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MEDICINE AND PHARMACY UNIVERSITY
”CAROL DAVILA”, BUCHAREST
DOCTORAL SCHOOL
FIELD OF MEDICINE

DOCTORAL THESIS

PhD Coordinator:

PROF. UNIV. DR. ANCA COLIȚĂ

PhD Student:

BELDIMAN (MARCU) ANDRA-DANIELA

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The importance of molecular diagnosis for personalized treatment in pediatric myeloid neoplasms

SUMMARY OF THE DOCTORAL THESIS

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Introduction

Pediatric myeloid neoplasms represent a rare and heterogeneous group of hematologic disorders, profoundly different from adult forms, marked by a distinct genomic architecture for each subtype. Recent advances in molecular technologies have enabled a shift beyond the classical morphological framework, fostering an integrative approach centered on genetic signatures, with fundamental implications for understanding hematopoiesis and advancing personalized therapy.

This thesis explores this complexity along three major axes: mutational characterization, assessment of the genetic impact on clinical evolution, and the optimal management approach in the context of hematopoietic stem cell transplantation (HSCT). The overarching goal is to anticipate disease aggressiveness, identify vulnerable subgroups, and select personalized treatments tailored to the molecular profile of these rare entities, which hold significant implications in modern pediatric hematology.

Current state of knowledge

The general section of the thesis provides a rigorous theoretical foundation on pediatric myeloid neoplasms through an integrative analysis of the biological, genetic, clinical and therapeutical characteristics of these rare entities. It begins with detailed epidemiological data for each entity: acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and myeloproliferative neoplasms (MPN), with a focus on juvenile myelomonocytic leukemia (JMML). These data are presented in comparison to adult population in order to highlight the necessity of tailoring diagnostic and staging criteria specifically for pediatric pathology (1, 2).

The classification of pediatric myeloid neoplasms has undergone a significant transformation in recent decades, shifting from an approach based solely on morphological and clinical criteria to an integrative, molecular, and adaptive model. The latest WHO-HAEM5 and ICC 2022 editions reflect this transition, acknowledging the distinct genetic and clinical features that differentiate pediatric cases from those in adults (3, 4). Pediatric AML has undergone particularly substantial revisions, with a redefinition of entities based on specific genetic rearrangements (e.g., KMT2A, NUP98, MECOM) and the introduction of new subtypes such as “AML with other defined genetic abnormalities.” These updates are complemented by the recognition of germline mutations with diagnostic and therapeutic implications (5).

Regarding MDS, recent classifications emphasize the importance of differentiating between low- and high-blast forms, integrating recurrent mutations and complex cytogenetic criteria for risk stratification and HSCT indication. The classical term “refractory cytopenia of childhood” has been replaced by more clearly defined entities, such as “MDS with low blast count” and “MDS with increased blast count,” reflecting a more accurate understanding of disease biology. Emphasis is also placed on excluding other causes of cytopenia and recognizing the role of germline mutations (6, 7).

JMML, previously categorized under MDS/MPN, has been reclassified in the WHO classification as a myeloproliferative neoplasm, while in the ICC it is associated with germline mutations or pediatric-specific forms. This reclassification is grounded in the recognition of canonical RAS pathway mutations, present in the majority of cases. It underscores the importance of identifying neoplasms associated with germline predisposition syndromes (3-5).

The following sections of the general part are dedicated to presenting the clinical and biological particularities in children, as well as the current diagnostic stages (7-14). Furthermore, it details the characterization of mutations with major pathogenetic roles, epigenetic dysfunctions, and chromosomal rearrangements associated with unfavorable prognosis (15-22). The identification and characterization of genetic predispositions in myeloid neoplasms represents a crucial component in the evaluation of persistent cytopenias in children and young adults. Understanding these genetic syndromes, whether they present with evident phenotypes or solely hematologic manifestations, enables personalization of therapeutic strategies, prognosis optimization and effective implementation of family counseling (10, 23, 24).

