

DETERMINATION OF MEIS1 (MYELOID ECOTROPHIC INSERTION SITE 1)
EXPRESSION PROFILE IN LEUKEMIA STEM CELLS AND INVESTIGATION OF
THE EFFECT OF MEIS1 INHIBITORS



by
Neslihan Meriç

Submitted to Graduate School of Natural and Applied Sciences
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in
Biotechnology

Yeditepe University
2021

DETERMINATION OF MEIS1 (MYELOID ECOTROPHIC INSERTION SITE 1)
EXPRESSION PROFILE IN LEUKEMIA STEM CELLS AND INVESTIGATION OF
THE EFFECT OF MEIS1 INHIBITORS

APPROVED BY:

Assoc. Prof. Dr. Fatih Kocabaş
(Thesis Supervisor)
(Yeditepe University)

.....

Prof. Dr. Gamze Torun Köse
(Yeditepe University)

.....

Assist. Prof. Dr. İbrahim Hatipoğlu
(Yeditepe University)

.....

Prof. Dr. İrfan Değirmenci
(Kütahya University of Health Sciences)

.....

Prof. Dr.Olga Meltem Akay
(Koç University)

.....

DATE OF APPROVAL:/....../2021

I hereby declare that this thesis is my own work and that all information in this thesis has been obtained and presented in accordance with academic rules and ethical conduct. I have fully cited and referenced all material and results as required by these rules and conduct, and this thesis study does not contain any plagiarism. If any material used in the thesis requires copyright, the necessary permissions have been obtained. No material from this thesis has been used for the award of another degree. I accept all kinds of legal liability that may arise in case contrary to these situations.



Name, Last name

Signature

ACKNOWLEDGEMENTS

In the first place, I'd want to express my thanks to Assoc. Prof. Dr. Fatih Kocabaş, my advisor, for his excellent guidance, steadfast support, confidence, encouragement, and patience during my Ph.D. and other research attempts. I am grateful to Prof. Dr. Zafer Gülbaş for providing me with experimental material. Prof. Dr. Gamze Torun Köse, Prof. Dr. İrfan Değirmenci, Prof. Dr. Olga Meltem Akay, Prof. Dr. Miriş Dikmen, Assist. Prof. Dr. İbrahim Hatipoğlu members of my thesis committee, for their thoughtful questions, comments, and support. In addition, I am indebted to my dear lab companion, Ph.D. Esra Albayrak, for her unwavering support, drive, and encouragement. I'd like to thank MSc. Sezer Akgöl and all of my colleagues at the Department of Genetics and Bioengineering at Yeditepe University.

I would also like to thank my colleague at Anadolu Medical Center, Biologist Serkan Demir, for her moral support and assistance. I'd want to convey my heartfelt gratitude to my father Ramazan Meriç, mother Şaziye Meriç, brothers Hakan and Gökhan Meriç and of course, my son Doruk Başkan for their unwavering faith, motivation, understanding, unconditional support, and commitment throughout my life.

ABSTRACT

DETERMINATION OF MEIS1 (MYELOID ECOTROPHIC INSERTION SITE 1) EXPRESSION PROFILE IN LEUKEMIA STEM CELLS AND INVESTIGATION OF THE EFFECT OF MEIS1 INHIBITORS

Acute leukemia (AL) is a poor progressive resistant hematological disease, which has different subtypes and immunophenotypic properties according to leukemic blasts. AL is a disease caused by genetic changes and probably by Leukemia Stem Cells (LSCs), which determine its prognosis and endurance. LSCs are Hematopoietic Stem Cells (HSCs)-derived cells that underwent a malignant transformation. Even though the number of LSCs is low, they have the characteristics of self-renewal, resistance to chemotherapy, and relapse of leukemia. The MEIS1 (Myeloid Ecotropic Integration Site-1) protein is a member of the TALE (Three Amino Acids Loop Extension) family of Homeodomain (HD) proteins and can bind to DNA. Studies have proved that overexpression of MEIS1 protein can reason tumorigenesis of many cancers. Increased expression of MEIS1 protein has been determined in Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML) patients. Resistance to conventional chemotherapy was observed in samples with high MEIS1 expression. Small molecule inhibitors of the MEIS1 protein can be used to remove the effect of chemotherapy resistance on the leukemia and LSCs of the MEIS1 protein. This study, it is aimed to investigate MEIS1 proteins which are known to be effective in leukemia development and relapsed in LSCs, and to target MEIS activity with newly developed drug structured MEIS1Inhibitors (MEIS1i). In this study of BM-PB samples from patients with AML/ALL (n=65), we discovered that MEIS1i had a negative impact on MEIS protein activity, LSCs cell survival, and the apoptotic pathway. MEIS1i-1 and MEIS1i-2 inhibitors also reduced the levels of expression of known genes linked to cancer growth and survival in patient samples. In this study, we also discovered that MEIS1 gene expression plays a significant role in leukemia relapse and resistance to chemotherapy treatment. The findings in these studies will be the basis for future animal models and preclinical studies. Successful completion of these goals will enable leukemia to develop effective MEIS1i; thus, it will contribute to the development of an alternative drug for leukemia treatments.

ÖZET

LÖSEMİ KÖK HÜCRELERİNDE MEIS1 (MYELOID ECOTROPHIC INSERTION SITE 1) İFADE PROFİLİNİN BELİRLENMESİ VE MEIS1 İNHİBİTÖRLERİN ETKİSİNİN İNCELENMESİ

Akut lösemi (AL), lösemik blastlara göre farklı alt tiplere ve immünofenotipik özelliklere sahip, zayıf, ilerleyici dirençli bir hematolojik hastalıktır. AL genetik değişikliklerin ve muhtemelen prognozunu ile dayanıklılığını belirleyen Lösemi Kök Hücre (LKH)'lerinin neden olduğu bir hastalıktır. LKH'ler, malign bir dönüşüm geçirmiş Hematopoietik Kök Hücre (HKH)'lerden türevli hücrelerdir. LKH sayısı az olsa da kendini yenileme, kemoterapiye direnç ve lösemi relapsı özelliklerine sahiptirler. MEIS1 (Miyeloid Ekotropik Entegrasyon Sitesi-1) proteini, Homeodomain (HD) proteinlerinin TALE (Üç Amino Asit Döngü Uzantısı) ailesinin bir üyesidir ve DNA'ya bağlanabilir. Çalışmalar, MEIS1 proteininin aşırı ekspresyonunun birçok kanserin tümör oluşumuna neden olabileceğini kanıtlamıştır. Akut Lenfoblastik Lösemi (ALL) ve Akut Miyeloid Lösemi (AML) hastalarında MEIS1 proteininin artan ekspresyonu belirlenmiştir. Yüksek MEIS1 ekspresyonu olan örneklerde konvansiyonel kemoterapiye direnç gözlenmiştir. MEIS1 proteininin küçük molekül inhibitörleri, kemoterapi direncinin MEIS1 proteininin lösemi ve LKH'leri üzerindeki etkisini ortadan kaldırmak için kullanılabilir. Bu çalışmada, lösemi gelişiminde etkili olduğu bilinen ve LKH'lerde tekrarlayan MEIS1 proteinlerinin araştırılması ve yeni geliştirilen ilaç yapılı MEIS1 İnhibitörleri (MEIS1i) ile MEIS aktivitesinin hedeflenmesi amaçlanmıştır. AML/ALL (n=65) hastalarından alınan Kİ-PK numunelerinin bu çalışmada, MEIS1i'nin MEIS protein aktivitesi, LKH'lerinin hayatta kalması ve apoptotik yol üzerinde olumsuz bir etkisi olduğunu keşfettik. MEIS1i-1 ve MEIS1i-2 inhibitörleri, hasta örneklerinde kanser büyümesi ve hayatta kalma ile bağlantılı olduğu bilinen genlerin ekspresyon seviyelerini de azalttı. Bu çalışmada ayrıca MEIS1 gen ekspresyonunun lösemi nüksü ve kemoterapi tedavisine dirençte önemli bir rol oynadığını keşfettik. Bu çalışmalarda elde edilen bulgular, gelecekteki hayvan modelleri ve klinik öncesi çalışmalar için temel oluşturacaktır. Bu hedeflerin başarıyla tamamlanması, lösemiye etkili MEIS1i geliştirmesini sağlayacaktır; böylece lösemi tedavileri için alternatif bir ilacın geliştirilmesine katkı sağlayacaktır.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	IV
ABSTRACT.....	V
ÖZET	VI
LIST OF FIGURES	X
LIST OF TABLES.....	XII
LIST OF SYMBOLS/ABBREVIATIONS.....	XV
1. INTRODUCTION.....	1
1.1. LEUKEMIA.....	1
1.1.1. Leukemia Stem Cells (LSCs) and Leukemia-Initiating Cells (LICs).....	2
1.1.2. Biomarkers of Leukemia Stem Cells in AML and ALL	4
1.1.3. Acute Myeloid Leukemia (AML) and LSC.....	6
1.1.4. Acute Lymphocytic Leukemia (ALL) and LSC	9
1.2. MEIS1	11
1.2.1. The Role of MEIS1 on Normal Hematopoiesis.....	12
1.2.2. The Oncogenic Role of MEIS1 in Leukemia	13
1.3. RELAPSE- REFRACTORY AML/ALL AND MEIS1.....	14
1.4. MOLECULAR MECHANISM OF LEUKEMIA ASSOCIATED WITH MEIS1.....	15
1.4.1. Relation of MLL with MEIS1 Protein and HoxA9/Pbx	15
1.4.2. Relation with FMS-Like Tyrosinekinase (FLT3) with MEIS1 and HoxA9 /Pbx.....	16
1.4.3. Regulation of Hypoxic Phenotype in Hematopoietic Cells with MEIS1	17
1.4.4. Effect of MEIS1 Protein on Cell Cycle and Apoptosis	19
1.5. LEUKEMIA STEM CELLS (LSCS) INHIBITION METHODS	19
1.5.1. Targeting Surface Markers Expressed by LSCs	19
1.5.2. Targeting the LSCs Microenvironment	20
1.5.3. Targeting the MEIS1 Protein via Small Molecules Called MEIS1i.....	20
1.5.4. Other Methods for the Elimination of LSCs.....	20
2. AIM OF THE STUDY	22

3. MATERIALS	23
3.1. INSTRUMENTS	23
3.2. BONE MARROW AND BLOOD SAMPLES.....	23
3.3. SMALL MOLECULES	27
3.4. CELL CULTURE MEDIA AND SUPPLEMENTS	27
3.5. SEPARATION MNCS FROM BONE MARROW- PERIPHERAL BLOOD.....	28
3.6. LSC SEPARATION	28
3.7. DETERMINATION OF LSCS MARKERS	29
3.8. ANNEXIN-V APOPTOSIS DETECTION ASSAY	29
3.9. VIABILITY ASSAY	30
3.10. GENE EXPRESSION ANALYSIS.....	30
4. METHODS.....	32
4.1. SEPARATION AND ANALYSIS OF LEUKEMIA STEM CELLS	32
4.2. LSCS SEPARATION WITH FLOW CYTOMETRY SELECTION (FCS) METHOD.....	32
4.3. LSCS SEPARATION WITH MAGNETIC SELECTION (MS) METHOD	33
4.4. PURITY, VIABILITY, AND CELL PHENOTYPING USING FLOW.....	33
4.5. ANALYSIS OF EXPRESSIONS OF THE MEIS GENE FAMILY AND RELATED GENES IN LCSS AND OTHER MNCS.....	33
4.6. ANALYSIS OF THE EFFECT OF MEIS INHIBITORS ON LSCS.....	34
4.7. ANALYSIS OF THE EFFECT OF MEIS1i ON EXPRESSION OF MEIS TARGET GENES IN LSCS	35
4.8. STATISTICAL ANALYSIS	35
5. RESULTS.....	36
5.1. DETERMINATION OF MEIS (+) IN WHOLE BONE MARROW MNCS OF HEALTHY INDIVIDUALS AND PATIENTS	36
5.2. COMPARISON OF MEIS (+) RATIO IN WHOLE BONE MARROW MNCS OF HEALTHY INDIVIDUALS AND PATIENTS.....	39
5.3. COMPARISON OF MEIS (+) RATIO OF HEALTHY INDIVIDUALS AND ALL / AML LSCS.....	40

5.4.	DETERMINATION OF EXPRESSION OF MEIS1/2/3 AND RELATED GENES IN AML/ALL	41
5.5.	DETERMINATION OF EXPRESSION OF MEIS1/2 AND AML/ALL RELATED GENES.....	43
5.6.	DETERMINING THE RELATIONSHIP BETWEEN MEIS AND LSCS MARKERS	43
5.7.	DETERMINATION OF LSC MARKERS IN AML / ALL.....	46
5.8.	DETERMINATION OF LSCS SEPARATION WITH MAGNETIC SELECTION (MS) METHOD	49
5.9.	LSCS SEPARATION WITH FLOW CYTOMETRY SELECTION (FCS) METHOD.....	51
5.10.	DETERMINATION OF CELL VIABILITY POST MEIS1i TREATMENT IN PATIENTS WITH HIGH-LOW MEIS PROTEIN PROFILE	53
5.11.	APOPTOSIS ASSAY DETERMINATION OF MNCS/LSCS POST MEIS1i TREATMENT.....	55
5.12.	DETERMINATION OF GENE EXPRESSION POST MEIS1i TREATMENT IN ALL/AML PATIENTS.....	59
5.13.	DETERMINATION OF MEIS (+) RATIO POST MEIS1i TREATMENT IN PATIENTS WITH HIGH/LOW MEIS PROTEIN PROFILE.....	64
5.14.	DETERMINATION OF LSC MARKERS 7 DAYS POST MEIS1i TREATMENT.....	66
5.15.	DETERMINATION OF THE EFFECT OF MEIS1i TREATMENT ON CELL CYCLE PROFILE	67
5.16.	DETERMINATION OF EXPRESSION OF MEIS1 AND RELATED GENES IN POST-CHEMOTHERAPY RELAPSE /REFRACTORY.....	69
5.17.	EVALUATION OF MUTATIONS AND MEIS RELATIONSHIP	72
6.	DISCUSSION.....	73
7.	CONCLUSION AND FUTURE PERSPECTIVES	78
	REFERENCES	79
	APPENDIX A.....	102

LIST OF FIGURES

Figure 1.1. Development stages of normal hematopoiesis and leukemogenesis.....	3
Figure 1.2. The TALE superfamily of homeodomain-containing transcription factors consists of three-amino-acid-loop-extension (TALE) factors.	11
Figure 1.3. Conventional chemotherapy and relapse/refractory formation	14
Figure 1.4. TALE protein communication with each other.....	17
Figure 5.1. MEIS (+) protein profile determination in BM-MNCs healthy individuals.. ...	37
Figure 5.2. MEIS (+) protein profile determination in AML/ALL patients' BM-MNCs)...	38
Figure 5.3. Quantification of flow cytometry analysis performed in BM-MNCs of AML/ALL patients and healthy individuals to compare MEIS (+) protein profiles	39
Figure 5.4. Quantification of flow cytometry analysis done in LSCs of AML/ALL patients and healthy individuals to compare MEIS (+) protein profile.....	41
Figure 5.5. Gene expression analysis of MEIS1/2/3 and related genes in MNCs of AML/ALL patients	42
Figure 5.6. Gene expression in BM-MNCs of ALL/AML patients compared to healthy individuals by Real-time PCR	43
Figure 5.7. Relationship between MEIS and LSC markers (CD123, CD34) in AML/ALL	45
Figure 5.8. Quantification of flow cytometry analysis which performed to determine LSC markers.....	47

Figure 5.9. LSCs separation using the Magnetic Selection (MS) method.....	50
Figure 5.10. LSC separation using the Flow Cytometry Selection (FCS) method.....	52
Figure 5.11. Cell viability post MEIS1i treatment.....	54
Figure 5.12. The effect of MEIS1i on apoptosis process on MNCs post 72h of treatment.....	57
Figure 5.13. The effect of MEIS1i on the apoptosis process on LSCs post 72h of treatment.	58
Figure 5.14. Gene expression analysis before MEIS1i treatment	60
Figure 5.15. Gene expression analysis post MEIS1i treatment.	61
Figure 5.16. Quantification of flow cytometry analysis performed to determine MEIS (+) ratio post MEIS1i treatment.....	65
Figure 5.17. Post 7 days of MEIS1i treatment on MNCs cells, the LSC marker profile was analyzed	66
Figure 5.18. Cell cycle analysis of LSCs after 4 days of MEIS1i treatment	68
Figure 5.19. Gene expression analysis of MEIS1/2/3 and associated genes	70

LIST OF TABLES

Table 1.1. The most common leukemia stem cell identification markers.....	4
Table 1.2. World Health Organization (WHO) classification of AML.....	7
Table 1.3. World Health Organization (WHO) classification of ALL.....	10
Table 3.1. Characteristics of AML/ALL patients. MS: Magnetic Sort, FCS: Flow Cytometry Sort, ND: Not Done, BM: Bone Marrow, PB: Peripheral Blood.....	24
Table 3.2. Cell culture media and supplements used in the research include catalog numbers/vendors.....	27
Table 3.3. Vendor /catalog number information of specific materials and chemicals used for MNCs separation.....	28
Table 3.4. Catalog numbers/vendor of LSCs isolation materials.....	28
Table 3.5. Surface markers for LSCs with their conjugated fluorochromes from Biolegend and eBioscience.....	29
Table 3.6. Apoptosis test components with vendor/catalog numbers.....	29
Table 3.7. The following is a list of specialized RNA isolation, cDNA synthesis, and RT-PCR kits, along with their vendor/catalog numbers.....	30
Table 3.8. LSCs related genes and sequences of human primers (5 prime to 3 prime) ordered from SENTEGEN, TURKEY.....	31

Table 5.1. The percentage and fold difference in average AML/ALL MNCs cell content quantification data corresponds to MEIS+.....	40
Table 5.2. The percentage and fold difference in the average AML/ALL MNCs cell content quantification results correspond to CD123+.....	48
Table 5.3. The percentage and fold difference that corresponds to CD34+ in the average AML/ALL MNCs cell content quantification results.....	48
Table 5.4. The percentage and fold difference that corresponds to CD33+ in the average AML/ALL MNCs cell content quantification results.....	49
Table 5.5. The percentage and fold difference in average AML/ALL MNCs cell content quantification data that relate to LSCs markers.....	51
Table 5.6. The percentage and fold difference in average AML/ALL MNCs cell content quantification data that relate to LSCs markers.....	53
Table 5.7. The percentage and fold difference that corresponds to cell viability post-treatment in average AML/ALL MNCs with a high MEIS protein profile cell content quantification results.....	55
Table 5.8. The percentage and fold difference that corresponds to cell viability post-treatment in average AML/ALL MNCs with a low MEIS protein profile cell content quantification results.....	55
Table 5.9. The percentage and fold difference that corresponds to apoptotic effect in average AML/ALL MNCs cell content quantification results.....	59
Table 5.10. The percentage and fold difference that corresponds to apoptotic effect in average AML/ALL LSCs cell content quantification results.....	59

Table 5.11. MEIS and associated gene expression and fold differences in average AML-R compared to average healthy PBs cell content quantified findings.....	62
Table 5.12. MEIS-related gene expression and fold differences in average patients compared to average healthy BMs cell content quantified data.....	62
Table 5.13. The expression and fold difference of MEIS-related genes in average AML-R cells post-treatment when compared to DMSO cell content quantified data.....	63
Table 5.14. The expression and fold difference that corresponds to MEIS and related genes post- treatment in average patients compared to DMSO cell content quantification data...	63
Table 5.15. The percent and fold difference in average AML/ALL MNCs cell content quantification data that matches post-treatment LSCs markers.....	67
Table 5.16. The expression and fold difference that corresponds to MEIS and related genes in average patients/average relapse/refractory BMs cell content quantified results when compared to average healthy BMs cell content.....	71
Table 5.17. The expression and fold difference that corresponds to MEIS and related genes in average relapse/ refractory compared to average patient BMs cell content quantification results.....	71
Table 5.18. The expression and fold difference that corresponds to MEIS1 expression in patients with mutation/ fusion gene compared to average healthy BMs cell content quantification results.....	72

LIST OF SYMBOLS/ABBREVIATIONS

AL	Acute leukemia
ALDH	Aldehyde dehydrogenase activity
AML-R	Acute myeloid leukemia relapse/refractory
ALL-R	Acute lymphoblastic leukemia relapse/refractory
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AML-IC	Aml-initiating cells
BCL-2	B-cell lymphoma-2
BM	Bone marrow
CSCs	Cancer stem cells
CXCR-4	C-x-c chemokine receptor type 4
DNMT3a	Dna methyltransferase 3 alpha
DFS	Disease free survival
DMSO	Dimethyl sulfoxide
FAB	French–american–british
FLT3	Fms-like tyrosine kinase
FLT3-ITD	Fms-like tyrosine kinase 3-Internal Tandem Duplication
FCS	Flow cytometry selection
HD	Homeodomain
HIF	Hypoxia- inducible factor
Hif-1 α	Hypoxia inducible factor 1 alpha subunit
Hif-2 α	Hypoxia inducible factor 2 alpha subunit
HoxA9	Homeobox a9
HSC	Hematopoietic stem cell
HSPCs	Hematopoietic stem and progenitor cells
LICs	Leukemia initiating cells
LSCs	Leukemia stem cells
MDS	Myelodysplastic syndromes
MEIS1	Myeloid ecotropic integration site-1
MEIS1i	MEIS1 inhibitors

MLL	Mixed lineage leukemia
MNC	Mononuclear cells
MRD	Minimal residual disease
MS	Magnetic selection
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency
NPM1	Nucleophosmin 1
OS	Overall survival
Pbx1	Pre-b-cell leukemia transcription factor 1
PB	Peripheral blood
ROS	Reactive oxygen species
TALE	Three amino acids loop extension
Tdt	Terminal deoxynucleotidyl transferase

1. INTRODUCTION

1.1. LEUKEMIA

Leukemia is a hematological malignant disease that is a life-threatening condition for patients carrying this disease [1]. There are 2 types of leukemia. Acute (fast progressive) and Chronic (more slowly progressing), are divided into two as myeloid and lymphoid origin. Acute and chronic leukemias have different mechanisms and different treatment options.

Acute myeloid (or myelogenous) leukemia (AML)

Acute lymphocytic (or lymphoblastic) leukemia (ALL)

Chronic myeloid (or myelogenous) leukemia (CML)

Chronic lymphocytic leukemia (CLL)

According to the 2021 data from the *American Cancer Society*, the number of newly diagnosed AML patients was 20240, while the number of patients diagnosed with ALL was 5690. According to the same Society, while the number of patients who died from AML was 11400 in 2021, the number of patients who died from ALL was 1580 [2]. The risk of most leukemias increases as people get older. The typical age of a patient with Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL), or Chronic Myeloid Leukemia (CML) is 65 years or older. People under the age of 20 are more likely to develop Acute Lymphocytic Leukemia (ALL). The median age of an ALL patient at the time of diagnosis is 15 [3]. As a result of studies on the cause of leukemia, mutagenic factors, genetic combinations, and environmental factors be among the known causes [4]. Besides all these reasons, somatic mutations are accepted as the major factor in the development of leukemia. Some somatic mutations are;

Chromosomal Translocation (Fusion protein, abnormal gene expression) [5,6]

Numerical Chromosomal Aberration (Deletion, aneuploidy) [7,8]

Mutation (Point-,ITD.) in known oncogenes or tumor suppressor genes [9,10].

1.1.1. Leukemia Stem Cells (LSCs) and Leukemia-Initiating Cells (LICs)

LSCs are usually characterized by their quiet nature, immature phenotype, distinct metabolic characteristics, and reliance on certain signaling pathways [11]. In addition to these characters, LSCs have properties such as self-renewal, undifferentiation, drug resistance, as well as initiating leukemia when transported to NOD / SCID mice [12,13].

Leukemia initiation means to engraft and reconstitute the heterogeneous leukemia disease, while self-renewal refers to the ability to transfer disease into secondary/tertiary recipients [14]. LSCs and HSCs share the same niche in the bone marrow (BM) [15]. Both LSCs and HSCs have the same CD34⁺ CD38⁻ immunophenotype. This strengthens the hypothesis that LSCs originate from HSCs and subsequently undergo a series of malignant transformations [16,17] (Figure 1.1). The LSCs originate from the myeloid series and also have more specific self-renewal and other biological properties of HSCs [16–19]. The most important difference between healthy and leukemic cells is their response to hematopoietic growth factors. The important point here is that it has a different sensitivity to cytokines in stem cells [22]. Interleukin-3 receptor alpha chain (CD123) is the most prominent marker of LSCs and is not highly expressed in CD34⁺/CD38⁻ hematopoietic cells [20–22]. In addition to this, studies support the presence of LSCs in the CD34⁺/CD38⁻ population [12]. Actually, leukemia stem cells are well understood for AML, while their existence and relevance for ALL are less evident [25]. Human AML-IC was first found in 1994 by Lapidot and colleagues in immunodeficient SCID mice [26] *in vivo* [27]. Later studies showed that the NOD/SCID model was more effective [28]. LSCs, defined as leukemia-initiating cells (LIC) from time to time, represent a subset of leukemia cells and are distinguished from other leukemia cells because they have some characteristics of the stem cell [14]. Ng et al., conducted a research in four patients with AML were identified as CD34⁺/CD38⁻ and injected into the mouse. Transfected mice were identified with LICs cells [29]. LSCs obtained from transplanted xenograft specimens were found to be similar to the original patient LSCs [30]. LSCs are present in CD34⁺/CD38⁻ cells and a small number of CD34⁺-cells and opposite with that in a large number of CD34⁺ cells [12]. Recent studies have confirmed that the prevalence of CD34⁺/CD38⁻ LSCs have parallel correlation with Minimal Residual Disease (MRD) levels after chemotherapy, disease responsiveness and poor survival [31]. In the presence of LSCs, the treatment of AML

patients is different and resistance to treatment increases [30,31]. In a broader gene study with 1047 AML patients, the expression of 52 genes representing LSCs and Non-LSCs was determined, and it was found to be related that patients with high-score LSCs had poor OS (overall survival) and DFS (disease-free survival) [34].

Although xenograft transplantation experiments confirmed the presence of LSCs in CD34+ / CD38- cells, on the other hand, Taussig et al. found that the presence of LSCs in the samples taken from NPM-mutated AMLs was observed in low CD34-expression [24,33]. In some instances, only the presence of LSCs in CD34- cells was observed, while in some samples the presence of LSCs in both CD34+ and CD34- cells emphasized that the phenotyping of LSCs was heterogeneous. It has also been shown that the phenotype of LSCs has changed and the existence of LSCs differs in CD34 and CD38 expressions [30]. LSCs observed different ratios of AML subtypes and the ratio of LSCs was found to be directly proportional to high-risk AML [34,35]. In addition, LSCs-associated microRNAs obtained from samples known to be LSCs are associated with OS [33]. All these studies allow the patient to determine the treatment modality and identify risky groups according to the genetic and other characteristics of AML LSCs.

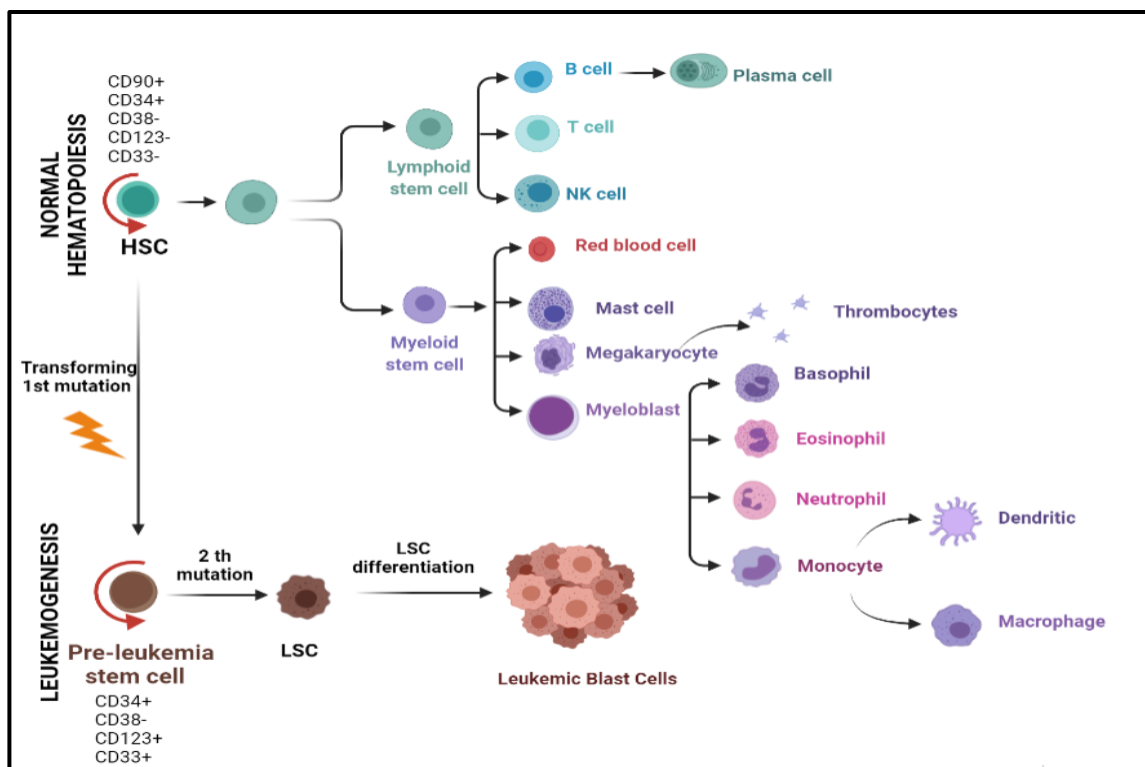


Figure 1.1. Development stages of normal hematopoiesis and leukemogenesis.

There are cell surface markers that characterize HSC (indicated in black). A small population of self-renewing HSC are transformed into a pre-leukemia stem cells with reduced self-renewal capacity. Pre-leukemia stem cells have altered differentiation hierarchies and few express some cell surface markers (indicated in black). HSCs form functionally mature blood cells. LSC is occurred by transforming mutations. As a result of the differentiation of LSCs, leukemic blast cells are formed (Figure 1.1).

1.1.2. Biomarkers of Leukemia Stem Cells in AML and ALL

The identification of LSCs in leukemia is particularly important in disease diagnosis, prognosis, monitoring, and drug screening of leukemia. Multicolor flow cytometry, side population assays, and ALDH assays are all used to identify LSCs. Despite the variability of AML/ALL, multiple cell surface markers that are increased on CD34+CD38- LSCs when compared to normal CD34+CD38- HSPCs have been found. The most common surface markers in the LSCs are shown in Table 1.1.

