

# What are you missing in your research?

Highly sensitive, flexible flow cytometry

## Amnis® CellStream® Flow Cytometry System



For Research Use Only. Not for use in diagnostic procedures.

**Luminex®**  
complexity simplified.

**BRIEF REPORT**



**CYTOMETRY**  
*Journal of Quantitative Cell Science* PART A

# CD56, HLA-DR, and CD45 recognize a subtype of childhood AML harboring CBFA2T3-GLIS2 fusion transcript

Andrea Zangrando<sup>1,2</sup> | Francesca Cavagnero<sup>1</sup> | Pamela Scarparo<sup>1</sup> |  
 Elena Varotto<sup>1</sup> | Samuela Francescato<sup>1</sup> | Claudia Tregnago<sup>1</sup> |  
 Rosanna Cuccurullo<sup>3</sup> | Franca Fagioli<sup>4</sup> | Luca Lo Nigro<sup>5</sup> | Riccardo Masetti<sup>6</sup> |  
 Maria Caterina Putti<sup>1</sup> | Carmelo Rizzari<sup>7</sup> | Nicola Santoro<sup>8</sup> | Andrea Pession<sup>6</sup> |  
 Martina Pigazzi<sup>1</sup> | Franco Locatelli<sup>9</sup> | Giuseppe Basso<sup>1,10†</sup> | Barbara Buldini<sup>1</sup>

<sup>1</sup>Pediatric Hemato Oncology, Maternal and Child Health Department, University of Padua, Padua, Italy

<sup>2</sup>Pediatric Hemato Oncology, Istituto di Ricerca Pediatrica Città della Speranza, Padua, Italy

<sup>3</sup>Pediatric Hemato Oncology, Pausillipon Hospital, Naples, Italy

<sup>4</sup>Pediatric Onco-Hematology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, Turin, Italy

<sup>5</sup>Center of Pediatric Hemato-Oncology, Azienda Policlinico-OVE, Catania, Italy

<sup>6</sup>Department of Pediatrics, "Lalla Seragnoli", Hematology-Oncology Unit, Sant'Orsola-Malpighi Hospital, University of Bologna, Bologna, Italy

<sup>7</sup>Pediatric Hematology-Oncology Unit, Department of Pediatrics, University of Milano-Bicocca, MBBM Foundation c/o ASST Monza, Milan, Italy

<sup>8</sup>Department of Pediatric Hemato-Oncology, University of Bari, Bari, Italy

<sup>9</sup>Department of Pediatric Hematology and Oncology, Scientific Institute for Research and Healthcare (IRCCS) Childrens' Hospital Bambino Gesù, Sapienza, University of Rome, Rome, Italy

<sup>10</sup>Italian Institute for Genomic Medicine, Turin, Italy

**Correspondence**

Barbara Buldini, Maternal and Child Health Department, University of Padua, Via Giustiniani 3, 35128 Padua, Italy.  
 Email: barbara.buldini@unipd.it

**Funding information**

Fondazione CARIPARO, Grant/Award  
 Numbers: Airc-Ig n.19186, Airc-Ig n.20562

## Abstract

The presence of CBFA2T3-GLIS2 fusion gene has been identified in childhood Acute Myeloid Leukemia (AML). In view of the genomic studies indicating a distinct gene expression profile, we evaluated the role of immunophenotyping in characterizing a rare subtype of AML-CBFA2T3-GLIS2 rearranged. Immunophenotypic data were obtained by studying a cohort of 20 pediatric CBFA2T3-GLIS2-AML and 77 AML patients not carrying the fusion transcript. Enrolled cases were included in the Associazione Italiana di Ematologia Oncologia Pediatrica (AIEOP) AML trials and immunophenotypes were compared using different statistical approaches. By multiple computational procedures, we identified two main core antigens responsible for the identification of the CBFA2T3-GLIS2-AML. CD56 showed the highest performance in single marker evaluation (AUC = 0.89) and granted the most accurate prediction when used in combination with HLA-DR (AUC = 0.97) displaying a 93% sensitivity and 99% specificity. We also observed a weak-to-negative CD45 expression, being exceptional in AML. We here provide evidence that the combination of HLA-DR negativity and intense bright CD56 expression detects a rare and aggressive pediatric AML genetic lesion improving the diagnosis performance.

**KEY WORDS**

acute myeloid leukemia, CBFA2T3-GLIS2, computational analysis, immunophenotyping

Giuseppe Basso and Barbara Buldini contributed equally to this work as last co-authors.

