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MEDICINE

The role of personalized medicine in assessing prognosis and therapeutic approaches in acute myeloid leukemia

ABSTRACT OF Ph.D. THESIS

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I. Background

In 2015, the President of the United States of America publicly announced the Initiative for Precision Medicine, aimed at fast-forwarding the development of novel therapies for diseases with high public healthcare burden, such as cancer [1]. This historical moment legitimized on a political scale the long-term efforts of many academic researchers during the past decade. Personalized medicine, or precision medicine is an approach that integrates genetic and phenotypic data of a given individual in the clinical decision-making process of prevention and personalized therapies. Such an approach contrasts with the status-quo of contemporary medicine – the large-scale use of "one size fits all" evidence-based guidelines to uniformly treat all individuals diagnosed with a certain disease.

In oncology, due to tumoral genetic heterogeneity of compared to the normal genome, personalized therapies could, at least theoretically, target cancer cells and spare healthy cells and, therefore, have high clinical utility. The tyrosine kinase inhibitor imatinib revolutionized the field of cancer therapies; imatinib showed clinical efficacy in the treatment of chronic myeloid leukemia, a disease characterized by the presence of Philadelphia chromosome and the mutant tyrosine kinase BCR-ABL, and dramatically improved survival of patients with a previously lethal malignancy [2]. Patients with acute promyelocytic leukemia, a subtype of acute leukemia associated with high-mortality complications, benefit today from therapies that target the pathogenesis of leukemia – all-trans retinoic acid and arsenic trioxide demonstrated impressive remission rates and even cure in a hematologic cancer historically considered incurable [3].

The progress of precision medicine was essentially facilitated by the many technical advances in the field of high-throughput multi-omics: DNA or RNA sequencing, proteomics or metabolomics, but also bioinformatics, much needed to properly make clinical sense of high volumes of data generated by such assays. These techniques have become increasingly prominent over the past years, culminating with the complete sequencing of the human genome within the Human Genome Research Project [4]. Cancer research is, however, the field where precision medicine techniques are the most applicable and was a major driver of the Presidential initiative for precision medicine, for a couple of reasons. First, it's widely accepted that pathogenesis of cancer involves various genetic lesions, either germline mutations that predispose to cancer, or somatic mutations identified only in tumoral tissues that lead to progression, immune evasion, proliferative advantages

and survival of cancer cells. Cancer is a genetically heterogeneous entity and harbors specific potential targets for personalized therapies. Second, cancer is still a leading cause of death worldwide, and identifying new therapies to improve survival or induce remissions is an area of unmet medical need.

The first cancer genome fully sequenced using next-generation sequencing techniques (NGS) was a normal karyotype acute myeloid leukemia (AML) sample [5]. AML is suitable disease model for precision medicine, due to its high genetic heterogeneity that subdivides this clinical entity into various subtypes according to the presence of specific genetic mutations [6].

AML is an aggressive blood cancer characterized by the malignant clonal proliferation of immature myeloid cells in the bone marrow and suppression of normal hematopoiesis. Leukemic clones are genetically, phenotypically and morphologically different from normal myeloid progenitors. From a pathogenesis perspective, AML is a heterogenous disease and multiple mechanisms responsible for malignant transformation have been described. [7,8]. High-throughput sequencing studies of large AML patient cohorts identified recurrent oncogenic mutations in a large proportion of patients [9,10]. Such mutations are heterogeneous in nature and alter genes with critical functions for hematopoiesis homeostasis, differentiation (PML-RARA), signaling pathways (FLT3, KIT, NRAS, PTPN11), transcription factors (CEBPA, RUNX1, GATA2), DNA methylation DNMT3A, TET2, IDH1/2), chromatin regulation (ASXL1, MLL rearrangements), RNA splicing (SRSF2, U2AF1) or tumor suppression (WT1, TP53).

Briefly, AML pathogenesis can be defined by genetic lesions that 1) cause accelerated proliferation of malignant hematopoietic stem cells and 2) induce a differentiation block of stem cells to mature, functional hematopoietic cells [11]. However, despite the extensive characterization of AML genome, the mainstay of treatment is still cytotoxic chemotherapy and hematopoietic stem cell transplant (HSCT) only if patients are deemed eligible. Although intensive chemotherapy can induce complete remissions (CR), they come at the cost of important toxicities and are oftentimes transient with post-chemotherapy relapse rates being a major pitfall in AML clinical management [12]. Moreover, the clonal composition of AML samples harvested from patients changes throughout the course and under the selective pressure of therapy, a process known as clonal evolution [13]. Development of novel personalized, targeted therapies that enhance remissions is therefore an area of critical unmet need to improve survival rates in this aggressive hematologic malignancy.

In this work, we sought to investigate the utility of precision medicine on multiple levels of AML management – refining the diagnosis, prognosis and optimizing treatments, with an emphasis on targeted, personalized therapies. In the first part of this paper we discuss precision medicine in AML in the context of the current status-quo of molecular pathogenesis, AML classifications incorporating genetic features and clinical prognosis groups, but also of currently approved or pre-clinically tested targeted therapies. In the second part, we aimed to validate through a series of experimental studies in an integrative approach, the implications of personalized medicine in AML diagnosis, prognosis and targeted therapies. We used high-throughput genomics (next-generation DNA sequencing), transcriptomics (RNA-seq) and bioinformatics, as well as preclinical in vitro and in vivo AML models to validate a system of hypotheses and demonstrate that molecular precision techniques hold the potential to be successfully implemented in improving outcomes and therapeutic approaches in AML.

II. Personal contributions

1. Hypothesis and objectives

AML is a genetically and phenotypically heterogeneous and dynamic clinical entity. Comprehensive molecular characterization of AML allows for a thorough risk stratification, but also for the possibility to use targeted inhibitors against clinically actionable mutations. Moreover, a good understanding of the complex genetic background of AML can lead to development of novel experimental therapeutics aimed at improving outcomes and overcome resistance to current therapies.

As discussed above, in this work, we sought to validate through experimental studies, in an integrative manner, the implications of personalized medicine in AML management, such as diagnosis, prognosis and targeted therapies. We used high-throughput multi-omics and preclinical in vivo and in vitro models to validate a hypotheses system and demonstrate that molecular precision techniques hold potential to improve outcomes and optimize therapeutic approaches in AML.

To validate our hypothesis, we followed four main goals to each we assigned an independent research project, as follows:

Goal 1 – Evaluate the role of intensive salvage chemotherapy in modern treatment of relapsed/refractory AML. We demonstrate that salvage cytotoxic chemotherapy has the same limited utility as it did two decades ago, underscoring the necessity of novel, personalized therapies to improve survival.

Goal 2 – Evaluate the utility of NGS in detection of clinically actionable somatic mutations in AML. Using a simple, in house NGS method, we identify mutations with prognostic value that can inform on selecting optimal targeted therapies.

Goal 3 – Preclinical evaluation of novel targeted therapies that address the limitations of genetically-guided therapies currently approved for clinical use. We demonstrate that a combination of small molecule inhibitors that co-target the RAS/MAPK-activating protein SHP2 and the antiapoptotic protein BCL2 have synergistic antileukemic activity in AML with mutations in receptor tyrosine kinases FLT3 and KIT, including in vitro and in vivo models of AML resistant to FLT3 inhibitors.

Goal 4 – Evaluate the differential expression of miRNAs to identify potential targets for future experimental novel therapies. We show that expression of miRNA-4328 is significantly upregulated in a specific subtype of AML – acute promyelocytic leukemia.