All these transformations reflect a profound reconfiguration of the diagnostic and therapeutic paradigm, oriented toward the integration of the genomic profile as a central element of clinical evaluation. In this context, the general section of the thesis conceptually underpins personalized management in pediatric hemato-oncology, providing the necessary support for the integrative interpretation of clinical and molecular data from the original studies.

PERSONAL CONTRIBUTION

Working hypotheses, general objectives, and research methodology

This thesis explores the biological and clinical complexity of pediatric myeloid neoplasms through a multidimensional approach focused on the integration of genetic data into therapeutic decision-making. The investigative endeavor is grounded in an in-depth analysis of the disease's molecular architecture, evaluating how mutations influence its clinical course and the optimal therapeutic approach within the HSCT setting. The formulated hypotheses support the existence of functional connections between genetic alterations and clinical phenotype, with the potential to guide personalized therapeutic strategies and generate new directions in translational research.

The methodology was based on an observational, cross-sectional study involving a cohort of 61 pediatric patients diagnosed and treated at the Fundeni Clinical Institute over a six-year period. The study encompassed three pathological groups: AML (including MPAL and Down syndrome-associated AML), MDS, and MPN, all diagnosed according to international criteria. HSCT was indicated based on evolutionary risk and was performed in patients who achieved complete remission. The investigation was structured in three phases: (1) molecular analysis and correlation of the genetic profile with clinical characteristics and survival until HSCT; (2) evaluation of the relationship between the initial mutational profile and early therapeutic response; (3) detailed analysis of transplanted patients, with emphasis on the impact of conditioning regimens on remission, post-HSCT complications, and one-year survival.

This integrative approach emphasizes the importance of tailoring therapeutic strategies according to clinical features and individual molecular profiles, thus promoting a personalized treatment model in pediatric hemato-oncology. The data were processed using robust statistical methods, including survival analyses and heatmap visualizations, all conducted in accordance with current ethical standards.

Chapter 6. Study 1 – Identification of molecular characteristics in pediatric myeloid neoplasms

This study investigates the molecular characteristics of pediatric myeloid neoplasms, based on the premise that each pathological entity (AML, MDS, MPN) exhibits a distinct genetic signature with functional and prognostic implications. It is grounded in the hypothesis that an

integrated analysis of immunophenotypic, cytogenetic, and molecular profiles enables the definition of specific pathogenetic pathways and early markers of disease aggressiveness. The central objective was the identification of clinically relevant molecular risk factors, with secondary objectives including the assessment of significant mutations, prognostic trends associated with genetic abnormalities, the predictive value of markers such as CD7, and the analysis of clinical aggressiveness signals at disease onset.

Working hypotheses for Study 1 were:

i. The molecular and biological heterogeneity of pediatric myeloid neoplasms defines distinct pathogenetic pathways, which can be captured through integrated analysis of clinical, immunophenotypic, and molecular characteristics, with potential implications for prognosis and therapeutic strategies.

ii. The expression of immunophenotypic markers such as CD7 or specific genetic abnormalities (e.g., FLT3-ITD, chromosome 7 aberrations, KMT2A rearrangements) constitutes early indicators of disease aggressiveness and may predict unfavorable prognosis, including post-transplant outcomes.

iii. Pediatric myeloid neoplasms (AML, MDS, MPN) exhibit distinct functional molecular signatures, correlated with specific biological behavior.

Main objective for Study 1 was:

i. To evaluate the clinical, immunophenotypic, cytogenetic, and molecular profile of pediatric myeloid neoplasms (AML, MDS, MPN) in order to identify risk factors and predictive biological signatures with prognostic impact.

Secondary objectives were:

i. To explore the distribution of mutations and functional pathways across diagnostic entities.

ii. To investigate the prognostic trends of genetic abnormalities identified at diagnosis.

iii. To assess immunophenotypic markers (CD7, CD56, CD123) as early predictors of poor prognosis.

iv. To identify clinical and biological baseline parameters that may indicate disease aggressiveness.