Table 1.1. The Most common leukemia stem cell identification markers

Marker	Description	References
CD34	Marker antigen for hematopoietic stem and progenitor cells (HSPCs) and identified in CD34- stem cell and LSCs.	[35]
CD38	Maturation marker, normal HSC should be negative for this marker. This marker was found negative in AML- LSCs.	[38]
CD123 (IL3RA)	LSCs are frequently identified by this marker	[24] [39]
CD33	Positive for patients with AML -LSCs	[40] [41] [42]
TIM3	Surface molecule expressed on LSCs in most types of	[43]

(T-cell Ig mucin3)	AML except for Acute Promyelocytic Leukemia, but not on HSCs.	[44]
Anti BCL-2	The BCL-2 is highly expressed in CLL patients	[45]
CD96	Cell surface marker present on many AML-LSCs	[40] [46] [47]
CD47	Up-regulated in murine and human myeloid leukemia	[48] [49]
CLL-1	C-type lectin-like molecule-1, a stem cell marker, also for myeloid LSCs.	[35] [40] [50] [51]
ALDH	ALDH activity is high in both myeloid LSCs and HSCs.	[52] [53]
CD133	CD133 expression on LICs in children with ALL.	[54]
CD99	Allows for the separation of functionally normal hematopoietic stem cells (HSCs) from leukemic stem cells (LSCs) in AML.	[55]
CD32	The expression is associated with LSC activity in some patients with AML	[56]
CD93	Novel biomarker of LSC in CML	[57]
CD22 CD19 Anti-Tdt CD10 CD20	These markers are used by the European Group for characterization in B-ALL classification.	[58]
CD2 CD5 CD7 CD8 CD10 Anti-Tdt	These markers are used by the European Group for characterization in T-ALL classification.	[58]

Many of these markers related to relapses of the disease have not been studied. But the combination of CD123+ CD34+ CD38- is known to increase disease relapsed [30,57]. In addition, CD123 positive cells and FLT3-internal tandem duplication (ITD) mutation come together in the LSC CD34+ CD38- population [60] suggests that CD123 is a strong LSCs marker in FLT3-ITD mutated AML. In many studies to identify myeloid cells, CD123 and CD33 were used as LSCs determinants [37–39,59].

All of these studies indicate that CD123 is an important determinant in the targeting and identification of LSCs. In other studies, it has been reported that TIM3 is highly expressed on the surface of a large number of LSCs but not in normal BM- HSCs [43]. TIM3 is defined as a new surface marker in the determination of AML-LSCs.

Saito et al., examined a research that CD32 and CD25 be highly expressed on the surface of human LSCs [62]. In recent studies, Although LSCs were defined in CD34+ CD38- phenotype, Taussing et al. showed that CD34+ CD38+ cells were engrafted in all seven AML samples in their investigation. However research authors noticed that CD34+ CD38+ cells were required for the engraftment of 106 cells in each animal, while CD34+ CD38- cells were significantly less required in animal models [63]. As evidenced by recent studies, AML disease in the clinic is associated with CD34+ CD38- LSCs. In the flow cytometer analysis, LSCs were observed to be quite high in Aldehyde Dehydrogenase Activity (ALDH) positive cells [64].

1.1.3. Acute Myeloid Leukemia (AML) and LSC

AML is defined as an aggressive neoplastic disease characterized by elevated proliferation, discontinuance of differentiation, and disruption of apoptosis mechanism [65]. Although the advantageous approaches to the treatment of AML have increased in recent years, it is still weak in determining the cause of the disease [66]. Although transplantation is still an important treatment modality in relapsed and high-risk AML disease, side effects associated with transplantation and refractory diseases cause morbidity and mortality [67]. AML patients are treated with various treatment methods and hematopoietic stem cell transplantation, but the relapsed of leukemia is still an insoluble problem of AML treatment [68]. The most important reason for the relapsed of the disease after the treatment is the presence of LSCs which enables the self-renewal of leukemia. For the

discovery of new drugs to be developed for the treatment of AML types (Table1.1), CD34⁺ CD38⁻LSCs with leukemic blasts belonging to AML patients in xenograft models can be examined by separating them with a special method, as LSCs may be the clinical targets for treatment [69]. With the disruption of hematopoiesis, the number of normal cells (red blood cells, platelets, and granulocytes) is reduced, which contributes to the development of the disease due to their resistance to chemotherapy drugs, LSCs have been forced to respond to treatment and OS rates have decreased in AML [68,69]. Beside CD34 is a trans-membrane sialomucine protein and as a cell-cell adhesion factor and is a protein that is expressed on the surface of HSCs, which allows stem cells to bind to the extracellular matrix [72]. The CD34 monoclonal antibody is important in the identification of immature and mature cells in LSCs and in identifying residual immature blasts for MRD disease [73].

Chromosomal structural differences and genetic defects play an important role in the pathogenesis of AML [74]. The five most common mutated genes in AML comprise NPM1, Idh1, Idh2, Dna methyltransferase 3 alpha (Dnmt 3a), and FLT3, according to The Cancer Genome Atlas Project [75]. In addition to all these , the classification of leukemia is an important issue. For this purpose, the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, fourth edition, was originally released in 2016, with the whole description of the classification being published in 2017.[72–74]. The AML classification has been revised in the 2016 WHO classification. (Table1.2).

Table 1.2. World Health Organization (WHO) classification of AML

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11
APL with PML-RARA
AML with t(9;11)(p21.3;q23.3);KMT2A-MLLT3
AML with t(6;9)(p23;q34.1);DEK-NUP214

AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM
AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1
Provisional entity: AML with BCR-ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, Not Otherwise Specified
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with down syndrome

CD34+ AML

The majority of AML-LCSs samples (75 percent) were positive for CD34 expression and the blast rate was above > 10 percent [79] . In CD34+ CD38-AML cells, a higher

percentage of LSCs are observed than CD34⁺ CD38⁺ cells, whereas these cells are immature cells with higher self-renewal properties [78,79].

CD34- AML

CD34- leukemia cells are defined as cells that are resistant to treatment and have the potential to initiate leukemia [12]. 20 percent of patients with AML have a lack of neoplastic CD34⁺ cells [80,81]. No LSCs were found in samples with CD34⁺ and a blast population below one percent [80,82,83]. Patients with CD34- except for CD34⁺ / CD38- or CD34⁺ / CD38⁺ leukemic populations potentially have a population of LSCs. Apart from all these, it is thought that more small cell groups in the AML patient group are not yet defined, which are more leukemic and much more resistant to treatment [12]. Quek and colleagues studied 49-sample CD34-AML cohort, they compared the mutation profile with that of a distinct cohort of 84 sequential AML samples with more than 2 percent CD34⁺ blasts. There was enrichment of CD34-versus CD34⁺ AML with NPM1, TET2 mutations [86]. In parallel with this study, the presence of NPM1 mutation was found to be inversely proportional to CD34 expression in the study by Falini et al., [87]. Cells identified by flow cytometry using Hoechst dye 33342 were found to be resistant to AML treatment, and these cells were found to have leukemia initiation when NOD / SCID mice were administered [86–89]. As a result of these studies, CD34⁺, and CD34-cells [48,90] were found to be neoplastic [50].

1.1.4. Acute Lymphocytic Leukemia (ALL) and LSC

ALL is defined as a hematological disease that is seen in 20 percent of all adult AL and 80 percent of children AL [91–93]. ALL disease is divided into T-lineage ALL and B-lineage ALL (Table 1.3). LSCs are found in a large proportion of AML but are less defined in ALL [96]. Satake and colleagues have developed a new method of separating ALL LSCs and found that they had *in vivo* LICs cells obtained by this new method. They also demonstrated that the LSCs isolated from the same investigation had different transcriptome profiles and also showed that LSCs had subclones [97].

There are genetic variations that can be observed by cytogenetic, FISH or molecular tests in around 80 percent of ALL patients. Seven significant genetic mutations in B-cell

leukemia/lymphoma, including hypodiploidy and hyperdiploidy, are established in the WHO 2008 classification: t(9;22) (q34;q11.2) BCR-ABL1, t(v;11q23) MLL rearranged, t(12;21) (p13;q22) TEL-AML1 (ETV6-RUNX1), t(5;14)(q31;q32) IL3-IGH, t(1;19) (q23;p13.3) E2A-PBX1 (TCF3-PBX1) [77]. Besides these, philadelphia chromosome-like B-lineage ALL is one of the latest high-risk subtypes characterized by genetic alterations that cause different signaling pathways, including those involving cytokine receptors, tyrosine kinases, and epigenetic modifiers [98].

Table 1.3. World Health Organization (WHO) classification of ALL

WHO Types	Immunophenotyping
B- ALL	
Early precursor B-ALL	(pro-B-ALL, CD10 ⁻ , CD19 ⁺ , cCD79a ⁺ , cCD22 ⁺ , TdT ⁺) (NCCN Clinical Practice Guidelines in Oncology-Acute Lymphoblastic Leukemia (2016 Version I). https://www.nccn.org . Accessed 6 May 2019)
Common B-ALL	CD10 ⁺ (NCCN Clinical Practice Guidelines in Oncology-Acute Lymphoblastic Leukemia (2016 Version I). https://www.nccn.org . Accessed 6 May 2019).
Precursor B-ALL (pre-B-ALL)	(pre-B-ALL, cytoplasmic μ^+ , sIg ⁻ , CD10 ^{+/}) (NCCN Clinical Practice Guidelines in Oncology-Acute Lymphoblastic Leukemia (2016 Version I). https://www.nccn.org . Accessed 6 May 2019).
T- ALL	
	CD1a, CD2, CD3 (membrane and cytoplasm), CD4, CD5, CD7,CD8 (99)

CD34 (age 1 to 10 years) is an important marker for B-ALL (100). In low-risk adult B-ALL patients, CD34 expression was correlated with higher WBC cell count, and high

peripheral blood (PB) LSCs [101]. In most of the ALL patients, CD34 is not expressed on the surface of LSCs [72].

1.2. MEIS1

Homeodomain transcription factors play an important function in embryogenesis and differentiation. The long homeodomain of 60 amino acids is unusual, characterizing the class of TALE (three loop extension of amino acids) that includes three extra amino acids between the first and the second α -helix. This class comprises two sub-families, PBC (Pbx1-4 in human) and MEINOX, further divided into sub-families PREP (PREP1-2) and MEIS (MEIS1-3) [102] (Figure 1.2).

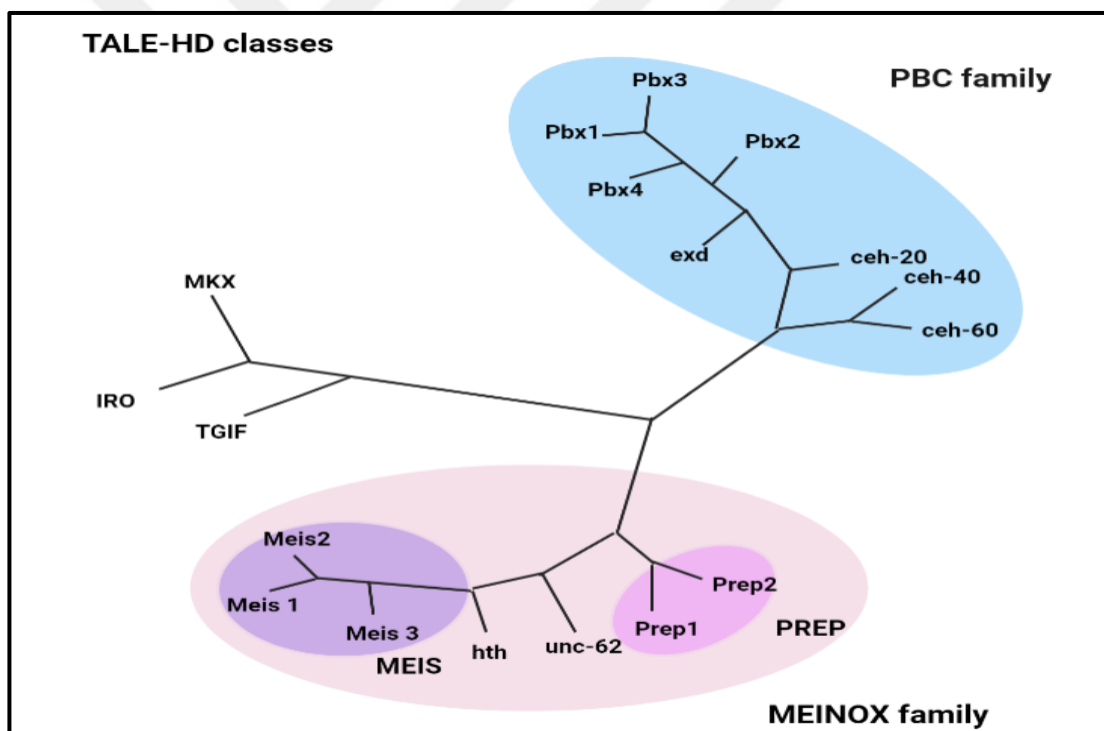


Figure 1.2. The TALE superfamily of homeodomain-containing transcription factors consists of three-amino-acid-loop-extension (TALE) factors [103].

MEINOX and PBC transcription factors can be seen separately (mammalian: Meis1, Meis2, Meis3, Prep1, Prep2, Pbx1, Pbx1, Pbx1 and Pbx4; *D. melanogaster*: exd and hth; *C.*

C. elegans: *ceh-20*, *ceh-40*, *ceh-60* and *unc-62*). Only the gene groups Iroquois (IRO), Mohawk homeobox (MKX), and TGIF are displayed (Figure 1.2) [103].

Belonging to the MEIS (MEIS1-3) subfamily, MEIS1 protein has important roles in both hematopoiesis and leukemogenesis, including differentiation, regulation of apoptosis mechanism [102,103]. The MEIS1 protein is a 43KDa protein of 137360 base-pair gene length, consisting of 13 exons and containing 390 amino acids [104,105]. The DNA binding sequence is TGACAG [108]. The MEIS1 protein plays an important role in many types of cancer including neuroblastomas, nephroblastomas, ovarian carcinomas, prostate cancer, nonsmall cell lung adenocarcinoma, and leukemia [107–112]. Moreover, in a recent research, it was found that MEIS1 controls both the development of papilloma and the malignant conversion from papilloma to carcinoma in papilloma. Despite all these studies, the role and mechanism of MEIS1 protein in tumor formation and development are not fully known [115]. MEIS1 protein was first discovered in the BXH-2 mouse leukemia model as a common viral integration site [104]. The normal MEIS1 mRNA or MEIS1 - proviral fusion mRNA level increases with viral integration. Recent studies have shown increased cytoplasmic glycolysis by overexpression of MEIS1 protein, which is thought to be involved in the spread and progression of cancer. The increase in resistance to chemotherapy treatment is more parallel to the expression of MEIS1 than normal. In addition, the MEIS1 protein increases stem cell regeneration while maintaining stem cell glycolytic metabolism [114–119].

1.2.1. The Role of MEIS1 on Normal Hematopoiesis

Recent studies suggest that MEIS1 plays an important role in the normal HSC self-renewal/proliferation. MEIS1 is co-work with the Hox genes, causing further differentiation stages in hematopoiesis [120–122]. This hypothesis is supported by a high rate of bleeding due to the lack of megakaryocytes in mouse 14.5 embryonic days, which has not to supplement the MEIS1 protein [125]. While the presence of myeloerythroids in dead embryos was observed, the total number of colony-forming cells was observed to be quite low. In the future, the illumination of the role of the MEIS1 protein in normal hematopoiesis will help to resolve the pathway of the LSCs [124].

1.2.2. The Oncogenic Role of MEIS1 in Leukemia

As a result of various genetic changes in the MEIS1 gene, the onset of some types of cancer is triggered by its overexpression. Among them, ovarian cancer [126] and neuroblastoma [110] are the most well-known. In studies, the excessive expression of MEIS1 causes cell proliferation and apoptosis resistance [105].

Cancer cells, unlike healthy cells, use cytoplasmic glycolysis to digest large amounts of glucose, a phenomenon known as the Warburg effect in biology. With this effect, cancer cells can survive in the hypoxic environment where the tumor exists. Pernilla Eliasson et al. revealed that when they performed hypoxic conditions to LSCs, they noticed that HSC proliferation had reduced by the accumulation of the cells to G0 phase [116,125,126]. The initial effect of MEIS1 protein on cancer has been demonstrated in studies of myeloid leukemia cells in AML patients [110,127–129]. Supporting this thesis with other studies, increased MEIS1 expression in the BMs of ALL and AML patients was observed [120,130]. In the experiments with murine AML models, it was found that high expression of MEIS1 allowed the onset of the disease [131,132]. MEIS1 and Hox proteins especially Homeobox A9 (HoxA9) and Pre-B-Cell Leukemia Transcription Factor 1 (Pbx1) work together in leukemia and accelerate leukemogenesis by activating responsible genes [110,122]. HoxA9 HD with transcription factor is important in HSCs replication and its regulation is mostly degenerated in AL [135]. A high number of expressions of MEIS1 and HoxA9 were observed in aggressive murine and human ALL samples [102,134,135]. In addition to all these, MEIS1 increases the proliferation of cell proliferation parallel to the nature of cancer and strengthens the resistance to apoptosis [105]. According to some researchers, decreased activity of fumarate hydratase (Fh1) (TriCarboxylic Acid (TCA) cycle and fumarate metabolism causes activation of MEIS1 / HoxA9 oncogenic pathway in LSCs [138]. In this case, the change in metabolic activity is thought to trigger the activity of MEIS1 during the development of leukemia. In another study, even in the absence of fusion, the Dntm3a mutation in AML has been shown to reactivate the oncogenic MEIS1 pathway [139]. Zhou et al. found that PU.1 (tissue-specific transcription factor that is expressed in cells of the hematopoietic lineage is a function of leukemia, and increases the expression of MEIS1 in non MLL U937 cells [138,139].

Although there is a correlation between the high expression of MEIS1 protein in cancer cells and cytoplasmic glycolysis, the effects of MEIS1 and its cofactors on cancer stem cells and cancer metabolism are still being investigated [116].

1.3. RELAPSE- REFRACTORY AML / ALL AND MEIS1

One of the biggest problems encountered in the treatment of leukemia disease in the clinic is the resistance of LSCs in the blast population of patients to traditional chemotherapy. As a result of this resistance, the disease appears to be relapsed, known as *relapse or recurrence*. There are still uncertainties regarding the relapse of the disease, and it is known that the MEIS1 gene and its family are resistant to traditional chemotherapy regimens. According to studies, patients with a high MEIS1 expression level were more resistant to chemotherapy than those with a low MEIS1 expression level. The connection between MEIS1 and relapse/refractory is strengthened as a result of this [68]. According to the investigation of Adamaki et al., high HoxA9 and MEIS1 gene expression are associated with relapse and overall survival in childhood AL [142].

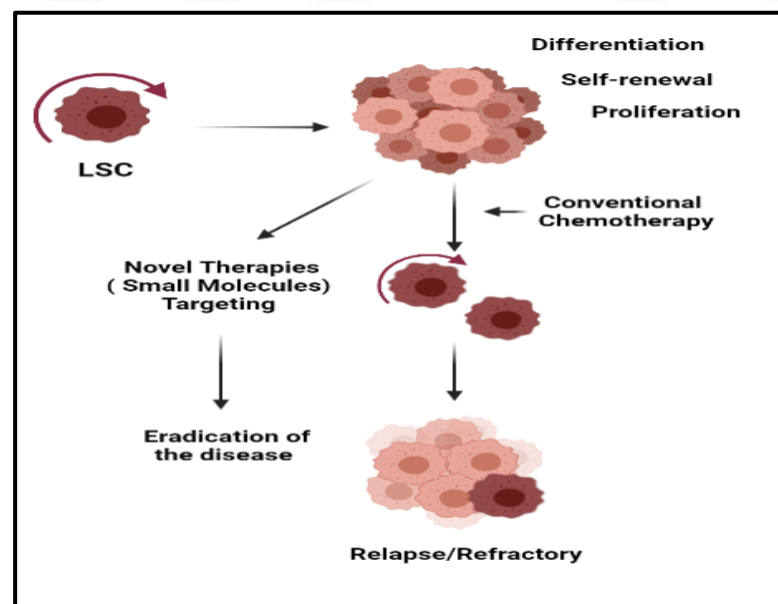


Figure 1.3. Conventional chemotherapy and relapse/refractory formation [143].

The goal of traditional leukemia treatment is to remove leukemic blasts. These treatments may not be able to eliminate the LSC population, which has the functional properties of

self-renewal, differentiation, and proliferation, and hence recapitulates the disease. The capacity to build newer medicines that target LSCs should result in more successful disease elimination (Figure 1.3) [143].

1.4. MOLECULAR MECHANISM OF LEUKEMIA ASSOCIATED WITH MEIS1

1.4.1. Relation of MLL with MEIS1 Protein and HoxA9 /Pbx

MLL contains chromosomal translocations that contain > 50 different genes associated with leukemia [142,143]. Chromosomal translocations involving the MLL gene (11q23) are found in 8-19 percent of ALL patients, 3 percent of AML patients. Chromosomal translocations involving the MLL gene (11q23) fuse N-terminal sequences of MLL to one of > 40 functionally diverse groups of C-terminal fusion partners [146]. AFL, AF9, or ENL translocation of the MLL gene in AML patients contributes to the development of both lymphoid and myeloid disease. LICs were characterized by using xenotransplantation assays in primary infants with MLL-abnormal leukemia [147]. Gene analysis of all leukemias associated with MLL fusion indicated that Hox gene dysregulation [129,145–147] was an integral factor for MLL fusion-related leukemias. In other studies, both the wild-type MLL and MLL oncogenic fusions have been associated with the Hox genes and active transcription of these genes [149–152]. Upregulation of HoxA9 and MEIS1 are seen in leukemias associated with MLL rearrangements. [16,147,153,154]. Recently, various genetic techniques are used to investigate the contribution of the TALE family of Pbx1, Pbx2, and Pbx3, and MEIS1 to the formation of MLL-induced AML. The presence of the relationship defines MLL fusion genes and their capacity to induce MEIS1 transcription was promising to find the contribution of MEIS1 to the formation of MLL oncogenesis [131]. This hypothesis is not valid in all myeloid leukemias. In the recent study with the AML mouse model, it was found that the MLL -AF9 oncogene [155,156] was effectively induced in FAB-M4 or M5 subtypes of human AML [159].

1.4.2. Relation with FMS-Like Tyrosinekinase (FLT3) with MEIS1 and HoxA9/Pbx

FLT3 belongs to a group of class III tyrosine kinase receptors. Although it is temporarily secreted during HSC maturation, it is much more expressed in AML disease [158,159]. FLT3 is expressed in leukemic blasts even in AL groups with negative CD34 [160–162]. In human ALs, the MEIS1 protein and Hox gene are high, which is associated with high expression of FLT3 mRNA [165]. On the other hand, Morgado et al. found that FLT3 was independent of MEIS1 and HoxA9 oncologic cooperation, and leukemogenesis was independent of FLT3 [166]. Garcia-Cuellar et al. conducted an important research on the regulation of Hox function with HoxA9-MEIS1 -Pbx3 interaction. According to this study; Pbx3 binds to MEIS1, protects it from ubiquitination, and then inhibits its proteasome-mediated degradation, greatly extending the half-life of MEIS1. When there is a mutation in MEIS1 (ie MEIS1 Δ) the association of Pbx3-MEIS1 is disrupted and the ubiquitination and degradation of MEIS1 is canceled, suggesting that the binding sites of Pbx3 and ubiquitin ligase in MEIS1 may be the same. Pbx3 -MEIS1 binding is required for MEIS1 - HoxA9 communication. As a result of Pbx3 stabilizing the MEIS1 protein level, MEIS1 - HoxA9 communication and gene regulation activity are increased (Figure 1.4) [167]. Similarly, Wang et al. suggested that Meis1-Pbx complexes control FLT3 transcription, expansion of normal sort term HSCs, and expansion of leukemia-initiating progenitors *in vivo* [168].

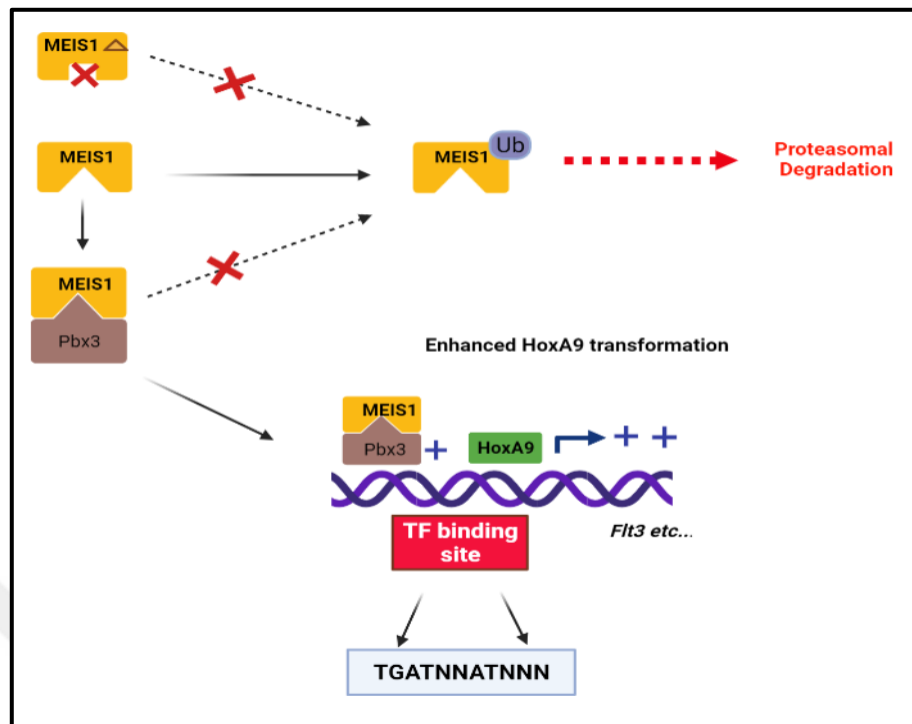


Figure 1.4. TALE protein communication with each other [169].

MEIS1-Pbx3 dimerization improves MEIS1 protein stability and Hox-mediated transcription of the MEIS1 protein's target genes (such as FLT3 and Trib2). MEIS1 is ubiquitinated and degraded by proteosomes when it is unbound. MEIS1 has been protected from ubiquitination thanks to a mutation in the Pbx-binding domain MEIS1 (MEIS1Δ). Ub: ubiquitin [169] (Figure 1.4).

1.4.3. Regulation of Hypoxic Phenotype in Hematopoietic Cells with MEIS1

One characteristic of the hematopoietic niche is the low oxygen partial pressure. Hypoxia provides long-term protection of HSPCs. Hypoxia is a powerful signal and is provided by members of the Hypoxia Inducible Factor (HIF) family [170]. High expression of Hif-1α was detected in leukemia and especially in AML, Acute Promyelocytic Leukemia (APL), ALL, and the response to chemotherapy decreased and poor prognosis was observed in these samples [169–173]. The severity and survival of AML and Myelodysplastic syndromes (MDS) diseases are affected by the level of Hif-1α [174–176]. Hif-2α expression has not been observed in normal cells and has been observed in AML and ALL

disease [173,177, 178]. Several studies have shown that when Hif-1 α was inhibited by RNAi targeting or small molecules, the primary cells did not form colonies in vitro, tumor growth and leukemic development reduced. In these studies, the disease was significantly regressed in AML xenograft samples and leukemia was eliminated by inhibition of Hif-1 α in second transplantation [168,170,174]. This is also supported by studies in ALL [179,180] and CML [181,182]. Similarly, in vitro limited cell proliferation, weak or incomplete engraftment was observed *in vivo* by knock-out of Hif-2 α with shRNA triggers leukemic inhibition [173,177, 183]. Hypoxia created by the HIFs family allows the progression of disease and maintenance through a series of mechanisms effective in the deregulated and normal physiology of cancer, such as energy metabolism, cycle, quiescence, and immune function [184,185]. Hif-1 α and Hif-2 α activate the signaling pathways involved in the protection and spread of leukemia. Hif-1 α influences the Notch1 pathway to activate the Wnt pathway that induces leukemia propagation and formation [179,180]. On the other hand, Hif-1 α inhibits the expression of tumor suppressor genes such as p15, p16, p19, and p53 [170,182]. Expressing HIFs allows cells to remain in the quiescence phase. The leukemia cells in the quiescence phase gain resistance to chemotherapy drugs. Here, hypoxia induces members of the HIF family and Hif-1 α keeps the cells in the G0 /G1 phase and prevents the AML cells from passing to the S phase [116,117,184].

MEIS1 protein has an important role in the transcriptional regulation of Hif-1 α and Hif-2 α genes [116–118,184]. MEIS1 and its cofactors (Pbx1, HoxA9) work together to activate Hif-1 α by binding to the reserved sites on the Hif-1 α gene in this mechanism [118].

With the parallel approach, the expression of Hif-1 α and Hif-2 α decreases with the penetration of the MEIS1 protein, thus increasing the ROS activity and the hematopoietic cells entering apoptosis [119]. ROS activity in metabolism plays an important role in the functioning of stem cells. ROS activity is also effective in the onset and progression of leukemia. ROS levels are lower in LSCs than HSCs, and agents that increase the activity of ROS provide the elimination of AML stem cells. In the knockout studies of MEIS1, the ROS level was observed to increase.

1.4.4. Effect of MEIS1 Protein on Cell Cycle and Apoptosis

Cell replication is a necessary process for the development of the organism. Apoptosis is the system that controls this proliferation mechanism. However, in cancer cells, cell proliferation goes out of control and the pathways that provides the apoptosis mechanism is disrupted. Likewise, uncontrolled cell proliferation in cancer cells causes a change in the activity of cell cycle regulations. Apoptotic pathways are targeted using engineered small molecules called anticancer agents. MEIS1, which is highly expressed in many cancer types, causes cell proliferation and apoptosis resistance [105]. In a research conducted by Kumar et al., inhibition of MEIS1 as a result of cell-cycle and apoptosis measurements reduced cell growth. The findings obtained as a result of PI-stained nuclei analysis in flow cytometry show that inhibition of MEIS1 caused cell-cycle arrest in the G0/G1 phase. It was observed that G0/G1 phase increased and S and G2/M phase decreased in M26-transduced cells in this study [188]. Other studies showed that the G0 phase decreased and the G1 phase doubled by silencing of the MEIS1 gene in the K562 cells. On the other hand, Wermuth et al. found that HoxA9 and MEIS1 eliminate the caspase-mediated apoptosis pathway [189].