This thesis comprise four independent research studies conducted over the course of the doctoral studies in labs affiliated with various institutes, such as the Department of Hematology and Bone Marrow Transplant of the Fundeni Clinical Institute, Bucharest, Romania, the National Institutes of Health, Bethesda, United States of America, and Division of Hematology/Oncology, Department of Medicine, University of California San Francisco, United States of America.

In the first study [14] we demonstrated that despite the current modern standards of care that include better prophylaxis and blood product transfusions, the use of cytotoxic chemotherapy as salvage regimens for relapsed/refractory (R/R) AML has a limited clinical utility, similarly to two decades ago when sequential chemotherapy was pioneered. Intensive salvage chemotherapy is associated with often fatal toxicities and should be reserved for patients ineligible for novel personalized therapies that exhibit a higher therapeutic index.

In order to benefit from such therapies, it is critical that AML samples obtained from patients are investigated using molecular biology techniques to identify clinically-actionable mutations. In the second study we demonstrate the utility of DNA next-generation sequencing to detect somatic oncogenic variants that can act as targets for selective inhibitors.

Although some selective inhibitors, specifically FLT3 inhibitors are clinically effective, their use in monotherapy leads to adaptive resistance through feedback reactivation of signaling pathway, such as the RAS/MAPK pathway [15]. Development of pharmacologic associations of compounds that synergistically inhibit oncogenic signaling associated to resistance is critical to maintain clinical responses and improve survival. In our third study [16], we evaluate the preclinical activity of the experimental SHP2 inhibitor RMC-4550 in combination with the BCL2 inhibitor venetoclax in in vitro and in vivo AML models with mutations in receptor tyrosine kinases FLT3 and KIT. We demonstrate that SHP2 inhibition suppresses signaling through the RAS/MAPK pathway and induces transcriptomic alterations that lead to an increase of apoptotic dependency of AML to BCL2 and augment the pharmacologic sensitivity to venetoclax. We therefore identified a synergistic combination of targeted inhibitors with a high therapeutic index that has the potential to be tested clinically.

Besides small molecule inhibitors, other experimental therapies that target cell-intrinsic oncogenic mechanisms, such as miRNA therapies, are being currently explored. In our fourth study [17], we aimed to investigate whether in a subgroup of AML with a well-defined genetic background, such as acute promyelocytic leukemia (APL), we could identify

a dysregulated expression of miRNA molecules that target genes involved in known pathogenic mechanisms. We demonstrate that has-mir-4328, a miRNA that targets the gene of retinoic acid receptor (RARA) is specifically downregulated in APL samples compared to samples derived from healthy donors. Experimental therapies with hsa-mir-4328 could, in theory, target RARA and lift the differentiation block pathognomonic for APL.

Taken together, our experimental data emerging form these studies support the general hypothesis of this thesis – the necessity of personalized medicine for optimal therapeutic approaches in AML. A schema of work frame and hypotheses of the research projects in this thesis is presented in figure 1 and the studies and summarized below.

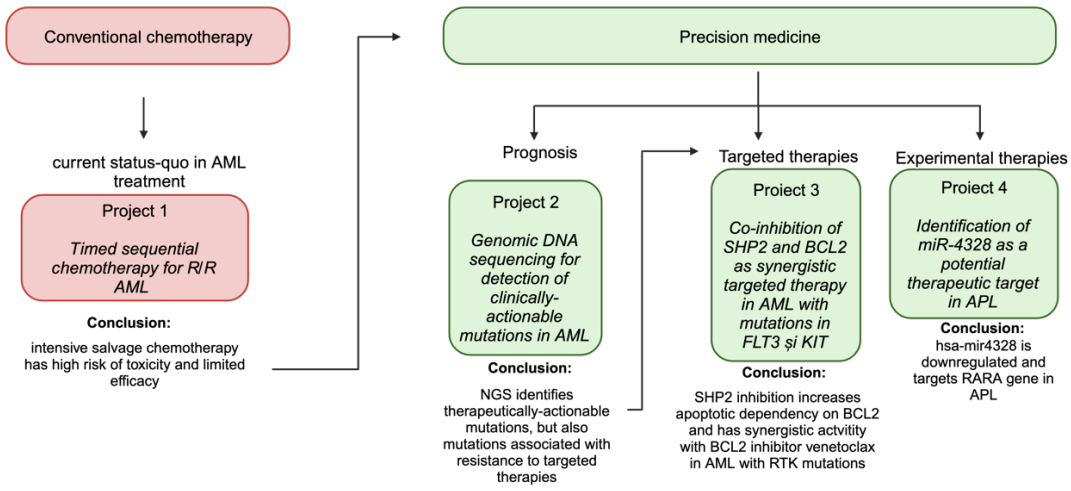

Figure 1 – Schematic representation of the work frame for the research studies

2. The place of cytotoxic chemotherapy in the current treatment of acute myeloid leukemia – timed sequential chemotherapy for relapsed/refractory acute myeloid leukemia

2.1. Introduction

Despite the last decade advances in development and clinical implementation of novel AML therapeutics, including molecular targeted therapies of biological therapies, cytotoxic chemotherapy is still the backbone of AML treatment. [18–21]. In the first study of this thesis, we aimed to evaluate if outcomes of patients who receive anthracycline-based standard chemotherapy changed after decades in which the supportive standards of care and anti-infectious prophylaxis have improved. Sequential chemotherapy (SC) is an approach that differs from standard regimen through the phased administration of cell-cycle active drugs and entails the administration of an initial sequence, followed by a secondary sequence during the maximal recruitment of leukemic blasts in the S phase of the cell cycle by the initial sequence [22–24] with the goal of maximizing efficacy and reduce toxicities associated with chemotherapy.

2.2. Methods

We conducted a unicentric retrospective study on a series of ten R/R AML patients who were treated with the EMA86 regimen at the National Heart Lung and Blood Institute (NHLBI) at the National Institutes of Health (NIH) of the United States of America, under protocols NCT00001397 și NCT02527447, NIH Clinical Center IRB-approved. The methods are detailed *in extenso* the thesis.

2.3. Results

Total CR rate after EMA86 administration was 40%, specifically 75% in relapsed AML subgroup and only 17% in the refractory AML subgroup. Within ELN risk groups, CR was ony 16.66% (n=1) in adverse-risk patients, 50% in favorable-risk patients and 100% (n=2) in intermediate-risk patients. Median overall survival was 80.5 days (30-1205); 41 days (30-166) in refractory subgroup and 332.5 days (42-1205) in relapsed subgroup. Two patients were primary refractory to EMA-86 and did not receive any additional therapy and two other refractory patients were included on other clinical trials but died at a later timepoint due to disease progression. Two patients received HSCT in CR. Of these, one died as a result of veno-occlusive disease, a common transplant complication, while the other patient stayed in CR for 1,000 days after HSCT. Only half of the patients achieved

recovery of circulant neutrophils counts of over 1,000/μL and only 40% recovered platelets over a value 100,000/μL. All patients developed febrile neutropenia and six of them had documented systemic or localized infections – fungal (n=4), bacterial (n=2) or mycobacterial (n=1), requiring prolonged large-spectrum antibiotic and antifungal therapies. Two patients developed invasive aspergillosis, one patient disseminated tuberculosis and one patient systemic infection with *Fusarium* spp (pulmonary, sensual and cutaneous) [25], and two patients died due to sepsis and multiple systems organ failure.

