the remainder of the pediatric AML cohort, revealed 119 differentially expressed genes in *RUNX1–CBFA2T3*, of which 76 genes were up-regulated (table S4). Because *RUNX1–CBFA2T3* clustered in close proximity to t(8;21)(q22;q22)

leading to the *RUNX1–RUNX1T1* fusion, we analyzed whether these two groups share a gene expression profile. To this purpose we detected differentially expressed genes in *RUNX1–CBFA2T3* using the pediatric AML cohort, excluding the t(8;21) cases. Of the 2786 differentially expressed genes (2507 in t(8;21) and 279 in *RUNX1–CBFA2T3*), 187 differentially expressed genes were shared between the two groups. A total of 112 genes were up-regulated in both groups, 70 were down-regulated in both groups and 5 genes were upregulated in *RUNX1–CBFA2T3* and downregulated in t(8;21) (table S5). Well known targets of t(8;21) like *POU4F1, TRH, PSD3, MEIS1* and *LAT2* were differentially expressed in *RUNX1-CBFA2T3* AML(24, 25).

Discussion

Within the framework of this international collaboration, we studied the clinical and biological features of two translocations involving chromosome 16 and 21. We identified 2 clinically relevant, distinct subtypes of pediatric AML patients with different t(16;21) rearrangements: *FUS–ERG* had poor outcome, whereas *RUNX1–CBFA2T3* had favorable outcome. Our data suggests that patients with *RUNX1–CBFA2T3*-rearranged AML might benefit from treatment protocol for standard risk AML without stem-cell transplantation, whereas those with FUS–ERG-rearranged AML seem to require high risk therapy, including HSCT, or even experimental therapy.

Although 87.1% of the patients with *FUS–ERG*-rearranged AML reached morphological complete remission, the 4-year CIR was 74% and most relapses occurred within the first year after diagnosis. Currently, early response to therapy is increasingly used for risk-group stratification of therapy in AML (3, 26, 27). The detection of minimal residual disease (MRD) through flow cytometry can provide a more accurate measure of therapy response, however the additional benefit of MRD measurement is inconsistent between AML subtypes and studies, and also depends on the sensitivity of the applied technique (3, 28-30). In our cohort, MRD data was reported in about half of the t(16;21) rearranged cases. Of those, almost 40% of the patients with *FUS–ERG*-rearranged AML, who had MRD data determined by flow cytometry, were MRD negative after the first course of treatment, and about half of the patients (7 out of 12) after the second course of treatment. However, EFS was very low in both MRD negative and MRD positive patients and no significant difference in EFS between the two groups could be found. Despite the fact that numbers are small, this may suggest that MRD does not adequately

predict relapse in this cytogenetic group. A reason for this might be that *FUS–ERG*-rearranged AML could be a leukemic stem cell driven disease which is not successfully eradicated with current treatment protocols.

Currently, in high-risk AML subgroups with an EFS below 30%, HSCT in CR1 is considered by some collaborative groups (31). As EFS of *FUS–ERG* rearranged AML is 7%, patients with this rearrangement should be considered for HSCT in CR1 despite the fact that benefit from HSCT seemed limited in our analysis. Therefore patients with *FUS–ERG* rearranged AML urgently require novel forms of therapy.

In contrast to *FUS–ERG*, no relapses were observed in *RUNX1–CBFA2T3* rearranged pediatric AML. The only events that occurred were toxic events as 5 patients died due to infections. Surprisingly, even patients with secondary AML did not suffer from relapse. To date 24 *RUNX1–CBFA2T3* cases have been reported, of whom only 5 concerned pediatric cases (15, 16, 20, 32-45) . In the literature, these patients were considered to be high risk. However, when we single out the pediatric cases, 2 died due to an infection and 3 were in complete remission for at least 1 year. This seems to be consistent with our results further supporting that *RUNX1–CBFA2T3* rearranged AML should be stratified as SR. This suggests that outcome for *RUNX1–CBFA2T3* rearranged AML differs between pediatric and adult patients. This may be related to the fact that this leukemia occurred often as second malignancy in the adult cases, and perhaps was not treated with curative intent. Moreover, in general outcome in pediatric AML is better than in adults, which may reflect issues such as organ-toxicity and tolerability for chemotherapy.