†deceased.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Cytometry Part A* published by Wiley Periodicals LLC on behalf of International Society for Advancement of Cytometry.

## 1 | INTRODUCTION

A recurrent cryptic inversion of chromosome 16 [inv (16) (p13.3q24.3)] fuses a portion of *CBFA2T3* gene in frame with a region of *GLIS2* gene encoding for *CBFA2T3-GLIS2* fusion transcript [1,2]. This chimeric oncogene accounts for 18% of pediatric non-Down syndrome acute megakaryoblastic leukemia (non-DS-AMKL) patients and 8% of pediatric cytogenetically normal AML (CN-AML) associated with different French-American-British (FAB) subtypes [1–3]. This genetic lesion confers a particularly poor prognosis, with event-free survival (EFS) ranging between 8% and 33% in different studies [1–5]. The gene expression profile of *CBFA2T3-GLIS2* AML has been associated with up-regulation of both Hedgehog (HH) and bone morphogenic protein (BMP) signaling [2,6,7]. In view of the genomic studies indicating a distinct gene expression profile compared with other genetic subtypes of childhood AML [2], we evaluated the immunophenotypic profile of pediatric AML *CBFA2T3-GLIS2* rearranged.

## 2 | MATERIALS AND METHODS

From April 2001 to March 2017, we identified 25 *CBFA2T3-GLIS2*-positive cases. Five samples were excluded from this analysis due to missing immunophenotypic data. The immunophenotype of 20 *CBFA2T3-GLIS2*-positive cases was compared with a cohort of 77 *CBFA2T3-GLIS2*-negative AML patients, including also two cases of Down syndrome myeloid leukemia (ML-DS) and nine cases of acute promyelocytic leukemia (APL), collected in the same time interval. All patients were enrolled in the Associazione Italiana di Ematologia Oncologia Pediatrica (AIEOP) AML trials (AIEOP AML 2002, AIEOP ICC APL Study 01). The biological material used in this study was centralized at the Pediatric Hematology Oncology reference AIEOP Laboratory of Padua. Clinical features are detailed in Table 1, data on molecular genetics in Table S1. Written informed consent to the use of the patient's data and biological material was obtained from parents/legal guardians in accordance with the Declaration of Helsinki. Local institutional ethical committees approved the study, including

**TABLE 1** Clinical characteristics for non-DS and non-APL AML patients (*CBFA2T3-GLIS2* positive and negative) enrolled in the study

	<i>CBFA2T3-GLIS2</i> positive		<i>CBFA2T3-GLIS2</i> negative		<i>p</i>
	N	%	N	%	
<b>Total cohort</b>	18	100	66	100	
<b>Age (years)</b>					
<10	18	100	36	54.5	<0.01
>10	0	0	30	45.5	<0.01
<b>Gender</b>					
Female	10	55.6	37	56.1	0.11
Male	8	44.4	29	43.9	0.11
<b>WBC</b>					
≤10,000	3	16.7	23	34.8	0.16
10,000 ≤ 100,000	15	83.3	37	56.1	0.05
>100,000	0	0	6	9.1	0.33
<b>FAB</b>					
M0	0	0	2	3	1
M1	1	5.5	6	9.1	1
M2	1	5.5	20	27.4	0.06
M2Eo	0	0	2	3	1
M4	0	0	17	25.8	<b>0.02</b>
M4Eo	0	0	1	1.5	1
M5	1	5.5	11	16.7	0.44
M6	0	0	1	1.5	1
M7	14	78.0	3	4.5	<0.01
Other	1	5.5	5	7.5	1
<b>BM blasts (median %)</b>	50		64		0.37
<b>Extramedullary involvement<sup>a</sup></b>	4	22.2	6	9.1	0.21

Note: *p* values have been calculated using Fisher's statistics. The bold values denote statistical significance at the *p* < 0.05 level.

<sup>a</sup>One patient with additional CNS involvement, three patients without CNS involvement.

the collection of bone marrow samples (BM) for diagnostic work-up, along with the national protocols. Diagnosis of *de novo* AML was performed according to WHO classification criteria [8,9]. Samples were screened for the presence of t(8;21), inv(16), t(16;16), t(15;17) with associated molecular transcripts, namely core-binding factor (CBF)- $\beta$  abnormalities and PML-RAR $\alpha$ ; moreover, KMT2A rearrangements [9,10], Internal Tandem Duplication of FLT3 mutation (FLT3-ITD) [11] and other rare mutations were screened (Table S1).