Clinical response		
	Relapsed (n=4)	Refractory (n=6)
Response, n (%)		
CR	2 (50)	1 (17)
CRi	1 (25)	0 (0)
PD	1 (25)	5 (83)
Hematologic recovery, n (%)		
ANC > 1000/ μL	4 (100)	1 (17)
PLT > 100000/ μL	2 (50)	2 (33)
Overall survival, days, median (range)	332,5 (42-105)	41 (33-116)
Cause of death, n (%)		
Sepsis	0 (0)	2 (33)
Progressive disease	1 (25)	3 (50)
HSCT complications	1 (25)	0 (0)
Hospitalization time, days, median (range)	42,5 (38-74)	41 (33-60)

Table 1 – Clinical response after salvage regimen EMA86

2.4. Discussions

R/R AML patients have a generally poor prognosis, with a 5-year survival rate of only 5-10% [7]. There is no consensus on the superiority of one chemotherapy-based salvage treatment or another. In our unicentric, retrospective study we showed that 40% of patients treated on EMA86 protocol achieved CR or CRi (17% among refractory AML and 75% among relapsed AML), results comparable with the ones of studies that investigate similar salvage regimens [26,27]. Notably, despite supportive care progresses in the last three decades, we observed significant toxicities associated to CS EMA86, similar to the ones reported by the authors of this regimen back in the early 1990s [28]. Prolonged pancytopenia and deaths due to sepsis occurred in 20% of the patients, while only 50% recovered neutrophils to a value above 1,000/μL. Therefore, the clinical benefit of timed sequential

salvage chemotherapy must be very carefully weighed against the risk of prolonged aplasia. In conclusion, salvage sequential chemotherapy has high toxicities and should be reserved on a case-by-case base only for selected patient where the ultimate goal is HSCT and for whom novel targeted therapies are not a viable option.

3. Next generation DNA sequencing identifies clinically actionable mutations in acute myeloid leukemia

3.1. Introduction

In the first study, we demonstrated that although cytotoxic chemotherapy is still the backbone of AML treatment, the clinical responses achieved in the R/R setting today are not superior to the ones observed during the past decades. Targeted therapies are increasingly frequent used and they improve survival and remission rates when associated to chemotherapy. Identifying therapeutic targets for selective inhibitors, as well as mutations associated with resistance to targeted therapies and mutations with prognostic value requires molecular testing of samples derived from patients with AML for the detection of mutant variants. Lately, DNA next generation sequencing (NGS) of hotspot regions of genes frequently mutated in AML became an elective method for sensitive detection of somatic mutations. Although NGS offers the potential to provide critical clinically-relevant information and inform on the optimal therapeutic choice, some technical limitations pertaining to the method per se but also to the bioinformatic analysis of NGS data raise significant challenges [29]. In this context, we aimed to evaluate in this study the efficacy of a simple, amplicon-based NGS method to detect mutations in a small panel of genes, with an emphasis on mutations in genes that can be pharmacologically targeted using selective inhibitors.

3.2. Methods

We conducted a unicentric, retrospective study on a cohort of 98 patients diagnosed with AML and with biological samples collected for research at the Department of Hematology and Bone Marrow Transplant of the Fundeni Clinical Institute in Bucharest, Romania. Additional cytogenetic, immunophenotypic and molecular testing were performed for complete diagnosis of AML. We used an amplicon-based NGS assay for detection of mutations in hotspot regions of myeloid neoplasia-related genes: DNMT3A, FLT3, KIT, KRAS, NRAS, IDH1, IDH2, RUNX1 and PCR tests to detect the internal tandem fusion of the FLT3 gene (FLT3-ITD) and fusion genes (RUNX1::RUNX1T1, CBFB::MYH11,

BCR::ABL1 and MLLT3::KMT2A). The methods are detailed in extenso in the thesis.

Results

Among the patients included in the study, 32,65% (n=32) had AML with AML-defining genetic abnormalities. The most frequent genetic abnormality was the fusion gene RUNX1::RUNX1T1 – 6.12% (n=6), followed by the CBFB::MYH11 fusion – 4.08% (n=4) and by BCR::ABL1 fusion and KMT2A rearrangements – 1.02% (n=1). The majority of patients (76.53%) had cytogenetic analysis performed at diagnosis. Among those, most of them harbored a normal karyotype (60%), while 4% had a complex, monosomal and hyperdiploid karyotypes. Frequent cytogenetic abnormalities included 8+ in 9.3% of patients, t(8;21) in 6.66%, 5-/5p- in 4%, inv(16) in 2.66%, 7-/7p-,17p- or t(9;11) in 1.33%. Regarding risk group inclusion according to ELN 2022 guidelines, the majority of patients were included in the intermediate risk group – 80.61%, 10.20% in the favorable risk group and 9.18% in the adverse risk group.

Of all the samples collected for the study, the majority (68,36%) carried somatic mutations in oncogenes associated with AML (figure 2). The most frequently mutated gene was FLT3 in almost half of the patients (48,9%). We detected the presence of the FLT3 ITD in 43% of the patients, but also mutations in the tyrosine kinase domain (TKD) of FLT3 in 6.12%. Most of these variants were substitutions at the 835 residue (D835E/Y/S/V) and a D839G substitution, all with variant allelic frequencies (VAF) between 2% and 28%. We identified the pathogenic D816Y variant in the KIT gene in one patient, but also a variant with unknown pathogenicity, M541L, in 11 patients. Moreover, we identified oncogenic mutations in NRAS gene in 7 patients and in KRAS in one patient. Those mutations are predominantly hyperactivating the RAS/MAPK signaling (NRAS G12D/S/V, Q61L/R, KRAS G12D) and have VAFs between 4 and 51%. Constitutively activating RAS mutations have a critical clinical relevance when they are associated with other targetable mutations (e.g. FLT3), because they can lead to selection of clones resistant to known targeted threapies [30]. We identified mutant variants in the genes IDH1 (R132C/H/P) and IDH2 (R140Q) with VAFs between 4 and 36%. Besides IDH1 and IDH2 mutations, we found mutations in another gene coding for epigenetic regulators, DNMT3A (R882C/H) in 8 patients with VAFs around 50%. Lastly, we detected the presence of mutations of the transcription factor RUNX1 (T111A/P, K110Q, G165C, W196R, R135K, R80H, F163Y, R107C, A149P, R201*) in 10 patients with VAFs between 6 and 75%.