Of note, most of the patients in our pediatric cohort had de novo AML, however 2 patients were diagnosed with Ewing sarcoma prior to AML development, and 3 additional patients had MDS prior to AML. The association between Ewing Sarcoma and MDS/AML in pediatrics has been described previously, but not in combination with *RUNX1-CBFA2T3* (46). Surprisingly, even though secondary AML is known to be a poor prognostic risk factor, there was no difference in outcome between patients with de novo or secondary AML, with no relapses in either group. According to the World Health Organization classification of myeloid neoplasms and acute leukemia, patients with a t(8;21)(q22;q22) rearrangement and less than 20% blasts in the bone marrow should be classified as AML and not as MDS (47, 48). This classification strategy could also be applied to *RUNX1–CBFA2T3*, as the 'MDS cases' (less than 20% blasts in the diagnostic marrow) were cured without SCT.

This study showed that the two fusions give rise to different gene expression signatures. The gene expression profile of *FUS–ERG* rearranged cases did not reveal any similarities with other cytogenetic subgroups. The t(16;21)(p11;q22) gives rise to a fusion of the N-terminal part of *FUS*, containing the transactivation domain of *FUS* and the C-terminal of *ERG*, containing the ETS DNA binding site of *ERG* (49). *FUS–ERG* is known to bind at genomic regions that are also bound by other transcription factors associated with stem cell programs like *RUNX1*, *FLI1* and *GATA2* (50). However, we found no differential expression of these associated genes when we compared gene expression of *FUS–ERG* rearranged AML to the other AML cases. Furthermore, Sotoca et al found that the nuclear receptor heterodimer RARA:RXR binds to *FUS–ERG* occupied genomic regions, suggesting possible modulation of the retinoic acid response in *FUS–ERG*

rearranged AML (50). This might make *FUS–ERG* rearranged AML a potential target for treatment with ATRA.

On the other hand, *RUNX1–CBFA2T3* can be classified as a core binding factor AML. The core binding factor (CBF) is a heterodimer, consisting of *RUNX1*, *RUNX2* or *RUNX3* and *CBFB.* The CBF attaches to DNA and activates genes involved in hematopoietic development (51). In leukemia, recurrent fusions of these genes have been described (52). When *CBFB* or *RUNX1* is part of a fusion gene, the function of the protein changes and instead of activating the genes it will repress them. In AML, two recurrent aberrations are currently classified as CBF AML: inv(16)(p11;q32)/t(16;16)(p11;q42) and t(8;21)(q22;q22), resulting in *CBFB-MYH11* and *RUNX1-RUNX1T1*, respectively(11). There are striking similarities between *RUNX1–CBFA2T3* and t(8;21) (also known as *RUNX1-RUNX1T1*) as both are mainly found in FAB M1/M2 AML and have a favorable outcome (6, 53). Cytogenetically, both show recurrent loss of a sex chromosome, which is rare in other types of pediatric AML (11). Both fusions also have similarities in biology. They do not only share the *RUNX1* gene, but *CBFA2T3* and *RUNX1T1* are paralogs and share 92% of their protein sequence (54). Furthermore, this study showed that *RUNX1–CBFA2T3* and t(8;21) cluster in close proximity of each other and that *RUNX1–CBFA2T3* and t(8;21) share 187 differentially expressed genes, among which target genes like *POU4F1*, *TRH* and *MEIS1*.These results are in line with the results Lavallee et al obtained when comparing gene expression profiles of t(8;21) and inv(16) (55). These findings provide additional support that *RUNX1–CBFA2T3* belongs to the CBF AML subgroup, similar to t(8;21).

In this study we relied on cytogenetic analysis to detect t(16;21). Due to the fact that cytogenetic analysis fails in about 10% of cases (56), there is risk of selection bias. Furthermore, as cytogenetic analysis can only detect large rearrangements, more subtle, complex rearrangements could be missed. More sensitive analysis like FISH, RT-PCR and RNAseq are more reliable to detect these rearrangements. *RUNX1–CBFA2T3*, for instance, can be detected through *RUNX1*-split FISH, which is usually performed as standard of care to detect *RUNX1-RUNX1T1* rearrangements. The difference in incidence between the BFM and COG AAML1031 cohort seems to confirm that RNAseq might be slightly more reliable. However, as RNAseq needs high quality RNA of samples with a high purity of blasts, not all patients can be analyzed by this method. We are also not informed on whether these cohorts were truly population-based.