The cohort of 61 subjects was selected based on inclusion criteria, and patients were diagnosed according to international standards, utilizing a multidimensional approach for

characterization: clinical, immunophenotypic, cytogenetic, and molecular data. Biological samples for next-generation sequencing (NGS) analysis were processed for genomic DNA extraction using standardized methods, with rigorous verification of DNA quality and concentration. Targeted sequencing was performed on 23 patients using the TruSight Myeloid Panel (MiSeq), while the remaining 38 were analyzed under a national extended sequencing program (TruSight Oncology 500 panel), covering 523 genes and including a gene fusion panel. Sample preparation for NGS analysis consisted of three stages: (1) DNA library preparation, (2) actual sequencing, and (3) bioinformatic analysis. Only mutations with an allelic frequency ≥2% and potential pathogenic significance were reported, classified according to international guidelines.

The study demonstrated the complexity and profound heterogeneity of pediatric myeloid neoplasms, highlighting significant differences among the analyzed entities not only at the clinical level, but also from a genomic, immunophenotypic, and functional perspective. Although the sample size limited the ability to obtain statistically robust conclusions for certain parameters, the observed clinical and molecular trends support the relevance of markers such as CD7, KMT2A and NUP98 fusions, *FLT3-ITD* mutations, and chromosome 7 abnormalities as factors associated with poor prognosis. The identification of cooperative genetic architectures and specific functional networks per entity provides a solid foundation for developing integrated and personalized therapeutic strategies, with an emphasis on dynamic risk stratification and early adaptation of treatment approaches according to the individual biological profile.

Chapter 7. Study 2 – The impact of molecular lesions on the clinical evolution of pediatric patients with myeloid neoplasms

Study 2 aims to explore the impact of molecular lesions on the clinical evolution of pediatric patients with malignant myeloid pathology or predisposition to it, based on three main directions. The first section analyzes the correlation between response to frontline therapy, patient evolution up to HSCT, and the genetic profile identified at diagnosis. The second part focuses on somatic mutations, emphasizing *TP53*, known for its association with poor prognosis, and the activation of the RAS/MAPK pathway with its multiple implications. Finally, the study highlights the relevance of genetic predispositions and germline mutations such as *GATA2* and *RUNX1*, which provide critical insight into hereditary risk in myeloid neoplasms and their impact in the context of HSCT.

By integrating molecular data with clinical and therapeutic information, this study aims to strengthen a personalized approach to the treatment of pediatric myeloid neoplasms, guided by the individual genomic profile of each patient.

Working Hypotheses for Study 2 were:

i. Pediatric patients achieving minimal resifual disease (MRD) negativity post-induction exhibit significantly improved overall survival and lower relapse rates following HSCT.

ii. In juvenile myelomonocytic leukemia (JMML) with RAS pathway mutations, clinical response to azacitidine correlates with molecular remission post-HSCT, indicating its predictive value.

iii. Conventional cytogenetic profiling lacks sufficient predictive capacity for induction therapy response or post-HSCT outcomes.

iv. *TP53* mutations are associated with a highly adverse prognosis regardless of therapeutic strategy, justifying early and intensified treatment approaches.

v. Germline mutations (e.g., *RUNX1*, *GATA2*) significantly increase the risk of post-transplant complications and necessitate an individualized therapeutic strategy.

The main objectives of Study 2 were:

i. To characterize the therapeutic response based on the induction regimen (AIE/MEC), MRD status, and initial cytogenetic profile in acute myeloid leukemia (AML).

ii. To determine the predictive value of the response to azacitidine (AZA) for the success of HSCT in patients with myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML), particularly in LMMCJ.

iii. To evaluate the correlation between somatic/germline molecular profiles, therapeutic response, complications, and post-transplant survival.

Secondary objectives:

i. Analyzing the impact of *TP53* mutations on treatment response and overall survival.

ii. Describing the clinical behavior of mutations in the RAS pathway, with particular focus on *NRAS* due to its high frequency in the studied cohort.

iii. Exploring the clinical significance of germline mutations in *RUNX1* and *GATA2*, emphasizing their influence on disease evolution and outcomes in HSCT.

iv. Systematizing cases with rare genetic syndromes to highlight the need for comprehensive genetic screening in pediatric myeloid neoplasms.