1.5. LEUKEMIA STEM CELLS (LSCS) INHIBITION METHODS

While developing strategies to selectively eliminate LSCs, it is believed that the maintenance of normal hematopoietic stem cells (HSCs) is necessary for the long-term survival of leukemia patients.

1.5.1. Targeting Surface Markers Expressed by LSCs

It is a very common approach to treat LCSs with monoclonal antibodies targeting markers on their surface. The effects of the newly developed humanized monoclonal anti-CD44 antibody, RG7356, on AML patients were investigated [190]. At the end of this investigation, it was shown that RG7356 increased macrophage uptake and decreased AML blasts especially in CD34[−]/CD38⁺ cells [191]. The anti-CD123 drugs developed for the CD123 antigen, the specific marker of LSC, was promising for the treatment of

leukemia [79]. IL-3 is a critical cytokine that plays a role in myeloid differentiation, and blocking the IL-3 receptor α chain (CD123) has been shown to reduce the growth of LSCs in NOD/SCID mice [24]. New drugs targeting LCSs are in phase 1-2 stages and continue to be clinically developed [79].

1.5.2. Targeting the LSCs Microenvironment

CSCs are believed to reside in niches, as is the case for normal stem cells. Niches are specialized micro-environments that regulate the fate of adult stem cells by providing indications in the form of both contacts between cells and secreted variables [192]. C-X-C chemokine receptor type 4 (CXCR-4) plays an important role in homing and quiescence of HSCs [193,194]. Blocking CXCR4 allows LCSs to move out of the protected stromal niche and allow the disease to be eliminated by chemotherapy. 52 AML patients were treated with CXCR4 antagonist *plerixafor* and salvage chemotherapy, and 46 percent of the patients showed improvement in one phase 1-2 study [195].

1.5.3. Targeting the MEIS1 Protein via Small Molecules Called MEIS1i

Inhibitors have been developed for MEIS1 protein, which has an important role in leukemia. Small molecules called MEIS1i-1 and MEIS1i-2 show an inhibition effect on MEIS1 protein activity over 95 percent of MEIS1-luciferase activity at doses of 0.1 μ M [119,196]. MEIS1i-1 has been found to lose the activity of MEIS1 by binding the small molecule to the MEIS1 protein and reduce the regulation of hypoxia-inducible factors (Hif-1 α and Hif-2 α) [116, 117]. As a result, the activity of glycolysis decreased and apoptosis started with increased ROS activity.

1.5.4. Other Methods for the Elimination of LSCs

Membrane markers, transcriptional factors, ROS activity, telomerase, and microRNAs have been targeted to eliminate LCSs in AML. In addition, transcription factors are therapeutic targets for the elimination of histone deacetylases (HDACs), NF- κ B, Hif-1 α , B-catenin [197]. Apart from these, LSCs relied on telomerase to continue to self-renew and

proliferate. Targeting telomerase as a therapeutic strategy for eradicating LSCs in AML may be ideal [198]. Furthermore, microRNAs such as miR-34a, miR-126, played important roles in the regulation of LSCs and could be used to create new anti-LSC therapies [31,199]. To achieve all these target treatments, different agents are used in chemotherapy in the clinic and stem cell transplantation is applied as the most effective therapeutic method after chemotherapy regimens are completed in the treatment of leukemia. [116,117,184].



2. AIM OF THE STUDY

LSCs in AML/ALL have important roles in the progression of leukemia and are considered the root of chemotherapeutic drug resistance and disease relapse. The relationship of MEIS1 with cancer was first demonstrated in a research of myeloid leukemia cells of AML patients. Increased expression of MEIS1 in BM was detected in patients with ALL and AML. Overexpression of MEIS1 accelerates the onset of disease in murine AML models. MEIS1 plays a role in the leukemic transformation with Hox proteins. Together with MEIS1, HoxA9 accelerates leukemogenesis. No studies are showing the effect of small molecule MEIS1i on MEIS1 protein, including recent studies. In addition, characterization of MEIS1 and related gene expression in LSCs is required. This research, it is aimed to investigate the MEIS1 protein known to be effective in leukemia development and relapsed in leukemia stem cells and to target MEIS1 activity with newly developed drug-based MEIS1i. For the first time, 1) it is aimed to investigate the effects on LSC number and viability 2) the effect of MEIS1i on LSCs. The findings in these studies will be the basis for future animal models and preclinical studies. Successful completion of these goals will enable the development of MEIS1i that are effective in leukemia; thus, it will contribute to the development of alternative approaches for leukemia treatments.

3. MATERIALS

3.1. INSTRUMENTS

Cytoflex S Flow Cytometry (Beckman, Cat. No: B47903, USA), S3E Cell Sorter (Life Science Research -Bio-Rad, Cat.No.1451008), FACS AriaIII (BD, Cat. No. 23-11539-00), Hettich® ROTINA 38/38R Centrifuge (Sigma, Cat. No: Z720119), Centrifuge (Labogene, Cat. No: 416), Mini centrifuge (Sigma, Cat. No: Sigma 1-14), BD Magnetic Bead System (EasySep™ Human CD34 Positive Selection Kit II, Cat.No.17856), PCR Thermal Cycler (BioRad, Cat. No: T100), LightCycler96™ Real-Time PCR System (Roche, Cat. No:12953), Nano-Drop 2000 (ThermoScientific, Cat.No: ND-2000), Carl Zeiss Inverted Microscopy (Zeiss, Cat. No:3849000464), Laminar Flow Cabinet, Incubator (ThermoScientific, Cat. No:3141), Shaking Water Bath (Witeg, Cat. No: DH.WSB03018), Vortex (Interlab, Cat. No:622.02.001), Mini Spin Centrifuge (Interlab, Cat. No:603.01.001), Autoclave, -200/+40 Refrigerators.

3.2. BONE MARROW AND BLOOD SAMPLES

BM and PB samples were taken from adult ALL and AML patients at initial diagnosis or at relapsed who applied to the Adult Bone Marrow Transplantation Department at Anadolu Medical Center.

A total of 65 patient samples were used in the research. These samples were selected from patient groups with diagnoses of AML, ALL, relapse/refractory AML/ALL according to the WHO classification. Seven relapse/refractory patients selected from these patient groups were used to demonstrate the relationship between chemotherapy and MEIS1. In addition, 59 healthy BM samples and 3 PB samples were used. The clinical information of the patients are given in the table (Table 3.1). All human studies were approved by the Institutional Clinical Studies Ethical Committee of Yeditepe University (Decision numbers 944).

Table 3.1. Characteristics of AML/ALL patients. MS:Magnetic Sort, FCS: Flow Cytometry Sort, ND : Not Done , BM: Bone Marrow, PB: Peripheral Blood

# Patients	Sample	Blast Percent	Diagnosis	Genetic Mutation	Sorting
1	BM	89	AML -M4	ITD and Asp835Tyr were not detected in the FLT3 gene INV 16 positive	CD34+ MS
1	PB	ND	AML -M4	ITD and Asp835Tyr were not detected in the FLT3 gene INV 16 positive	CD34+ MS
2	PB	ND	AML - Monositik	ITD and Asp835Tyr were not detected in the FLT3 gene	CD34+ MS
2	BM	34	AML - Monositik	ND	CD34+ MS
3	BM	63	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene	CD34+ MS
4	BM	83	Relapse T-ALL	ND	CD123 + MS
5	BM	87	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene t (8;21) positive	CD34+ MS
6	PB	47	B-ALL	BCR/ABL t (9; 22) was negative (PB)	ND
7	PB	32	B-ALL	BCR/ABL t (9; 22) was negative (BM)	ND
8	PB	23	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene (BM)	ND
9	PB		B-ALL	BCR/ABL t (9; 22) was negative	ND
10	PB	18	Relapse-B ALL	BCR/ABL p190 t (9; 22) was positive (20percent)	ND
11	PB	28(BM)	Relapse-AML -M2	ITD and Asp835Tyr were not detected in the FLT3 gene (BM) AML 1 / ETO t (8; 21) was negative (BM) Inv (16) and t (16; 16) was negative (BM) 4bp insertion in the exon 12 region of the NPM1 gene (BM)	ND
12	PB	NA	Relapse-ALL	BCR/ABL p210 t (9; 22) was positive (BM)	ND
13	BM	68	Relapse-AML	c2522A> T, (p N8411) was positive in FLT3 gene (BM) ITD and Asp835Tyr were not detected in the FLT3 gene (BM) No 956-959 mutations were not observed in exon 12 region of the NPM1 gene PML / RARA fusion t (15; 17) was positive AML1 / ETO t (8; 21) was negative The fusion of inv (16) and t (16; 16) was negative	CD34+ MS
13	PB	NA	Relapse -AML	ND	ND
14	PB	10	Relapse -AML Patient # 8 same	ITD and Asp835Tyr were not detected in the FLT3 gene (BM)	ND

15	PB	NA	Relapse-AML -M4	ITD and Asp835Tyr were not detected in the FLT3 gene (BM) Inv (16) is positive NPM1 gene exon 11 region was negative (BM)	ND
16	BM	58	Relapse-AML	ITD was detected in the FLT3 gene	ND
17	BM	56	Relapse - AML	ITD and Asp835Tyr were not detected in the FLT3 gene (BM)	ND
18	BM	65	Relapse B-ALL		ND
20	BM	29	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene (PB)	ND
21	BM	28	Relapse AML	ITD and Asp835Tyr were not detected in the FLT3 gene	ND
22	BM	43	Relapse B ALL Patient # 6-18 same	ND	ND
23	BM	29	Relapse AML Patient # 20 same	ND	ND
24	PB	15	Relapse -ALL		ND
25	BM	70	Relapse -ALL	The IGH / FGFR3 t (4; 14) fusion was negative The BCR/ABL fusion was t (9; 22) negative	
26	BM	49	AML -M5	ITD and Asp835Tyr were not detected in the FLT3 gene t (8; 21)-Inv 16 negative 4bp insertion in the exon 11 region of the NPM1 gene.	
27	BM	72	Comman ALL	BCR/ABL fusion was t (9; 22) negative (PB) IGH / MYC t (8; 14) fusion was negative (PB)	CD34+ FCS S
28	BM	84	Comman B-ALL	The BCR/ABL fusion was t (9; 22) negative	ND
29	BM	60	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene	ND
30	BM	87	T-ALL	ND	
31	BM	46	Relapse AML	ITD and Asp835Tyr were not detected in the FLT3 gene	CD123+ FCS S
32	BM	51	Comman B-ALL	ND	CD34+ FCS S
33	BM	21	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene 4bp insertion was observed in exon 11 region of the NPM1 gene	CD34+ FCS S
34	PB	67	AML	ITD and Asp835Tyr were not detected in the FLT3 gene	CD34+ FCS S
35	BM	36	AML	42 percent trisomy 8 in the AML1 / ETO (8q22) region ITD and Asp835Tyr were not detected in the FLT3 gene	
36	BM	35	Relapse - AML	c2503G> T, (pD835Y) in the FLT3 gene (rs121913488)	
37	BM	52	Relapse -AML	ITD mutation was detected in the FLT3 gene	
38	BM	62	Relapse-AML	ND	CD123+ FCS S

39	BM	83	Relapse -B-ALL	ND	CD34+ FCS S
40	BM	57	Relapse-AML	ITD and Asp835Tyr were not detected in the FLT3 gene PML/RARA (t(15;17)) fusion was negative	CD33+ FCS S
41	BM	55	Relapse-AML Patient # 38 same	ND	CD123+ FCS S
42	BM	32	Relapse-AML	ND	CD34+ FCS S
43	BM	65	Relapse-AML	ND	CD34+ FCS S
44	BM	94	Relapse-AML Patient #38,41 same	ND	CD34+ FCS S
45	BM	97	Relapse -B-ALL	BCR/ABL p190 gene was positive	CD34+ FCS S
46	BM	53	AML -M1	AML1 / ETO (t (8; 21)) fusion was negative	CD34+ FCS S
47	BM	72	AML	NPM1 gene exon 12 region was negative AML1 / ETO (t (8; 21)) fusion was negative	CD34+ FCS S
48	BM	62	Relapse-AML Patient # 16, 43 same	ND	ND
49	BM	14	Relapse-AML	ND	ND
50	BM	28	Relapse- AML	ITD and D835 were not detected in the FLT3 gene	ND
51	BM	13	Relapse -AML	ITD and Asp835Tyr were not detected in the FLT3 gene	ND
52	BM	51	Relapse B-ALL	The BCR/ABL p190 fusion was positive (BM) The BCR/ABL1 (t (9; 22) fusion was negative (PB)	ND
53	BM	53	AML -M5	MHY11 / CBFEB fusion was positive AML1 / ETO (t (8; 21)) fusion was negative NPM1 gene exon 12 region was negative	ND
54	BM	58	AML	c1775T> G, (pV592G) positive in FLT3 gene 4bp insertion was observed in exon 12 region of the NPM1 gene AML1 / ETO (t (8; 21) fusion was negative	ND
55	BM	68	AML	ND	ND
56	PB	25	Relapse -AML	5q and 7q deletion ITD and Asp835Tyr were not detected in the FLT3 gene	ND
57	BM	58	Refractory -AML Patient # 56 same	ND	ND
58	BM	80	B-ALL	The BCR/ABL1 (t (9; 22) fusion was negative	ND
59	BM	46	Relapse AML Patient # 26 same	ITD and Asp835Tyr were not detected in the FLT3 gene	ND
60	PB	84	AML	ND	ND
61	PB	44(BM)	Refractory -AML Patient # 60 same	ND	ND
62	BM	64	Refractory -B-ALL Patient # 58 same	ND	ND

63	BM	80	Relapse AML Patient # 50 same	ITD and Asp835Tyr were not detected in the FLT3 gene	ND
64	BM	50	Refractory -AML	ITD was detected in the FLT3 gene	ND
65	BM	24	Refractory -AML Patient # 64 same	ITD was detected in the FLT3 gene	ND

3.3. SMALL MOLECULES

The newly identified small molecule MEIS1i; MEIS1i-1 (MW: 388.467) (Cas # 446306-43-0) and MEIS1i-2 (MW:306.32) (Cas # 2250156-71-7) were used. These inhibitors are provided by Meinox Technologies (www.meinoxtech.com, Cat # MEIS1i-1-25mg and Cat # MEIS1i-2-25mg). Lyophilized MEIS1i-1 and MEIS1i-2 small molecules were prepared by dissolving in sterile dimethyl sulfoxide (DMSO, Calbiochem, 317275). DMSO (0.25 percent) was used as a positive control (DMSO, Santa Cruz Biotech, Cat. No: Sc-3590329).

3.4. CELL CULTURE MEDIA AND SUPPLEMENTS

Cell culture media and their supplements are provided by Gibco. The list of cell culture components with an ordered number was listed in Table 3.2.

Table 3.2. Cell culture media and supplements used in the research include catalog numbers/vendors

Materials	Vendor/Catalog Number
Roswell Park Memorial Institute Medium (RPMI) 1640 Medium	Gibco 12001-534
Fetal Bovine Serum, qualified, heat-inactivated, Canada origin	Gibco 12483020
Antibiotic-Antimycotic (100X)	Gibco 15240062

3.5. SEPARATION MNCS FROM BONE MARROW- PERIPHERAL BLOOD

BM-PB Mononuclear cells (MNCs) were isolated by using ficoll gradient centrifuge technique. Table 3.3 shows the required chemicals used for isolation with vendor and catalog numbers.

Table 3.3. Vendor /catalog number information of specific materials and chemicals used for MNCs' separation

Specific Chemicals	Vendor/Catalog Number	Aim of Utilization
Ficoll-Paque Histopaque™	Sigma 10831	Isolation of MNCs
Plastic spray-coated K2EDTA Tube	BD 367835	Collection of BM-PB
Dulbecco's Phosphate Buffer Saline (DPBS)	Thermofisher Scientific 14190144	General use

3.6. LSC SEPARATION

MNCs were isolated from the patient BM/PB samples. Two different kits (Table 3.4) were used to separate LSC from MNCs.

Table 3.4. Catalog numbers/vendor of LSCs isolation materials

Specific Chemicals	Vendor/Catalog Number	Aim of Utilization
EasySep Human Cord Blood CD34+ Selection Kit II	Stem Cell Technologies 17896	LSCs Separation
EasySep Human FITC Pozitive Selection Kit	Stem Cell Technologies 17662	LSCs Separation

3.7. DETERMINATION OF LSCS MARKER

LSCs surface markers were determined in MNC by flow cytometry. Table 3.5 shows LSCs surface markers used for the study with their vendor and catalog numbers.

Table 3.5. Surface markers for LSCs with their conjugated fluorochromes from Biolegend and eBioscience

Antigens	Conjugated Florochromes	Catalog Number
Anti-human CD34	PE	Biolegend 343505
Anti-human CD33	FITC	Biolegend 303304
Anti-human CD123	FITC	Biolegend 306013
Anti-human CD133	APC	eBioscience 12-0349-42

3.8. ANNEXIN-V APOPTOSIS DETECTION ASSAY

The kit given in Table 3.6 was used to determine apoptosis assay with their vendor and catalog numbers.

Table 3.6. Apoptosis test components with vendor/catalog numbers

Specific Kits and Chemicals	Vendor/Catalog Number	Catalog Number
eBioscience™ Annexin V-FITC Apoptosis Detection Kit	ThermoFisher Scientific BMS500FI-20	Apoptosis Assay

3.9. VIABILITY ASSAY

Flow cytometry was used to measure the vitality of expanded cells after they were labeled with 7AAD (7-Amino-Actinomycin D) (BD, Cat.No:559925).

3.10. GENE EXPRESSION ANALYSIS

The changes of the gene expression levels were analyzed by Real-Time Polymerase Chain Reaction (RT-PCR). Gene expression analysis was performed post cDNA synthesis following RNA isolation from expanded MNCs. Table 3.7 shows specific kits for RNA isolation, cDNA synthesis, and quantitative PCR analysis.

Table 3.7. The following is a list of specialized RNA isolation, cDNA synthesis, and RT-PCR kits, along with their vendor/catalog numbers.

Specific Chemicals, Kits or other components	Brand/Catalog Number	Aim of Utilization
Invitrogen PureLink RNA Mini Kit	ThermoFisher Scientific 12183025	RNA Isolation
RevertAid First Strand cDNA Synthesis Kit	ThermoFisher Scientific K1622	cDNA synthesis
SYBR™ Green PCR Master Mix	Applied Biosystem 4309155	Real-Time PCR
Trizol	Sigma T9424	RNA Isolation

For RT-PCR, predesigned human primers obtained from diverse databases (Gene Cards etc.) (Table 3.8).

Table 3.8. LSCs related genes and sequences of human primers (5 prime to 3 prime)
ordered from SENTEGEN, TURKEY

Genes	Purpose	Primer Sequences
GAPDH	Housekeeping Genes	F 5'-AATGAAGGGGTCATTGATGG-3'
		R 5'-AAGGTGAAGGTCGGAGTCAA-3'
β -ACTIN		F 5'-AGGCACCAGGGCGTGAT-3'
		R 5'-GCCCACATAGGAATCCTTCTGAC-3'
MEIS1	LSCs Related Genes	F 5'-GGACAACAGCAGTGAGCAAG-3'
		R 5'-CACGCTTTTTGTGACGCTT-3'
MEIS2		F 5'-GAAAAGGTCCACGAACTGTGC-3'
		R 5'-CTTTCATCAATGACGAGGTCGAT-3'
MEIS3		F 5'-ATGGCCCGGAGGTATGATGA-3'
		R 5'-GAAGAGCGGGTGTCCATAGA-3'
HIF1 α		F 5'-TTCCCGACTAGGCCCATTC-3'
		R 5'-CAGGTATTCAAGGTCCCATTTC-3'
PBX1		F 5'-CATGCTGTTAGCGGAAGGC-3'
		R 5'-CTCCACTGAGTTGTCTGAACC-3'
HOXA9		F 5'-TACGTGGACTCGTTCCTGCT-3'
		R 5'-CGTCGCCTTGGACTGGAAG-3'
CD34		F 5'-AATGAGGCCACAACAAACATCACA-3'
		R 5'-CTGTCCTTCTTAAACTCCGCACAGC-3'
mDNMT3A	Leukemia Related Genes	F 5'- GCTTTCTTCTCAGCCTCCCT -3'
		R 5'- CCATGCCAAGACTCACCTTC -3'
BCL-2		F 5'- GGTGGGGTCATGTGTGTGG -3'
		R 5'- CGGTTCAAGTACTCAGTCATCC -3'
FLT-3		F 5'- AGGGACAGTGTACGAAGCTG -3'
		R 5'- GCTGTGCTTAAAGACCCAGAG -3'

4. METHODS

4.1. SEPARATION AND ANALYSIS OF LEUKEMIA STEM CELLS

LSC and mononuclear cells (MNC) were be isolated by density gradient centrifugation as described previously in Ficoll-Paque. Briefly, 5 ml patient blood (up to 15 ml) was diluted in 1: 1 ratio with Dulbecco's Phosphate-Buffered Saline (DPBS) in a 50 ml falcon tube and mixed by gently inverting. The patient blood samples were covered with 5 ml Ficoll-Paque and centrifuged at 3000 g for 30 minutes without brake. After centrifugation, the supernatant was removed and the cloudy interphase was transferred to the new 50 ml falcon tube. MNCs were washed with 3x volume DPBS and mixed gently by inverting. The cells were centrifuged by braking at 1500 rpm for 5 minutes and then the supernatant was removed. The cell pellet was suspended in 10 ml of DPBS and the cell count was counted with a hemacytometer. MNCs were seeded in a 96-well plate in RPMI medium with 50,000 cells per well for flow cytometry analysis. Leukemia blood MNCs were labeled with; CD34PE, CD123FITC, CD133 APC, CD33FITC. Magnetic Selection (MS) and Flow Cytometry Selection (FCS) are the most accepted methods used for cell sorting. To separate LSCs of primary leukemia BM, we used S3e™ Cell Sorter and FACS Aria III. Alternatively, the BD Magnetic Bead System was used.

4.2. LSCS SEPARATION WITH FLOW CYTOMETRY SELECTION (FCS) METHOD

FCS is used in high-purity applications, but is challenged by sluggish sorting speeds and decreased cell viability. In this research, S3e™ Cell Sorter and FACS Aria III were used after marking with blast surface marker determined according to clinical diagnosis to distinguish LSCs from primary leukemia PB-BM (Tablo 3.1).

4.3. LSCS SEPARATION WITH MAGNETIC SELECTION (MS) METHOD

MS is highly enriched, simpler, conveniently available, cost-effective, and does not need specialized facilities. BD Magnetic Bead System was used for the separation of LCSs following the manufacturer's protocol in our research.

4.4. PURITY, VIABILITY, AND CELL PHENOTYPING USING FLOW

Before and after both isolation methods, cells were analyzed on the flow cytometer to determine the percentage of cells. Cells were labeled with CD34, CD123, CD133, CD33 for flow cytometry analysis (Cytoflex S Flow Cytometry (Beckman, Cat. No: B47903, USA).

4.5. ANALYSIS OF EXPRESSIONS OF THE MEIS GENE FAMILY AND RELATED GENES IN LCSS AND OTHER MNCS

The MNC population was collected for gene expression analysis. Analysis of the expression of the MEIS gene family and related genes were performed.

RNA isolation

MNCs from AML patient blood samples to be used in the study were collected by the Ficoll-Paque method. RNA isolation kit was used for RNA isolation. Samples were homogenized by syringe method in a 1 mL solution of guanid thiocyanate (trizol). Ethanol was added according to protocol and binding to RNA columns was achieved by centrifugation. After three washes RNA products with RNA elution solution bound to columns were obtained. For cDNA synthesis and quality measurement, an amount of RNA was separated and the rest was stored at - 80°C until use. The RNA concentration was determined in micro volumes with the nanodrop device.

cDNA Synthesis

1 µg of RNA from each tissue was taken with cDNA synthesis kit and converted to cDNA with random primers. Briefly, RNA was incubated with random hexamers for 5 minutes at

65°C. Random mixture of hexamer and RNA was added to the mixture of cDNA enzymes and first incubated at;

25°C for 5 min

42°C for 60 min

85°C for 5 min,+4°C... endless and placed on ice or stored at -20°C.

Real-Time PCR

Pre-designed primers (Table 3.8) obtained from databases (Gene Card etc.) and ordered from Sentegen Biotechnology. Real-Time PCR Bio-Rad FX96 Touch™ Real-Time PCR detection system and the PCR parameters previously used. Expression comparisons of each putative modulator genes amplified were done by GAPDH-β-Actin normalization.

4.6. ANALYSIS OF THE EFFECT OF MEIS INHIBITORS ON LSCS

Following separation, LSCs were seeded at a density of 50,000 cells/well in a 96-well plate. LSCs and MNCs were then be treated at a concentration of 20 uM MEIS1i-1 and MEIS1i-2. The cells used as positive control were treated with DMSO (0,25 percent). The treated cells were incubated for 5 days at 37°C, in 5 percent CO₂ and under normoxic conditions. Five days of post-treatment cells were labeled with (CD34PE, CD123FITC, CD133 APC, CD33 FITC) antibodies according to the manufacturer's manual (1:1000 dilution ratio). Labeled cells were analyzed by flow cytometry.

Apoptosis Detection Assay

The isolated LSCs and MNCs were seeded at a density of 50,000 cells / well in a 96-well plate. LSCs and MNCs were treated at a concentration of 20 uM MEIS1i-1 and MEIS1i-2. The treated cells were incubated for 5 days at 37°C, in 5 percent CO₂. Three days after the procedure, the cells were collected and centrifuged at 2000 rpm for 5 min.

The pellet was resuspended in 50 µl of 1X binding buffer, then stained with 1ul of FITC Annexin V and PI (Propidium Iodide) according to the modified manufacturer's manual. The stained cells were incubated for five minutes at room temperature in the dark. Cell

apoptosis, Annexin V-FITC, and PI staining were analyzed by flow cytometry compared to DMSO control (0,25 percent).

Day-dependent Cell Viability

The isolated LSCs and MNCs were seeded at a density of 30,000 cells / well in a 96-well plate. LSCs and MNCs were then be treated at a concentration of 20 uM MEIS1i-1 and MEIS1i-2. The treated cells were incubated for 5 days at 37°C, in 5 percent CO₂. Cells marked with 7-Amino-Actinomycin (7AAD) viability dye on the 3rd and 5th days post-treatment with inhibitors were analyzed in flow cytometry and the cell was obtained.

Cell Cycle

To be able to analyze the effect of MEIS1i on LSCs, CD34⁺ cells were sorted from the MNC population by the FCS method after labeling with anti-human PE-conjugated CD34. The CD34⁺ cells were seeded at a density of 10,000 cells per well in 96 well plates. The cells were treated with MEIS1i. After 4 days of the treatment, LSCs were labeled with PI. Following the staining, the cells were analyzed by flow cytometry compared to DMSO control (0,25 percent)

4.7. ANALYSIS OF THE EFFECT OF MEIS1I ON EXPRESSION OF MEIS TARGET GENES IN LSCS

After the separation of LSCs and MNCs, cells were seeded at 1-2 million/ well. To investigate the effect of MEIS1i on MEIS 1/2 and target genes, cells were harvested 5 days after MEIS1i (20 µM dose) treatment, and RNA was isolated. Then, cDNA synthesis and real-time PCR were performed, respectively.

4.8. STATISTICAL ANALYSIS

Dunnett's Multiple Comparison Test was performed to examine the data, and p values were employed. These results were judged statistically significant if the values were $p < 0.05$, and statistically more significant if the values were $p < 0.01$. The degree of significance was determined using a "2-tailed Student t-test" in addition to the "2-tailed Student t-test."

5. RESULTS

5.1. DETERMINATION OF MEIS (+) IN WHOLE BONE MARROW MNCS OF HEALTHY INDIVIDUALS AND PATIENTS

To determine the basal level of MEIS protein in patient samples and healthy individuals, the intracellular MEIS1-2-3 protein was labeled by fluorochrome-conjugated antibody and analyzed in flow cytometry. An average of 42,90 percent MEIS (+) protein profile was observed in the BM of 8 healthy individuals included in the research. A small group, which we call *MEIS+ high* was observed apart from the MEIS (+) group with an average rate of 24,28 percent in 2 of 8 healthy individuals (Figure 5.1b). An average of 76,24 percent MEIS (+) protein profile was observed in the BM of patients (n=6 AML, n=11 AML-R, n=6 ALL-R) included in the study. A small group, *MEIS+ high* was observed apart from the MEIS (+) group with an average rate of 16,12 percent in 8 of 23 patients. MEIS (+) protein profile was observed at a rate of average 82,75 percent in AML, 68,40 percent in AML-R and 77,59 percent in ALL-R. *MEIS+ high* rate was average 4,02 percent in AML, average 42,62 percent in AML-R, and average 1,73 percent in ALL-R (Figure 5.2b).