## 2.1 | Flow cytometry

Initial evaluation of qualitative results from the whole immunophenotypic characterization was performed at diagnosis according to WHO criteria and the latest consensus guidelines for flow-cytometry immunophenotyping published by the AIEOP-BFM working group [12]. Quantitative values of mean fluorescence intensity (MFI) was retrieved for 14 antigens: CD45 ECD, HLA-DR PE-Cy7, CD56 PE, CD11a PE, CD11b PE, CD13 FITC, CD15 FITC, CD41 FITC, CD61 PE-Cy7, CD38 PE-Cy5, Myeloperoxidase (MPO) FITC, CD33 PE-Cy5, CD34 PE-Cy5, and CD117 PE. Samples were processed and analyzed in the Hemato Oncology Laboratory of Padua, according to standardized operating procedures (SOP) previously described [8,12]. Flow cytometry methods have been detailed in a Supplementary document according to MIFlowCyt standards.

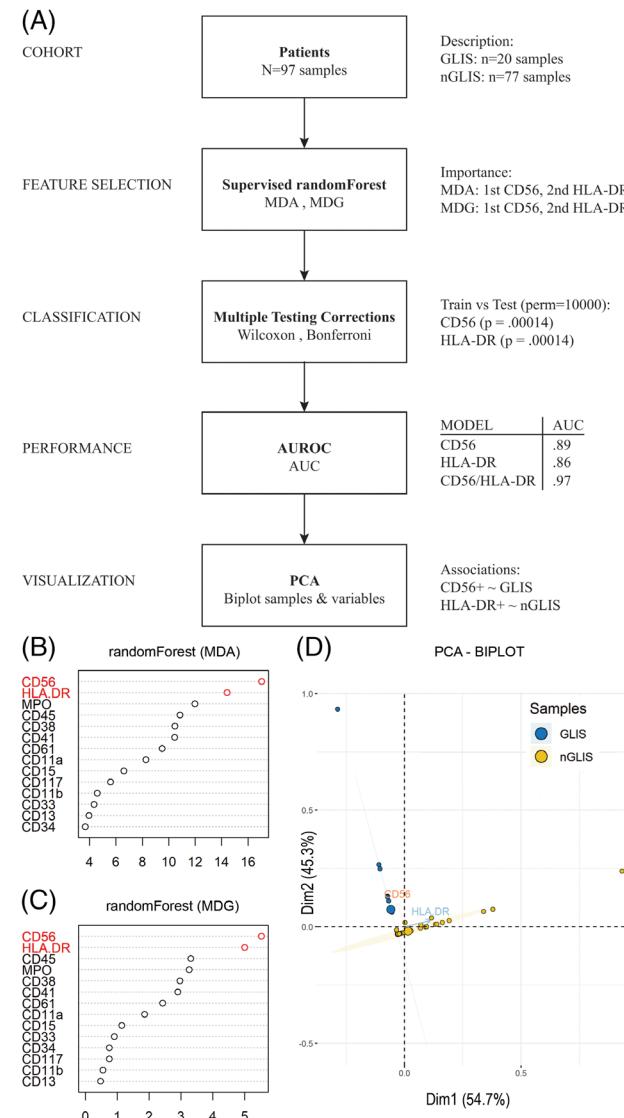
## 2.2 | Statistical analyses

Statistical procedures were performed to identify putative antigens correlated with the presence of CBFA2T3-GLIS2 mutation and estimate their accuracy in classifying patients as “GLIS” (with mutation) or “nGLIS” (without mutation) samples. All statistical analyses were performed using R software [13]: Random Forest analyses including Mean Decrease in Accuracy (MDA) and Mean Decrease in Gini coefficient (MDG) procedures were performed using “randomForest” package [14]; multiple testing corrections were evaluated with “multtest” package [15]; permutation tests were retrieved using k-Nearest Neighbor classifier and “caret” package; classification model’s performance was evaluated using Area Under Receiver Operating Characteristics (AUROC or AUC) calculated with “ROCR” package; principal component analysis (PCA) was obtained using “factoextra” and “factoMiner” packages. Figure 1A summarizes the analysis steps performed in the whole study starting from cohort description through feature selection, classification, and performance, ending to spatial visualization of the results.