This observational study included pediatric patients diagnosed with AML, MDS, and MPN between 2020 and 2025, who received first-line treatment according to international therapeutic protocols. The primary objective of the analysis was to evaluate the correlation between treatment response and the genetic profile identified at diagnosis.

For the diagnosis of AML, patients were treated according to current international protocols: AIE (cytarabine, idarubicin, etoposide) under the AML-BFM protocol, and MEC (mitoxantrone, etoposide, cytarabine) according to the NOPHO protocol. For diagnoses of MDS and MPN, azacitidine (AZA) was administered at a dose of 75 mg/m²/day intravenously for 7 consecutive days, in 28-day cycles, with careful monitoring of hematologic toxicities and adjustment of the regimen based on tolerance.

All patients underwent conventional cytogenetic analysis and extensive molecular testing (NGS). The overall survival (OS) analysis included subgroups defined by the presence of specific genetic abnormalities: complex karyotype, monosomy 7, KMT2A rearrangements (KMT2A-r), and/or FLT3-ITD mutation.

To highlight the specific clinical impact of certain genetic alterations associated with increased prognostic risk, representative cases were selected illustrating: the effects of adverse somatic mutations (TP53 and mutations in the RAS pathway), as well as the clinical consequences of germline mutations documented in the context of hereditary predisposition syndromes, with longitudinal follow-up from molecular diagnosis to allogeneic transplant.

The results obtained emphasize the importance of dynamic therapeutic and molecular assessment in the management of pediatric myeloid neoplasms (25). The efficacy of the induction regimen, post-induction MRD status, and the germline or somatic mutational profile emerge as critical factors for long-term prognosis. Germline mutations (*GATA2*, *RUNX1*) and adverse mutations (*TP53*) define biologically high-risk subgroups that require intensive monitoring, tailored therapy, and careful management both before and after transplantation (21). The limitations of conventional cytogenetics necessitate the integration of molecular and functional biomarkers to achieve precise prediction of treatment response and survival outcomes.

Chapter 8. Study 3 – Comparative analysis of conditioning strategies used in pediatric patients with myeloid neoplasms undergoing HSCT

HSCT represents a crucial therapeutic option for high-risk, relapsed, or refractory pediatric myeloid neoplasms, with the decision to proceed based on molecular stratification and MRD status. Despite improved HSCT efficacy, selecting the optimal conditioning regimen remains a significant challenge, requiring a balance between therapeutic intensity and systemic toxicity. Reduced-toxicity or reduced-intensity conditioning regimens have been progressively adopted in pediatric practice, aiming to minimize severe complications and post-transplant mortality while preserving the immunologic benefits of the graft-versus-leukemia (GvL) effect. These strategies underscore the necessity of therapeutic personalization grounded in individual risk profiles and treatment tolerability.

Working Hypotheses for Study 3 were:

i. Reduced-toxicity conditioning (RTC) and reduced-intensity conditioning (RIC) regimens provide therapeutic efficacy comparable to myeloablative conditioning (MAC) at 1 year post-HSCT, while significantly reducing toxicity in pediatric patients with myeloid neoplasms.

ii. Post-HSCT bacterial infections and viral reactivations occur more frequently and with greater severity in patients treated with MAC compared to those receiving RTC or RIC.

iii. Clostridioides difficile infection is significantly correlated with severe gastrointestinal acute graft-versus-host disease (aGvHD), playing an active pathogenic role and contributing to poor prognosis.

iv. Age ≥10 years is associated with superior progression-free survival (PFS) and a lower relapse rate post-transplant, particularly in patients treated with RTC regimens.

The main objectives of Study 3 were:

i. To compare the efficacy and toxicity profiles of MAC, RTC, and RIC conditioning regimens in pediatric patients with myeloid neoplasms undergoing HSCT.

ii. To evaluate one-year post-HSCT efficacy parameters, including incidence of acute and chronic GvHD, relapse rates, progression-free survival (PFS), overall survival (OS), overall mortality, and causes of death.

iii. To analyze early post-transplant events such as incidence of oral mucositis, graft dynamics, viral reactivations, incidence of Clostridioides difficile infection, length of hospitalization, and transfusion requirements.

iv. To assess the impact of recurrent mutations identified in the transplanted patient cohort on early post-HSCT survival and achievement of complete remission, aiming to define a potential molecular risk profile.