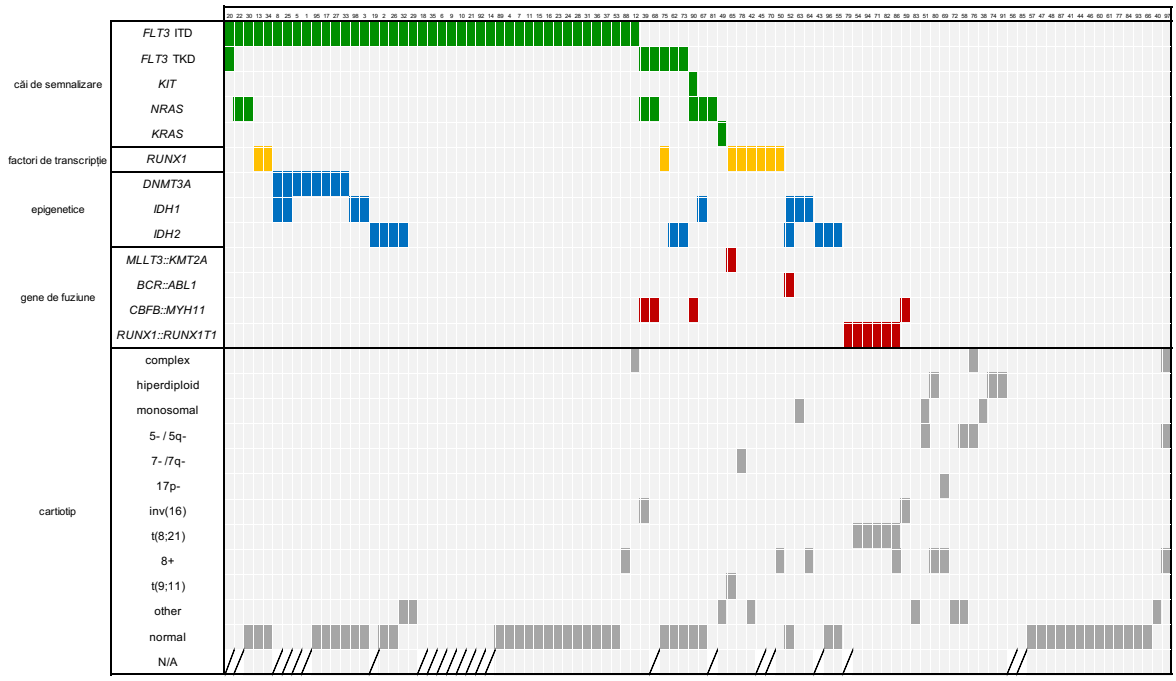

Figure 2 – Oncoplot showing the distribution of mutations in oncogenes, fusion transcripts and cytogenetic abnormalities identified in the study cohort

3.3. Discussions

Recent guidelines, including ELN 2022 and NCCN recommend the use of NGS to identify recurrent mutations and integrating this information in diagnostic and treatment algorithms. Moreover, ELN guidelines includes the presence of mutations in genes as RUNX1, ASXL1, TP53, EZH2, SF3B1, SRSF2, STAG2, U2AF1 or ZRSR2 in the adverse risk group [31]. NGS assays, albeit useful, raise some technical and financial challenges that limit their large scale use in the clinical practice. In this study, we evaluated an amplicon-based DNA sequencing assay build upon the in house design of a panel including a limited amount of genes with a myeloid bias, focusing on those genes with mutations that are clinically actionable with small molecule inhibitors. The simplicity of this panel allowed us to investigate the feasibility of NGS while reducing prohibitive costs that come with commercial kits and addressing some of the resource limitations mentioned above. Focusing on clinically actionable genes had the goal of maximizing the translational benefit in a limited-resource center like ours. The results of our study, one of the few of its kind conducted exclusively in Romania, are in line with data reported in larger cohorts [9,10]. We identified mutations in FLT3, KIT, NRAS, and IDH1/2 genes, which inform on using clinically-approved targeted therapies and can predict clinical response to such therapeutics.

This finding supports the idea that bulk DNA sequencing provides data with clinical utility, at least in identifying actionable targets using personalized therapies.

4. Novel personalized therapies in acute myeloid leukemia – allosteric SHP2 inhibition has synergistic efficacy with the BCL2 inhibitor venetoclax in acute myeloid leukemia with mutations in FLT3 and KIT

4.1.Introduction

In the second study, we demonstrated that genotypic analysis using NGS can identify with high sensitivity clinically-actionable oncogenic mutations. Of those, mutations in the gene coding for the receptor tyrosine kinase FLT3 are the most commonly found, but also benefit from the most clinically approved targeted therapies. Although FLT3 inhibitors significantly improve survival in AML, their clinical utility is limited by development of secondary resistance characterized by emergent clones that constitutively hyperactivate the Ras/MAPK signaling pathway. Protein-tyrosine phosphatase non-receptor 11 (SHP2, encoded by PTPN11 gene) is a tyrosine phosphatase that serves as a molecular relay to activate Ras/MAPK downstream of receptor tyrosine kinases (RTK). Novel allosteric inhibitors of SHP2 (SHP2i) stabilize the protein in a sterically closed, auto-inhibited conformation, hampering both exposing the catalytic domain to its substrate, but also the assembly of the molecular scaffold required by RAS loading with GTP [32,33] and demonstrated preclinical activity in multiple models of neoplasia driven by RTK mutations [33–35]. Considering the pivotal role of SHP2 in signaling downstream of RTK, we hypothesized that allosteric SHP2 inhibition is active in multiple models of AML with RTK mutations and suppressed key mechanisms of survival and resistance to FLT3 inhibitors, specifically through activation of RAS signaling.

4.2. Methods

We conducted a preclinical study to determine the antiproliferative and pro-apoptotic activity of the allosteric SHP2 inhibitor RMC-4550 as monotherapy and in synergistic combination therapy with the BCL2 inhibitor venetoclax in multiple in vitro and in vivo models of AML with FLT3 and KIT mutations, but also in models with co-mutations in NRAS that drive resistance to FLT3 inhibitors. We used cell line-based models, constructs

with doxycycline-inducible expression of various mutations via lentiviral vector transfections, CRISPR/Cas9 genome editing constructs, transcriptomics studies using RNA-seq and qPCR, BH3 profiling assays, flow-cytometric apoptosis assays, luminescent cell viability assays, immunoblots and immunofluorescent imaging studies to investigate the molecular mechanisms responsible for the anti-leukemic activity of RMC-4550, but also for the pro-apoptotic synergy with venetoclax. Ultimately, we performed in vivo studies using murine models engrafted with cell-line derived xenografts and patient-derived xenografts to for the preclinical assessment of the combination therapy of RMC-4550 and venetoclax to support further investigation of this combination in clinical trials. The methods are detailed in extenso in the thesis.

4.3. Results

We demonstrate that treatment with the SHP2 inhibitor RMC-4550 suppresses the proliferation of AML cell lines with mutations in FLT3 and KIT and inhibits RAS-GTP loading and signaling through the Ras/Raf/MEK/ERK pathway. This activity is preserved in the presence of conditions that induce resistance to FLT3 inhibitors, such as the protective effect of the bone marrow microenvironment cytokines, but also the presence of the NRAS G12C mutation, but not NRAS Q61K that constitutively activates RAS independently of GTP loading. On a transcriptomic level, SHP2 inhibition upregulated a gene expression signature consisting of interferon targets, of which some have been shown to induce apoptotic cell death in cancer. RMC-4550 upregulated the transcription and protein expression of the pro-apoptotic Bcl2 family member BMF that is responsible for BCL2 inhibition and also downregulated the expression of MCL1. Such changes led to an alteration in apoptotic dependency of AML in the sense of increased apoptotic priming and dependency on BCL2 for cell death. As a consequence, SHP2 inhibition sensitized all tested cell lines to the pro-apoptotic activity of BCL2 inhibition. We observed, using pharmacologic drug synergy algorithms that RMC-4550 and venetoclax have synergistic activity in both inhibiting proliferation and stimulating apoptosis in AML models with mutations in FLT3 and KIT, but also NRAS G12C and Ras/MAPK signaling suppression through SHP2 blockade is exclusively responsible for this effect. Next, we demonstrate that combinatorial therapy of RMC-4550 and venetoclax improved survival and decreased tumor burden in murine models engrafted with cell line-derived xenografts and patient-derived xenografts.

4.4. Discussions

Acquired resistance to FLT3 inhibitors remains a major pitfall of targeted therapies in FLT3-mutant AML. Preclinical data suggest the involvement of RAS signaling as the key mediator for survival and resistance to FLT3 inhibitors. SHP2 inhibition diminishes oncogenic signaling through the RAS/MAPK pathway by disrupting the RAS GTP loading [15]. In this study, we demonstrate that pharmacologic targeting of SHP2 unveils a vulnerability of AML by increasing the apoptotic dependency to BCL2 through RAS/MAPK-mediated mechanisms, resulting in an increased sensitivity to apoptotic cell death induced by the BCL2 inhibitor venetoclax. Our in vitro and in vivo data provide solid preclinical evidence that combination therapy with RMC-4550 and venetoclax is a synergistic and effective approach to RTK-driven AML treatment. This study advocates for the idea that inhibiting SHP2 effectively suppresses MAPK signaling and triggers a series of pro-apoptotic changes and an augmentation of BCL2 dependency that can be opportunistically exploited by simultaneous targeting SHP2 and BCL2. Our data supports clearly that a combination treatment of SHP2 and BCL2 inhibitors should be investigated in a clinical trial in patients with RTK-mutant AML.

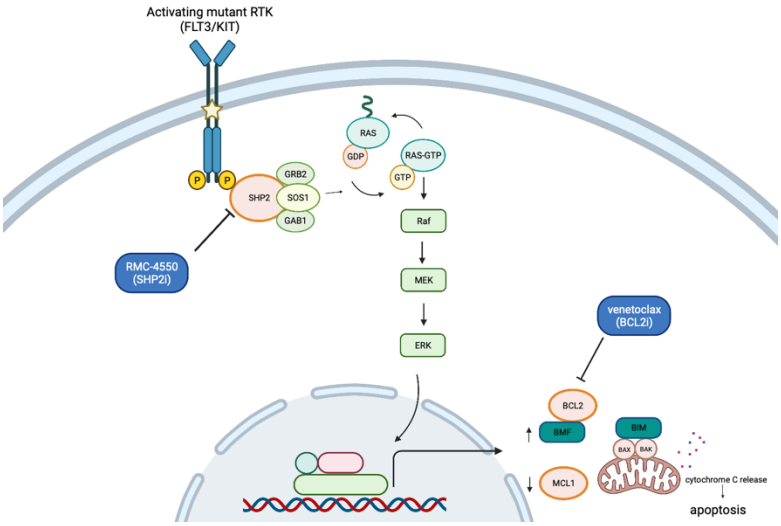

Figure 3 – Schematic representation of the proposed mechanism of synergy between SHP2 and BCL2 inhibitors

5. The therapeutic potential of micro RNAs in personalized medicine – expression and molecular targets of miRNA-4328 in acute promyelocytic leukemia

5.1. Introduction

In the third study, we demonstrated that one of the personalized medicine applications with high translational potential is the development of novel therapies that hold the potential to address the limitations of conventional treatments. Apart from targeted therapies with small molecule inhibitors, other innovative personalized therapies are being developed. Cellular therapies or micro RNA (miRNA) therapies have shown promising results in preclinical trials. miRNA-based therapies exploit cancer cell-intrinsic biological mechanisms to inhibit pro-survival or anti-differentiation oncogenic signals. In the fourth study we aimed to investigate if there are any miRNA with differential expression in a genetically well-characterized subtype of AML, acute promyelocytic leukemia (APL) and that can be exploited for the development of new therapies. We selected APL for this study because it is a disease model with a clearly defined pathogenesis and that already benefits from highly effective personalized therapies. In this study we evaluate if miRNA molecules with tropism for the RARA gene that encodes for the retinoic acid receptor, have a differential expression in samples derived from patients with APL and if they could be used as biomarkers for this AML subtype.

5.2. Methods

We performed an observational study that included 20 patients diagnosed with APL and treated under clinical protocols at the Fundeni Clinical Institute in Bucharest, Romania, and 20 control subjects without an acute leukemia diagnosis. Peripheral blood samples were subjected to qPCR to assess the expression of 6 miRNAs resulting from a bioinformatic prediction analysis of tropism for the retinoic acid-encoding gene RARA. The methods are detailed in extenso in the thesis.

5.3. Results

We performed a in silico analysis of tropism prediction for RARA gene, pathognomonic mutated in APL as a result of chromosomal translocations. Following bioinformatic filtering, we selected 6 candidate miRNAs that met ad-hoc prediction score criteria. We next performed qPCR tests to evaluate their expression in the APL group comparative to the control group. Of all targets, hsa-mir-4328 had a significantly lower expression in the study group ($p=0.02$, unpaired two-tailed Mann-Whitney U test). Functional analysis of signaling pathways of hsa-mir-4328 targets revealed that this miRNA

is involved in target gene transcription and in transcription regulation, but also in functional pathway associated with oncogenic signaling in AML, such as tyrosine kinase receptors (RTK) signaling, growth factors and interleukin signaling, RAS/MAPK signaling or RUNX1 transcription factor regulation. Protein-protein interaction network analysis of molecular targets of hsa-mir-4328 identified an association with processes of myeloid differentiation, suggesting an implication of hsa-mir-4328 in APL pathogenesis, a diseases defined essentially by an alteration of myeloid progenitor differentiation.

5.4. Discussions

The hallmark feature of APL is the PML-RARA fusion gene. In this study we investigated the expression of miRNAs predicted in silico to target RARA and found that hsa-mir-4328, implicated in transcription regulation and transcription factors, but also in signaling pathways attributed to myeloid differentiation and cell survival and proliferation, has a significantly downregulated expression in APL patients. According to our results, the identification of a new miRNA differentially expressed in APL can represent a stepping stone in future development of genetic therapies, but also can be used as a marker for assessing response to current therapies. Due to the sensitive variation of miRNA expression during the disease evolution, relapse sunder ATRA and arsenic-based therapies could be anticipated by a downregulation of hsa-mir-4328, even preceding the detection of PML-RARA fusion. Ultimately, our results can also represent a starting point for developing a multiplex miRNA panel to monitor clinical response and a framework to develop novel personalized therapies. Using novel non-coding RNA delivery methods, testing miRNA-4328 in vivo in preclinical or clinical trials alone or in association with ATRA and arsenic trioxide can be a feasible goal for further studies. Restoring baseline endogenous expression of hsa-mir-4328 has the potential, at least theoretically, to target the mutant variant of the retinoic acid receptor and lift the differentiation block pathognomonic for APL in patients for whom the current standard of care fails to induce CR and achieve cure.

6. Conclusions

As a result of accelerated progress in the field of multi-omics, but also of bioinformatics and large-volume data processing, we are witnessing today a paradigm shift in the management of acute myeloid leukemia. According to the most recent guidelines, the mere identification of molecular lesions in the leukemic cells genome can influence diagnosis criteria, risk group stratification and treatment choices. Some of the AML-specific genetic abnormalities can be target with precision therapeutics that include small molecule inhibitors, immunotherapies, cell therapies and genetic therapies based on non-coding RNA. A myriad of targeted therapies, mostly small molecule inhibitors, are already in clinical use and many others are investigated in randomized clinical trials. Furthermore, the response to therapies can be evaluated at a molecular level through the sensitive detection of a very low-abundance leukemic cells. All these applications are multiple facets of personalized medicine, an innovative approach that aims to ultimately improve survival and quality of life of people diagnosed with AML.

In this work, we aimed to evaluated through several scientific studies, the utility of personalized medicine in the management of AML. In summary, the conclusions of our studies are:

1) Cytotoxic chemotherapy for treatment of relapses in AML has, today, a similar low efficacy and high toxicity profile as two decades ago; novel therapies with improved efficacy and safety are an unmet need.
2) Selective therapies target certain oncogenic mutations, but their identification through genomic assays is critical for molecularly-guided treatments. Amplicon-based DNA next generation sequencing is a simple method that can successfully identify such clinically-actionable mutations.
3) One of the caveats of targeted therapies is resistance via clonal selection of sub-clones with mutations insensitive to the activity of targeted inhibitors, but personalized medicine approaches can address this sort of resistance mechanisms. A combination of SHP2 and BCL2 inhibitors synergistically induces apoptotic cell death in AML with mutations in FLT3 and KIT.
4) Non-coding RNA therapies, although not clinically available, have the potential to target genes specifically mutated in AML, while keeping a low toxicity profile. Bioinformatics can successfully predict miRNA with complementarity and transcriptional repressing activity against RARA gene, pathognomonic mutated in

APL. miR-4328 is transcriptionally downregulated in APL patients, suggesting a potential application of therapeutic development.

Altogether, the conclusions of our studies highlight the importance of personalized medicine in the modern therapeutic approach of AML. We consider that in that respect, the overarching goals of our research have been achieved. However, some of the challenges we encountered should be mentioned.

First, although the benefit of multi-omics are beyond doubt, the economic feasibility of large-scale implementation of such methods is limited. Identification of a complex genetic landscape that includes karyotype, fusion transcripts resulting from chromosomal translocations and somatic mutations in genes responsible for AML pathogenesis is a desirable goal for every patient diagnosed with AML. However, in real life, genetic testing, specifically NGS, entails expensive equipment and reagents, trained specialists, non-standardized and often convoluted bioinformatics. Resource-limited centers have a restricted accessibility to such tests that ultimately restrict the access of patients to optimal care from a personalized medicine standpoint. In our second study, we overcame this pitfall using a simple, cost-effective amplicon-based sequencing technique to identify somatic mutations in AML patients. Although our results are encouraging, we believe efforts to standardize NGS methods and the optimal gene panels to diagnose and monitor residual AML are necessary.

Second, despite the obvious clinical benefit of targeted therapies, resistance to such therapies is a major clinical problem. FLT3 inhibitors generated a lot of interest in the targeted inhibitors world. Despite initial efficacy, these inhibitors are limited by the resistance phenomena and patients ultimately relapse. Resistance occur mainly due to selection under the pressure of inhibitors of clones that constitutively activate signaling pathways downstream of mutated FLT3 receptors, predominantly the RAS/MAPK pathway. RAS activation occurs biochemically as a result of GDP cycling to GTP, a process that involves many adjuvant proteins, including SHP2. In our third study we demonstrate that allosteric SHP2 inhibitor RMC-4550 stabilizes the inactive conformation of SHP2 protein and impairs RAS loading with GTP, inhibiting thus the leukemic proliferation of AML cell lines with signaling mutations in FLT3 and KIT. Targeting RAS activation in this manner, we hypothesized that RMC-4550 can overcome adaptive resistance to FLT3 inhibitors, mediated by RAS activation through constitutive active mutations and through the humoral protective effect of the microenvironmental cytokines. In in vivo validation studies, we

demonstrated preclinically that RMC-4550 plus venetoclax combinatorial treatment improves survival and reduces significantly tumor burden in murine models with FLT3-mutant AML. These data support the investigation of this combinatorial treatment in a clinical trial. Leveraging personalized medicine, we identified an Achille heel of FLT3-mutant AML that we exploited to propose new candidate targets. However, future studies to investigate the feedback adaptive resistance to SHP2 inhibitors are needed. Moreover, novel targeted therapies with activity against RAS-GTP cycling-independent RAS mutations are necessary toc over the whole spectrum of resistance to FLT3 inhibitors.

Third, studies that prove the preclinical efficacy of miRNA therapies are challenging to perform in vivo, mostly due to the difficulties to effectively deliver in vivo non-coding RNAs with minimal degradation. In our fourth study, we identified in APL patients a significant downregulation of hsa-mir-4328, a molecule that targets the RARA gene. Hypothetically, restoring the physiologic levels of hsa-mir-4328 by exogenous therapeutic delivery has the potential to lift the differentiation block specific for APL by targeting the mutant PML-RARA transcript. We consider that such an approach is in line with the general idea of this work, that personalized medicine can enhance current therapies, but this hypothesis needs to be verified preclinically in future studies that include optimizations of in vivo delivery methods of miR-4328.

The results of our four studies have, in our opinion, noteworthy contributions in the field of personalized medicine applications in AML. Some of our conclusions can generate, in return new hypotheses that remain to be explored in future studies. Consistent efforts coming from the international AML workgroups are needed towards an evidence-based, consensus-standardized recommendations regarding the use of NGS in AML management, to maximize accessibility and the number of patients that can benefit from personalized therapies. Moreover, we hope that our very promising preclinical results of SHP2 inhibitor and venetoclax combination therapy will generate enthusiasm and eventually translate to a clinical trial. Despite the new sort of challenges generated by personalized treatments in AML, discussed above, our work concludes that personalized medicine is the proper direction in the long journey towards improved survival in AML, a hematologic cancer with devastating prognosis.

References

1. Collins FS, Varmus H. A new initiative on precision medicine. N Engl J Med. 2015 Feb 26;372(9):793–5.
2. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001 Apr 5;344(14):1031–7.
3. Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S, Ferrara F, Fazi P, Cicconi L, Di Bona E, Specchia G, Sica S, Divona M, Levis A, Fiedler W, Cerqui E, Breccia M, Fioritoni G, Salih HR, Cazzola M, Melillo L, Carella AM, Brandts CH, Morra E, von Lilienfeld-Toal M, Hertenstein B, Wattad M, Lübbert M, Hänel M, Schmitz N, Link H, Kropp MG, Rambaldi A, La Nasa G, Luppi M, Ciceri F, Finizio O, Venditti A, Fabbiano F, Döhner K, Sauer M, Ganser A, Amadori S, Mandelli F, Döhner H, Ehninger G, Schlenk RF, Platzbecker U. Retinoic Acid and Arsenic Trioxide for Acute Promyelocytic Leukemia. New England Journal of Medicine. 2013 Jul 11;369(2):111–21.
4. Green ED, Watson JD, Collins FS. Human Genome Project: Twenty-five years of big biology. Nature. 2015 Oct 1;526(7571):29–31.
5. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, Dooling D, Dunford-Shore BH, McGrath S, Hickenbotham M, Cook L, Abbott R, Larson DE, Koboldt DC, Pohl C, Smith S, Hawkins A, Abbott S, Locke D, Hillier LW, Miner T, Fulton L, Magrini V, Wylie T, Glasscock J, Conyers J, Sander N, Shi X, Osborne JR, Minx P, Gordon D, Chinwalla A, Zhao Y, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson M, Baty J, Ivanovich J, Heath S, Shannon WD, Nagarajan R, Walter MJ, Link DC, Graubert TA, DiPersio JF, Wilson RK. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature. 2008 Nov 6;456(7218):66–72.
6. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, Bejar R, Berti E, Busque L, Chan JKC, Chen W, Chen X, Chng WJ, Choi JK, Colmenero I, Coupland SE, Cross NCP, De Jong D, Elghetany MT, Takahashi E, Emile JF, Ferry J, Fogelstrand L, Fontenay M, Germing U, Gujral S, Haferlach T, Harrison C, Hodge JC, Hu S, Jansen JH, Kanagal-Shamanna R, Kantarjian HM, Kratz CP, Li XQ, Lim MS, Loeb K, Loghavi S, Marcogliese A, Meshinchi S, Michaels P, Naresh KN, Natkunam Y, Nejati R, Ott G, Padron E, Patel KP, Patkar N, Picarsic J, Platzbecker U, Roberts I, Schuh A, Sewell W, Siebert R, Tembhare P, Tyner J, Verstovsek S, Wang W, Wood B, Xiao W, Yeung C, Hochhaus A. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul 22;36(7):1703–19.
7. Döhner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med. 2015 Sep 17;373(12):1136–52.
8. Khwaja A, Bjorkholm M, Gale RE, Levine RL, Jordan CT, Ehninger G, Bloomfield CD, Estey E, Burnett A, Cornelissen JJ, Scheinberg DA, Bouscary D, Linch DC. Acute myeloid leukaemia. Nat Rev Dis Primers. 2016 Mar 10;2:16010.
9. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson AG, Hoadley K, Triche TJ, Laird PW, Baty JD, Fulton LL, Fulton R, Heath SE, Kalicki-Veizer J, Kandoth C, Klco JM, Koboldt DC, Kanchi KL, Kulkarni S, Lamprecht TL, Larson DE, Lin L, Lu C, McLellan MD, McMichael JF, Payton J, Schmidt H, Spencer DH, Tomasson MH, Wallis JW, Wartman LD, Watson MA, Welch J, Wendl MC, Ally A, Balasundaram M, Birol I,