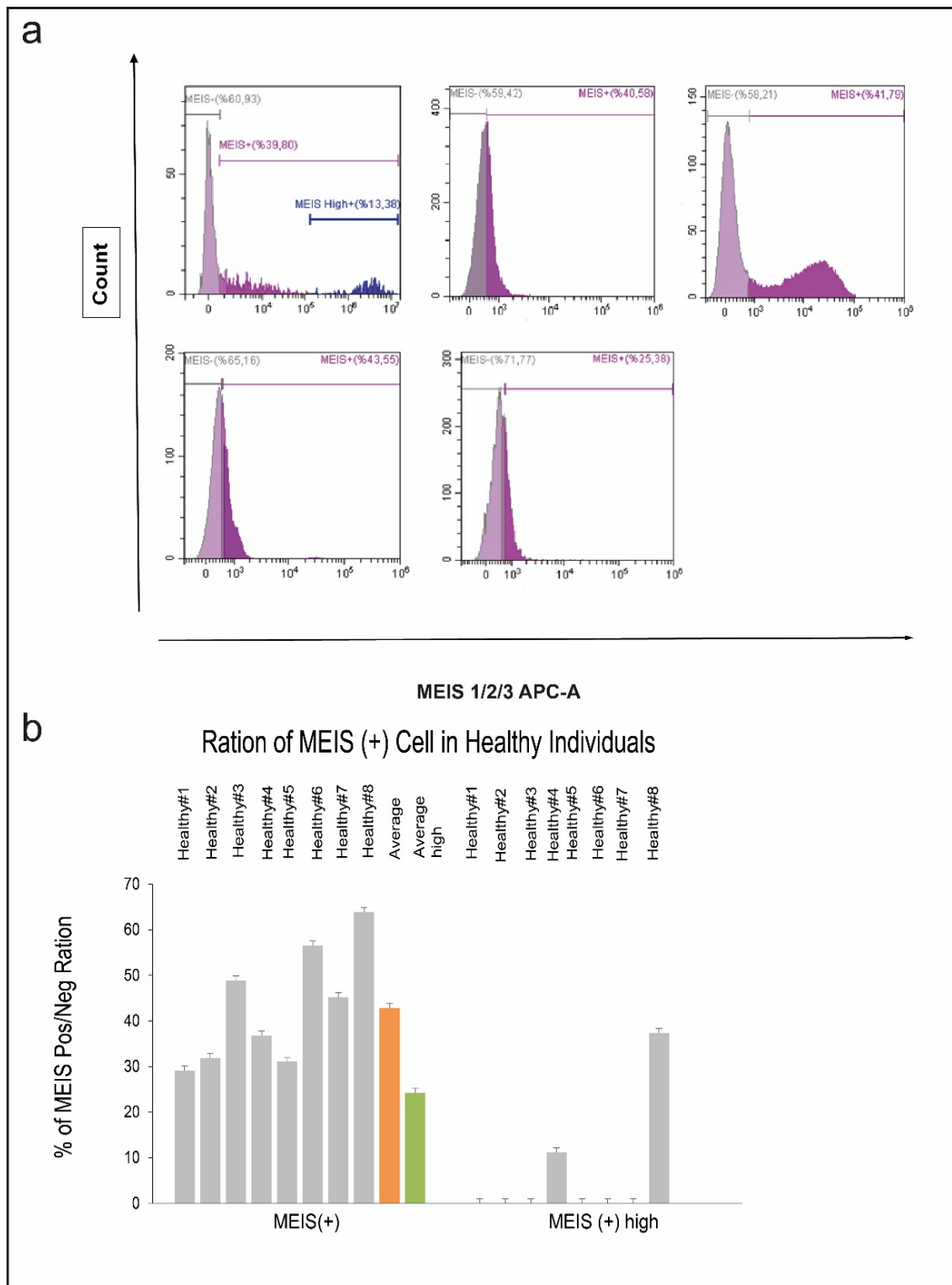


Figure 5.1. MEIS (+) protein profile determination in BM-MNCs healthy individuals. a) Flow cytometry analysis gating strategy for determining MEIS (+), b) Flow cytometry analysis quantification for determining MEIS (+).

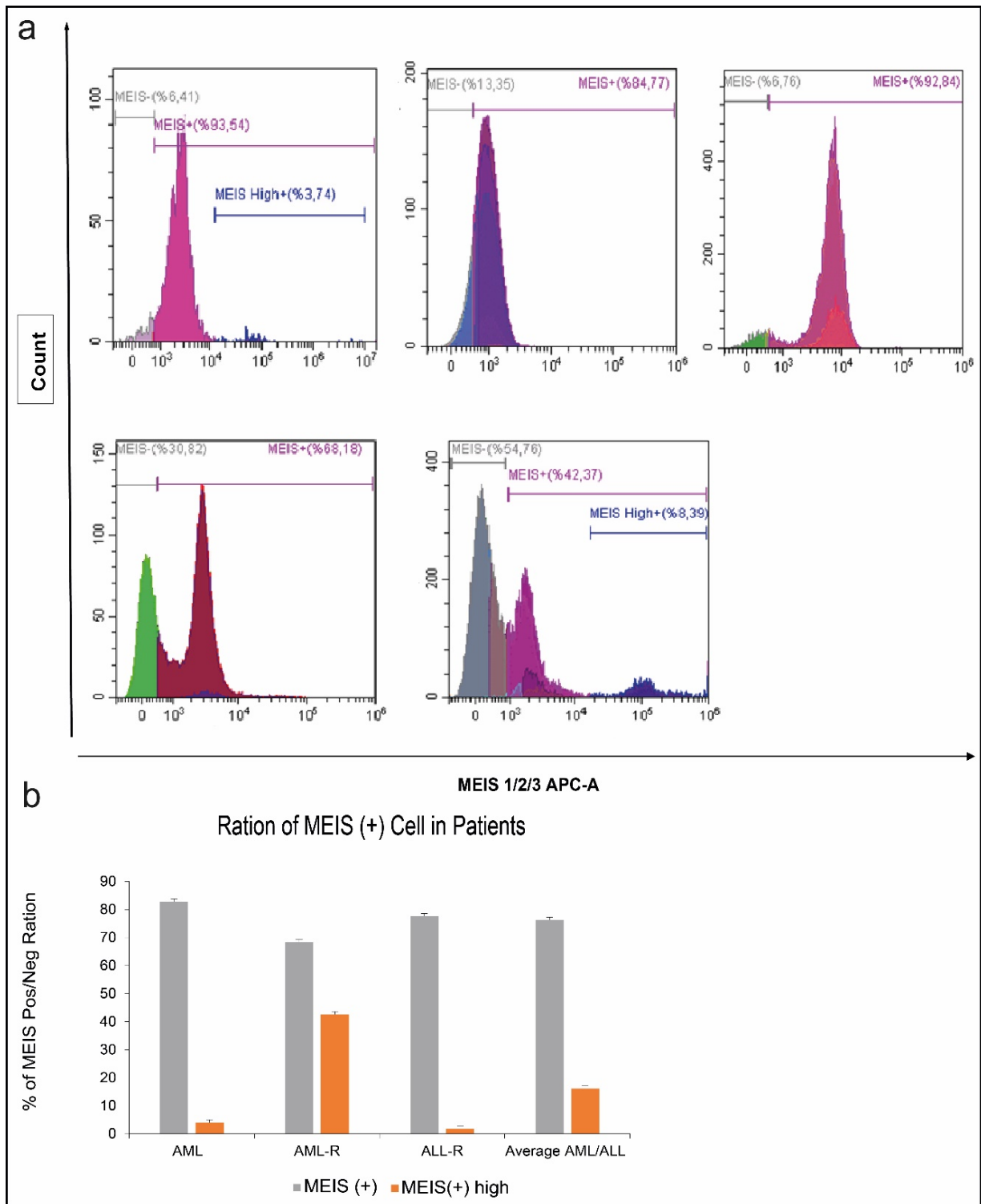


Figure 5.2. MEIS (+) protein profile determination in AML/ALL patients' BM-MNCs a) Flow cytometry analysis gating strategy for determining MEIS (+), b) Flow cytometry analysis quantification for determining MEIS (+).

5.2. COMPARISON OF MEIS (+) RATIO IN WHOLE BONE MARROW MNCs OF HEALTHY INDIVIDUALS AND PATIENTS

The MEIS (+) rate in patients was compared with the MEIS (+) rate in healthy individuals. When patients were compared with healthy individuals (n=8), It was observed that the rate of MEIS(+) in average AML, AML-R, ALL-R patients (n=23) was considerably higher than in average healthy individuals (Figure 5.3) The Fold difference table is also shown below (Table 5.1).

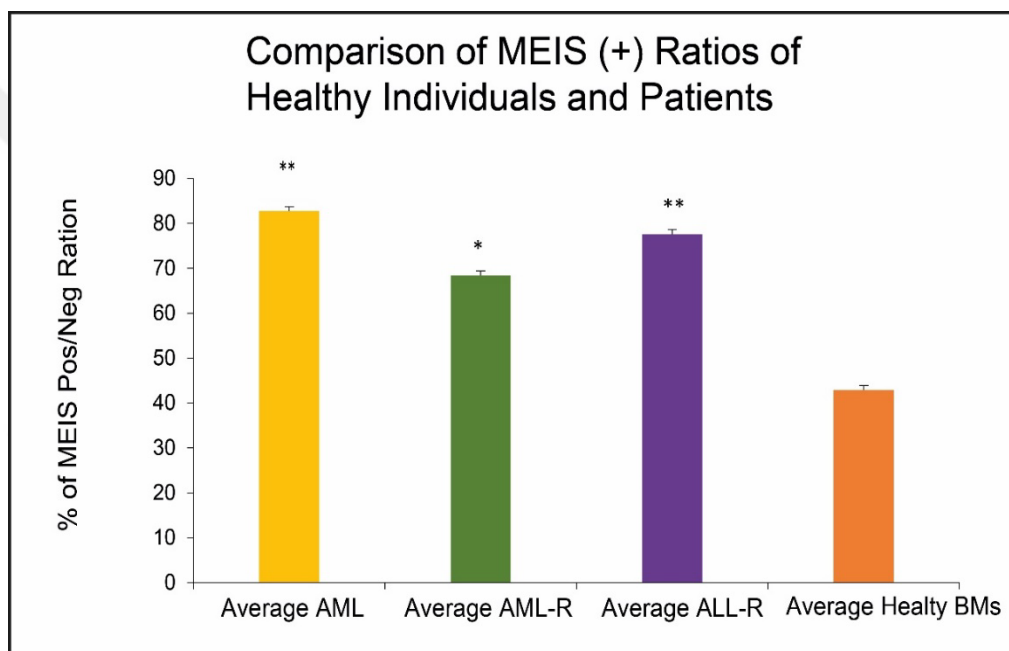


Figure 5.3. Quantification of flow cytometry analysis performed in BM-MNCs of AML/ALL patients and healthy individuals to compare MEIS (+) protein profiles (* $p < 0,05$, ** $p < 0,01$).

Table 5.1. The percentage and fold difference in average AML/ALL MNCs cell content quantification data corresponds to MEIS+

Patient Grups	MEIS+ Percent in Average Patients MNCs Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
AML	82,76 vs 42,90	≈ 2 -fold
AML-R	68,40 vs 42,90	≈ 1,5-fold
ALL-R	77,59 vs 42,90	≈ 2-fold

5.3. COMPARISON OF MEIS (+) RATIO OF HEALTHY INDIVIDUALS AND ALL / AML LSCS

To determine the MEIS (+) ratio in LSCs, MNCs were isolated by the FCS method after marking with the surface marker determined according to the clinical diagnosis from AML/ALL BM-MNCs. In terms of separation performance, MNCs and LSCs after separation were assessed by flow cytometry and it was concluded that the separation was more than 90 percent efficient and then the intracellular MEIS1-2-3 protein was labeled by fluorochrome-conjugated antibody and analyzed in flow cytometry. An average of 42,90 percent MEIS (+) protein profile was observed in the BM of healthy individuals (n=8) included in the investigation. An average of 80,55 percent MEIS (+) protein profile was observed in the BM-LSCs of patients (n=3) included in the research. It was observed that the average MEIS(+) rate of the patients was significantly higher than the average healthy individuals (Figure 5.4).

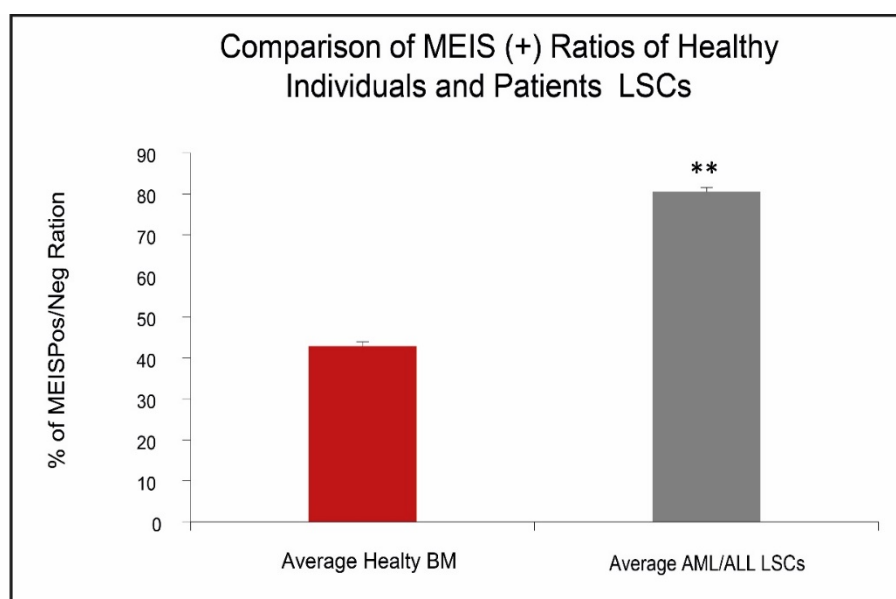


Figure 5.4. Quantification of flow cytometry analysis done in LSCs of AML/ALL patients and healthy individuals to compare MEIS (+) protein profiles (** $p < 0,01$).

5.4. DETERMINATION OF EXPRESSION OF MEIS1/2/3 AND RELATED GENES IN AML/ALL

Real-time PCR testing was performed to determine the expression of MEIS 1/2/3 and related genes in AML and ALL patients. It was shown that MEIS1 and Hif-1 α expression in BM of AML/ALL patients (n=11) were extensively higher than gene expressions in the BM of healthy individuals (n=3). Expressions of HoxA9 and Pbx1 genes were found to be significantly lower compared to average healthy individuals (Figure 5.5a). In addition, MEIS1 and CD34 expression were found to be considerably higher in PB of ALL patients (n=3) compared to PB of healthy individuals. It was found that Hif-1 α was notably higher expressed in AML(n=1), AML-R (n=3) and ALL-R (n=1) patients compared to healthy individuals (n= 3) (Figure 5.5b).

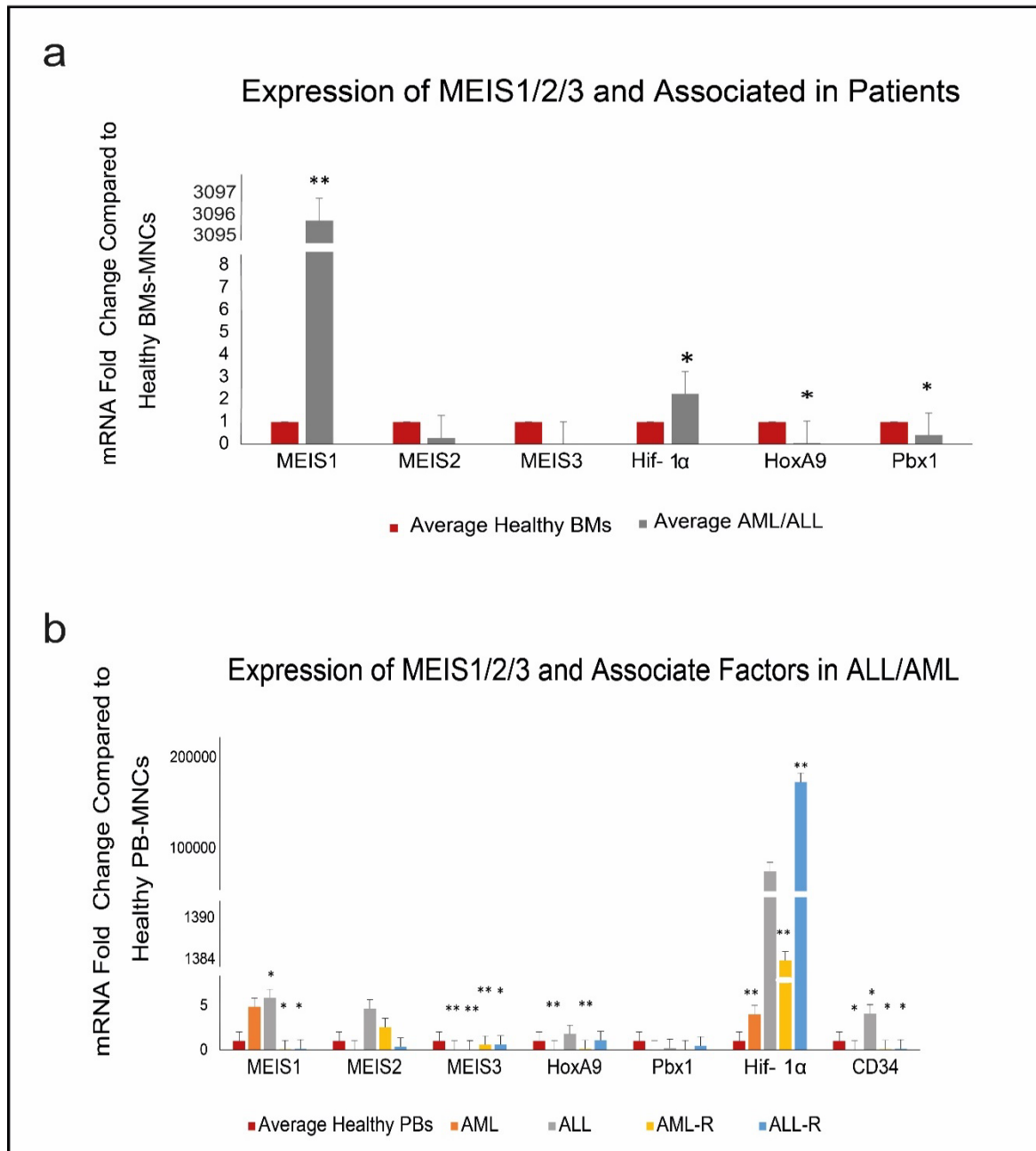


Figure 5.5. Gene expression analysis of MEIS1/2/3 and related genes in MNCs of AML/ALL patients a) Gene expression in BM-MNCs of AML/ALL patients compared to healthy individuals by Real-time PCR (n=11; *p<0,05, **p<0,01), b) Gene expression in PB-MNCs of AML/ALL patients compared to healthy individuals by Real-time PCR (n=8; *p<0,05, **p<0,01).

5.5. DETERMINATION OF EXPRESSION OF MEIS1/2 AND AML/ALL RELATED GENES

The expression of MEIS1/2 and AML/ALL associated genes in AML/ALL patients was determined using real-time PCR testing. It was shown that MEIS1, CD34, BCL-2, and FLT3 expression in BM of average AML patients (n=3) were significantly higher than gene expressions in the BM of average healthy individuals (n=3). MEIS1, MEIS2 gene expressions were higher in the ALL patient (n=1) compared to BM of average healthy individuals. In addition, it was observed that MEIS2, BCL-2, FLT3 gene expressions were higher in the AML-R patients (n=7) compared to the healthy individuals (Figure 5.6).

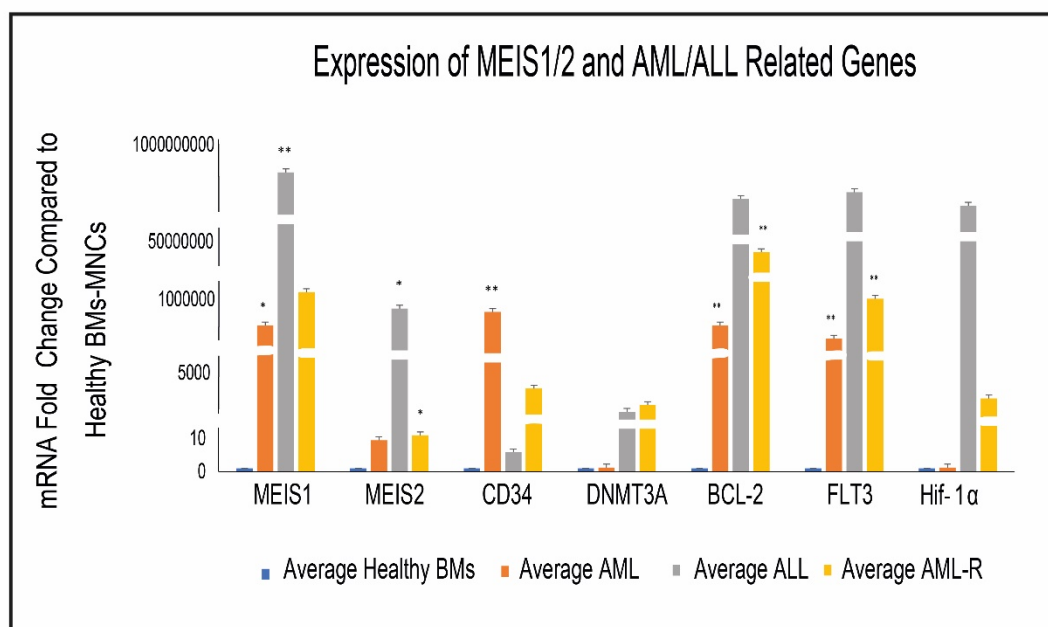


Figure 5.6. Gene expression in BM-MNCs of ALL/AML patients compared to healthy individuals by Real-time PCR (*p<0,05, **p<0,01).

5.6. DETERMINING THE RELATIONSHIP BETWEEN MEIS AND LSCS MARKERS

To understand the relationship between LSC markers (CD123, CD34) and MEIS protein, MNCs were labeled by fluorochrome-conjugated antibodies (CD123, CD34) and analyzed

in flow cytometry. Accordingly, it was shown that the rate of average MNCs with MEIS(+) ratio in cells with CD123+ (n=6) was 51,42 percent. It was found that the proportion of average MNCs with MEIS(+) ratio in cells with CD123+ was considerably higher than the group of average MNCs with CD123+ who were also MEIS(-) (*p<0,05). Average MNCs with CD123+ ratio in cells with MEIS(+) (n=16) was 6,5 percent. It was found that the proportion of average MNCs with CD123- ratio in cells with MEIS(+) was significantly higher than the group of average MNCs with MEIS(+) who were also CD123+ (**p<0,01) (Figure 5.7b). It was shown that the rate of average MNCs with MEIS(+) ratio in cells with CD34+ (n=5) at the same time was 25,54 percent. The average MNCs with MEIS(-) ratio in cells with CD34+ was 12,67 percent. It was found that the proportion of average MNCs with CD34+ ratio in cells with MEIS(+) was 8,21 percent (n=14). Average MNCs with CD34- ratio in cells with MEIS(+) was remarkably higher than the group of average MNCs with MEIS(+) who were also CD34+ (**p<0,01) (Figure 5.7d)

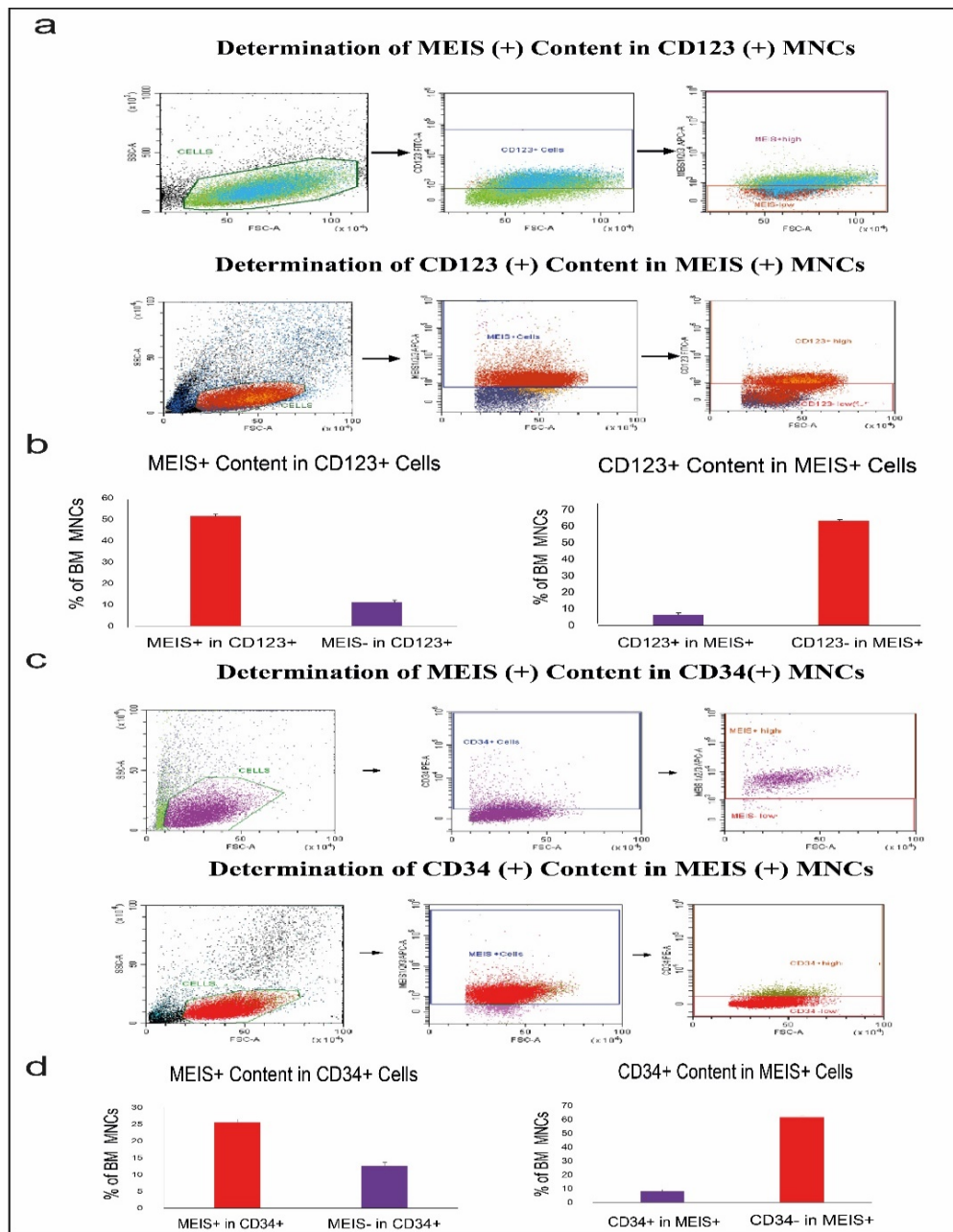


Figure 5.7. Relationship between MEIS and LSC markers (CD123, CD34) in AML/ALL

a) Flow cytometry analysis gating strategy to establish the relationship between MEIS and CD123, b) Association between MEIS and CD123, c) Gating strategy establish the relationship between MEIS and CD34, d) Quantification of flow cytometry analysis which performed for determining the relationship between MEIS and CD34 (* $p < 0.05$, ** $p < 0.01$).

5.7. DETERMINATION OF LSC MARKERS IN AML / ALL

We determined the LSC markers in AML/ALL patients by flow cytometry. After MNC cells were isolated from the BM of the patients, they were stained with LSC markers (CD34+, CD33+, CD123+) and analyzed by flow cytometry. It was observed that the CD123+ ratio in MNCs of AML(n=5), ALL(n=4), AML-R (n=27) and ALL-R(n=9) patients was significantly higher when compared to MNCs of average healthy individuals (n=13) (*p<0,05, **p<0,01). CD34+ ratio in MNCs of AML (n=6), ALL(n=3), AML-R (n=26) and ALL-R(n=8) patients was considerably higher when compared to MNCs of healthy individuals (n= 35) (**p<0,01). CD33+ ratio in MNCs of AML (n=7), ALL(n=4), AML-R (n=24) and ALL-R(n=9) patients was notably higher when compared to MNCs of healthy individuals (n= 35) (**p<0,01) (Figure 5.8). The Fold difference table is also shown below (Table 5.2,5.3,5.4).

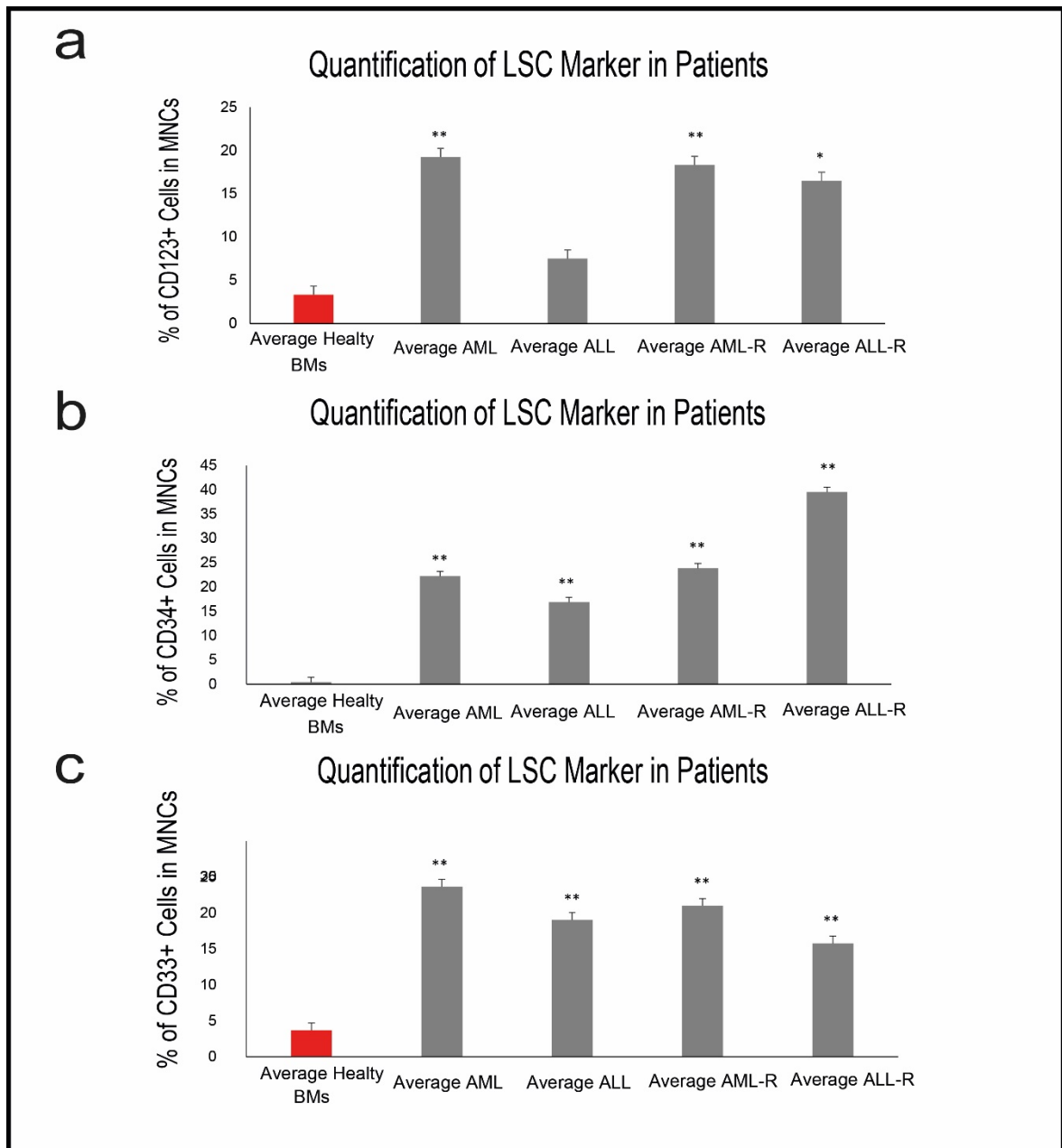


Figure 5.8. Quantification of flow cytometry analysis which performed to determine LSC markers a) CD123+, b) CD34+, c) CD33+ in AML/ALL (* $p < 0,05$, ** $p < 0,01$).