Secondary objectives were:

i. To evaluate the differences in progression between de novo and secondary acute myeloid leukemia (AML) forms.

ii. To assess post-HSCT outcomes according to age groups (<10 years vs. ≥10 years).

iii. To determine the response to azacitidine (AZA) administered post-HSCT as monotherapy or in combination with donor lymphocyte infusion (DLI).

This retrospective study analyzed 33 pediatric patients diagnosed with AML, MDS, and MPN who underwent HSCT between 2020 and 2025 in Fundeni Clinical Institute. All patients had documented molecular profiles at diagnosis, allowing integration of these data in the post-HSCT evaluation. Transplantation was indicated based on disease risk and response to induction therapy, preceded by assessment of MRD and remission status.

Patients were divided into three groups according to the conditioning regimen used: myeloablative conditioning (MAC), reduced toxicity conditioning (RTC), and reduced intensity conditioning (RIC). Regimens were selected based on clinical profile, donor compatibility, and therapeutic goals. Graft-versus-host disease (GvHD) prophylaxis was personalized, varying according to donor type (matched sibling donor, matched unrelated donor, haploidentical donor). The main monitored parameters included hematologic engraftment, full donor chimerism, infectious complications (CMV, EBV, BKV), incidence of acute and chronic GvHD, as well as progression-free survival (PFS) and overall survival (OS).

A subgroup of patients received post-transplant therapy with azacitidine (AZA) and donor lymphocyte infusion (DLI), administered both prophylactically and therapeutically, depending on MRD status or the presence of mixed chimerism. These interventions aimed to consolidate remission and prevent relapse. The study provides a detailed overview of the efficacy and safety of various conditioning regimens and post-transplant adjuvant strategies in the context of pediatric myeloid neoplasms.

The analysis of conditioning regimens in HSCT for pediatric myeloid neoplasms highlights the need for a personalized therapeutic strategy that balances oncologic efficacy with toxicity risks (26). Reduced-intensity conditioning regimens (RTC/RIC) prove to be viable options for vulnerable patients, providing increased tolerability and disease control comparable to MAC, but with a significantly lower toxicity profile and fewer infectious complications (27). Factors such as age, pre-transplant status, molecular mutations, and conditioning regimen type influence post-transplant outcomes, supporting the use of an integrative decision-making algorithm based on clinical and genetic biomarkers to optimize results and minimize risks (28-31).

Chapter 9. Conclusions and Personal Contributions

Conclusions for Study 1:

1. The study highlights the profound heterogeneity of pediatric myeloid neoplasms, both biologically and clinically.
2. The patient cohort included three main entities, with significantly younger median ages in MDS and MPN groups compared to AML (3.5 and 5.7 years vs. 9 years; p=0.008).
3. History of perinatal complications and recurrent infections was significantly more frequent in MDS (42.9%), suggesting a biologically fragile substrate in these subgroups.
4. Clinical and paraclinical features showed clear differences: MPN characterized by multiorgan infiltration (hepatomegaly, splenomegaly, lymphadenopathy); AML demonstrated an intense proliferative profile supported by tumor lysis syndrome (31.8%, p=0.036), inflammatory markers, and high combined transfusion requirements (>60%, multifactorial cytopenias), indicating major oncogenic activation; MDS showed slow progression.
5. Secondary cases were more frequent in MDS (57.1%) and AML (38.6%), with all secondary cases in MDS/MPN and 41.2% in AML linked to genetic predisposition, supporting the importance of early genetic evaluation and family screening.
6. Multilineage dysplasia was not a significant predictor of adverse prognosis.
7. MPAL was identified in 15.9% of AML cases and associated with poor prognosis.
8. Co-expression of immunophenotypic markers and phenotypic heterogeneity were frequent, reflecting plasticity and immunophenotypic instability, though statistically nonsignificant.

9. CD7 expression emerged as an early immunophenotypic marker with significant negative prognostic impact in pediatric AML, reducing overall survival (14.4 months vs. median not reached; p=0.005) and maintaining this trend post-HSCT. CD56 and CD123 did not significantly influence survival.
10. Chromosomal instability and karyotype abnormalities were significantly more frequent in AML and MDS compared to MPN (p=0.038 and p=0.034), supporting genomic stability differences among entities.
11. Gene fusions are central in the molecular architecture of pediatric AML, directly impacting prognosis and therapeutic strategies. The detected fusion frequency was relatively low (36.8%), with RUNX1::RUNX1T1 and KMT2A::AF9 most frequent, along with prognostically adverse fusions (e.g., NUP98, KMT2A partners), demonstrating both diversity and clinical relevance.
12. Functional mutation classification highlighted dominant molecular pathways per entity: AML with broad distribution (RAS and FLT3 predominance), associated with proliferative, anti-apoptotic, and epigenetic activation linked to higher aggressiveness; MPN dominated by RAS/MAPK mutations (e.g., KRAS, PTPN11), defining a hyperproliferative, resistant biology; MDS featured transcription factor mutations and germline predispositions (SAMD9/SAMD9L, GATA2).
13. Distinct genomic architecture by entity suggests differentiated pathogenic mechanisms: AML showed a hypermutated, strongly mitogenic profile (NRAS, KRAS, FLT3-ITD, KMT2A), MPN exhibited RAS pathway activation without major genomic complexity, and MDS presented a narrower profile suggestive of early genetic predisposition and slow progression.
14. Mutation co-occurrence patterns reflect cooperative functional networks: correlations among epigenetic mutations (STAG2, KDM6A, ASXL1), transcriptional fusions (RUNX1::AFF3, NUP98::DENR), and proliferative genes (WT1, MYC, STAT3) indicate a cooperative genetic architecture with potential to guide combined and personalized therapeutic strategies for distinct disease subgroups.
15. MPN and MPAL presented the shortest median overall survival (16.5 months and 9.2 months, respectively).

16. OS analysis revealed relevant age-related trends, with visibly worse prognosis in children under 1 year, though statistically nonsignificant likely due to small sample size (p=0.577).
17. Patients with MPAL had shorter OS, indicating more aggressive biology; prognosis was favorably influenced by early treatment response, MRD negativity, and use of ALL-type therapeutic regimens.
18. Secondary AML did not show reduced OS compared to de novo cases, except those with genetic predisposition (median 18.5 months), where prognosis was poorer.
19. Certain clinical and paraclinical signs at diagnosis (sepsis, ascites, extreme leukocytosis, blast percentage >70% or >50%, transfusion dependence) correlated with significantly reduced survival, indicating an aggressive biological phenotype; these signs may serve as early indicators of severe disease evolution, guiding risk stratification and early therapeutic adjustment.
20. Personal and family history, as well as type and grade of dysplasia on morphological exam, did not predict disease outcome.Although evaluated genetic parameters (FLT3-ITD, KMT2A-r, chromosome 7 abnormalities, complex karyotype) did not reach statistical significance in survival, clinical trends suggest a negative impact of FLT3-ITD mutations and 7q deletions, underscoring the need for validation in larger cohorts.

Conclusions for Study 2:

1. The efficacy of the induction regimen directly influences overall survival (OS), with patients treated with MEC and those achieving complete remission (CR) post-induction showing significantly higher survival rates.
2. MRD status at the end of induction is a major independent prognostic predictor, associated with a significant reduction in relapse risk and longer survival.
3. Conventional cytogenetics does not reliably predict induction response or early mortality, necessitating supplementation with dynamic molecular markers.
4. AZA treatment in MDS/MPNp provides an effective preparative window for HSCT, and early response may serve as a predictive biomarker for post-transplant success.
5. Molecular subgroups harboring TP53 mutations exhibit a profoundly adverse profile, characterized by reduced survival and resistance to combined therapies.