In conclusion, this international collaborative study describes two clinically relevant distinct subtypes of pediatric AML. Although numbers are small, reflecting the rarity of the diseases, *FUS–ERG* represents an extremely poor prognostic subgroup, whereas *RUNX1–CBFA2T3* has a favorable outcome. Patients with *RUNX1–CBFA2T3* rearranged AML might benefit from risk-stratification to standard intensive therapy, as for CBF AML, whereas *FUS–ERG* rearranged AML patients should be considered high risk and offered HSCT in CR1, even though the effect of HSCT in *FUS–ERG* rearranged AML may be limited in this retrospective series. Although unfortunately we have no data on surface marker expression in these specific cases, more experimental therapy like flotetuzumab or CAR T-cells may offer opportunities to circumvent chemotherapy drug resistance and need to be explored in these high risk *FUS–ERG* patients, certainly after relapse-(57-60).

Authorship

Contribution: S.N., C.M.Z. and M.M.v.d.H.-E. designed the study; M.Z., D.R., M.P., J.S., R.E.R., T.A.A., B.H., D.T., F.L., T.A.G., S.R., E.S., D.K.C., M.D., J.S., J.A., N.A.-C., M.C., B.D.M., H.H., S.M. contributed materials and clinical data; M.Z., M.P., W.C. J.S., R.E.R., S.N., C.M.Z. and M.M.v.d.H.-E analyzed data; S.N., M.Z., J.S. and R.E.R. performed statistical analysis; S.N., C.M.Z. and M.M.v.d.H.-E. wrote the paper; C.M.Z. and M.M.v.d.H.-E. supervised the study; and all coauthors performed critical review of the manuscript and gave their final approval.

Conflict-of-interest disclosure: the authors declare no competing financial interests.

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Figures and tables

Table 1. Clinical characteristics of pediatric cases with t(16;21).

	FUS–ERG	*RUNX1–CBFA2T3*	I-BFM reference cohort
N	31	23	1326
Median age (range)	8.5 (2.0-17.5)	6.8 (1-17)	8.7 (0-20.3)
Gender			
% male	61	46	51.6
Median WBC x10^9/l (range)	14.0 (1-203)	12.5 (0.01-185) *	19.5 (0.01-190)
<20 x 10^9/L, N (%)	17 (54.8)	13 (61.9)	670 (50.5)
20-100 x 10^9/L, N (%)	10 (32.3)	7 (33.3)	414 (31.2)
>100 x 10^9/L, N (%)	5 (12.9)	1 (4.8)	242 (18.3)
FAB-type, N (%)			
M0	3 (9.6)	1 (4.3)	46 (3.7)
M1	8 (25.8)	3 (13.0)	178 (14.5)
M2	8 (25.8)	10 (43.5) *	339 (27.6)
M4	6 (19.3)	2 (8.7)	293 (23.9)
M5	4 (12.9)	1 (4.3)	258 (21.6)
M6	-	-	29 (2.4)
M7	1 (3.2)	1 (4.3)	85 (6.9)
NOS	1 (3.2)	5 (21.7)	-
CNS involvement, N (%)	6 (18.1)	5 (22.7)	
Cytogenetics, N (%)			
Sole abnormality	12 (36.4)	4 (21.1)	
Trisomy 8	6 (18.1)	7 (36.8)	
Trisomy 10	4 (12.1)	-	
Complex karyotype	10 (30.3)	5 (26.3)	
Treatment, N (%)			
CR obtained	28 (87.5)	22 (95.6)	
Refractory disease	3 (9.4)	-	
HSCT in CR1	13 (40.6)	8 (34.8)	
Survival, % (SE)			
4-year EFS	13 (5) **	77 (9) **	51 (1)
4-year OS	26 (8) **	81 (8) **	68 (1)
4-year CIR	69 (8) **	0 (0) **	32 (1)