Butterfield Y, Chiu R, Chu A, Chuah E, Chun HJ, Corbett R, Dhalla N, Guin R, He A, Hirst C, Hirst M, Holt RA, Jones S, Karsan A, Lee D, Li HI, Marra MA, Mayo M, Moore RA, Mungall K, Parker J, Pleasance E, Plettner P, Schein J, Stoll D, Swanson L, Tam A, Thiessen N, Varhol R, Wye N, Zhao Y, Gabriel S, Getz G, Sougnez C, Zou L, Leiserson MDM, Vandin F, Wu HT, Applebaum F, Baylin SB, Akbani R, Broom BM, Chen K, Motter TC, Nguyen K, Weinstein JN, Zhang N, Ferguson ML, Adams C, Black A, Bowen J, Gastier-Foster J, Grossman T, Lichtenberg T, Wise L, Davidsen T, Demchok JA, Shaw KRM, Sheth M, Sofia HJ, Yang L, Downing JR, Eley G. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013 May 30;368(22):2059–74.

10. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Döhner K, Schlenk RF, Döhner H, Campbell PJ. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 2016 Jun 9;374(23):2209–21.
11. Takahashi S. Current findings for recurring mutations in acute myeloid leukemia. J Hematol Oncol. 2011 Sep 14;4:36.
12. Walter RB, Othus M, Burnett AK, Löwenberg B, Kantarjian HM, Ossenkoppele GJ, Hills RK, Ravandi F, Pabst T, Evans A, Pierce SR, Vekemans MC, Appelbaum FR, Estey EH. Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. Leukemia. 2015 Feb 12;29(2):312–20.
13. Morita K, Wang F, Jahn K, Hu T, Tanaka T, Sasaki Y, Kuipers J, Loghavi S, Wang SA, Yan Y, Furudate K, Matthews J, Little L, Gumbs C, Zhang J, Song X, Thompson E, Patel KP, Bueso-Ramos CE, DiNardo CD, Ravandi F, Jabbour E, Andreeff M, Cortes J, Bhalla K, Garcia-Manero G, Kantarjian H, Konopleva M, Nakada D, Navin N, Beerenwinkel N, Futreal PA, Takahashi K. Clonal evolution of acute myeloid leukemia revealed by high-throughput single-cell genomics. Nat Commun. 2020 Oct 21;11(1):5327.
14. Popescu B, Sheela S, Thompson J, Grasmeder S, Intrater T, DeStefano CB, Hourigan CS, Lai C. Timed sequential salvage chemotherapy for relapsed or refractory acute myeloid leukemia. Clin Hematol Int. 2020 Mar;2(1):27–31.
15. Popescu B, Shannon K. Sidestepping SHP2 inhibition. Journal of Experimental Medicine. 2023 May 1;220(5).
16. Popescu B, Stahlhut C, Tarver TC, Wishner S, Lee BJ, Peretz CA, Luck C, Phojanakong P, Camara Serrano JA, Hongo H, Rivera JM, Xirenayi S, Chukinas A, Steri V, Tasian SK, Stieglitz E, Smith CC. Allosteric SHP2 Inhibition Increases Apoptotic Dependency on BCL2 and Synergizes with Venetoclax in FLT3- and KIT- Mutant AML. Cell Rep Med. 2023 Nov 21;4(11).
17. Lupu OT, Popescu B, Avram E, Dragomir M, Cimponeriu GD, Mighiu I, Aposteanu S, Coriu D. Downregulation of hsa-miR-4328 and target gene prediction in Acute Promyelocytic Leukemia. Rev Rom Med Lab. 2022 Jul 1;30(3):261–72.
18. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017 Jan 26;129(4):424–47.

19. DeStefano CB, Hourigan CS. Personalizing initial therapy in acute myeloid leukemia: incorporating novel agents into clinical practice. Ther Adv Hematol. 2018 May 27;9(5):109–21.
20. DiNardo CD, Perl AE. Advances in patient care through increasingly individualized therapy. Nat Rev Clin Oncol. 2019 Feb 2;16(2):73–4.
21. Lai C, Karp JE, Hourigan CS. Precision medicine for acute myeloid leukemia. Expert Rev Hematol. 2016 Jan 2;9(1):1–3.
22. Vaughan WP, Karp JE, Burke PJ. Long chemotherapy-free remissions after single-cycle timed-sequential chemotherapy for acute myelocytic leukemia. Cancer. 1980 Mar 1;45(5):859–65.
23. Karp J, Donehower R, Enterline J, Dole G, Fox M, Burke P. In vivo cell growth and pharmacologic determinants of clinical response in acute myelogenous leukemia. Blood. 1989 Jan 1;73(1):24–30.
24. Norsworthy KJ, DeZern AE, Tsai HL, Hand WA, Varadhan R, Gore SD, Gojo I, Pratz K, Carraway HE, Showel M, McDevitt MA, Gladstone D, Ghiaur G, Prince G, Seung AH, Benani D, Levis MJ, Karp JE, Smith BD. Timed sequential therapy for acute myelogenous leukemia: Results of a retrospective study of 301 patients and review of the literature. Leuk Res. 2017 Oct;61:25–32.
25. Sheela S, Ito S, Strich JR, Manion M, Montemayor-Garcia C, Wang HW, Oetjen KA, West KA, Barrett AJ, Parta M, Gea-Banacloche J, Holland SM, Hourigan CS, Lai C. Successful salvage chemotherapy and allogeneic transplantation of an acute myeloid leukemia patient with disseminated Fusarium solani infection. Leuk Res Rep. 2017;8:4–6.
26. Roboz GJ, Rosenblat T, Arellano M, Gobbi M, Altman JK, Montesinos P, O'Connell C, Solomon SR, Pigneux A, Vey N, Hills R, Jacobsen TF, Gianella-Borradori A, Foss Ø, Vetrhusand S, Giles FJ. International Randomized Phase III Study of Elacytarabine Versus Investigator Choice in Patients With Relapsed/Refractory Acute Myeloid Leukemia. Journal of Clinical Oncology. 2014 Jun 20;32(18):1919–26.
27. Litzow MR, Wang X V., Carroll MP, Karp JE, Ketterling RP, Zhang Y, Kaufmann SH, Lazarus HM, Luger SM, Paietta EM, Pratz KW, Tun HW, Altman JK, Broun ER, Rybka WB, Rowe JM, Tallman MS. A randomized trial of three novel regimens for recurrent acute myeloid leukemia demonstrates the continuing challenge of treating this difficult disease. Am J Hematol. 2019 Jan 15;94(1):111–7.
28. Archimbaud E, Leblond V, Michallet M, Cordonnier C, Fenaux P, Travade P, Dreyfus F, Jaubert J, Devaux Y, Fiere D. Intensive sequential chemotherapy with mitoxantrone and continuous infusion etoposide and cytarabine for previously treated acute myelogenous leukemia. Blood. 1991 May 1;77(9):1894–900.
29. Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. Detection of ultra-rare mutations by next-generation sequencing. Proceedings of the National Academy of Sciences. 2012 Sep 4;109(36):14508–13.
30. Kennedy VE, Smith CC. FLT3 Mutations in Acute Myeloid Leukemia: Key Concepts and Emerging Controversies. Front Oncol. 2020;10:612880.
31. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, Ebert BL, Fenaux P, Godley LA, Hasserjian RP, Larson RA, Levine RL, Miyazaki Y, Niederwieser D, Ossenkoppele G, Röllig C, Sierra J, Stein EM, Tallman MS, Tien HF, Wang J, Wierzbowska A, Löwenberg B. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022 Sep 22;140(12):1345–77.

32. Nichols RJ, Haderk F, Stahlhut C, Schulze CJ, Hemmati G, Wildes D, Tzitzilonis C, Mordec K, Marquez A, Romero J, Hsieh T, Zaman A, Olivas V, McCoach C, Blakely CM, Wang Z, Kiss G, Koltun ES, Gill AL, Singh M, Goldsmith MA, Smith JAM, Bivona TG. RAS nucleotide cycling underlies the SHP2 phosphatase dependence of mutant BRAF-, NF1- and RAS-driven cancers. Nat Cell Biol. 2018 Sep;20(9):1064–73.
33. Chen YNP, LaMarche MJ, Chan HM, Fekkes P, Garcia-Fortanet J, Acker MG, Antonakos B, Chen CHT, Chen Z, Cooke VG, Dobson JR, Deng Z, Fei F, Firestone B, Fodor M, Fridrich C, Gao H, Grunenfelder D, Hao HX, Jacob J, Ho S, Hsiao K, Kang ZB, Karki R, Kato M, Larrow J, La Bonte LR, Lenoir F, Liu G, Liu S, Majumdar D, Meyer MJ, Palermo M, Perez L, Pu M, Price E, Quinn C, Shakya S, Shultz MD, Slisz J, Venkatesan K, Wang P, Warmuth M, Williams S, Yang G, Yuan J, Zhang JH, Zhu P, Ramsey T, Keen NJ, Sellers WR, Stams T, Fortin PD. Allosteric inhibition of SHP2 phosphatase inhibits cancers driven by receptor tyrosine kinases. Nature. 2016 Jul 7;535(7610):148–52.
34. Mainardi S, Mulero-Sánchez A, Prahallad A, Germano G, Bosma A, Krimpenfort P, Lieftink C, Steinberg JD, de Wit N, Gonçalves-Ribeiro S, Nadal E, Bardelli A, Villanueva A, Bernards R. SHP2 is required for growth of KRAS-mutant non-small-cell lung cancer in vivo. Nat Med. 2018 Jul;24(7):961–7.
35. Pandey R, Ramdas B, Wan C, Sandusky G, Mohseni M, Zhang C, Kapur R. SHP2 inhibition reduces leukemogenesis in models of combined genetic and epigenetic mutations. J Clin Invest. 2019 Dec 2;129(12):5468–73.

Published work

In extenso articles:

1. **Popescu B**, Sheela S, Thompson J, Grasmeder S, Intrater T, DeStefano CB, Hourigan CS, Lai C. Timed sequential salvage chemotherapy for relapsed or refractory acute myeloid leukemia. Clin Hematol Int. 2020;2(1):27-31. (Chapter 5 – pp. 49-56) https://doi.org/10.2991/chi.d.191128.001
2. **Popescu B**, Stahlhut C, Tarver TC, Wishner S, Lee BJ, Peretz CAC, Luck C, Phojanakong P, Camara Serrano JA, Hongo H, Rivera JM, Xirenayi S, Chukinas AJ, Steri V, Tasian SK, Stieglitz E, Smith CC. Allosteric SHP2 Inhibition Increases Apoptotic Dependency on BCL2 and Synergizes with Venetoclax in FLT3- and KIT-Mutant AML. Cell Rep Med. 2023;4(11). Impact factor: 14,3. (Chapter 7 – pp. 69-99) https://doi.org/10.1016/j.xcrm.2023.101290
   Pre-print: https://doi.org/10.1101/2022.12.01.518665
3. **Popescu B**, Shannon K. Sidestepping SHP2 inhibition. J Exp Med. 2023;220(5). Impact factor: 15,3 (Chapter 7 – pp. 69-99) https://doi.org/10.1084/jem.20230082
4. Lupu O, **Popescu B**, Avram E, Dragomir M, Cimponeriu G, Mighiu I, Aposteanu S, Coriu D. Downregulation of hsa-miR-4328 and target gene prediction in Acute Promyelocytic Leukemia. Revista Romana de Medicina de Laborator. 2022;30(3): 261-272. Impact factor: 0,5 (Chapter 8 – pp. 100-113) https://doi.org/10.2478/rrlm-2022-0022
5. Celik H, Lindblad KE, **Popescu B**, Gui G, Goswami M, Valdez J, DeStefano CB, Lai C, Thompson J, Ghannam JY, Fantoni G, Biancotto A, Candia J, Cheung F, Sukumar G, Dalgard C, Smith RH, Larochelle A, Dillon LW, Hourigan CS. Highly multiplexed proteomic assessment of human bone marrow in acute myeloid leukemia. Blood Adv. 2020;4(2):367-79 Impact factor: 7,5 (Chapter 1 – pp. 22) https://10.1182/bloodadvances.2019001124

Published abstracts:

1. **Popescu B**, Lindblad K, Fantoni G, Gui G, Valdez J, Goswami M, DeStefano CB, Lai C, Biancotto A, Candia J, Cheung F, Thompson J, Dillon LW, Hourigan CS. Abstract 3756: Highly multiplexed proteomic assessment of the human acute myeloid leukemia bone marrow microenvironment. Cancer Research. 2019;79(13_Supplement):3756-. (Chapter 1 – pp. 22)
   https://doi.org/10.1158/1538-7445.Am2019-3756
2. Celik H, Lindblad KE, **Popescu B**, Fantoni G, Gui G, Valdez J, Goswami M, DeStefano CB, Lai C, Biancotto A, Candia J, Cheung F, Thompson J, Ghannam JY, Sukumar G, Smith RH, Dalgard C, Larochelle A, Dillon LW, Hourigan CS. A Novel Proteomic Profiling of the Bone Marrow Microenvironment Reveals Elevated Levels of the Chemokine CCL23 Isoforms in Acute Myeloid Leukemia. Blood. 2019;134(Supplement_1):2709-. (Chapter 1 – pp. 22)
   https://doi.org/10.1182/blood-2019-122121
3. **Popescu B**, Stahlhut C, Tarver TC, III, Ferng TT, Peretz C, Wishner S, et al. Allosteric SHP2 Inhibitor RMC4550 Synergizes with Venetoclax in FLT3 and KIT Mutant Acute Myeloid Leukemia. Blood. 2021;138(Supplement 1):2231-.(Chapter 7 – pp. 69-99)
   https://doi.org/10.1182/blood-2021-145964