## 3 | RESULTS AND DISCUSSION

Ninety-seven patients were investigated for antigen ( $N = 14$ ) associations using different computational analyses (Figure 1A). First, supervised random forests analysis was performed to measure the

importance of all variables and their ability in classifying the data appropriately [14]. MDA reflects the impact of each antigen on the accuracy of the prediction, model-antigens with a large mean decrease in accuracy, being more important for classification. As shown in Figure 1B, CD56 and HLA-DR antigens were the most accurate markers for the identification of “GLIS” AML cases in MDA evaluation; MDG (Figure 1C) confirmed MDA results, as detailed in Table S2. The same markers were identified by antigen selection



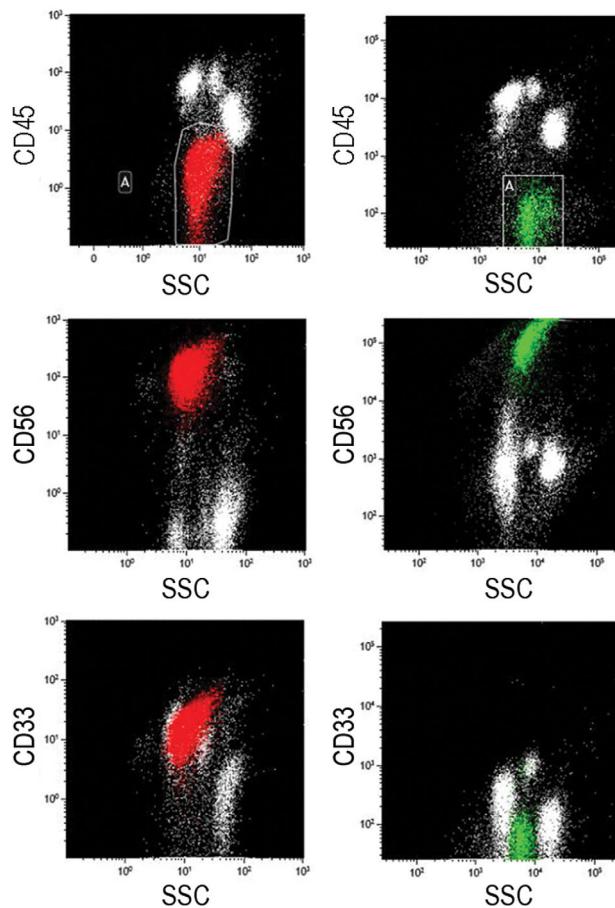
**FIGURE 1** Variable selection procedures and data presentation. (A) Analysis steps performed from cohort description to spatial visualization of the results. (B) Mean decrease in accuracy (MDA) results using “randomForest” package in R software. CD56 and HLA-DR have the largest mean decrease scores and are considered the most accurate antigens for classification of the data. (C) Mean decrease in Gini (MDG) coefficient confirms CD56 and HLA-DR antigens as the most accurate markers in separating “GLIS” and “nGLIS” patients. (D) Principal component analysis (PCA) results using “factoextra” and “factoMiner” packages. “GLIS” patients (in red) are associated with CD56 expression, while “nGLIS” group (in blue) with HLA-DR [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

based on Wilcoxon's test and Bonferroni procedure for multiple testing corrections [15]. As shown in Table S3, CD56 ( $p = 0.00014$ ) and HLA-DR ( $p = 0.00014$ ) were the most discriminating markers followed by CD45 ( $p = 0.00686$ ), MPO ( $p = 0.00882$ ), CD38 ( $p = 0.01932$ ), and CD11a ( $p = 0.02282$ ). To test the classification strength of identified markers, for each of the 10,000 permutations we randomly assigned patients either to training (total  $N = 64$ :  $N = 13$  "GLIS,"  $N = 51$  "nGLIS") or validation (total  $N = 33$ :  $N = 7$  "GLIS,"  $N = 26$  "nGLIS") sets. We further calculated the performance of the two markers and their combination in classifying "GLIS" and "nGLIS" patients using ROC curves. Weighted mean (WM) was calculated for accuracy measures including sensitivity, specificity, PPV (positive predictive value), and NPV (negative predictive value) as well as for AUC permutations. CD56 held the highest score in single-marker model evaluation (AUC = 0.89) followed by HLA-DR (AUC = 0.86); remarkably, their combination granted the most accurate model prediction with AUC = 0.97, as well as the highest weighted mean sensitivity (0.93), specificity (0.99), PPV (0.96), and NPV (0.98). Biplot (PCA) was performed on all patients using only CD56 and HLA-DR to clearly show the correlation between groups and antigens supporting the strength of the selected signature (Figure 1D): "GLIS" group was strongly associated with expression of CD56, while "nGLIS" correlated mainly with HLA-DR antigen expression (details in Table S4).