WBC, white blood cell count; FAB, French American British morphology classification; CNS, central nervous system; CR, complete remission; HSCT, hematopoietic stem cell transplant; EFS, event free survival; OS, overall survival; CIR, cumulative incidence of relapse. * $P < 0.05$, ** $P < 0.001$

Table 2. Multivariate analysis of survival parameters of t(16;21) rearranged AML.

	pEFS			pOS		
	Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
FUS–ERG	2.85	1.93-4.21	<0.001	2.61	1.71-4.00	<0.001
WBC >10*10^9	1.40	1.15-1.70	<0.001	1.27	1.00-1.60	0.046
Age >10 years	1.14	0.98-1.34	0.087	1.38	1.14-1.67	0.001
Time to HSCT	0.84	0.63-1.12	0.23	0.97	0.70-1.33	0.834
Cytogenetic SR group	0.36	0.28-0.47	<0.001	0.25	0.17-0.36	<0.001
RUNX1–CBFA2T3	0.32	0.12-0.87	0.025	0.42	0.14-1.33	0.140

pEFS indicates the probability of event free survival; pOS, probability of overall survival; CI confidence interval; WBC white blood cell count; HSCT hematopoietic stem cell transplant; and SR standard risk.

Figure 1. Survival of *FUS–ERG* and *CBFA2T3/RUNX1* AML as compared to a pediatric AML reference cohort. Survival curves of patients with *FUS–ERG* rearranged AML and *CBFA2T3/RUNX1* rearranged AML, as compared to the BFM reference cohort. In D, E and F, the reference cohort is split up according to high risk and standard risk.

Figure 2. Unsupervised clustering analysis. Pairwise sample correlations of 1037 samples of pediatric AML. The cells in the visualization are colored by Pearson's correlation coefficient values. Cytogenetic subgroups are depicted in the first column. Presence of *FUS–ERG* or *RUNX1–CBFA2T3* is depicted in the second column.

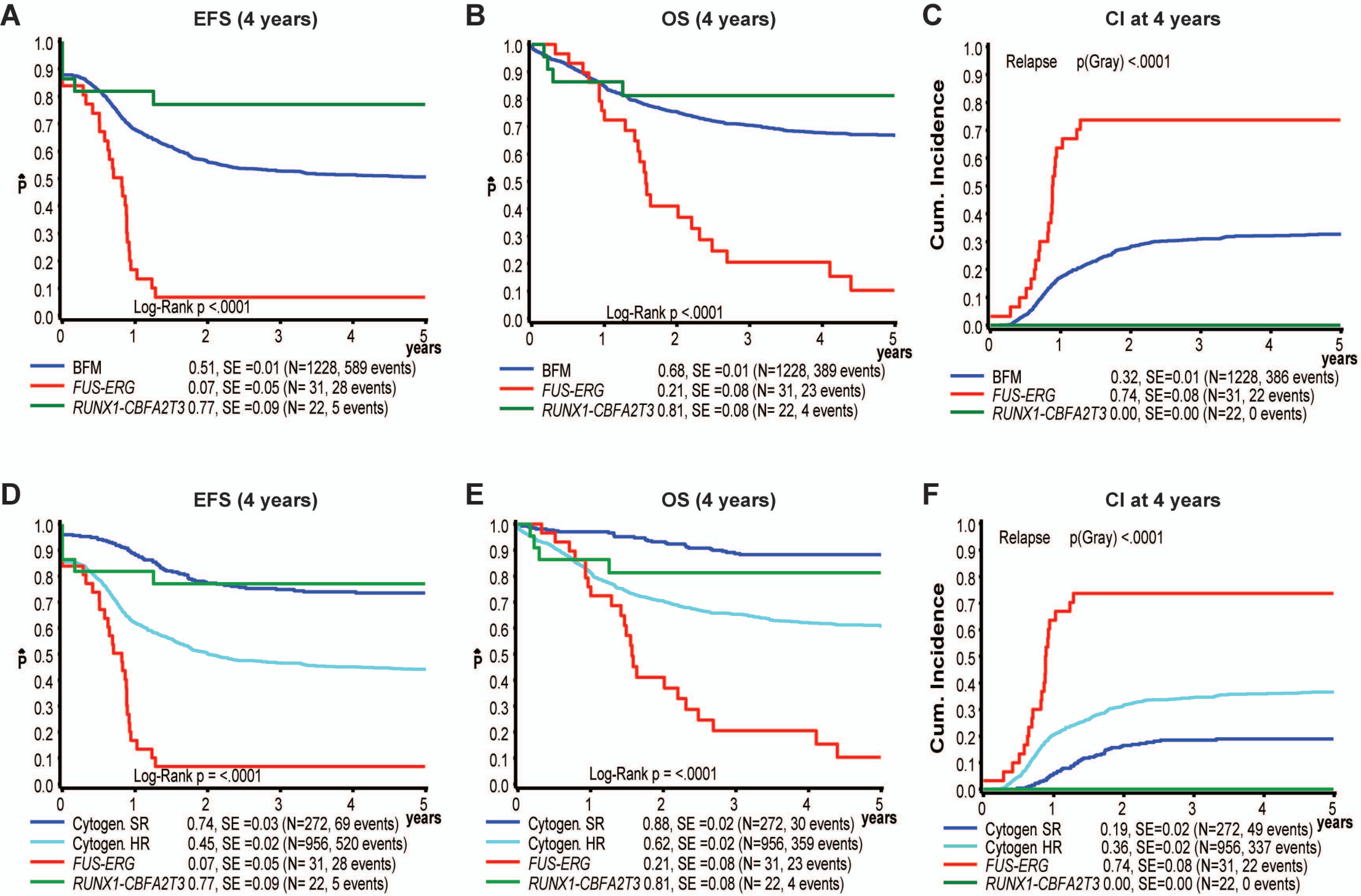
A
EFS (4 years)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
P̂
Log-Rank p <.0001
0
1
2
3
4
5
years
BFM 0.51, SE =0.01 (N=1228, 589 events)
FUS-ERG 0.07, SE =0.05 (N= 31, 28 events)
RUNX1-CBFA2T3 0.77, SE =0.09 (N= 22, 5 events)
B
OS (4 years)
Log-Rank p <.0001
BFM 0.68, SE =0.01 (N=1228, 389 events)
FUS-ERG 0.21, SE =0.08 (N= 31, 23 events)
RUNX1-CBFA2T3 0.81, SE =0.08 (N= 22, 4 events)
C
CI at 4 years
Relapse p(Gray) <.0001
Cum. Incidence
BFM 0.32, SE=0.01 (N=1228, 386 events)
FUS-ERG 0.74, SE=0.08 (N=31, 22 events)
RUNX1-CBFA2T3 0.00, SE=0.00 (N=22, 0 events)
D
EFS (4 years)
Log-Rank p = <.0001
Cytogen. SR 0.74, SE =0.03 (N=272, 69 events)
Cytogen. HR 0.45, SE =0.02 (N=956, 520 events)
FUS-ERG 0.07, SE =0.05 (N= 31, 28 events)
RUNX1-CBFA2T3 0.77, SE =0.09 (N= 22, 5 events)
E
OS (4 years)
Log-Rank p = <.0001
Cytogen. SR 0.88, SE =0.02 (N=272, 30 events)
Cytogen. HR 0.62, SE =0.02 (N=956, 359 events)
FUS-ERG 0.21, SE =0.08 (N= 31, 23 events)
RUNX1-CBFA2T3 0.81, SE =0.08 (N= 22, 4 events)
F
CI at 4 years
Relapse p(Gray) <.0001
Cum. Incidence
Cytogen SR 0.19, SE=0.02 (N=272, 49 events)
Cytogen HR 0.36, SE=0.02 (N=956, 337 events)
FUS-ERG 0.74, SE=0.08 (N=31, 22 events)
RUNX1-CBFA2T3 0.00, SE=0.00 (N=22, 0 events)