Table 5.2. The percentage and fold difference in the average AML/ALL MNCs cell content quantification results correspond to CD123+

Patient Groups	CD123+ Percent in Average Patients MNCs Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
AML	19,24 vs 3,33	≈ 6 -fold
ALL	7,52 vs 3,33	≈ 2 -fold
AML-R	18,32 vs 3,33	≈ 5 -fold
ALL-R	16,49 vs 3,33	≈ 5 -fold

Table 5.3. The percentage and fold difference that corresponds to CD34+ in the average AML/ALL MNCs cell content quantification results

Patient Groups	CD34+ Percent in Average Patients MNCs Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
AML	22,21 vs 0,45	≈ 50-fold
ALL	16,87 vs 0,45	≈ 40 -fold
AML-R	23,83 vs 0,45	≈ 53 -fold
ALL-R	39,55 vs 0,45	≈ 88 -fold

Table 5.4. The percentage and fold difference that corresponds to CD33+ in the average AML/ALL MNCs cell content quantification results

Patient Groups	CD33+ Percent in Average Patients MNCs Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
AML	23,66 vs 3,70	≈ 6-fold
ALL	19,05 vs 3,70	≈ 5-fold
AML-R	20,99 vs 3,70	≈ 6-fold
ALL-R	15,77 vs 3,70	≈ 4-fold

5.8. DETERMINATION OF LSCS SEPARATION WITH MAGNETIC SELECTION (MS) METHOD

The MNCs were isolated by magnetic isolation technique (EasySep™ Human CD34 Positive Selection Kit II) after marking with the surface marker determined according to the clinical diagnosis from BM-MNCs of AML/ALL patients. In terms of separation performance, MNCs and LSCs after separation were assessed by flow cytometry and it was concluded that the separation was more than 90 percent efficient. LSC markers (CD34+, CD33+, CD123+, CD133+) staining was performed before and after separation to determine whether separation was successful. According to this, While the CD34+ rate was 32,80 percent in AML/ALL patients (n= 7) with CD34+ separation before separation, this rate was 89,87 percent in LSCs after separation. No significant change was observed in the marker analyzes of other LSCs after separation (Figure 5.9b). The Fold difference table is also shown below (Table 5.5).

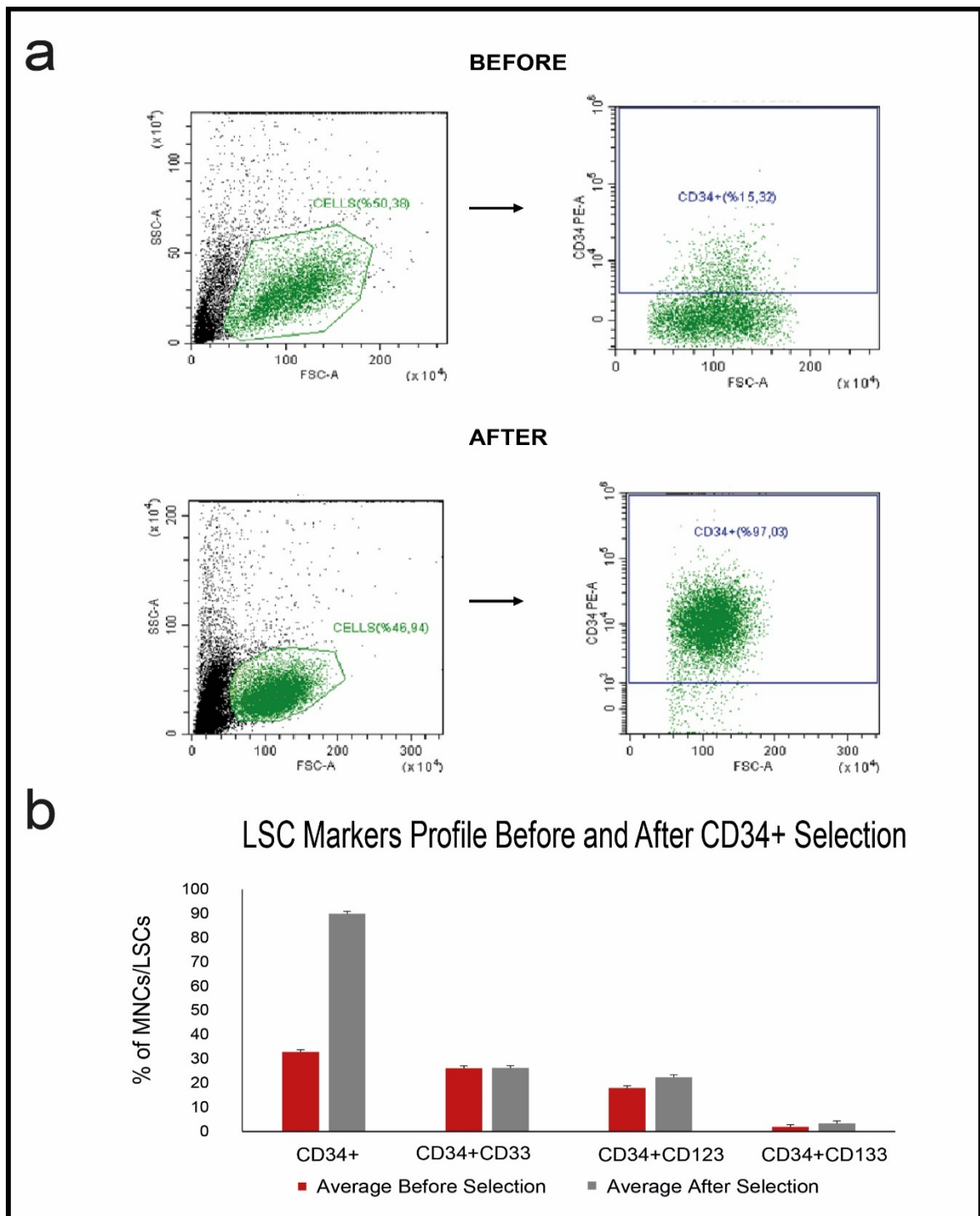


Figure 5.9. LSCs separation using the Magnetic Selection (MS) method a) Flow cytometry analysis gating strategy to determine LSC markers profile before and after CD34+ separation, b) Flow cytometry analysis quantification to determine LSC markers profile before and after CD34+ separation.

Table 5.5. The percentage and fold difference in average AML/ALL MNCs cell content quantification data that relate to LSCs markers

LSCs Markers	LSCs Markers Percent in Average Patients MNCs Compared to Before Selection	Fold Difference Compared to Before Selection
CD34+	89,87 vs 32,80	≈ 3 -fold
CD34+CD33+	26,26 vs 26,12	≈ 1-fold
CD34+CD123+	22,37 vs 17,91	≈ 1,2-fold
CD34+CD133+	3,35 vs 1,88	≈ 1,7-fold

5.9. LSCS SEPARATION WITH FLOW CYTOMETRY SELECTION (FCS) METHOD

The MNCs were isolated by Flow Cytometry Selection (FCS) after marking with the surface marker determined according to the clinical diagnosis from BM-MNCs of AML/ALL patients (n= 8). In terms of separation performance, MNCs and LSCs after separation were assessed by flow cytometry and it was concluded that the separation was more than 90 percent efficient. LSC markers (CD34+, CD33+, CD123+, CD133+) staining was performed before and after separation to determine whether separation was successful. Among the patients who were separated, only one patient had CD123+ separation and the other patients had CD34+ separation. While LSC markers were CD34+ (31,13 percent), CD123+ (17,03 percent), CD33+ (11,73 percent), CD133+ (24,36 percent) before separation, it was observed to be CD34+ (85,61 percent), CD123+ (52,73 percent), CD33+ (47,61 percent), CD133+ (73,43 percent) after separation. It was found that the ratio of other LSCs markers increased compared to before separation. This proves the reliability of the FCS selection method (Figure 5.10b). The Fold difference table is also shown below (Table 5.6).

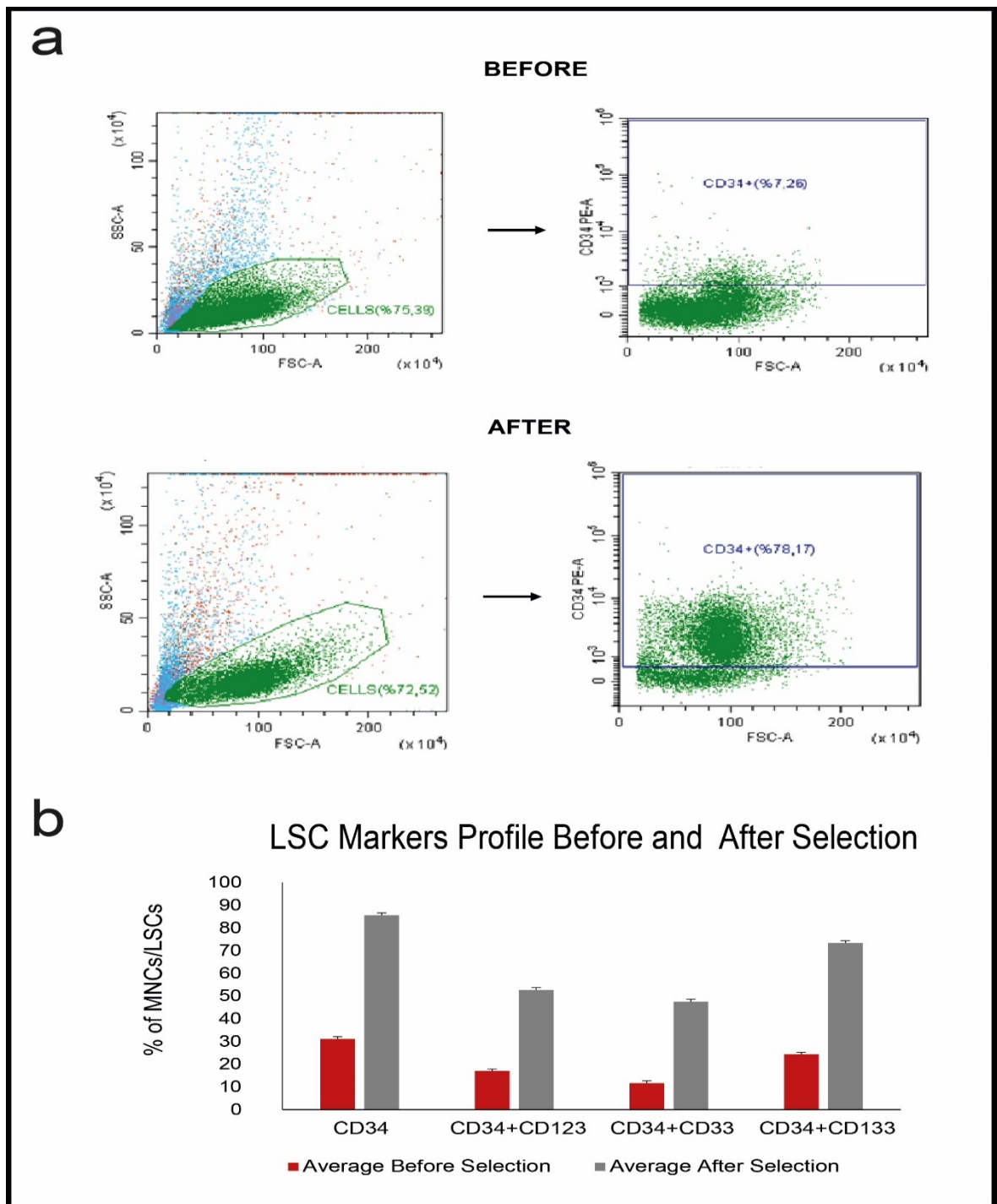


Figure 5.10. LSC separation using the Flow Cytometry Selection (FCS) method a) Flow cytometry analysis gating strategy to establish LSC markers profile before and after separation, b) Flow cytometry analysis quantification to establish LSC markers profile before and after separation.

Table 5.6. The percentage and fold difference in average AML/ALL MNCs cell content quantification data that relate to LSCs markers

LSCs Markers	LSCs Markers Percent in Average Patients MNCs Compared to Before Selection	Fold Difference Compared to Before Selection
CD34+	85,61 vs 31,13	≈ 3-fold
CD34+CD33+	47,61 vs 17,03	≈ 4-fold
CD34+CD123+	52,73 vs 17,91	≈ 3-fold
CD34+CD133+	10,71 vs 7,31	≈ 3-fold

5.10. DETERMINATION OF CELL VIABILITY POST MEIS1I TREATMENT IN PATIENTS WITH HIGH-LOW MEIS PROTEIN PROFILE

MNCs of AML/ALL patients with high and low MEIS protein profile were stained with 7 AAD stains to evaluate cell viability 3 and 5 days post MEIS1i treatment. In patients with a high MEIS protein profile (n=6), MEIS1i-1 was observed to reduce cell viability by approximately -1,4-fold compared with DMSO control at 3 and 5 days after treatment with MEIS1i-1 (Figure 5.11a). In patients with a low MEIS protein profile (n=4), MEIS1i-2 was found to reduce cell viability by approximately -1,6-fold 3 days post-treatment and -1,2-fold 5 days post-treatment compared with DMSO control (Figure 5.11b). The Fold difference table is also shown below (Table 5.7,5.8).

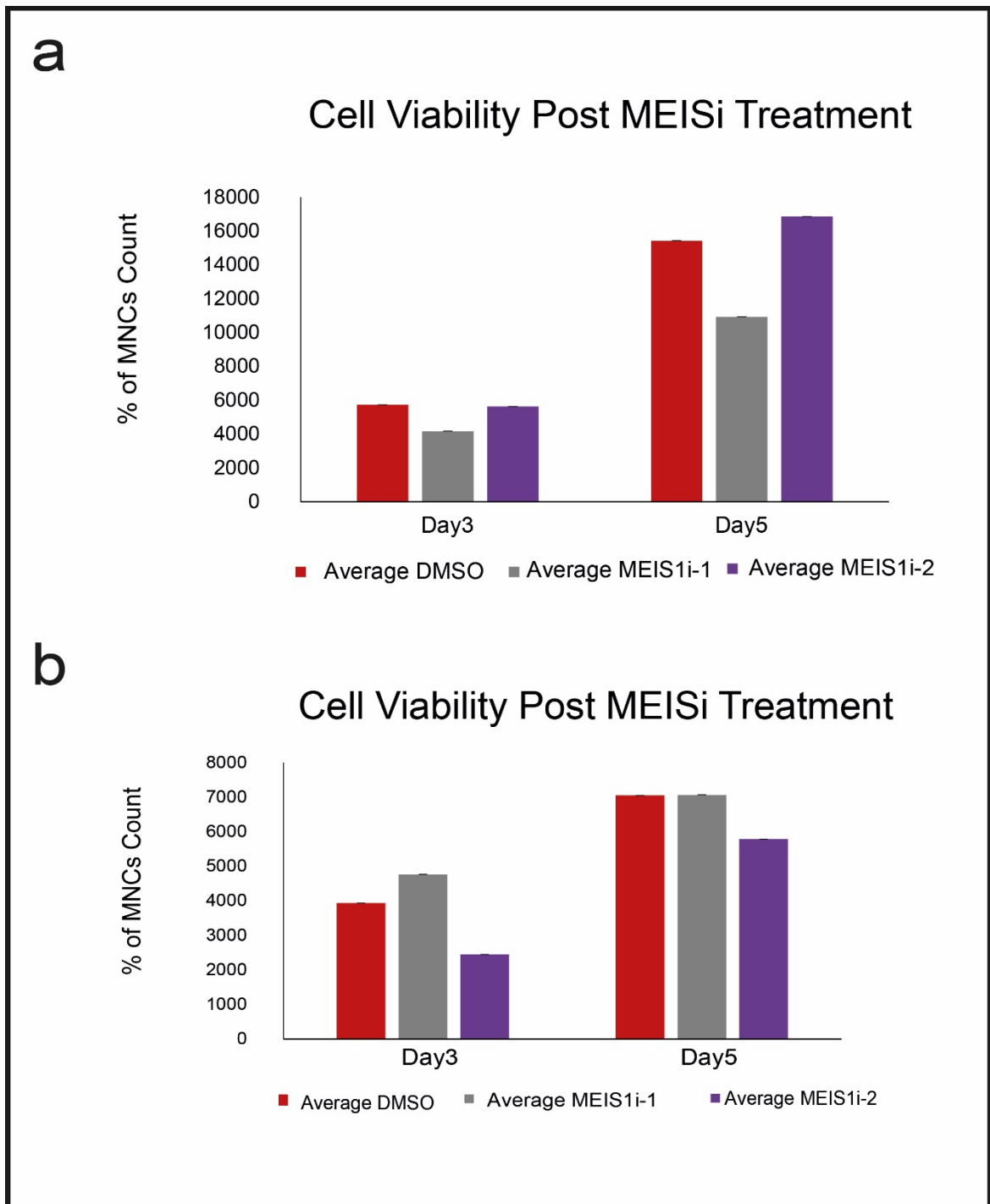


Figure 5.11. Cell viability post MEIS1i treatment a) Quantification of flow cytometry analysis which performed to determine cell viability post MEIS1i treatment in patients with high MEIS protein profile, b) Quantification of flow cytometry analysis which performed to determine cell viability post MEIS1i treatment in patients with low MEIS protein profile.

Table 5.7. The percentage and fold difference that corresponds to cell viability post-treatment in average AML/ALL MNCs with a high MEIS protein profile cell content quantification results

Cell Viability	MEIS1i	Cell Viability Post Treatment in Average Patients MNCs Compared to DMSO	Fold Difference Compared to DMSO
Day3	MEIS1i-1	4174 vs 5732	≈ -1,4 -fold
Day5	MEIS1i-1	10934 vs 15423	≈ -1,4-fold

Table 5.8. The percentage and fold difference that corresponds to cell viability post-treatment in average AML/ALL MNCs with a low MEIS protein profile cell content quantification results

Cell Viability	MEIS1i	Cell Viability Post Treatment in Average Patients MNCs Compared to DMSO	Fold Difference Compared to DMSO
Day3	MEIS1i-2	2449 vs 3937	≈ -1,6 -fold
Day5	MEIS1i-2	5786 vs 7055	≈ -1,2-fold

5.11. APOPTOSIS ASSAY DETERMINATION OF MNCS/LSCS POST MEIS1I TREATMENT

Knowing the tolerance effect of MEIS1 against the apoptosis process in cancer cells caused an idea that the use of MEIS1i may have apoptotic effects on cells. For this reason, apoptosis analysis was performed post 5 days of MEIS1i treatment in AML/ALL patients. It was observed that MEIS1i-1 had an apoptotic effect in the MNC of AML/ALL patients (n=9). In MNCs, MEIS1i-1 has been observed to increase post-pre-apoptosis by approximately 1.5-fold compared to DMSO control (Figure 5.12b). In LSCs cells isolated

from AML/ALL patients (n=5), it was observed that MEIS1i-2 increased post apoptosis approximately 1.8-fold and pre-apoptosis 1.5-fold compared to DMSO control (Figure 5.13b). The Fold difference table is also shown below (Table 5.9,5.10).



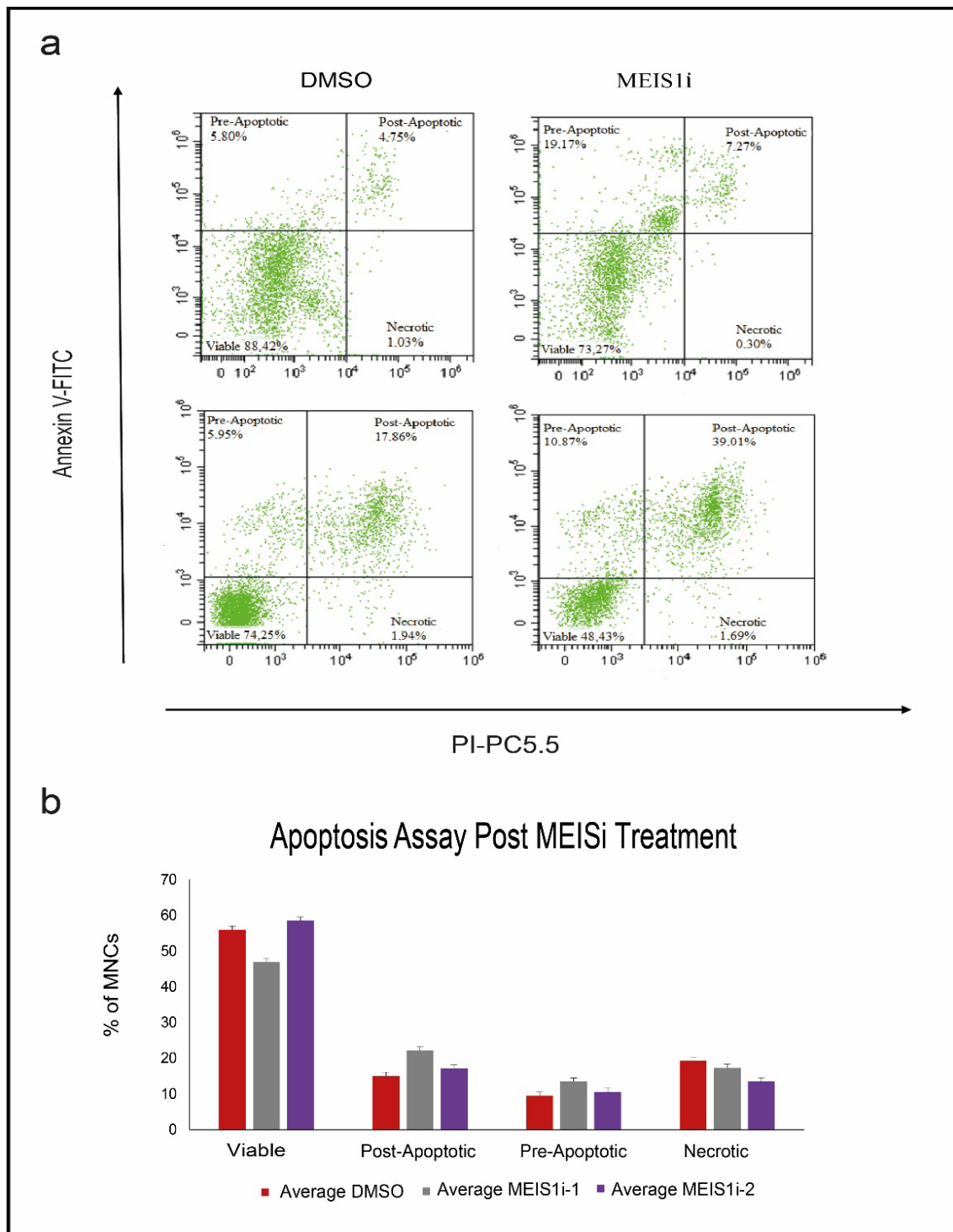


Figure 5.12. The effect of MEIS1i on apoptosis process on MNCs post 72h of treatment a)

The plots of flow cytometry analysis that belonged to the assay were shown, b) The quantification results were presented (n=9, *p<0,05, **p<0,01).

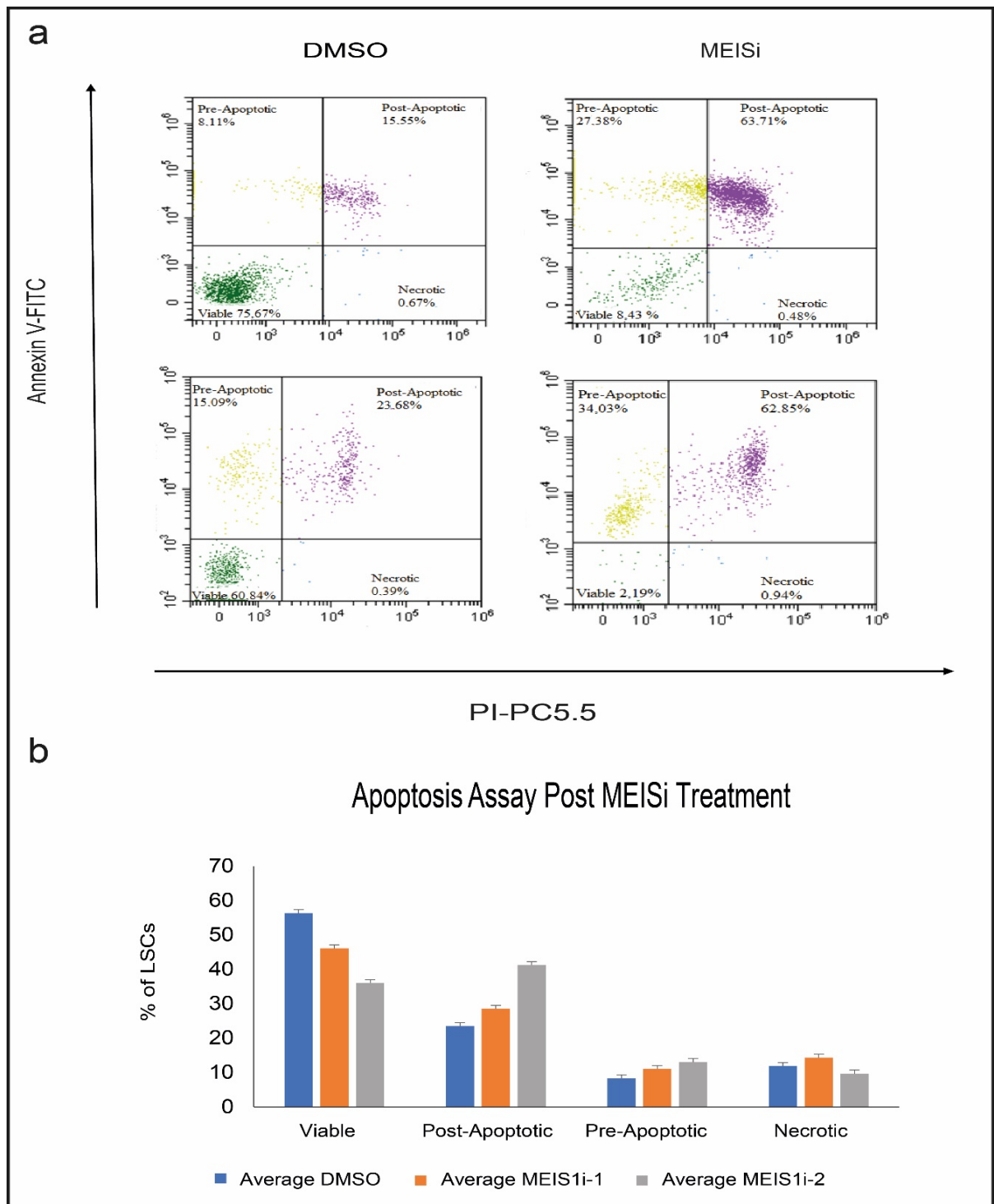


Figure 5.13. The effect of MEIS1i on the apoptosis process on LSCs post 72h of treatment

a) The plots of flow cytometry analysis that belonged to the assay were shown, b) The quantification results were presented (n=9, *p<0,05, **p<0,01).

Table 5.9. The percentage and fold difference that corresponds to apoptotic effect in average AML/ALL MNCs cell content quantification results

Apoptotic Effect	MEIS1i	Apoptotic Effect in Average Patients MNCs Compared to DMSO	Fold Difference Compared to DMSO
Post-Apoptotic	MEIS1i-1	22 vs 15	≈ 1,5-fold
Pre-Apoptotic	MEIS1i-1	13 vs 9	≈ 1,5-fold

Table 5.10. The percentage and fold difference that corresponds to apoptotic effect in average AML/ALL LSCs cell content quantification results

Apoptotic Effect	MEIS1i	Apoptotic Effect in Average Patients LSCs Compared to DMSO	Fold Difference Compared to DMSO
Post-Apoptotic	MEIS1i-2	41 vs 23	≈ 1,7-fold
Pre-Apoptotic	MEIS1i-2	13 vs 8	≈ 1,5-fold

5.12. DETERMINATION OF GENE EXPRESSION POST MEIS1I TREATMENT IN ALL/AML PATIENTS

The RT-PCR assay was performed to observe changes of the gene expression levels in AML/ALL patients 5 days post MEIS1i treatment. It was shown that MEIS1 gene expression was 323-fold higher, Hif-1 α 12-fold higher, and HoxA9 and Pbx substantially lower in PB-MNCs derived from average AML-R (n=2) compared to average healthy individuals before MEIS1i treatment (Figure 5.14a). Likewise, MEIS1, CD34, BCL-2, FLT3 extensively higher in BM-MNCs derived from AML/ALL patients (n=6) compared to healthy individuals (n=3) before MEIS1i treatment (Figure 5.14b). After MEIS1i treatment, MEIS1i-1 significantly decreased the expression of MEIS1, HoxA9 and MEIS1i-2 notably reduced the expression of HoxA9 compared to DMSO control (Figure

5.15a). In the other patient group, after MEIS1i treatment, MEIS1i-2 considerably decreased the expression of MEIS1, MEIS1i-1 significantly decreased the expression of BCL-2 in BM-MNCs derived from AML/ALL patients (n=6). In addition to these results, MEIS1i-1 was found to reduce FLT3(-10-fold) expressions compared to DMSO control (Figure 5.15b). The Fold difference table is also shown below (Table 5.11, 5.12, 5.13, 5.14).