The aim of this study was to evaluate the role of multiparametric flow cytometry analysis in the identification of CBFA2T3-GLIS2 positive AML as previously reported for other genetic subtypes. Our results show that the combination of low HLA-DR together with CD56 as intensive bright provides the highest accuracy in classifying an AML case as "GLIS." The lack of HLA-DR is one of the most sensitive feature typical of APL with respect to other AML subtypes [16] and is described as negative also in some NPM1-mutated AMLs [17–19]. Antibodies against CD56 are primarily used to detect natural killer (NK) cells but are also useful in the evaluation of pediatric solid tumors, such as neuroendocrine and neuroectodermal malignancies (e.g., neuroblastoma, primitive neuroectodermal tumor) [20, 21]. We attempted a CD56 intensity comparison in GLIS-AML and a solid tumor finding a comparable CD56 bright expression (Figures 2 and S1A,B). In AML, CD56 expression has been frequently associated with t(8;21) RUNX1-RUNX1T1 [22] but with a lower intensity than compared with "GLIS" cases. Thiollier et al. previously showed that CBFA2T3-GLIS2+ AMKL exhibits a distinct gene-expression signature: surface marker NCAM1 (CD56) presented a mean differential expression of 35-fold by microarray and of more than 200-fold by RNA-seq with respect to other non-DS AMKL. Using flow-cytometry, they also observed that AMKL blasts carrying CBFA2T3-GLIS2 fusion were CD41+/CD56+ [6]. In our cohort, we observed by flow cytometry a CD56 median differential expression of 29-fold with respect to AML nGLIS (Figure S1B). Moreover, in diagnostic immunophenotyping either lack of or low expression of CD45 is uncommon in AML (Figure S1A); negativity for CD45 associated with positivity for CD56 could be suggestive of non-hematological malignancies [20,21]. In the group of "GLIS" patients here described, CD45 was under-expressed with respect to other AML subtypes (not statistically relevant).

**Case 1**  
CBFA2T3-GLIS2 AML  
positive

**Case 2**  
Neuroblastoma  
invading BM



**FIGURE 2** Flow cytometry analyses of BM samples. CBFA2T3-GLIS2 positive AML immunophenotype (Case 1) may be misinterpreted with non-hematopoietic tumors BM involvement (Case 2) due to the comparable high expression of CD56 and downregulation of CD45 [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

The peculiar immunophenotype we discuss also matches the RAM phenotype described by Eidenschink Brodersen et al. [23]. The RAM phenotype was characterized by bright CD56, dim-to-negative CD45 and CD38, lack of HLA-DR; it was primarily restricted to very young patients and associated with an extremely poor outcome [23,24]. Pardo et al., investigated the relationship between CD56 expression and prognosis in AML patients enrolled in the Children's Oncology Group Trial AAML0531 [25]. In particular, their Cohort 3 was characterized by a unique immunophenotype (RAM phenotype) and a high prevalence of patients with CBFA2T3-GLIS2 fusion transcript. In our study, we corroborate a highly specific immunophenotype for this fusion transcript in an independent cohort of patients enrolled in the AIEOP study. Analysis of our patients' characteristics confirmed that the CBFA2T3-GLIS2 rearrangement is typical of very young children [26] and is not restricted to M7 subtype (Table 1) [1]. Although, despite this characteristic immunophenotype, no morphological aspects were

suggestive for “GLIS”: BM smears could also mimic BM invasion by non-hematopoietic tumors (Figure 3A–D). Clinical features at leukemia presentation could include extramedullary involvement: frequency of extramedullary leukemia in our CBFA2T3-GLIS2 patients was higher (22.2%, Table 1) than that described in pediatric AML [27]. Notably, in two of four cases, extramedullary involvement (including massive cranial bone, ribs, and lumbosacral column involvement, respectively) primarily raised the suspicion of non-hematopoietic tumor instead of acute leukemia.

Accurate diagnosis of childhood hematological malignancies is often challenging and based on the combination of morphology, flow cytometry, cytogenetics, and molecular biology data. Here, we describe an immunophenotypic profile specific for a rare cytogenetic lesion (CBFA2T3-GLIS2), clearly discriminated from other AML subtypes and characterized by weak-to-negative CD45 expression unusual in AML, HLA-DR negativity (a finding uncommon in AML apart from APL and NPM1-mutated cases), and intense bright CD56 expression (more suggestive of non-hematological malignancies). This immunophenotypic profile not suggestive of AML could be associated with clinical presentation and BM morphologic features that overlap non-hematopoietic tumors as shown in some of our patients.