Figure 1

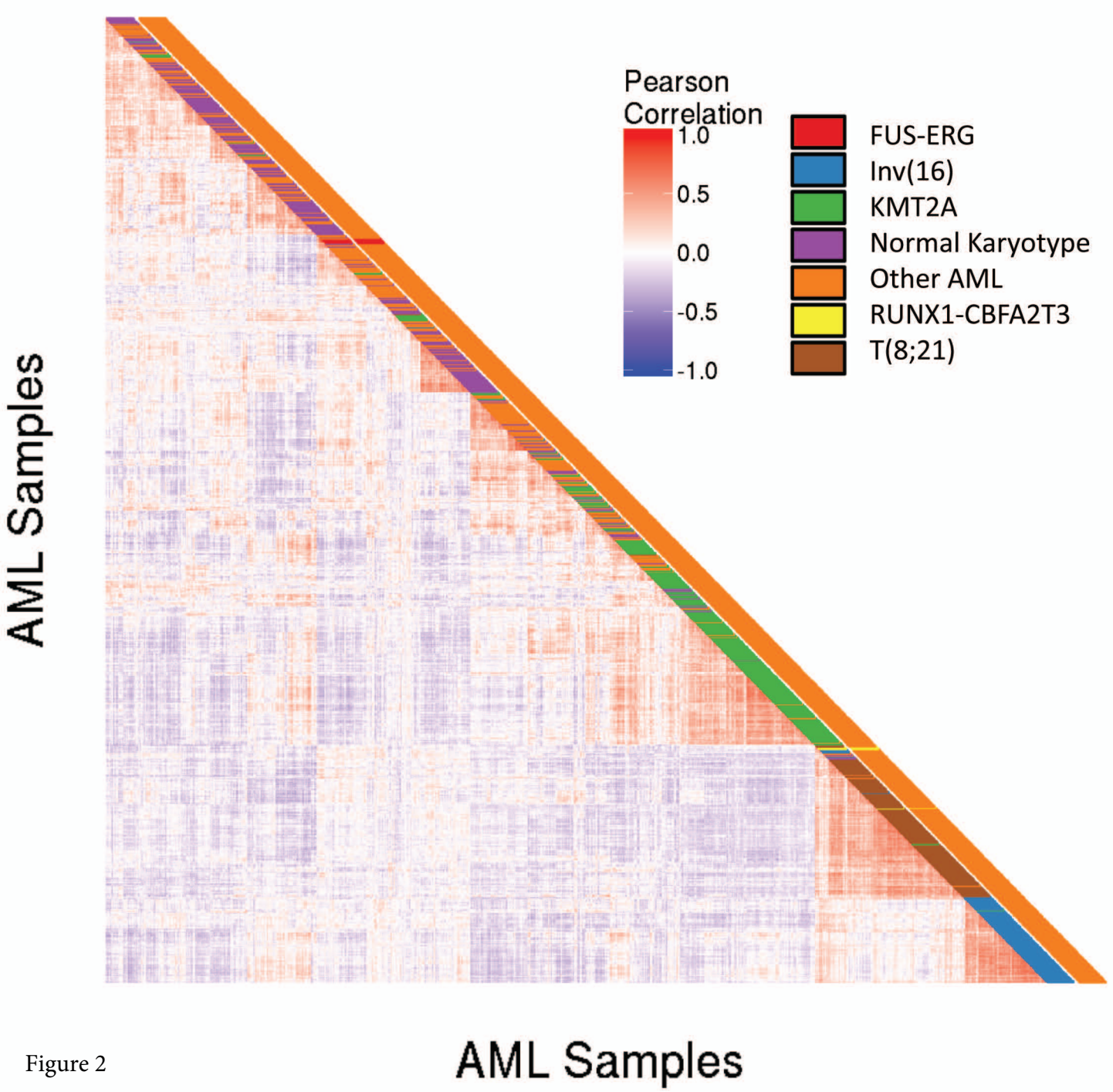
Pearson
Correlation
1.0
0.5
0.0
-0.5
-1.0
FUS-ERG
Inv(16)
KMT2A
Normal Karyotype
Other AML
RUNX1-CBFA2T3
T(8;21)
AML Samples
AML Samples

Figure 2

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Prognostic impact of t(16;21)(p11;q22) and t(16;21)(q24;q22) in pediatric AML: a retrospective study by the I-BFM Study Group

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