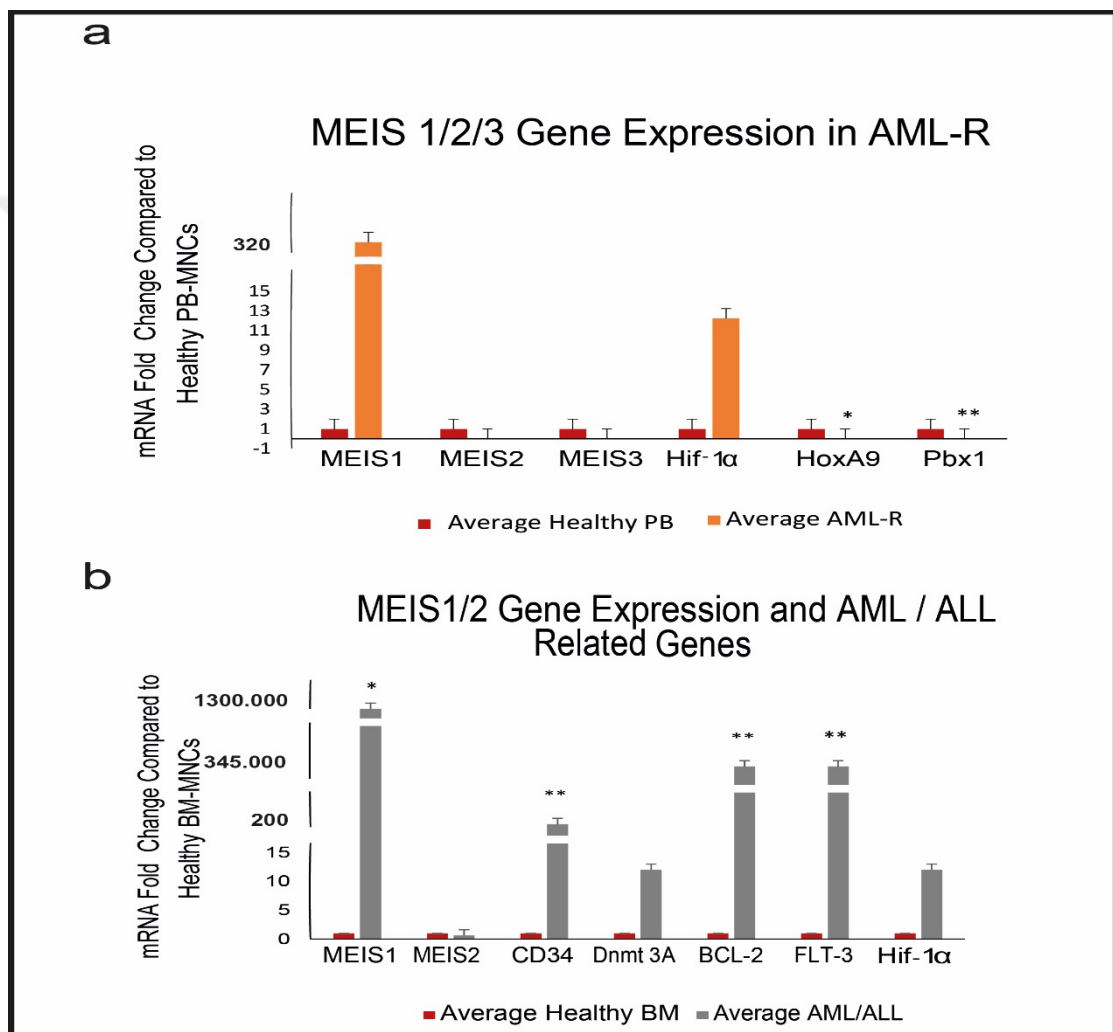


Figure 5.14. Gene expression analysis before MEIS1i treatment on a) PB- MNCs of AML-R patients, b) BM-MNCs of AML/ALL patients compared to healthy individuals by Real-time PCR (* $p < 0,05$, ** $p < 0,01$).

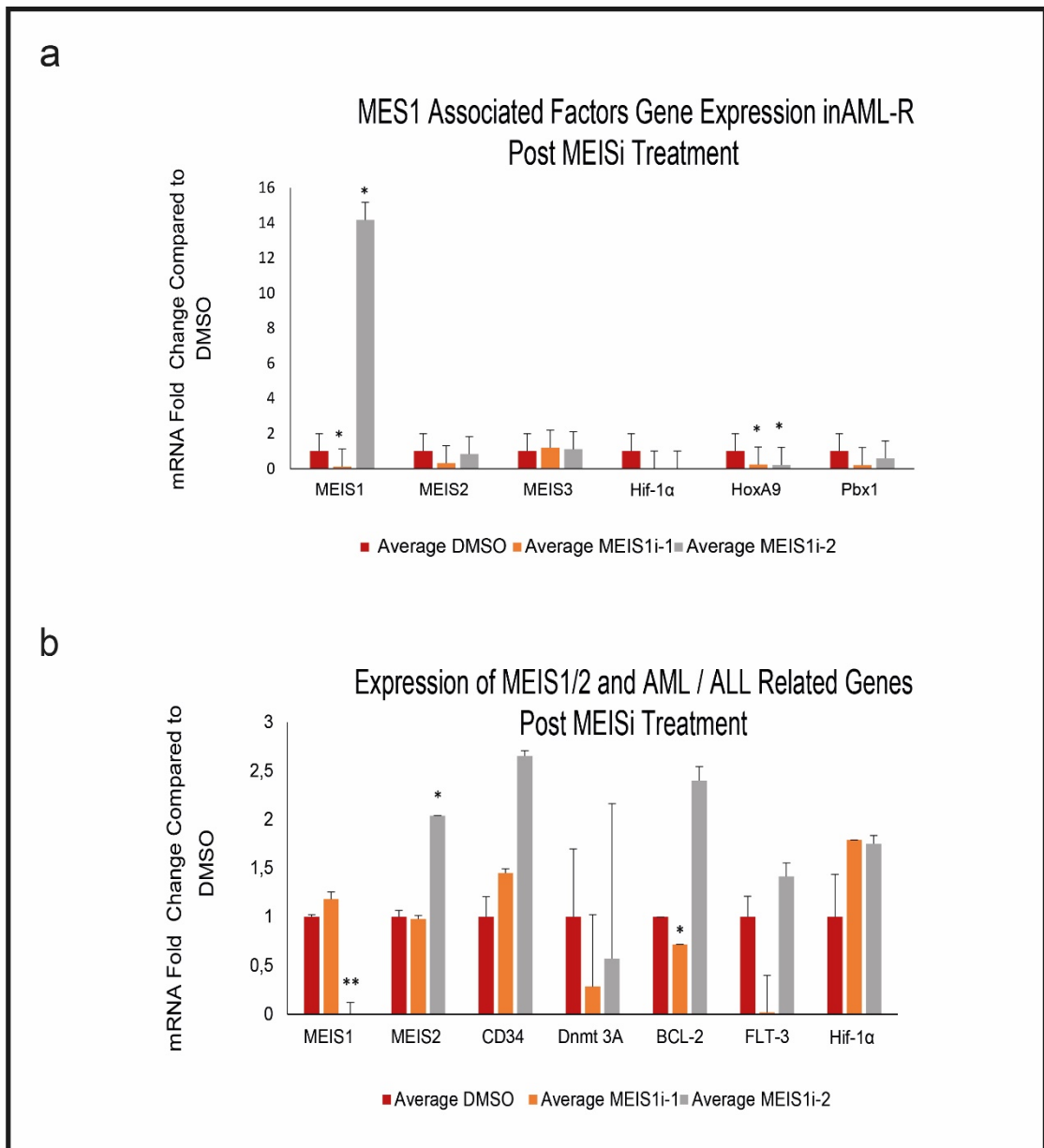


Figure 5.15. Gene expression analysis post MEIS1i treatment on a) PB- MNCs of AML-R patients, b) BM-MNCs of AML/ALL patients compared to DMSO control by Real-time PCR (* $p < 0,05$, ** $p < 0,01$).

Table 5.11. MEIS and associated gene expression and fold differences in average AML-R compared to average healthy PBs cell content quantified findings

Gene Groups	MEIS and Related Genes in Average AML-R Compared to Average Healthy PBs	Fold Difference Compared to Average Healthy PBs
MEIS1	323 vs 1	≈ 323 -fold
MEIS2	0 vs 1	≈ 0-fold
MEIS3	0 vs 1	≈0-fold
HoxA9	0 vs 1	≈ 0-fold
Pbx1	0 vs 1	≈ 0-fold
Hif-1 α	12 vs 1	≈ 12-fold

Table 5.12. MEIS-related gene expression and fold differences in average patients compared to average healthy BMs cell content quantified data

Gene Groups	MEIS and Related Genes in Average Patients Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
MEIS1	1,262,663 vs 1	≈ 1,2 million-fold
MEIS2	0,6 vs 1	≈ 0,6-fold
CD34	201 vs 1	≈200-fold
Dnmt3a	12 vs 1	≈ 12-fold
BCL-2	341,143 vs 1	≈ 340 thousand-fold
FLT3	344,306 vs 1	≈ 344 thousand-fold
Hif-1 α	12 vs 1	≈ 12-fold

Table 5.13. The expression and fold difference of MEIS- related genes in average AML-R cells post-treatment when compared to DMSO cell content quantified data

Gene Groups	MEIS1i	MEIS and Related Genes Post Treatment in Average AML-R Compared to DMSO	Fold Difference Compared to DMSO
MEIS1	MEIS1i-1	0,11 vs 1	≈ -10-fold
MEIS2	MEIS1i-1	0,3 vs 1	≈ -3,3-fold
MEIS3	MEIS1i-1	1,2 vs 1	≈ 1,2-fold
HoxA9	MEIS1i-1	0,2 vs 1	≈ -5-fold
Pbx1	MEIS1i-1	0,2 vs 1	≈ -5-fold
Hif-1α	MEIS1i-1	0 vs 1	≈ -10-fold

Table 5.14. The expression and fold difference that corresponds to MEIS and related genes post- treatment in average patients compared to DMSO cell content quantification data

Gene Groups	MEIS1i	MEIS and Related Genes Post Treatment in Average Patients Compared to DMSO	Fold Difference Compared to DMSO
MEIS1	MEIS1i-1 / MEIS1i-2	1,1/0 vs 1	≈ 1,1/-10-fold
MEIS2	MEIS1i-1 / MEIS1i-2	0,9/2 vs 1	≈ 1/ 2-fold
CD34	MEIS1i-1 / MEIS1i-2	1,4/2,6 vs 1	≈ 1,4/2,6-fold
Dnmt3a	MEIS1i-1 / MEIS1i-2	0,2/0,5 vs 1	≈ -5/-2-fold
BCL-2	MEIS1i-1 / MEIS1i-2	0,7/2,4 vs 1	≈ -1,4/2,4-fold
FLT3	MEIS1i-1 / MEIS1i-2	0/1,4 vs 1	≈ -10/1,4-fold
Hif-1α	MEIS1i-1 / MEIS1i-2	1,79/1,7 vs 1	≈ 1,8/1,7-fold

5.13. DETERMINATION OF MEIS (+) RATIO POST MEIS1I TREATMENT IN PATIENTS WITH HIGH/LOW MEIS PROTEIN PROFILE

To observe the effects of MEIS1i on the MEIS profile in ALL/AML patients with a high/low MEIS protein profile, 5 days post MEIS1i treatment, cells MEIS1-2-3 protein was labeled by fluorochrome-conjugated antibody and analyzed in flow cytometry. 5 days post MEIS1i-1 and MEIS1i-2 treatment, MEIS(+) ratio was significantly reduced in patients with high (AML-R, # patient 21), and low (ALL-R, # patient 24) MEIS protein profile compared to DMSO control (Figure 5.16a,b).



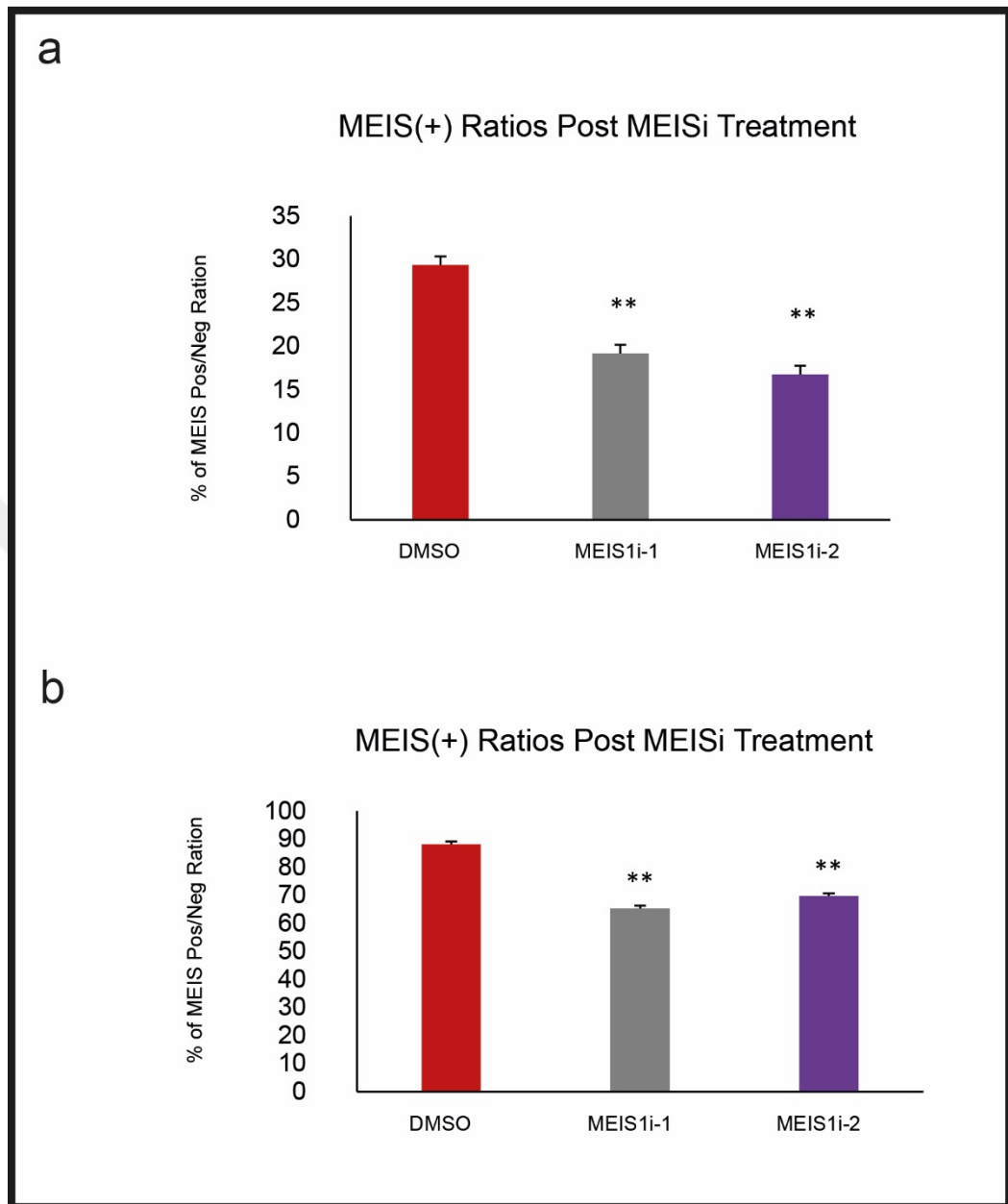


Figure 5.16. Quantification of flow cytometry analysis performed to determine MEIS (+) ratio post MEIS1i treatment on a) MEIS high patients and, b) MEIS low patients after MEIS1i treatment in comparison to DMSO control (** $p < 0.01$).

5.14. DETERMINATION OF LSC MARKERS 7 DAYS POST MEIS1I TREATMENT

To observe the change caused by MEIS1i in LSC markers, 7 days post MEIS1i treatment, MNCs from AML/ALL patients (n=3) were stained with LSC markers (CD34+, CD33+, CD123+, CD133+) and analyzed in flow cytometry. All of the LSC markers were used in combination with CD34. LSC markers were reduced compared to DMSO control, 7 days post MEIS1i treatment (Figure 5.17). The fold difference table is shown below (Table 5.15).

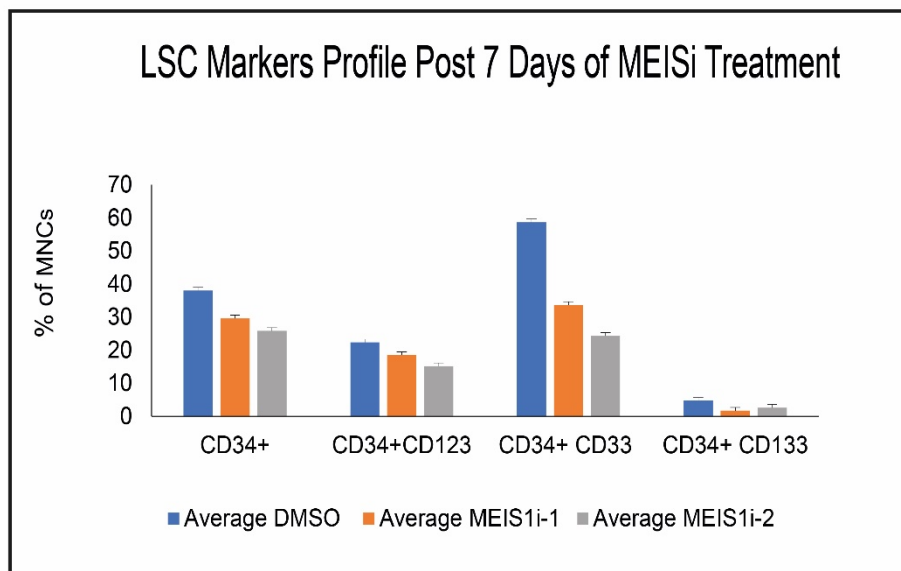


Figure 5.17. Post 7 days of MEIS1i treatment on MNCs cells, the LSC marker profile was analyzed

Table 5.14. The percent and fold difference in average AML/ALL MNCs cell content quantification data that matches post-treatment LSCs markers

LSCs Markers	MEIS1i	LSCs Markers Percent Post Treatment in Average Patients MNCs Compared to DMSO	Fold Difference Compared to DMSO
CD34+	MEIS1i-1 / MEIS1i-2	29/25 vs 38	≈ -1,4/-1,6 -fold
CD34+CD33+	MEIS1i-1 / MEIS1i-2	33/24 vs 58	≈ -2/-2,5-fold
CD34+CD123+	MEIS1i-1 / MEIS1i-2	18/15 vs 22	≈ -1,2/-1,6-fold
CD34+CD133+	MEIS1i-1 / MEIS1i-2	1/2 vs 4	≈ -3,3/-2-fold

5.15. DETERMINATION OF THE EFFECT OF MEIS1I TREATMENT ON CELL CYCLE PROFILE

CD34+ LSCs were separated using the MS method in an AML patient (# patient 1) with blasts in both BM and PB. 4 days post MEIS1i treatment, LSCs were labeled with PI and compared with DMSO control (0,25 percent), cell cycle analysis was performed in a flow cytometer. The BM analysis of the patient post MEIS1i treatment showed that the G0-G1 and S phase of the cells were higher and the G2-M phase was low, compared to the DMSO control of MEIS1i-1 and MEIS1i-2 (Figure 5.18a). In the PB analysis, the G0-G1 phase of the cells was found to be significantly higher compared to the DMSO control of MEIS1i-1, and the G2-M phase of the cells was found to be low compared to the DMSO control of MEIS1i-1 and MEIS1i-2 (Figure 5.18b).

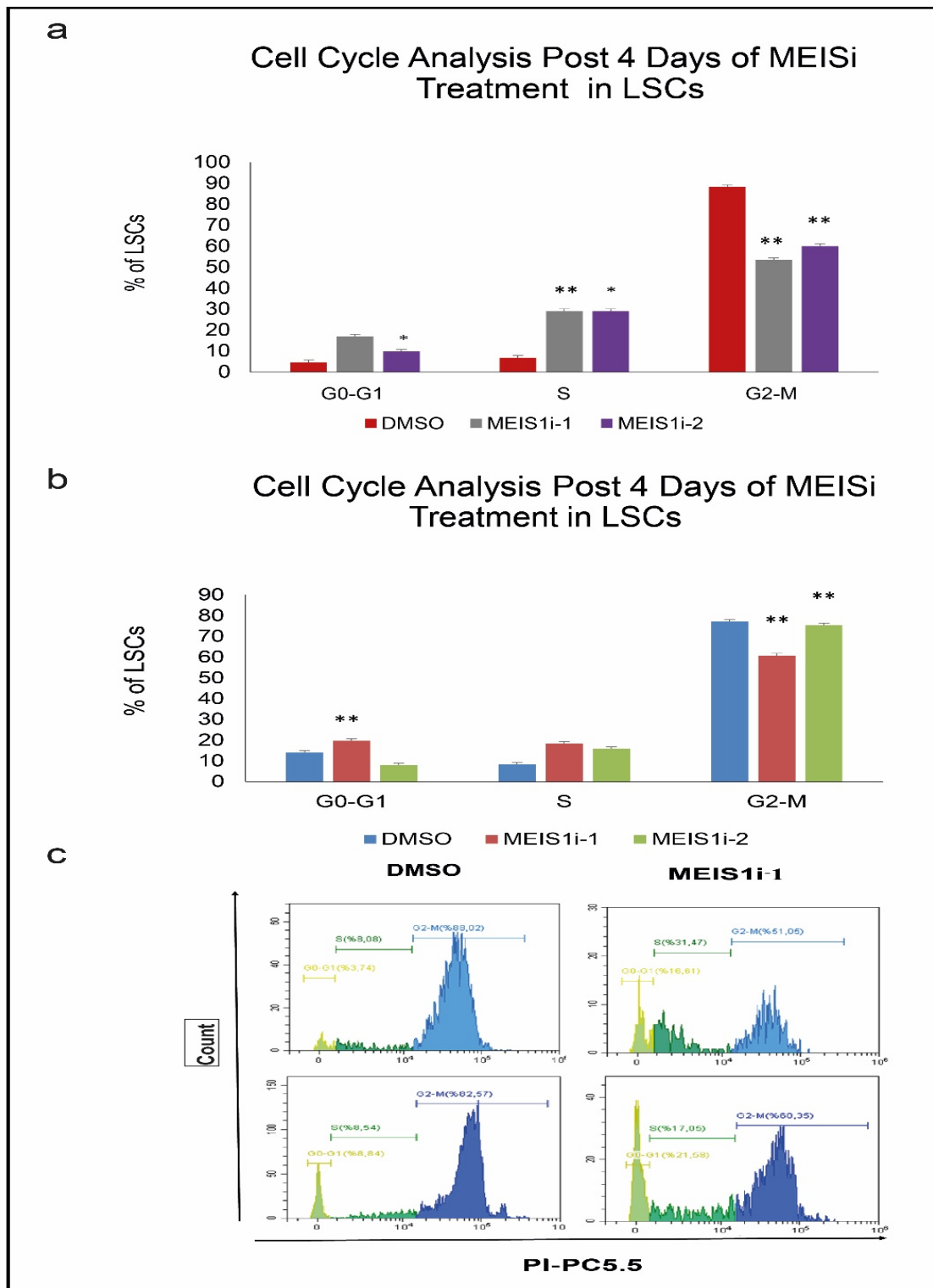


Figure 5.18. Cell cycle analysis of LSCs after 4 days of MEIS1i treatment on a) BM-MNC, b) PB-MNC (n=1, *p<0,05, **p<0,01) c) The assay flow plots were displayed.

5.16. DETERMINATION OF EXPRESSION OF MEIS1 AND RELATED GENES IN POST-CHEMOTHERAPY RELAPSE /REFRACTORY

We collected RNA and cDNA from BM samples from patients with AML/ALL before and after relapse/refractory. MEIS1 and its gene family were analyzed via RT-PCR. Chemotherapy regimens received by patients until relapse/refractory were recorded. The following is the chemotherapy drugs; Neupogen, Fludara, Alexan, Zavedos, Blinatumomab, Cyclophosphamide, Clofarabine, Mitoxantrone, Etoposide, Cytarabine, Alkeran, Endoxan, Vidaza, Dacogen, Idarubicin, Vincristine, Adriamycin, Prednisone, L-asparaginase, Leunase, Oncovin.

Gene expression analyzes in BMs of AML/ALL patients (n=14) were compared with BM of healthy individuals before and after chemotherapy. It has been shown that the expression of MEIS1 (about 390 thousand-fold), CD34 (about 5700-fold), and Hif-1 α (5-fold) in average AML/ALL patients (n=7) is increased compared to average healthy individuals (n= 3). It has been shown that after relapse/refractory in the same patient group, MEIS1 (about 1 million 900-fold), MEIS2 (92-fold), Pbx1 (24-fold), Hif-1 α (about 8250-fold), CD34 (6088-fold) expressions in relapse/refractory (n=7) were higher than those of average healthy individuals (Figure 5.19a). Relapse/refractory patients (n=7) compared to AML/ALL patients (n=7) MEIS1 (about 5-fold), MEIS2 (210-fold), MEIS3 (2-fold), HoxA9 (about 1.4-fold), Pbx1 (1656-fold), Hif-1 α (1613-fold), CD34 (1-fold) expression increased (Figure 5.19b). The fold difference table is shown below (Table 5.16, 5.17).

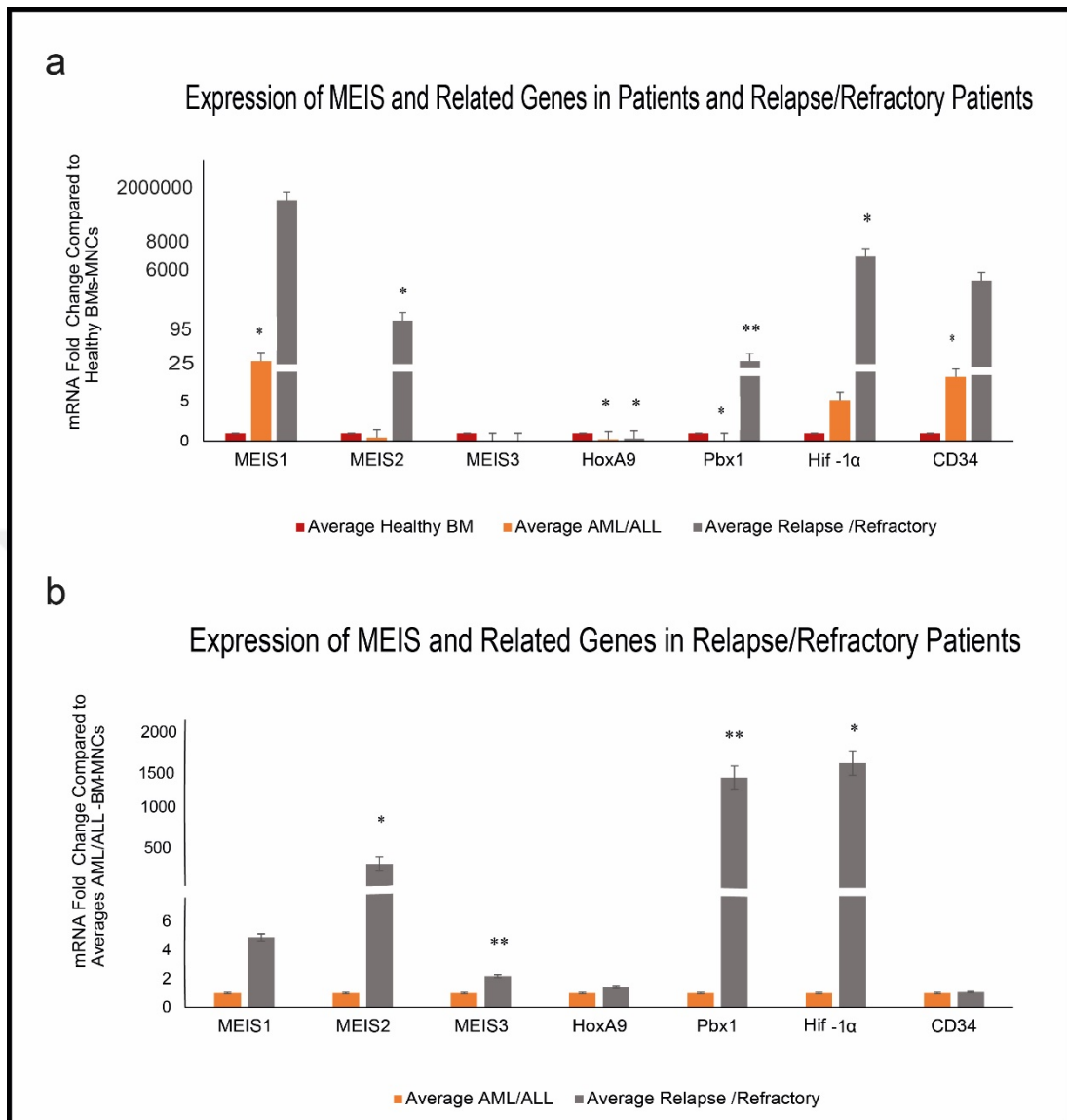


Figure 5.19. Gene expression analysis of MEIS1/2/3 and associated genes in a) MNCs of average patients and average relapse/refractory patients compared to healthy individuals, and b) MNCs of relapse/refractory patients compared to average patients by Real-time PCR (* $p < 0,05$, ** $p < 0,01$).

Table 5.15. The expression and fold difference that corresponds to MEIS and related genes in average patients/average relapse/refractory BMs cell content quantified results when compared to average healthy BMs cell content

Gene Groups	MEIS and Related Genes in Average Patients /Average Relapse/ Refractory Compared to Average Healthy BMs	Fold Difference Compared to Average Healthy BMs
MEIS 1	388,660 / 1,899,663 vs 1	≈ 39 thousand/1,9 million-fold
MEIS 2	0,43/92 vs 1	≈ 0,4/92-fold
MEIS 3	0/0 vs 1	≈0/0 fold
HoxA9	0,2/0,3 vs 1	≈0,2/0,3-fold
Pbx1	0 /24 vs 1	≈ 0/24-fold
Hif-1α	5,1/8235 vs 1	≈ 5,1/8235-fold
CD34	5658/6088 vs 1	≈5658/6088-fold

Table 5.16. The expression and fold difference that corresponds to MEIS and related genes in average relapse/ refractory compared to average patient BMs cell content quantification results

Gene Groups	MEIS and Related Genes in Average Relapse/ Refractory Compared to Average Patient BMs	Fold Difference Compared to Average Patient BMs
MEIS 1	4,8 vs 1	≈ 4.8-fold
MEIS 2	210 vs 1	≈ 210-fold
MEIS 3	2 vs 1	≈2-fold
HoxA9	1,3 vs 1	≈1,3-fold
Pbx1	1656 vs 1	≈ 1656-fold
Hif-1α	1613 vs 1	≈ 1613-fold
CD34	1 vs 1	≈1-fold

5.17. EVALUATION OF MUTATIONS AND MEIS RELATIONSHIP

The correct diagnosis was made and treatment was started by considering the abnormalities in some fusion genes (AML1/ETO, PML/RARA, MHY11/CBFB) as well as mutations such as BCR/ABL, NPM1, FLT3 in AML/ALL patients in this research. it was observed that MEIS1i did not have a lethal effect on LSCs and could not lead cells to apoptosis in a patient with positive FLT3 and NPM1 mutations (#patient 54) in our study, among these two mutations that guide drugs and the prognosis of the disease. In addition to these results, no negative effect of MEIS1i on LSCs cells was observed in the patient groups with mutation and fusion genes positive and resistant to drugs. Overexpression of the MEIS1 gene was also found in patients with mutation and fusion gene in patients who underwent gene expression analysis. Since the subject of this study is not mutations and MEIS relationship, the MEIS1 gene and mutation relationship we encountered during the research are shown in the table as fold difference (Table 5.18).