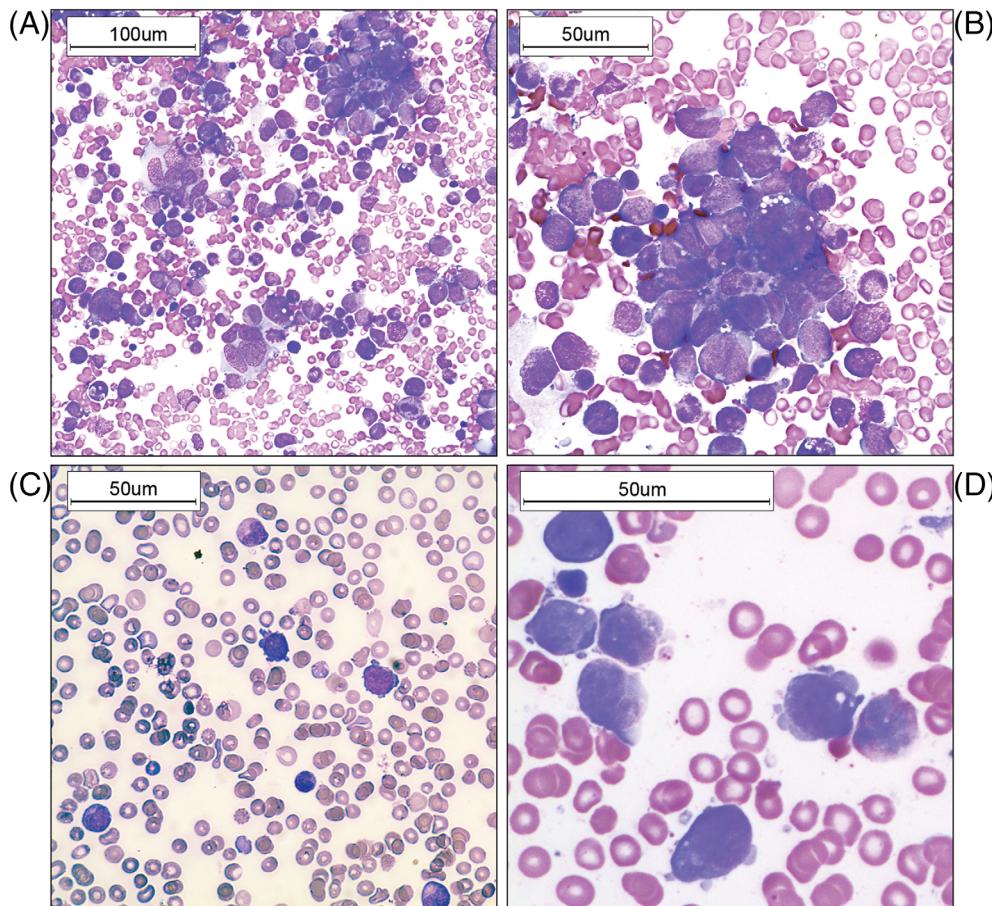
In the presence of symptoms related to organ failure or extramedullary involvement, in young children with no clear diagnosis

of solid tumors, we propose to add a BM aspirate to the diagnostic workup, even if peripheral blood count does not portend leukemia diagnosis. Whenever suspicious tumor cells are detected, further comprehensive phenotypic characterization must be performed. Immunophenotyping by flow cytometry is mandatory for a correct AML diagnosis and can provide the indication of molecular biology before other invasive diagnostic procedures are planned.

Overall, our study supports a specific immunophenotype highly suggestive of the AML subtype carrying CBFA2T3-GLIS2 rearrangement. Our results indicate that an accurate flow cytometry evaluation of leukemia cells at diagnosis allows a rapid identification of this AML subtype even when AML morphological aspect and clinical presentation may not be suggestive of this rare and aggressive pediatric AML entity.

#### ACKNOWLEDGMENTS

Dedicated to the memory of Prof. Giuseppe Basso (1948–2021). We are grateful to patients, families, Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) and clinical teams who have been involved in this study. We thank our colleagues of the Hemato Oncology lab at the University of Padua for sample preparation and analysis; we sincerely thank Marco Giordan and Carlo Zanon for statistical suggestions. This work was supported by Fondazione CARIPARO



**FIGURE 3** Examples of morphological presentation of bone marrow aspirates in CBFA2T3-GLIS2 positive patients. (A) Bone marrow aspirate showing AML infiltration overlapping BM involvement by non-hematopoietic tumors; (B) High-power magnification of leukemia cells clump; (C) infant with clinical presentation suggestive of non-hematopoietic tumor: in normal regenerating BM aspirates, isolated cells of medium size with large nuclei and prominent nucleoli, basophilic cytoplasm with blebs, no granules or Auer rods may be observed; (D) Leukemia cells suggestive of FAB-M7 morphology [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

(to G. B. and M. P.), AIRC (Airc-Ig n.19186 to G. B., Airc-Ig n.20562 to M. P.), Istituto di Ricerca Pediatrica Città della Speranza and Fondazione Città della Speranza.

## AUTHOR CONTRIBUTIONS

**Andrea Zangrando:** Conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing-original draft; writing-review & editing. **Francesca Cavagnero:** Formal analysis; investigation; methodology. **Pamela Scarparo:** Data curation; formal analysis; investigation; visualization. **Elena Varotto:** Data curation; formal analysis; investigation; visualization; writing-review & editing. **Samuela Francescato:** Investigation; visualization. **Claudia Tregnago:** Data curation. **Rosanna Cuccurullo:** Resources. **Franca Fagioli:** Resources. **Luca Lo Nigro:** Resources. **Riccardo Mastretta:** Resources. **Maria Caterina Putti:** Resources. **Carmelo Rizzari:** Resources. **Nicola Santoro:** Resources. **Andrea Pession:** Data curation; resources. **Martina Pigazzi:** Conceptualization; data curation; funding acquisition; investigation; methodology; writing-original draft; writing-review & editing. **Franco Locatelli:** Conceptualization; data curation; methodology; resources; supervision; writing-original draft. **Giuseppe Basso:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing-original draft; writing-review & editing. **Barbara Baldini:** Conceptualization; data curation; investigation; methodology; project administration; resources; supervision; validation; writing-original draft; writing-review & editing.

## CONFLICT OF INTEREST

The authors declare no competing financial interests.

## ORCID

Andrea Zangrando  <https://orcid.org/0000-0002-9816-2299>

## REFERENCES

1. Masetti R, Pigazzi M, Togni M, Astolfi A, Indio V, Manara E, et al. CBFA2T3-GLIS2 fusion transcript is a novel common feature in pediatric, cytogenetically normal AML, not restricted to FAB M7 subtype. *Blood*. 2013;121(17):3469–72.
2. Gruber TA, Larson Gedman A, Zhang J, Koss CS, Marada S, Ta HQ, et al. An Inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. *Cancer Cell*. 2012;22(5):683–97.
3. Gruber TA, Downing JR. The biology of pediatric acute megakaryoblastic leukemia. *Blood*. 2015;126(8):943–9.
4. de Rooij JD, Masetti R, van den Heuvel-Eibrink MM, Cayuela JM, Trka J, Reinhardt D, et al. Recurrent abnormalities can be used for risk group stratification in pediatric AMKL: a retrospective intergroup study. *Blood*. 2016;127(26):3424–30.
5. de Rooij JD, Branstetter C, Ma J, Li Y, Walsh MP, Cheng J, et al. Pediatric non-down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. *Nat Genet*. 2017;49(3):451–6.
6. Thiollier C, Lopez CK, Gerby B, Ignacimouttou C, Poglio S, Duffourd Y, et al. Characterization of novel genomic alterations and therapeutic approaches using acute megakaryoblastic leukemia xenograft models. *J Exp Med*. 2012;209(11):2017–31.
7. Masetti R, Bertuccio SN, Astolfi A, Chiarini F, Lonetti A, Indio V, et al. Hh/Gli antagonist in acute myeloid leukemia with CBFA2T3-GLIS2 fusion gene. *J Hematol Oncol*. 2017;10(1):26.
8. Basso G, Baldini B, De Zen L, Orfao A. New methodologic approaches for immunophenotyping acute leukemias. *Haematologica*. 2001;86(7):675–92.
9. Swerdlow SH, Campo E, Harris NL, Harris NL, Jaffe ES, Pileri SA, et al. WHO classification of Tumours of Haematopoietic and lymphoid tissues. Lyon, France: IARC Press; 2008.
10. Pigazzi M, Masetti R, Bresolin S, Beghin A, Di Meglio A, Gelain S, et al. MLL partner genes drive distinct gene expression profiles and genomic alterations in pediatric acute myeloid leukemia: an AIEOP study. *Leukemia*. 2011;25(3):560–3.
11. Manara E, Basso G, Zampini M, Baldini B, Tregnago C, Rondelli R, et al. Characterization of children with FLT3-ITD acute myeloid leukemia: a report from the AIEOP AML-2002 study group. *Leukemia*. 2017;31(1):18–25.
12. Dworzak MN, Baldini B, Gaipa G, Ratei R, Hrusak O, Luria D, et al. AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of pediatric acute lymphoblastic leukemia. *Cytometry B Clin Cytom*. 2018;94:82–93.
13. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org>.
14. Classification and Regression by randomForest by Andy Liaw and Matthew Wiener in R News (ISSN 1609–3631), 2007.
15. Pollard KS, Dudoit S, van der Laan MJ. Multiple testing procedures: R multtest package and applications to genomics. *Bioinformatics and computational biology solutions using R and bioconductor*. New York: Springer; 2005.
16. Orfao A, Chillón MC, Bortoluci AM, López-Berges MC, García-Sanz R, Gonzalez M, et al. The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML-RARalpha gene rearrangements. *Haematologica*. 1999;84(5):405–12.
17. Kern W, Haferlach C, Bacher U, Haferlach T, Schnittger S. Flow cytometric identification of acute myeloid leukemia with limited differentiation and NPM1 type a mutation: a new biologically defined entity. *Leukemia*. 2009;23(7):1361–4.
18. Syampurnawati M, Tatsumi E, Ardianto B, Takenokuchi M, Nakamachi Y, Kawano S, et al. DR negativity is a distinctive feature of M1/M2 AML cases with NPM1 mutation. *Leuk Res*. 2008;32(7):1141–3.
19. Chen W, Konoplev S, Medeiros LJ, Koeppen H, Leventaki V, Vadhan-Raj S, et al. Cuplike nuclei (prominent nuclear invaginations) in acute myeloid leukemia are highly associated with FLT3 internal tandem duplication and NPM1 mutation. *Cancer*. 2009;115(23):5481–9.
20. Bryson GJ, Lear D, Williamson R, Wong RCW. Detection of the CD56+/CD45– immunophenotype by flow cytometry in neuroendocrine malignancies. *J Clin Pathol*. 2002 Jul;55(7):535–7.
21. Ferreira-Facio CS, Milito C, Botafogo V, Fontana M, Thiago LS, Oliveira E, et al. Contribution of multiparameter flow cytometry immunophenotyping to the diagnostic screening and classification of pediatric cancer. *PLoS One*. 2013;8(3):e55534.
22. Hurwitz CA, Loken MR, Graham ML, Karp JE, Borowitz MJ, Pullen DJ, et al. Asynchronous antigen expression in B lineage acute lymphoblastic leukemia. *Blood*. 1988;72(1):299–307.
23. Eidenschink Brodersen L, Alonzo TA, Menssen AJ, Gerbing RB, Pardo L, Voigt AP, et al. A recurrent immunophenotype at diagnosis independently identifies high-risk pediatric acute myeloid leukemia: a report from Children's oncology group. *Leukemia*. 2016;30(10):2077–80.
24. Voigt AP, Eidenschink Brodersen L, Alonzo TA, Gerbing RB, Menssen AJ, Wilson ER, et al. Phenotype in combination with genotype improves outcome prediction in acute myeloid leukemia: a

- report from Children's oncology group protocol AAML0531. *Haematologica*. 2017;102(12):2058–68.
25. Pardo LM, Voigt AP, Alonso TA, Wilson ER, Gerbing RB, Paine DJ, et al. Deciphering the significance of CD56 expression in pediatric acute myeloid leukemia: a report from the Children's oncology group. *Cytometry B Clin Cytom*. 2020;98(1):52–6.
26. Schuback HL, Alonso TA, Gerbing RB, Miller KL, Kahwash S, Heerema-Mckenney A, et al. CBFA2T3-GLIS2 fusion is prevalent in younger patients with acute myeloid leukemia and associated with high-risk of relapse and poor outcome: a Children's oncology group report. *Blood*. 2014;124(21):13.
27. Pession A, Masetti R, Rizzari C, Putti MC, Casale F, Fagioli F, et al. Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. *Blood*. 2013;122(2):170–8.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Zangrando A, Cavagnero F, Scarparo P, et al. CD56, HLA-DR, and CD45 recognize a subtype of childhood AML harboring CBFA2T3-GLIS2 fusion transcript. *Cytometry*. 2021;99:844–850. <https://doi.org/10.1002/cyto.a.24339>