6. NRAS mutations display heterogeneous behavior, ranging from indolent courses to aggressive forms of juvenile chronic myelomonocytic leukemia (JMML).
7. Germline mutations, especially in GATA2 and RUNX1, along with other predisposition syndromes, define distinct risk subgroups with major implications for patient monitoring, family screening, and crucial therapeutic decision-making.
8. Post-transplant complications, particularly GvHD and severe infections, are exacerbated in the context of germline predispositions.

Conclusions for Study 3:

1. The comparative analysis of conditioning regimens used in pediatric HSCT for myeloid neoplasms underscores the need for a personalized therapeutic strategy tailored to biological and clinical complexity.
2. Reduced-toxicity conditioning (RTC) and reduced-intensity conditioning (RIC) regimens emerge as viable therapeutic alternatives for vulnerable patients, offering lower toxicity and increased tolerability without compromising oncologic efficacy—overall survival (OS) and progression-free survival (PFS) at one year showed no statistically significant differences between regimens.
3. Post-transplant infections, especially bacterial infections and viral reactivations (CMV, BKV), were common. The myeloablative conditioning (MAC) regimen was associated with a higher risk of severe infections.
4. Clostridioides difficile infection correlated with severe and persistent gastrointestinal acute graft-versus-host disease (aGvHD), indicating a bidirectional interaction between digestive colonization and immune-mediated inflammation.
5. RIC regimens were linked to a higher incidence of chronic GvHD (cGvHD).
6. Post-HSCT azacitidine showed a favorable safety profile; however, its survival benefit in the current cohort was not statistically significant, likely due to the selection of patients with a high risk of relapse.
7. Patient age significantly influenced post-transplant outcomes: those under 10 years had higher relapse rates, while patients aged ≥10 years showed superior PFS and a favorable trend in OS.

8. The RTC regimen was associated with reduced systemic toxicity, faster engraftment, and a more favorable immunological profile in selected patients (over 10 years old, in first complete remission).
9. Molecular analysis revealed an increased risk of death within the first year post-HSCT among patients with mutations in EGFR, MET, GATA2, RUNX1, FLT3, CDK4, KRAS, and PTPN11. Among RAS family mutations, NRAS did not influence survival and was predominantly found in patients who survived beyond one year post-HSCT.
10. These findings support the implementation of an integrative therapeutic algorithm that allows the selection of a conditioning regimen with reduced toxicity under safe conditions, incorporating pre-transplant remission status, molecular and immunologic stratification, infection risk assessment, and toxicity profile.

Several limitations must be considered when interpreting the results of this scientific endeavor. The small cohort size, characteristic of pediatric oncology research, limits statistical power and the generalizability of conclusions. Furthermore, the pronounced biological heterogeneity of pediatric myeloid neoplasms, reflected by increased molecular diversity and the presence of rare entities, generates variability in the data and necessitates caution in generalizing findings. The inherent technological variability from using two different sequencing panels, differences in detection sensitivity, and types of biological samples can affect the comparative interpretation of mutations. These limitations underscore the need for validation in large multicenter cohorts and for ongoing research within international consortia dedicated to pediatric myeloid pathologies.

Personal contributions:

This work reflects my direct contribution to the implementation and consolidation of molecular analysis through NGS sequencing in the pediatric practice at the Fundeni Clinical Institute, starting in 2019, within an interdisciplinary collaboration between the "Ștefan S. Nicolau" Institute of Virology and the MedLife network. My active involvement in developing and expanding these partnerships enabled the integration of genetic profiling in the evaluation of pediatric patients with malignant hematologic diseases. I contributed to establishing collaboration with the EWOG group from Freiburg, Germany, facilitating access to international research networks, and participated in initiating the biobanking process of biological samples in

collaboration with the Center for Translational Medicine (CEMT), thus strengthening the infrastructure necessary for precision medicine. All these achievements were possible thanks to the scientific coordination and continuous support provided by Prof. Univ. Dr. Anca Coliță and the multidisciplinary team at the Fundeni Clinical Institute, to whom I am deeply grateful.

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