Table 5.17. The expression and fold difference that corresponds to MEIS1 expression in patients with mutation/ fusion gene compared to average healthy BMs cell content quantification results

# Patients	Mutation/ Fusion Genes	MEIS1 Expression in Patients with Mutation/ Fusion Gene Compared to Average Healthy BMs	Fold Difference compared to Average Healthy BMs
#16	FLT3	59,377 vs 1	≈ 59 million -fold
#10	BCL-2	10 vs 1	≈ 10-fold
#11	NPM1	13 vs 1	≈ 13-fold
#53	MHY11/CBFB Fusion Gene	2,779,000 vs 1	≈ 2.7 million-fold
#26	NPM1	350,000 vs 1	≈ 350 thousand -fold
#64	FLT3	11,511,000 vs 1	≈ 11 million-fold
#65	FLT3	7,543,000 vs 1	≈ 7.5 million-fold

6. DISCUSSION

Recent studies have been stated that MEIS1 plays an important role in the self-renewal/proliferation of normal HSCs. It is known that MEIS1 cooperates with Hox genes that cause more differentiation stages in hematopoiesis [120–122]. This hypothesis has been supported by detecting a high bleeding rate due to megakaryocyte deficiency in 14.5 mouse embryos lacking MEIS1 protein [125]. Our findings suggest that there is a 42,90 percent MEIS protein profile in healthy individuals (Figure 5.1b). The normal blood cell distributions in these healthy individuals in flow cytometry analyzes indicate the role of MEIS in hematopoiesis. In the future, elucidation of the role of the MEIS1 protein in normal hematopoiesis will help to analyze the pathway of LSCs [124].

On the other hand, it is been demonstrated that MEIS1 protein plays an effective role in cancer metabolism. 64 of 95 (67.4 percent) AML patients showed abnormally high levels of MEIS1 expression in the study by Liu et al. In comparison, MEIS1 levels were considerably low in 31 of 95 (32,6 percent) patients [68]. In research on myeloid leukemia cells in AML patients, the MEIS1 protein's first influence on malignancy was demonstrated [110,127,129,200]. Other investigations have found elevated MEIS1 expression in the BMs of ALL and AML patients, supporting this hypothesis [120,130]. We confirmed a similar relationship between MEIS and leukemia. The expression of MEIS1 protein level was shown to be higher in average ALL-AML patients (76,25 percent) than in average healthy individuals (42,90 percent). In addition to this finding, According to the patient groups, the highest MEIS protein level was observed in the AML patient group (82,75 percent) (Figure 5.2b). Another group of cells, which we name a small group of *MEIS+ high*, was detected at a rate of 16,12 percent in the AML, AML-R, and ALL-R patient groups (Figure 5.2b).

There have been studies showing that the expression of MEIS1 and its related genes is high in leukemia. High MEIS1 expression was revealed to play a role in the onset of AML in mouse models of the disease [132,201]. Furthermore, elevated HoxA9 gene expression is a strong predictor of poor prognosis in AML [202]. It has been reported that MEIS1 and HoxA9, as well as Pbx1, work together in leukemia to speed up leukemogenesis by activating the genes involved [110,122,196]. Several studies confirmed that high levels of

MEIS1 and HoxA9 expression have been seen in aggressive murine and human ALL samples [134,135,203]. It is also known that Hif-1 α is a key regulator of low oxygen levels in AML. Meanwhile, Hif-1 α was involved in the self-renewal of HSCs and LSCs in AML [204]. In addition, high expression of Hif-1 α has a negative prognosis effect on leukemia patients with decreased response to chemotherapy [170,172,205–207]. According to Bonnet and colleagues, Hif-1 or Hif-2 is required for LSC survival and could be used as a therapeutic target for eradicating LSCs in AML [208]. In parallel with these results in our investigation, it was observed that BM-PB-MNC samples from average AML/ALL patients expressed the MEIS1 and Hif-1 α were extensively higher level (* $p < 0,05$, ** $p < 0,01$) compared to the average healthy individuals as a result of RT-PCR analysis (Figure 5.5a,b). Besides, HoxA9 expression was higher (1,7-fold) in the PB-MNC sample from average ALL patients compared to the average healthy individuals in PB-MNC samples (Figure 5.5b). Similarly, in other BM patient group samples, it was observed that MEIS1 expression was significantly higher in average AML and average ALL BM compared to average healthy individuals (* $p < 0,05$, ** $p < 0,01$) (Figure 5.6). In the same group, high expression of Hif-1 α was found in average ALL and average AML-R BM compared to average healthy individuals (Figure 5.6).

The B-cell lymphoma 2 (BCL-2) protein family is important in the intrinsic apoptotic pathway. This protein family is also significantly overexpressed in a variety of malignancies. Abnormal BCL-2 family protein overexpression causes treatment resistance and is linked to a poor prognosis. Furthermore, the expression of BCL-2 family proteins is generally dysregulated and overexpressed in hematologic malignancies, particularly AML, results in a poor rate of complete remission in patients [214,209,210–213]. It has been reported that besides BCL-2, FLT3 is highly expressed in AML patients [209–211]. As with BCL-2, high expression of FLT3 affects the prognosis of the disease. According to the analysis of Wang et al., there is an increase in leukemia when MEIS1 and HoxA9 work together. They provide this with the activation of FLT3 and CD34 expression [168]. Our gene expression analysis revealed that MEIS1, CD34, BCL-2 and FLT3 expression in BM of average AML patients (n=3) were notably higher than gene expressions in the BM of average healthy individuals (n=3) in this research (Figure 5.6). In addition, it was also stated that BCL-2, FLT3 gene expressions were higher in the average AML-R patients (n=7) compared to the average healthy individuals (* $p < 0,05$, ** $p < 0,01$) (Figure 5.6). The

findings of this study also support the hypothesis that high co-expression of MEIS1, FLT3, and CD34 plays an important role in the formation and development of leukemia.

LSCs are defined with special surface markers as stated in many studies. In many studies to identify myeloid cells, CD123 and CD33 markers have been used as determinants of LSCs [39,59,218,219]. According to our flow cytometry analysis, it was observed that CD123+ and CD33+ cell populations were found in the blast category in many patient samples with a diagnosis of leukemia. In the first studies, it was shown that LSCs are in CD34+ and 38- cells [214]. In other studies, CD123+ was found in CD34+ and CD38- cells [20,22]. Another research showed that the CD133 rate is high in Leukemia Initiating cells (LICs) in childhood ALL [54]. According to the results of our analysis with AML, ALL, AML-R, and ALL-R patient groups, the CD123, CD34, and 33 ratios in the patient groups were found to be considerably higher than average healthy individuals (Figure 5.8a,b,c). The fact that LSC markers are higher in patients with leukemia than in healthy individuals proves the accuracy of the LSC markers used.

The relationship between MEIS and LSC markers (CD123 and CD34) was demonstrated for the first time in this latest investigation. According to the study, it was found that the proportion of average LSCs with MEIS(+) ratio in cells with CD123+ was significantly higher than the group of average LSCs with CD123+ who were also MEIS(-) (* $p < 0,05$) (Figure 5.7b). In addition to that, the rate of average LSCs with MEIS(+) ratio in cells with CD34+ was higher (2-fold) than the group of average LSCs with CD34+ who were also MEIS(-) (Figure 5.7d). Our findings confirmed that LSCs also have MEIS(+) at a certain rate indicates that MEIS has an active role in the presence of leukemia.

MS, FCS methods were used for the separation of LSCs in the studies. In our research, we have validated that LSC marker ratios were higher than before separation as a result of separation with FCS made us think that the FCS separation method is safer (Table 5.6).

Targeting the MEIS1 protein expressed in LSC using various agents may reduce the function of LSCs [117,187]. Therefore, negative effects of MEIS1i on LSC were demonstrated for the first time in a series of experiments in this study. MNCs from AML/ALL patients with MEIS high and low protein profile showed a gradual but non-significant decrease in cell viability compared with DMSO at 3 and 5 days post-treatment with MEIS1i (Figure 5.11a, b). On the other hand, Wermuth and colleagues found that

HoxA9 and MEIS1 abolished caspase-mediated apoptosis [216]. LSCs and MNCs obtained from AML/ALL patients were analyzed for apoptosis 3 days post MEIS1i treatment and it was seen that MEIS1i-1 increased pre and post apoptosis approximately 1.5-1.7-fold compared to DMSO. We confirmed that MEIS1i-1 activates the apoptotic pathway in AML/ALL patient samples (Table 5.9, 5.10). The fact that the cell viability and apoptosis analyzes performed to observe the effect of MEIS1i were not statistically significant led us to conclude that it varies according to the physiology of the patients, the drugs they take, the subtypes of leukemia they have, and whether they have mutations or not. Different results were obtained in the evaluation made as to the average of the patients compared to the individual evaluation of the patients in the research. Besides all these, the negative effect of MEIS1i on leukemia cells also manifests itself at the level of gene expression. It has been observed that MEIS1i-1 and MEIS1i-2 inhibitors decrease the expression levels of known genes related to the development and survival of cancer in some patient samples in our research (Figure 5.15a,b). It was shown in our investigation that the MEIS protein level decreased as a result of the negative effect of MEIS1i in the patient group with high and low MEIS protein levels (Figure 5.16a,b). The reducing effect of MEIS1i treatment on LSC markers was demonstrated in our results (Figure 5.17). The effects of MEIS1i were also confirmed in the cell cycle analysis results, and it was observed that the LSCs were kept in the G0-G1 phase after the treatment and did not enter the cell cycle seen in cancer cells (Figure 5.18a,b).

Recent studies have shown that LSCs are biologically different from mature leukemic blast cells and do not respond to conventional chemotherapy drugs [222–224]. High expression of MEIS1 and related genes in LSCs increases resistance to chemotherapy. Therefore, it is important to evaluate the expression of MEIS1 and related genes in AML/ALL patients before chemotherapy and after disease relapse/refractory. Pre-and post-chemotherapy expression of MEIS1 and related gene family, which is thought to be the cause of relapse/refractory, was compared for the first time in this research. One study found that high MEIS1 expression showed greater resistance to the response to conventional chemotherapy compared to the low MEIS1 group [68]. Moreover, up-regulation of MEIS1 is required for the maintenance of leukemic cell proliferation. According to Rosales-Avia et al., down-regulation of MEIS1 or up-regulation of Prep1 and Pbx genes may be involved in modifying the cellular response to chemotherapeutic-induced apoptosis [220]. Our gene

expression analysis revealed that the high MEIS1 gene and its gene family in patients with relapse/refractory after chemotherapy suggests that the MEIS1 gene and its gene family are resistant to chemotherapy drugs and promote the survival of LSCs (Figure 5.19a,b). We further analyzed that the high level of MEIS1 and related gene family even after chemotherapy can be shown as the cause of relapse/refractory of the disease. In addition to these findings, it can be stated that different chemotherapy regimens tend to increase the expression of MEIS1 and related gene family in the relapse/refractory patient group. These findings also emphasize the importance of gene expression levels of MEIS1 and related gene family during cancer development after chemotherapy.

Genetic tests were performed for the presence of some mutations (BCR/ABL, NPM1, FLT3) and fusion genes (AML1/ETO, PML/RARA, MLY11/CBFB) to diagnose and treat the AML/ALL patients included in our research accurately in the clinic. Our findings demonstrated that MEIS1i had no negative effect on apoptotic and cell survival in the group of patients who had significantly higher levels of MEIS1 gene expression and also had various mutations and fusion genes. These findings confirmed that the association of MEIS1 and mutation increases resistance against MEIS1i. More research is needed relating to a mutation in patients with MEIS and leukemia to prove the accuracy of these findings.

In the light of all these findings, it has been stated that MEIS1 inhibitors can be used to suppress MEIS1 protein activity, which plays an important role in the development and recurrence of leukemia. As a result of our research, it was found that MEIS1i was effective on LCSs and could be used as a potential drug precursor in future studies.

With the knowledge of the MEIS protein and related regulatory pathways and its gene family, it will increase the possibility of being a potential treatment target for leukemia treatment in the clinic.

7. CONCLUSION AND FUTURE PERSPECTIVE

With the irresistible development in drug technology, the fact that the treatment of many types of cancer is not yet known is one of the most important problems encountered today. The ineffectiveness of traditional chemotherapy methods and drugs used in cancer treatment has necessitated the development of new drug precursors. One of them, MEISi's have been developed to meet this need in the future to be used in the treatment of various types of cancer.

We aimed to investigate MEIS1 proteins which are known to be effective in leukemia development and relapsed in LSCs, and to target MEIS activity with newly developed MEIS1i in this investigation. MEIS1i showed a detrimental effect on MEIS protein activity, LSCs cell survival, and apoptotic pathway in a study performed on BM-PB samples of patients diagnosed with AML/ALL. In addition to these findings, MEIS1i-1 and MEIS1i-2 inhibitors decreased the level of expression of known genes associated with cancer growth and survival in patient samples. In addition, we showed that MEIS1 gene expression plays an important role in the relapse of leukemia disease and its resistance to chemotherapy treatment in this research.

Finally, considering the other clinical features of the leukemia patient groups to be included in the studies, the genetic abnormalities they have, all the treatments they receive, and different secondary diseases will increase the accuracy of the data obtained in the studies.

Future studies on resolving the relationship between MEIS and cancer will illuminate unknown issues about the cancer mechanism of MEIS and thus contribute to the development of drug precursors to reduce the resistance of MEIS in cancer. Our findings will be aided by developing different MEISi and evaluating their impact on leukemia cells, as well as *in vivo* investigations on preclinical and mouse models.

REFERENCES

1. Juliusson G, Hough R. Leukemia. *Tumors in adolescents and young adults*. 2016;87-100.
2. Analysis Tool | American Cancer Society - Cancer Facts & Statistics [cited 2021 12 April]. Available from: <https://cancerstatisticscenter.cancer.org/#!/data-analysis/NewCaseEstimates>.
3. Top Leukemia Causes & Factors That Put You at Risk CTCA. [cited 2021 13 April]. Available from: <https://www.cancercenter.com/cancer-types/leukemia/risk-factors>.
4. Kreile M, Piekuse L, Rots D, Dobeles Z, Kovalova Z, Lace B. Analysis of possible genetic risk factors contributing to development of childhood acute lymphoblastic leukaemia in the Latvian population. *Arch. Med. Sci.* 2016;12(3): 479–485.
5. Kumari P, Madhumathi D, Appaji L, Mazloumi SH. Rare and recurrent chromosomal abnormalities and their clinical relevance in pediatric acute leukemia of south Indian population. *Indian J. Med. Paediatr. Oncol.* 2012;33(3):166-9.
6. Nickoloff JA, De Haro LP, Wray J, Hromas R. Mechanisms of leukemia translocations. *Curr. Opin. Hematol.* 2008;15(4): 338–345.
7. Zhang L, Lan Q, Guo W, Hubbard AE, Li G, Rappaport SM, et al. Chromosome-wide aneuploidy study (CWAS) in workers exposed to an established leukemogen, benzene. *Carcinogenesis*. 2011;32(4):605-12.
8. Simonetti G, Padella A, Ferrari A, Guadagnuolo V, Zago E, Griggio F, et al. Dissecting the molecular mechanisms of aneuploidy in acute myeloid leukemia by next generation sequencing. *Blood*. 2014;124 (21):1028.
9. Panuzzo C, Signorino E, Calabrese C, Ali MS, Petiti J, Bracco E, Cilloni D. Landscape of tumor suppressor mutations in acute myeloid leukemia. *J. Clin. Med.* 2020;9(3): 802.
10. Osato M. Point mutations in the RUNX1/AML1 gene: Another actor in RUNX

- leukemia. *Oncogene*. 2004;23(24):4284-96.
11. Polak A, Bialopiotrowicz E, Krzymieniewska B, Wozniak J, Stojak M, Cybulska M, et al. SYK inhibition targets acute myeloid leukemia stem cells by blocking their oxidative metabolism. *Cell Death & Disease*. 2020;11:956.
 12. Hanekamp D, Cloos J, Schuurhuis GJ. Leukemic stem cells: identification and clinical application. *International Journal of Hematology*. 2017;105(5):549-557.
 13. Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: Mirage or reality? *Nature Medicine*. 2009;15(9):1010-2.
 14. Chung SS, Park CY. Acute Myeloid Leukemia Stem Cells-Updates and Controversies. *Cancer Stem Cells*. 2014;143-160
 15. Vormoor J, Lapidot T, Pflumio F, Risdon G, Patterson B, Broxmeyer HE, et al. Immature human cord blood progenitors engraft and proliferate to high levels in severe combined immunodeficient mice. *Blood*. 1994;83(9):2489-97.
 16. Weissman I. Stem cell research: Paths to cancer therapies and regenerative medicine. *J. Am. Med. Assoc*. 2005;294(11):1359-66.
 17. Passegué E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J. Exp. Med*. 2005;202(11):1599-611.
 18. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. *Genes. Dev*. 2003;17(18):2298-307.
 19. Kumar AR, Hudson WA, Chen W, Nishiuchi R, Yao Q, Kersey JH. Hoxa9 influences the phenotype but not the incidence of Mll-AF9 fusion gene leukemia. *Blood*. 2004; 103(5):1823-8.
 20. Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, et al. hDOT1L links histone methylation to leukemogenesis. *Cell*. 2005;121(2):167-78.
 21. Wang J, Iwasaki H, Krivtsov A, Febbo PG, Thorner AR, Ernst P, et al. Conditional MLL-CBP targets GMP and models therapy-related myeloproliferative disease.

Embo J. 2005;24(2):368-81.

22. Jordan CT, Upchurch D, Szilvassy SJ, Guzman ML, Howard DS, Pettigrew AL, et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stems cells. *Leukemia*. 2000;14, pages1777–1784.
23. Vergez F, Green AS, Tamburini J, Sarry JE, Gaillard B, Cornillet-Lefebvre P, et al. High levels of CD34 +CD38 low/-CD123 + blasts are predictive of an adverse outcome in acute myeloid leukemia: A groupe ouest-est des leucémies aiguës et maladies du sang (GOELAMS) study. *Haematologica*. 2011;96(12):1792-8.
24. Jin L, Lee EM, Ramshaw HS, Busfield SJ, Peoppl AG, Wilkinson L, et al. Monoclonal Antibody-Mediated Targeting of CD123, IL-3 Receptor α Chain, Eliminates Human Acute Myeloid Leukemic Stem Cells. *Cell Stem Cell*. 2009; 5(1):31-42.
25. Bernt KM, Armstrong SA. Leukemia Stem Cells and Human Acute Lymphoblastic Leukemia. *Semin. Hematol*. 2009;46(1):33–8.
26. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nature Reviews Immunology*. 2007;7,118–130.
27. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature*. 1994;367(6464):645.
28. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat. Med*. 1997;3(7):730-7.
29. Ng SWK, Mitchell A, Kennedy JA, Chen WC, McLeod J, Ibrahimova N, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature*. 2016; 540,433–437.
30. Buss EC, Ho AD. Leukemia stem cells. *Int. J. Cancer*. 2011;129(10):2328-36.
31. Terwijn M, Zeijlemaker W, Kelder A, Rutten AP, Snel AN, Scholten WJ, et al. Leukemic stem cell frequency: A strong biomarker for clinical outcome in acute myeloid leukemia. *PLoS One*. 2014;9(9):e107587.

32. Ho TC, LaMere M, Stevens BM, Ashton JM, Myers JR, O'Dwyer KM, et al. Evolution of acute myelogenous leukemia stem cell properties after treatment and progression. *Blood*. 2016;128(13):1671-8.
33. Lechman ER, Gentner B, Ng SWK, Schoof EM, van Galen P, Kennedy JA, et al. MiR-126 Regulates Distinct Self-Renewal Outcomes in Normal and Malignant Hematopoietic Stem Cells. *Cancer Cell*. 2016;29(2):214-28.
34. Gentles AJ, Plevritis SK, Page P, Majeti R, Alizadeh AA. Association of a Leukemic Stem Cell Gene in Acute Myeloid Leukemia. *Jama*. 2010;304(24):2706-15.
35. Taussig DC, Vargaftig J, Miraki-Moud F, Griessinger E, Sharrock K, Luke T, et al. Leukemia-initiating cells from some acute myeloid leukemia patients with mutated nucleophosmin reside in the CD34- fraction. *Blood*. 2010;115(10):1976-84.
36. Heuser M, Sly LM, Argiropoulos B, Kuchenbauer F, Lai C, Weng A, et al. Modeling the functional heterogeneity of leukemia stem cells: Role of STAT5 in leukemia stem cell self-renewal. *Blood*. 2009;114(19):3983-93.
37. Somervaille TCP, Matheny CJ, Spencer GJ, Iwasaki M, Rinn JL, Witten DM, et al. Hierarchical Maintenance of MLL Myeloid Leukemia Stem Cells Employs a Transcriptional Program Shared with Embryonic Rather Than Adult Stem Cells. *Cell Stem Cell*. 2009;4(2):129-40.
38. Takagi S, Yoneyama A, Uchida N, Mitsuki T, Yuasa M. Proportion of CD34+ CD38-Leukemia Stem Cell Fraction Predicts Relapse Rate after Umbilical Cord Blood Transplantation in Acute Myeloid Leukemia with Adverse. *Blood*. 2017;130(1):5082.
39. Han L, Jorgensen JL, Brooks C, Shi C, Zhang Q, Nogueras Gonzalez GM, et al. Antileukemia efficacy and mechanisms of action of SL-101, a novel anti-CD123 antibody conjugate, in acute myeloid leukemia. *Clin. Cancer Res*. 2017;23(13):3385-3395.
40. Zeijlemaker W, Kelder A, Oussoren-Brockhoff YJM, Scholten WJ, Snel AN, Veldhuizen D, et al. A simple one-tube assay for immunophenotypical

- quantification of leukemic stem cells in acute myeloid leukemia. *Leukemia*. 2016;30(2):439-46.
41. Ehninger A, Kramer M, Röllig C, Thiede C, Bornhäuser M, Von Bonin M, et al. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. *Blood Cancer J*. 2014;4(6):e218.
 42. Walter RB, Appelbaum FR, Estey EH, Bernstein ID. Acute myeloid leukemia stem cells and CD33-targeted immunotherapy. *Blood*. 2012;119(26):6198-208.
 43. Jan M, Chao MP, Cha AC, Alizadeh AA, Gentlese AJ, Weissmana IL, et al. Prospective separation of normal and leukemic stem cells based on differential expression of TIM3, a human acute myeloid leukemia stem cell marker. *Proc. Natl. Acad Sci. U S A*. 2011;108(12):5009-14.
 44. Kikushige Y, Shima T, Takayanagi SI, Urata S, Miyamoto T, Iwasaki H, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell*. 2010;7(6):708-17.
 45. Marschitz I, Tinhofer I, Hittmair A, Egle A, Kos M, Greil R. Analysis of Bcl-2 Protein Expression in Chronic Lymphocytic Leukemia. *Am. J. Clin. Pathol*. 2000;113(2):219-29.
 46. Hosen N, Park CY, Tatsumi N, Oji Y, Sugiyama H, Gramatzki M, et al. CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. *Proc. Natl. Acad Sci. U S A*. 2007;104(26):11008-13.
 47. Du W, Hu Y, Lu C, Li J, Liu W, He Y, et al. Cluster of differentiation 96 as a leukemia stem cell-specific marker and a factor for prognosis evaluation in leukemia. *Mol. Clin. Oncol*. 2015;3(4): 833–838.
 48. Jaiswal S, Jamieson CHM, Pang WW, Park CY, Chao MP, Majeti R, et al. CD47 Is Upregulated on Circulating Hematopoietic Stem Cells and Leukemia Cells to Avoid Phagocytosis. *Cell*. 2009;138(2):271-85.
 49. Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD, et al. CD47 Is an Adverse Prognostic Factor and Therapeutic Antibody Target on Human Acute Myeloid Leukemia Stem Cells. *Cell*. 2009;138(2):286-99.

50. Moshaver B, van Rhenen A, Kelder A, van der Pol M, Terwijn M, Bachas C, et al. Identification of a Small Subpopulation of Candidate Leukemia-Initiating Cells in the Side Population of Patients with Acute Myeloid Leukemia. *Stem Cells*. 2008; 26(12):3059-67.
51. Van Rhenen A, Van Dongen GAMS, Kelder A Le, Rombouts EJ, Feller N, Moshaver B, et al. The novel AML stem cell-associated antigen CLL-1 aids in discrimination between normal and leukemic stem cells. *Blood*. 2007;110(7):2659-66.
52. Cheung AMS, Wan TSK, Leung JCK, Chan LYY, Huang H, Kwong YL, et al. Aldehyde dehydrogenase activity in leukemic blasts defines a subgroup of acute myeloid leukemia with adverse prognosis and superior NOD/SCID engrafting potential. *Leukemia*. 2007;21(7):1423-30.
53. Ran D, Schubert M, Pietsch L, Taubert I, Wuchter P, Eckstein V, et al. Aldehyde dehydrogenase activity among primary leukemia cells is associated with stem cell features and correlates with adverse clinical outcomes. *Exp. Hematol*. 2009; 37(12):1423-34.
54. Cox C V, Diamanti P, Evely RS, Kearns PR, Blair A. Expression of CD133 on leukemia-initiating cells in childhood ALL. *Blood*. 2009;113(14):3287-96.
55. Chung SS, Eng WS, Hu W, Khalaj M, Garrett-Bakelman FE, Tavakkoli M, et al. CD99 is a therapeutic target on disease stem cells in myeloid malignancies. *Sci. Transl. Med*. 2017;9(374):eaaj2025.
56. Saito Y, Kitamura H, Hijikata A, Tomizawa-Murasawa M, Tanaka S, Takagi S, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci. Transl. Med*. 2010;2(17):17ra9.
57. Kinstrie R, Horne GA, Morrison H, Moka HA, Cassels J, Dunn K, et al. CD93 Is a Novel Biomarker of Leukemia Stem Cells in Chronic Myeloid Leukemia. *Blood* 2015;126 (23): 49.
58. Mannelli F. Immunophenotyping of Acute Leukemias – From Biology to Clinical Application. *Flow Cytometry-Select Topics*. InTech. 2016.

59. Testa U, Riccioni R, Militi S, Coccia E, Stellacci E, Samoggia P, et al. Elevated expression of IL-3R α in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. *Blood*. 2002;100 (8): 2980–2988.
60. Al-Mawali A, Gillis D, Lewis I. Immunoprofiling of leukemic stem cells CD34+/CD38-/CD123+ delineate FLT3/ITD-positive clones. *J. Hematol. Oncol.* 2016;9:61.
61. van Rhenen A, Moshaver B, Kelder A, Feller N, Nieuwint AWM, Zweegman S, et al. Aberrant marker expression patterns on the CD34+CD38- stem cell compartment in acute myeloid leukemia allows to distinguish the malignant from the normal stem cell compartment both at diagnosis and in remission. *Leukemia*. 2007;21(8):1700-7.
62. Saito Y, Uchida N, Tanaka S, Suzuki N, Tomizawa-Murasawa M, Sone A, et al. Induction of cell cycle entry eliminates human leukemia stem cells in a mouse model of AML. *Nat. Biotechnol.* 2010;28(3):275-80.
63. Taussig DC, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K, Ridler C, et al. Anti-CD38 antibody - Mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. *Blood*. 2008;112(3):568-75.
64. Gerber JM, Smith BD, Ngwang B, Zhang H, Vala MS, Morsberger L, et al. A clinically relevant population of leukemic CD34 -CD38 - cells in acute myeloid leukemia. *Blood*. 2012;119(15):3571-7.
65. Weinstein JN, Collisson EA, Mills GB, Shaw KRM, Ozenberger BA, Ellrott K, et al. The cancer genome atlas pan-cancer analysis project. *Nature Genetics*. 2013; 45,1113–1120.
66. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129 (4): 424–447.
67. Breems DA, Van Putten WLJ, Huijgens PC, Ossenkoppele GJ, Verhoef GEG, Verdonck LF, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J. Clin. Oncol.* 2005;23(9):1969-78.

68. Liu J, Qin YZ, Yang S, Wang Y, Chang YJ, Zhao T, et al. Meis1 is critical to the maintenance of human acute myeloid leukemia cells independent of MLL rearrangements. *Ann. Hematol.* 2017; 96(4):567-574.
69. Zhang L, Hofmann S, Bullinger L, Goetz M, Wiesneth M, Rojewski M, et al. Efficiency of Leukemic Stem Cell Separation From Patients with Acute Myeloid Leukemia. *Blood.* 2011;118(21):4997.
70. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *New England Journal of Medicine.* 2015;373:1136-1152.
71. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, et al. Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood.* 2010;115(3):453-74.
72. Aljurf M, Chaudhri NA, Almhareb F, Walter CU, Nounou R, Khalil S, et al. CD34 Expression in Adult Acute Lymphoblastic Leukemia (ALL) Is a Favorable Prognostic Factor in Patients Treated with Stem Cell Transplant in First Remission. *Blood.* 2011;118(21):4091.
73. Basso G, Lanza F, Orfao A, Moretti S, Castoldi G. Clinical and biological significance of CD34 expression in acute leukemia. *J. Biol. Regul. Homeost. Agents.* 2001;15(1):68-78.
74. Rowley JD. Chromosomal translocations: Revisited yet again. *Blood.* 2008; 112(6):2183–9.
75. Ley TJ, Miller C, Ding L, Raphael BJ, Mungall, AJ, Robertson AG, Hoadley K, et al. Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. *N. Engl. J. Med.* 2013;368(22):2059–74.
76. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-405.
77. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edition, *WHO*

Classification of Tumours. 2017.

78. Swerdlow SH, Campo E, Pileri SA, Lee Harris N, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127(20):2375-2390.
79. Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. *Blood*. 2017;129(12):1577-158.
80. Sarry JE, Murphy K, Perry R, Sanchez P V., Secreto A, Keefer C, et al. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD/SCID/IL2R γ c-deficient mice. *J. Clin. Invest*. 2011;121(1):384-95.
81. Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, Van Galen P, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat. Med*. 2011;17,1086–1093.
82. Zeijlemaker W, Kelder A, Wouters R, Valk PJM, Witte BI, Cloos J, et al. Absence of leukaemic CD34⁺ cells in acute myeloid leukaemia is of high prognostic value: A longstanding controversy deciphered. *Br. J. Haematol*. 2015;171(2):227-238.
83. Zhu HH, Liu YR, Jiang H, Lu J, Qin YZ, Jiang Q, et al. CD34 expression on bone marrow blasts is a novel predictor of poor prognosis independent of FLT3-ITD in acute myeloid leukemia with the NPM1-mutation. *Leuk. Res*. 2013;37(6):624-630.
84. Van Der Pol MA, Feller N, Roseboom M, Moshaver B, Westra G, Broxterman HJ, et al. Assessment of the normal or leukemic nature of CD34⁺ cells in acute myeloid leukemia with low percentages of CD34 cells. *Haematologica*. 2003;88(9):983-93.
85. Schuurhuis GJ, Meel MH, Wouters F, Min LA, Terwijn M, De Jonge NA, et al. Normal hematopoietic stem cells within the AML bone marrow have a distinct and higher ALDH activity level than co-existing leukemic stem cells. *PLoS One*. 2013; 8(11):e78897.
86. Quek L, Otto GW, Garnett C, Lhermitte L, Karamitros D, Stoilova B, et al. Genetically distinct leukemic stem cells in human CD34-acute myeloid leukemia are arrested at a hemopoietic precursor-like stage. *J. Exp. Med*. 2016;213(8):1513-35.

87. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N. Engl. J. Med.* 2005;352(3):254-66.
88. Boesch M, Reimer D, Rumpold H, Zeimet AG, Sopper S, Wolf D. DyeCycle violet used for side population detection is a substrate of P-glycoprotein. *Cytom. Part A.* 2012;81(6):517-22.
89. Richard V, Nair MG, Santhosh Kumar TR, Pillai MR. Side population cells as prototype of chemoresistant, tumor-initiating cells. *BioMed Research International.* 2013;2013:517237.
90. Wang Y, Yin C, Feng L, Ma L, Wei Y, Sheng G. Sorting, identification and enrichment of side population cells in THP-1 acute monocytic leukemia cells. *Oncol. Rep.* 2013;29(5):1923-1931.
91. Roshal M, Chien S, Othus M, Wood BL, Fang M, Appelbaum FR, et al. The proportion of CD34⁺ CD38^{low} or neg myeloblasts, but not side population frequency, predicts initial response to induction therapy in patients with newly diagnosed acute myeloid leukemia. *Leukemia.* 2013;27:728–731.
92. Feuring-Buske M, Hogge DE. Hoechst 33342 efflux identifies a subpopulation of cytogenetically normal CD34⁺CD38⁻ progenitor cells from patients with acute myeloid leukemia. *Blood.* 2001;97(12):3882-3889.
93. Liew E, Atenafu EG, Schimmer AD, Yee KWL, Schuh AC, Minden MD, et al. Outcomes of adult patients with relapsed acute lymphoblastic leukemia following frontline treatment with a pediatric regimen. *Leuk. Res.* 2012;36(12):1517-20.
94. Larson S, Stock W. Progress in the treatment of adults with acute lymphoblastic leukemia. *Current Opinion in Hematology.* 2008;15(4):400-7.
95. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *New England Journal of Medicine.* 2006;354:166-178.
96. Bernt KM, Armstrong SA. Leukemia Stem Cells and Human Acute Lymphoblastic Leukemia. *Semin. Hematol.* 2009;46(1):33-8.

97. Satake N, Duong C, Yoshida S, Davis R, Liu S, Van Dyke J, et al. Leukemia Stem Cells in Acute Lymphoblastic Leukemia: Unveiling Hierarchical Structure at Single Cell Resolution. *Blood*. 2014;124 (21):4786.
98. Yokota T, Kanakura Y. Genetic abnormalities associated with acute lymphoblastic leukemia. *Cancer Science*. 2016;107(6): 721–725.
99. Chiaretti S, Zini G, Bassan R. Diagnosis and subclassification of acute lymphoblastic leukemia. *Mediterranean Journal of Hematology and Infectious Diseases*. 2014; 6(1):e2014073.
100. Pui CH, Hancock ML, Head DR, Rivera GK, Look AT, Sandlund JT, et al. Clinical significance of CD34 expression in childhood acute lymphoblastic leukemia. *Blood*. 1993;82(3):889-94.
101. Thomas X, Archimbaud E, Charrin C, Magaud JP, Fiere D. CD34 expression is associated with major adverse prognostic factors in adult acute lymphoblastic leukemia. *Leukemia*. 1995;9(2):249-53.
102. Blasi F, Bruckmann C, Penkov D, Dardaei L. A tale of TALE, PREP1, PBX1, and MEIS1: Interconnections and competition in cancer. *BioEssays*. 2017;39(5).
103. Schulte D, Geerts D. MEIS transcription factors in development and disease. *Development*. 2019;146:16.
104. Moskow JJ, Bullrich F, Huebner K, Daar IO, Buchberg AM. Meis1, a PBX1-related homeobox gene involved in myeloid leukemia in BXH-2 mice. *Mol. Cell Biol*. 1995; 15(10):5434-43.
105. Rosales-Avía JA, Torres-Flores J, Aguilar-Lemarroy A, Gurrola-Díaz C, Hernández-Flores G, Ortiz-Lazareno PC, et al. MEIS1, PREP1, and PBX4 are differentially expressed in acute lymphoblastic leukemia: Association of MEIS1 expression with higher proliferation and chemotherapy resistance. *J. Exp. Clin. Cancer Res*. 2011;30:112.
106. Steelman S, Moskow JJ, Muzynski K, North C, Druck T, Montgomery JC, et al. Identification of a conserved family of Meis1-related homeobox genes. *Genome Res*. 1997;7:142-15.

107. Sitwala K V, Dandekar MN, Hess JL. HOX proteins and leukemia. *Int. J. Clin. Exp. Pathol.* 2008;1(6): 461–474.
108. Shen WF, Montgomery JC, Rozenfeld S, Moskow JJ, Lawrence HJ, Buchberg AM, et al. AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. *Mol. Cell Biol.* 1997;17(11): 6448–6458.
109. Geerts D, Schilderink N, Jorritsma G, Versteeg R. The role of the MEIS homeobox genes in neuroblastoma. *Cancer Letters.* 2003;197(1-2):87-92.
110. Jones TA, Flomen RH, Senger G, Nižeti D, Sheer D. The homeobox gene MEIS1 is amplified in IMR-32 and highly expressed in other neuroblastoma cell lines. *Eur J Cancer.* 2000;36(18):2368-74.
111. Spieker N, Van Sluis P, Beitsma M, Boon K, Van Schaik BDC, Van Kampen AHC, et al. The MEIS1 oncogene is highly expressed in neuroblastoma and amplified in cell line IMR32. *Genomics.* 2001;71(2):214-21.
112. Kroon E, Kros J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageau G. Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *Embo J.* 1998;17(13):3714–3725.
113. Dekel B, Metsuyanin S, Schmidt-Ott KM, Fridman E, Jacob-Hirsch J, Simon A, et al. Multiple imprinted and stemness genes provide a link between normal and tumor progenitor cells of the developing human kidney. *Cancer Res.* 2006; 66(12):6040-9.
114. Crijns APG, de Graeff P, Geerts D, ten Hoor KA, Hollema H, van der Sluis T, et al. MEIS and PBX homeobox proteins in ovarian cancer. *Eur. J. Cancer.* 2007; 43(17):2495-505.
115. Okumura K, Saito M, Isogai E, Aoto Y, Hachiya T, Sakakibara Y, et al. Meis1 regulates epidermal stem cells and is required for skin tumorigenesis. *PLoS One.* 2014; 9(7): e102111.
116. Aksoz M, Turan RD, Albayrak E, Kocabas F. Emerging Roles of Meis1 in Cardiac Regeneration, Stem Cells, and Cancer. *Curr. Drug Targets.* 2018;19(2):181-190.

117. Kocabas F, Mahmoud AI, Sosic D, Porrello ER, Chen R, Garcia JA, et al. The hypoxic epicardial and subepicardial microenvironment. *J. Cardiovasc. Transl. Res.* 2012; 5(5):654-65.
118. Kocabas F, Xie L, Xie J, Yu Z, DeBerardinis RJ, Kimura W, et al. Hypoxic metabolism in human hematopoietic stem cells. *Cell. Biosci.* 2015;5:39.
119. Kocabas F, Zheng J, Thet S, Copeland NG, Jenkins NA, DeBerardinis RJ, et al. Meis1 regulates the metabolic phenotype and oxidant defense of hematopoietic stem cells. *Blood.* 2012;120(25):4963-72.
120. Kocabas F, Zheng J, Zhang C, Sadek H, Sadek HA. Metabolic Characterization of Hematopoietic Stem Cells. *Methods in Mol. Biol.* 2014:155-164.
121. Mahmoud AI, Kocabas F, Muralidhar SA, Kimura W, Koura AS, Thet S, et al. Meis1 regulates postnatal cardiomyocyte cell cycle arrest. *Nature.* 2013;497(7448):249-253.
122. Imamura T, Morimoto A, Takanashi M, Hibi S, Sugimoto T, Ishii E, et al. Frequent co-expression of HoxA9 and Meis1 genes in infant acute lymphoblastic leukaemia with MLL rearrangement. *Br. J. Haematol.* 2002;119(1):119-21.
123. Pineault N, Helgason CD, Lawrence HJ, Humphries RK. Differential expression of Hox, Meis1, and Pbx1 genes in primitive cells throughout murine hematopoietic ontogeny. *Exp. Hematol.* 2002;30(1):49-57.
124. Argiropoulos B, Yung E, Humphries RK. Unraveling the crucial roles of Meis1 in leukemogenesis and normal hematopoiesis. *Genes and Development.* 2007; 21(22):2845-9.
125. Hisa T, Spence SE, Rachel RA, Fujita M, Nakamura T, Ward JM, et al. Hematopoietic, angiogenic and eye defects in Meis1 mutant animals. *Embo J.* 2004; 23(2):450-9.
126. Crijns APG, de Graeff P, Geerts D, ten Hoor KA, Hollema H, van der Sluis T, et al. MEIS and PBX homeobox proteins in ovarian cancer. *Eur. J. Cancer.* 2007 43(17):2495–2505.

127. Takubo K, Goda N, Yamada W, Iriuchishima H, Ikeda E, Kubota Y, et al. Regulation of the HIF-1 α level is essential for hematopoietic stem cells. *Cell Stem Cell*. 2010; 7(3):391-402.
128. Eliasson P, Rehn M, Hammar P, Larsson P, Sirenko O, Flippin LA, et al. Hypoxia mediates low cell-cycle activity and increases the proportion of long-term-reconstituting hematopoietic stem cells during in vitro culture. *Exp. Hematol*. 2010; 38(4):301-310.e2.
129. Kawagoe H, Humphries RK, Blair A, Sutherland HJ, Hogge DD. Expression of HOX genes, HOX cofactors, and MLL in phenotypically and functionally defined subpopulations of leukemic and normal human hematopoietic cells. *Leukemia*. 1999;13(5):687-98.
130. Thorsteinsdottir U, Kroon E, Jerome L, Blasi F, Sauvageau G. Defining Roles for HOX and MEIS1 Genes in Induction of Acute Myeloid Leukemia. *Mol. Cell Biol*. 2001;21(1):224-34.
131. Wong P, Iwasaki M, Somervaille TCP, So CWE, Cleary ML. Meis1 is an essential and rate-limiting regulator of MLL leukemia stem cell potential. *Genes Dev*. 2007; 21(21):2762-74.
132. Rozovskaia T, Feinstein E, Mor O, Foa R, Blechman J, Nakamura T, et al. Upregulation of Meis1 and HoxA9 in acute lymphocytic leukemias with the t(4 : 11) abnormality. *Oncogene*. 2001;20(7):874-8.
133. Fischbach NA, Rozenfeld S, Shen W, Fong S, Chrobak D, Ginzinger D, et al. HOXB6 overexpression in murine bone marrow immortalizes a myelomonocytic precursor in vitro and causes hematopoietic stem cell expansion and acute myeloid leukemia in vivo. *Blood*. 2005;105(4):1456-66.
134. Pineault N, Abramovich C, Ohta H, Humphries RK. Differential and Common Leukemogenic Potentials of Multiple NUP98-Hox Fusion Proteins Alone or with Meis1. *Mol. Cell Biol*. 2004;24(5):1907-17.
135. Collins CT, Hess JL. Role of HOXA9 in leukemia: Dysregulation, cofactors and essential targets. *Oncogene*. 2016;35(9):1090-8.

136. Nakamura T, Largaespada DA, Shaughnessy JD, Jenkins NA, Copeland NG. Cooperative activation of Hoxa and Pbx1-related genes in murine myeloid leukaemias. *Nat Genet.* 1996;12:149–153.
137. Shen W-F, Rozenfeld S, Kwong A, Kömüves LG, Lawrence HJ, Largman C. HOXA9 Forms Triple Complexes with PBX2 and MEIS1 in Myeloid Cells. *Mol. Cell Biol.* 1999;19(4):3051-61.
138. Guitart A V., Panagopoulou TI, Villacreces A, Vukovic M, Sepulveda C, Allen L, et al. Fumarate hydratase is a critical metabolic regulator of hematopoietic stem cell functions. *J. Exp. Med.* 2017;214(3):719-735.
139. Ferreira HJ, Heyn H, Vizoso M, Moutinho C, Vidal E, Gomez A, et al. DNMT3A mutations mediate the epigenetic reactivation of the leukemogenic factor MEIS1 in acute myeloid leukemia. *Oncogene.* 2016;35(23):3079-82.
140. Zhou J, Zhang X, Wang Y, Guan Y. PU.1 affects proliferation of the human acute myeloid leukemia U937 cell line by directly regulating MEIS1. *Oncol. Lett.* 2015; 10(3):1912-1918.
141. Celada A, Borràs FE, Soler C, Lloberas J, Klemsz M, Van Beveren C, et al. The transcription factor PU.1 is involved in macrophage proliferation. *J. Exp. Med.* 1996;184:61-69.
142. Adamaki M, Lambrou GI, Athanasiadou A, Vlahopoulos S, Papavassiliou AG, Moschovi M. HOXA9 and MEIS1 gene overexpression in the diagnosis of childhood acute leukemias: Significant correlation with relapse and overall survival. *Leuk. Res.* 2015;39(8):874-82.
143. Guzman ML, Jordan CT. Considerations for Targeting Malignant Stem Cells in Leukemia. *Cancer Control.* 2004;11(2):97-104.
144. Rowley JD. The role of chromosome translocations in leukemogenesis. *Semin Hematol.* 1999;36(4 Suppl 7):59-72.
145. Ernst P, Wang J, Korsmeyer SJ. The role of MLL in hematopoiesis and leukemia. *Current Opinion in Hematology.* 2002;9(4):282-7.

146. Daser A, Rabbitts TH. The versatile mixed lineage leukaemia gene MLL and its many associations in leukaemogenesis. *Seminars in Cancer Biology*. 2005;15(3):175-88.
147. Aoki Y, Watanabe T, Saito Y, Kuroki Y, Hijikata A, Takagi M, et al. Identification of CD34 + and CD34 - Leukemia-initiating cells in MLL-rearranged human acute lymphoblastic Leukemia. *Blood*. 2015;125(6):967-80.
148. Armstrong SA, Staunton JE, Silverman LB, Pieters R, Den Boer ML, Minden MD, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat. Genet*. 2002;30:41–47.
149. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell*. 2002;1(2):133-43.
150. Ferrando AA, Armstrong SA, Neuberg DS, Sallan SE, Silverman LB, Korsmeyer SJ, et al. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: Dominance of HOX dysregulation. *Blood*. 2003;102(1):262-8.
151. Yu BD, Hess JL, Horning SE, Brown GAJ, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. *Nature*. 1995;378(6556):505-8.
152. Yu BD, Hanson RD, Hess JL, Horning SE, Korsmeyer SJ. MLL, a mammalian trithorax-group gene, functions as a transcriptional maintenance factor in morphogenesis. *Proc. Natl. Acad. Sci. U.S.A*. 1998;95(18):10632-10636.
153. Milne TA, Dou Y, Martin ME, Brock HW, Roeder RG, Hess JL. MLL associates specifically with a subset of transcriptionally active target genes. *Proc. Natl. Acad. Sci. U.S.A*. 2005;102(41):14765-70.
154. Caslini C, Yang Z, El-Osta M, Milne TA, Slany RK, Hess JL. Interaction of MLL amino terminal sequences with menin is required for transformation. *Cancer Res*. 2007;67(15):7275–83.

155. Kohlmann A, Schoch C, Schnittger S, Dugas M, Hiddemann W, Kern W, et al. Molecular characterization of acute leukemias by use of microarray technology. *Genes Chromosomes Cancer*. 2003;37(4):396-405.
156. Tsutsumi S, Taketani T, Nishimura K, Ge X, Taki T, Sugita K, et al. Two distinct gene expression signatures in pediatric acute lymphoblastic leukemia with MLL rearrangements. *Cancer Res*. 2003;63(16):4882-7.
157. Krivtsov AV, Twomey D, Feng Z, Stubbs MC, Wang Y, Faber J, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. *Nature*. 2006;442:818-822.
158. Somervaille TCP, Cleary ML. Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia. *Cancer Cell*. 2006;10(4):257-68.
159. Swansbury GJ, Slater R, Bain BJ, Moorman a V, Secker-Walker LM. Hematological malignancies with t(9;11)(p21-22;q23)--a laboratory and clinical study of 125 cases. European 11q23 Workshop participants. *Leukemia*. 1998;12(5):792-800.
160. Ozeki K, Kiyoi H, Hirose Y, Iwai M, Ninomiya M, Kodera Y, et al. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. *Blood*. 2004;103(5):1901-8.
161. Gary Gilliland D, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood*. 2002;100(5):1532-42.
162. Drexler HG. Expression of FLT3 receptor and response to FLT3 ligand by leukemic cells. *Leukemia*. 1996;10(4):588-99.
163. Meierhoff G, Dehmel U, Gruss HJ, Rosnet O, Birnbaum D, Quentmeier H, et al. Expression of FLT3 receptor and FLT3-ligand in human leukemia-lymphoma cell lines. *Leukemia*. 1995;9(8):1368-72.
164. Al-Mawali A, Gillis D, Lewis I. Characteristics and prognosis of adult acute myeloid leukemia with internal tandem duplication in the FLT3 gene. *Oman Med. J*. 2013; 28(6):432-440.

165. Roche J, Zeng C, Barón A, Gadgil S, Gemmill RM, Tigaud I, et al. Hox expression in AML identifies a distinct subset of patients with intermediate cytogenetics. *Leukemia*. 2004;18:1059–1063.
166. Morgado E, Phanie Albouhair S, Lavau C. Flt3 is dispensable to the Hoxa9/Meis1 leukemogenic cooperation. *Blood*. 2007;109 (9): 4020–4022.
167. Garcia-Cuellar MP, Steger J, Füller E, Hetzner K, Slany RK. Pbx3 and Meis1 cooperate through multiple mechanisms to support Hox-induced murine leukemia. *Haematologica*. 2015;100(7):905-913.
168. Wang GG, Pasillas MP, Kamps MP. Meis1 programs transcription of FLT3 and cancer stem cell character, using a mechanism that requires interaction with Pbx and a novel function of the Meis1 C-terminus. *Blood*. 2005;106(1):254-64.
169. Thorne RMW, Milne TA. Dangerous liaisons: Cooperation between Pbx3, Meis1 and Hoxa9 in leukemia. *Haematologica*. 2015;100(7):850-853.
170. Deynoux M, Sunter N, Hérault O, Mazurier F. Hypoxia and hypoxia-inducible factors in leukemias. *Frontiers in Oncology*. 2016;6:41.
171. Deeb G, Vaughan MM, McInnis I, Ford LA, Sait SNJ, Starostik P, et al. Hypoxia-inducible factor-1 α protein expression is associated with poor survival in normal karyotype adult acute myeloid leukemia. *Leuk. Res*. 2011;35(5):579-84.
172. Gao XN, Yan F, Lin J, Gao L, Lu XL, Wei SC, et al. AML1/ETO cooperates with HIF1 α to promote leukemogenesis through DNMT3a transactivation. *Leukemia*. 2015;29(8):1730-40.
173. Li Y, Ye D. Cancer Therapy By Targeting Hypoxia-Inducible Factor-1. *Curr. Cancer Drug Targets*. 2010;10(7):782-96.
174. Zhe N, Wang J, Chen S, Lin X, Chai Q, Zhang Y, et al. Heme oxygenase-1 plays a crucial role in chemoresistance in acute myeloid leukemia. *Hematology*. 2015; 20(7):384-91.
175. Forristal C, Brown A, Helwani F, Leukemia IW-, 2015 undefined. Hypoxia inducible factor (HIF)-2 α accelerates disease progression in mouse models of

- leukemia and lymphoma but is not a poor prognosis factor in human AML. *Leukemia*. 2015; 29:2075–2085.
176. Wang Y, Liu Y, Malek SN, Zheng P, Liu Y. Targeting HIF1 α eliminates cancer stem cells in hematological malignancies. *Cell Stem Cell*. 2011;8(4):399-411.
 177. Song K, Li M, Xu XJ, Xuan L, Huang GN, Song XL, et al. HIF-1 α and GLUT1 gene expression is associated with chemoresistance of acute myeloid leukemia. *Asian Pacific J. Cancer Prev*. 2014;15(4):1823-9.
 178. Tong H, Hu C, Zhuang Z, Wang L, Jin J. Hypoxia-inducible factor-1 α expression indicates poor prognosis in myelodysplastic syndromes. *Leuk. Lymphoma*. 2012; 53(12):2412-8.
 179. Rouault-Pierre K, Lopez-Onieva L, Foster K, Anjos-Afonso F, Lamrissi-Garcia I, Serrano-Sanchez M, Mitter R, et al. HIF-2 α protects human hematopoietic stem/progenitors and acute myeloid leukemic cells from apoptosis induced by endoplasmic reticulum stress. *Cell Stem Cell*. 2013;13(5):549-63.
 180. Wellman S, Guschmann M, Griethe W, Eckert C, Stackelberg A, Lottaz C, et al. Activation of the HIF pathway in childhood ALL, prognostic implications of VEGF. *Leukemia*. 2004;18(5):926-33.
 181. Giambra V, Jenkins CE, Lam SH, Hoofd C, Belmonte M, Wang X, et al. Leukemia stem cells in T-ALL require active Hif1 α and Wnt signaling. *Blood*. 2015; 125(25):3917-27.
 182. Zou J, Li P, Lu F, Liu N, Dai J, Ye J, et al. Notch1 is required for hypoxia-induced proliferation, invasion and chemoresistance of T-cell acute lymphoblastic leukemia cells. *J. Hematol. Oncol*. 2013;6(1):3.
 183. Kawada H, Kaneko M, Sawanobori M, Uno T, Matsuzawa H, Nakamura Y, et al. High Concentrations of L-Ascorbic Acid Specifically Inhibit the Growth of Human Leukemic Cells via Downregulation of HIF-1 α Transcription. *PLoS One*. 2013; 8(4):e62717.
 184. Zhang H, Li H, Xi HS, Li S. HIF1 α is required for survival maintenance of chronic myeloid leukemia stem cells. *Blood*. 2012;119(11):2595-2607.

185. Coltella N, Percio S, Valsecchi R, Cuttano R, Guarnerio J, Ponzoni M, et al. HIF factors cooperate with PML-RAR α to promote acute promyelocytic leukemia progression and relapse. *Embo Mol. Med.* 2014;6(5):640-50.
186. Simsek T, Kocabas F, Zheng J, Deberardinis RJ, Mahmoud AI, Olson EN, et al. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. *Cell Stem Cell.* 2010;7(3):380-90.
187. Suda T, Takubo K, Semenza GL. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell.* 2011;9(4): 298-310.
188. Kumar AR, Li Q, Hudson WA, Chen W, Sam T, Yao Q, et al. A role for MEIS1 in MLL-fusion gene leukemia. *Blood.* 2009;113(8):1756-8.
189. Wermuth PJ, Buchberg AM. Meis1-mediated apoptosis is caspase dependent and can be suppressed by coexpression of HoxA9 in murine and human cell lines. *Blood.* 2005;105(3):1222-30.
190. Vey N, Delaunay J, Martinelli G, Fiedler W, Raffoux E, Prebet T, et al. Phase I clinical study of RG7356, an anti-CD44 humanized antibody, in patients with acute myeloid leukemia. *Oncotarget.* 2016;7(22):32532-42.
191. Saygin C, Matei D, Majeti R, Reizes O, Lathia JD. Targeting Cancer Stemness in the Clinic: From Hype to Hope. *Cell Stem Cell.* 2019;24(1):25-40.
192. Plaks V, Kong N, Werb Z. The cancer stem cell niche: How essential is the niche in regulating stemness of tumor cells?. *Cell Stem Cell.* 2015;16(3):225-238.
193. Moriuchi M, Moriuchi H, Turner W, Fauci AS. Cloning and analysis of the promoter region of CXCR4, a coreceptor for HIV-1 entry. *J. Immunol.* 1997;159(9):4322-9.
194. Saini V, Marchese A, Majetschak M. CXC chemokine receptor 4 is a cell surface receptor for extracellular ubiquitin. *J. Biol. Chem.* 2010;285(20):15566-15576.
195. Uy GL, Rettig MP, Motabi IH, McFarland K, Trinkaus KM, Hladnik LM, et al. A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in

- relapsed or refractory acute myeloid leukemia. *Blood*. 2012;119(17):3917-24.
196. Collins CT, Hess JL. Deregulation of the HOXA9/MEIS1 axis in acute leukemia. *Current Opinion in Hematology*. 2016;23(4):354-61.
 197. Ding Y, Gao H, Zhang Q. The biomarkers of leukemia stem cells in acute myeloid leukemia. *Stem Cell Investigation*. 2017;4:19.
 198. Kuo YH, Bhatia R. Pushing the limits: Defeating leukemia stem cells by depleting telomerase. *Cell Stem Cell*. 2014;15(6):673-675.
 199. Wang S, Wang T, Li MZ, Cheng XL, Li XL. Expression of microRNA miR-34a inhibits leukemia stem cells and its metastasis. *Eur. Rev. Med. Pharmacol. Sci*. 2016; 20(13):2878-83.
 200. Thorsteinsdottir U, Kroon E, Jerome L, Blasi F, Sauvageau G. Defining Roles for HOX and MEIS1 Genes in Induction of Acute Myeloid Leukemia . *Mol. Cell Biol*. 2001;21(1):224-34.
 201. Fischbach NA, Rozenfeld S, Shen W, Fong S, Chrobak D, Ginzinger D, et al. HOXB6 overexpression in murine bone marrow immortalizes a myelomonocytic precursor in vitro and causes hematopoietic stem cell expansion and acute myeloid leukemia in vivo. *Blood*. 2005;105(4):1456-66.
 202. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring. *Science*. 1999; 286(5439):531-7.
 203. Moskowitz JJ, Bullrich F, Huebner K, Daar IO, Buchberg AM. Meis1, a PBX1-related homeobox gene involved in myeloid leukemia in BXH-2 mice. *Mol. Cell Biol*. 1995; 15(10): 5434–5443.
 204. Wang Y, Liu Y, Malek SN, Zheng P, Liu Y. Targeting HIF1 α eliminates cancer stem cells in hematological malignancies. *Cell Stem Cell*. 2011;8(4):399-411.
 205. Deeb G, Vaughan MM, McInnis I, Ford LA, Sait SNJ, Starostik P, et al. Hypoxia-inducible factor-1 α protein expression is associated with poor survival in normal karyotype adult acute myeloid leukemia. *Leuk. Res*. 2011;35(5):579-84.

206. Forristal CE, Brown AL, Helwani FM, Winkler IG, Nowlan B, Barbier V, et al. Hypoxia inducible factor (HIF)-2 α accelerates disease progression in mouse models of leukemia and lymphoma but is not a poor prognosis factor in human AML. *Leukemia*. 2015;29(10):2075-85.
207. Li Y, Ye D. Cancer Therapy By Targeting Hypoxia-Inducible Factor-1. *Curr. Cancer Drug Targets*. 2010;10(7):782-96.
208. Rouault-Pierre K, Hamilton A, Bonnet D. Effect of hypoxia-inducible factors in normal and leukemic stem cell regulation and their potential therapeutic impact. *Expert Opinion on Biological Therapy*. 2016;16:4.
209. Kiyoi H, Kawashima N, Ishikawa Y. FLT3 mutations in acute myeloid leukemia: Therapeutic paradigm beyond inhibitor development. *Cancer Science*. 2020;111(2):312–322.
210. Levis M. Midostaurin approved for FLT3-mutated AML. *Blood*. 2017;129(26):3403–3406.
211. Zhou JD, Zhang TJ, Xu ZJ, Gu Y, Ma JC, Li XX, et al. BCL2 overexpression: Clinical implication and biological insights in acute myeloid leukemia. *Diagn. Pathol*. 2019; 14(1):68.
212. Han L, Jorgensen JL, Brooks C, Shi C, Zhang Q, Nogueras Gonzalez GM, et al. Antileukemia efficacy and mechanisms of action of SL-101, a novel anti-CD123 antibody conjugate, in acute myeloid leukemia. *Clin. Cancer Res*. 2017; 23(13):3385-3395.
213. Zeijlemaker W, Kelder A, Oussoren-Brockhoff YJM, Scholten WJ, Snel AN, Veldhuizen D, et al. A simple one-tube assay for immunophenotypical quantification of leukemic stem cells in acute myeloid leukemia. *Leukemia*. 2016;30(2):439-46.
214. Ding Y, Gao H, Zhang Q. The biomarkers of leukemia stem cells in acute myeloid leukemia. *Stem Cell Investigation*. 2017;4:19.
215. Lagadinou ED, Sach A, Callahan K, Rossi RM, Neering SJ, Minhajuddin M, et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates

- quiescent human leukemia stem cells. *Cell Stem Cell*. 2013;12(3):329-41.
216. Wermuth PJ, Buchberg AM. Meis1-mediated apoptosis is caspase dependent and can be suppressed by coexpression of HoxA9 in murine and human cell lines. *Blood*. 2005;105(3):1222-30.
 217. Al-Mawali A, To LB, Gillis D, Hissaria P, Mundy J, Lewis I. The presence of leukaemia-associated phenotypes is an independent predictor of induction failure in acute myeloid leukaemia. *Int. J. Lab Hematol*. 2009;31(1):61-8.
 218. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat. Biotechnol*. 2007;25(11):1315-21.
 219. Terpstra W, Ploemacher RE, Prins A, Van Lom K, Pouwels K, Wognum AW, et al. Fluorouracil selectively spares acute myeloid leukemia cells with long-term growth abilities in immunodeficient mice and in culture. *Blood*. 1996;88(6):1944-50.
 220. Rosales-Avía JA, Torres-Flores J, Aguilar-Lemarroy A, Gurrola-Díaz C, Hernández-Flores G, Ortiz-Lazareno PC, et al. MEIS1, PREP1, and PBX4 are differentially expressed in acute lymphoblastic leukemia: Association of MEIS1 expression with higher proliferation and chemotherapy resistance. *J. Exp. Clin Cancer Res*. 2011;30:112.

APPENDIX A: ETHICAL APPROVAL FORM



T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1592

31/01/2019

Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makarna (Neslihan Meriç)

Anadolu Sağlık Merkezi Hastanesi Hematoloji Anabilim Dalı Prof. Dr. Zafer Gülbaş'ın sorumlu olduğu “**Lösemi Kök Hücrelerinde MEIS Ekspresyon Profiline Belirlenmesi ve MEIS İnhibitörlerinin Etkisinin** İncelenmesi” isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (**1556** kayıt Numaralı KAEK Başvuru Dosyası), Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **30.01.2019** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 944**).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı