NEW THERAPEUTIC TARGETS IN HUMAN ACUTE MYELOID LEUKEMIA

HÅKON REIKVAM

2012

Dissertation for the degree philosophiae doctor (PhD) at the University of Bergen

SCIENTIFIC ENVIRONMENT

This study was performed at the Division for Hematology, Institute of Medicine, University of Bergen and Department of Medicine, Haukeland University Hospital.

The work was founded by University of Bergen, Helse-Vest and the Norwegian Cancer Society.

ACKNOWLEDGMENTS

First and foremost my thanks go to my supervisor Øystein Bruserud. I am very grateful for his willingness to take a chance on me, for his believes in me and his tremendous support during this process. We are all impressed over his enormous work capacity, despite his many responsibilities both in clinical work, teaching and research; he still always has time for supervision.

Second, my gratitude goes to my co-supervisor Kimberly Joanne Hatfield. Her patience in teaching my things in the laboratory as well as data analyzing and graphical presentation has been exemplary. Although not always too optimistic, but ever realistic and thoroughly, she has dutifully and carefully proofread many manuscripts and made several comments for improvements.

Special thanks also to Tor Hervig for introducing me for scientific work and research in the field of hematology and transfusion medicine. His ever positive attitude, along with his knowledge and great network, have been a trough inspiration and help for me to start academically work.

The scientific environment at the Division of Hematology at the Institute of Medicine has been a fantastic place to spend as PhD-student. I am very grateful for my colleagues and very good friends in our research group; Elisabeth Ersvær, Hanne Fredly, Astrid Olsnes Kittang, Anita Ryningen, Kristin Paulsen Rye, Anne-Kristin Johannessen and Karen Marie Hagen. Without their support this work would never been realized. Special thanks to Kjell Petersen for introducing and helping me with bioinformatical analyzes. Thanks to all my other coauthors who have contributed to the work and made these thesis possible; Anne Margrethe Øyan, Karl Henning Kalland, Philippe Lasalle, Randi Hovland, Jørn Skavland, Bjørn Tore Gjertsen, Jerome Tamburini, Laury Poulain, Knut Anders Mosevoll, Guro Melve, Clara-Cecilie Günther, Malvin Sjo and Pål Tore Bentsen.

At last, but not a least, my thanks go to my family for supporting and encouraging me trough this process. My parents, Åse and Kjell-Olav, my brother Tore and sister Anne-Grete have through the whole life been the corner stones of my life. Finally, but most importantly, my wife Anette deserves all the praise she could get, for bearing with me and being a tremendous support.

Bergen- February 2012

Håkon Reikvam

TABLE OF CONTENTS

ACKNOWLEDGMENTS 4

TABLE OF CONTENTS 5

ABBREVIATIONS 7

LIST OF PAPERS 10

INTRODUCTION 11

- AML-EPIDEMIOLOGY AND ETIOLOGY 11
- CLINICAL PRESENTATION OF AML 11
- DIAGNOSIS AND CLASSIFICATION OF AML 12
- CYTOGENETIC ANALYSIS IN AML 14
- MOLECULAR GENETICS OF AML 15
- COVENTIONAL TREATMENT OF AML 22
- SPECIAL SITUATIONS IN THE TREATMENT OF AML 24
- THE BIOLOGY OF HUMAN AML CELLS: NEW THERAPEUTIC APPROACHES TARGETING INTRACELLULAR PATHWAYS 26
- THE BIOLOGY OF HUMAN AML CELLS: FUNCTION AND THERAPEUTIC TARGETING OF THE HEAT SHOCK PROTEIN SYSTEM 33
- THE BIOLOGY OF HUMAN AML CELLS: FUNCTION AND THERAPEUTIC TARGETING OF NF-KB MEDIATED INTRACELLULAR SIGNALING 40
- THE BONE MARROW MICROENVIRONMENT: NORMAL AND LEUKEMIC BONE MARROW 44
- THE BONE MARROW MICROENVIRONMENT: ANGIOGENESIS AND THE ANGIOREGULATORY NETWORK IN AML 47

AIMS OF THE THESIS 54

MATERIAL AND METHODOLOGICAL CONSIDERATIONS 55

SUMMARY OF THE RESULTS 59

GENERAL DISCUSSION 64

- AML HETEROGENEITY 64
- “ONE SIZE FITS ALL” – AML THERAPY REVISITED 64
- CURATIVE VS DISEASE STABILIZING AML THERAPY 65

ANTIANGIOGENIC THERAPY IN HUMAN AML 66

CONCLUDING REMARKS 67

REFERENCES 69

ABBREVIATIONS

4E-BP1	Eukaryotic initiation factor 4E-binding protein 1
ALL	Acute lymphoblastic leukemia
Allo-SCT	Allogeneic stem cell transplantation
AML	Acute myeloid leukemia
Ang	Angiopoietin
APL	Acute promyelocytic leukemia
ASXL1	Additional Sex Comb-Like 1
ATO	Arsenic trioxide
ATRA	All-trans retinoic acid
Auto-SCT	Autologous stem cell transplantation
BAALC	Brain and Acute Leukemia, Cytoplasmatic
CBF	Core binding factor
CEBPA	CCAAT enhancer binding protein alpha
CML	Chronic myeloid leukemia
CNS	Central nervous system
DIC	Disseminated intravascular coagulation
DNMT3A	DNA methyltransferase 3A
ERG	ETS-related gene
FAB	French-American-British
FISH	Fluorescence in situ hybridization
FLT3	FMS-like thyrosin kinase 3
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor
GPCR	G-protein coupled receptor
GVHD	Graft versus host disease
HDAC	Histone deactylase
HGF	Hepatocyte growth factor
HIF-1	Hypoxia-inducible factor-1

HSC	Hematopoietic stem cell
HSF	Heat shock transcription factor
HSP	Heat shock protein
IDH	Isocitrate dehydrogenase
IGF-1	Insulin-like growth factor-1
IKK	IκB kinase
IL	Interleukin
JAK	Janus kinase
LSC	Leukemic stem cell
MAP	Mitogen-activated protein
MDS	Myelodysplastic syndrome
MET	Mesenchymal-epithelial transition factor
MLL	Mixed-Lineage Leukemia
MM	Multiple myeloma
MMP	Matrix metalloprotease
MMPI	MMP inhibitor
MN1	Meningioma 1
MPD	Myeloproliferative disease
MRD	Minimal residual disease
mTOR	Mammalian target of rapamycin
MVD	Microvessel density
NF-κB	Nuclear factor κ B
NIK	NF-κB inducing kinase
NOS	NO synthase
NPM1	Nuclophosmin 1
P70S6K	N ribosomal protein S6 kinase
PDK1	Phosphoinositide-dependent kinase 1
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
PtdIns	Phosphatidylinositol

PV	Polycythemia vera
RIC	Reduced-intensity conditioning
RTK	Receptor tyrosine kinase
RUNX1	Runt-related transcription factor 1
SCF	Stem cell factor
STAT	Signal transducer and activator
TET2	Tet oncogene family member 2
TIMP	Tissue inhibitor of matrix metalloprotease
TKD	Tyrosine kinase domain
TKI	Tyrosine kinase inhibitor
TNF-α	Tumor necrosis factor-α
TP53	Tumor protein 53
VDA	Vascular disrupting agent
VEGF	Vascular endothelial growth factor
WBC	White blood cell count
WHO	World health organization
WT1	Wills tumor 1

LIST OF PAPERS

Paper I

Primary human acute myelogenous leukemia cells release matrix metalloproteases and their inhibitors: release profile and pharmacological modulation.

Reikvam H, Hatfield KJ, Oyan AM, Kalland KH, Kittang AO, Bruserud Ø.

European Journal of Haematology 2010 Mar; 84(3): 239-251

Paper II

Targeting the angiopoietin (Ang)/Tie-2 pathway in the crosstalk between acute myeloid leukaemia and endothelial cells: studies of Tie-2 blocking antibodies, exogenous Ang-2 and inhibition of constitutive agonistic Ang-1 release.

Reikvam H, Hatfield KJ, Lassalle P, Kittang AO, Ersvaer E, Bruserud Ø.

Expert Opin Investig Drugs 2010 Feb; 19(2): 169-183.

Paper III

Heat shock proteins expression profile for AML patients reveals a distinct signature strongly associated with FLT3 mutation status - consequences and potentials for pharmacological intervention.

Reikvam H, Hatfield KJ, Ersvær E, Hovland R, Skavalnd J, Gjertsen BT, Petersen K, Bruserud Ø.

British Journal of Haemtology 2012 Feb;156(4): 468-80.

Paper IV:

Angiogenic signature for AML patients and the possible effects of different pharmacological agents acting on the PI3K-mTOR pathway.

Reikvam H, Hatfield KJ, Tamurini J, Poulain L, Ersvær E, Ryningen A, Bruserud Ø.

Manuscript

Paper V:

The pretransplant serum cytokine profile in allogeneic stem cell recipients differ from healthy individuals and different profiles are associated with different risk of posttransplant complications

Reikvam H, Mosevoll KA, Melve G, Günther CC, Sjo M, Bentsen PT, Bruserud Ø.

Biol Blood Marrow Transplant. 2012 Feb; 18(2): 190-9.

INTRODUCTION

AML- EPIDEMIOLOGY AND ETIOLOGY

Acute myeloid leukemia (AML) is the most common myeloid malignancy; the median age at the time of diagnosis is 70 years and men have a slightly higher incidence (ratio 3:2) [1]. The etiology of the disease can be identified only for a minority of patients, even though several risk factors are known. Firstly, exposure to ionizing radiation increases the risk of cancer in general and especially of myeloid malignancies; this has been studied in detail among survivors of the atomic bombs in Japan at the end of the Second World War [2]. Radiation is probably also the cause of an increased incidence among cockpit crew [3]. Secondly, exposure to environment toxins is associated with AML; occupational benzene exposure is well established as a risk factor [4], and the benzene content in cigarette smoke may also explain the slight increase in AML incidence among smokers [5]. Thirdly, treatment of other malignancies with chemotherapy and/or radiotherapy increases the risk of a second malignancies, especially AML or myelodysplastic syndromes (MDS). Approximately 10-15% of AML cases are reported to be therapy related [6], and especially treatment with topoisomerase II inhibitors (e.g. doxorubicin, mitoxantrone and etoposide) and alkylating agents (e.g. cyklophosphamides and its derivates) seems to have a leukemogenic effect [6]. Finally, inherited genetic abnormalities (e.g. Downs syndrome, Li-Fraumeni syndrome, certain forms of congenital neutropenia) also increase the risk of AML.

CLINICAL PRESENTATION OF AML

The clinical signs and symptoms of AML are diverse and nonspecific, they are usually caused by the leukemic bone marrow infiltration or more seldom by infiltration in other organs. The leukemic cell population blocks normal hematopoiesis and thereby causes cytopenia. This will usually affect all three myeloid lineages and cause anemia leading to fatigue, neutropenia causing infections and thrombocytopenia causing hemorrhages. Leukemic infiltration of other organs is less common and can manifest as hepatospelenomegaly, lymphadenopathy, gingival hyperplasia or central nervous system

(CNS) symptoms. Hyperleukocytosis with leukostatsis can lead to acute multiorgan failure. Exceptional patients present as a myeloid sarcoma, i.e. a single extra medullar mass of leukemic cells.

DIAGNOSIS AND CLASSIFICATION OF AML

Although new diagnostic tools have been introduced during the last decades, the primary diagnosis of AML still rests on the morphological identification of leukemic blast in blood and/or bone marrow smears for most patients. These smears are examined after May-Grünwald-Giemsa or Wright-Giemsa staining. Bone marrow biopsy is not considered necessary for the diagnosis, but should be considered in patients with dry tap (punctio sicca). Leukemic blasts typical have a round-to-irregular nucleus, distinct nucleoli and very little fine granular cytoplasm. Auer rods could be seen in some cases. The diagnosis of acute leukemia requires the presence of >20% leukemic blast in the bone marrow, but for certain subtypes (e.g. t(8;21), t(16;16), inv(16;16)) this is not required [7]. The WHO criteria reduced the blast criteria from 30% in the FAB (French-American-British) classification to 20%, even though several investigators have argued that there may be a biological difference between patients with 20-30% and more than 30% myeloblasts in the bone marrow at the time of diagnosis [8].

The first subclassification in AML was the FAB-system based on leukemic cell morphology and histochemistry (Table 1). This system did not define prognostically relevant subsets except for the acute promyelocytic leukemia (APL) variant [9]. Morphological characteristics are still important in the more recent and generally accepted WHO classification (Table 2) [8], but this classification is in addition based on clinical characteristics (previous MDS or therapy related AML) and genetic abnormalities as will be described below (Table 2).

Table 1. The FAB classification based on AML cell morphology [1, 10].

Classification	Characteristics
M0	Immature blasts without signs of differentiation
M1	Without maturation
M2	Evidence of granulocyte differentiation
M3	Acute promyelocytic leukemia
M4	Acute myelomonocytic leukemia
M5	Acute monocytic leukemia
M6	Acute erythroleukemia
M7	Acute megakaryoblastic leukemia

Table 2. The WHO classification of AML [1, 8, 11]; a classification based on clinical, morphological and genetic abnormalities.

Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)
- AML with inv(16) or t(16;16)
- APL with t(15;17)
- AML with t(9;11)
- AML with t(6;9)
- AML with inv(3) or t(3;3)
- AML (megakaryoblastic) with t(1;22)
- AML with mutated NPM1
- AML with mutated CEBPA

Acute myeloid leukemia with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukemia, not otherwise specified
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic/monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

Acute leukemias of ambiguous lineage
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)
- Mixed phenotype acute leukemia with t(v;11q23)
- Mixed phenotype acute leukemia, B/myeloid
- Mixed phenotype acute leukemia, T/myeloid

Immunophenotyping is mandatory to distinguish between AML and acute lymphoblastic leukemia (ALL) [12]. Especially expression of the CD34 stem cell marker is important [13]; it represents an additional independent prognostic factor and differences in CD34 expression are associated with distinct gene expression profiles [14]. Conventional cytogenetic analysis is a mandatory screening strategy for detection of genetic abnormalities; at least 20 metaphases should be analyzed and abnormalities are then detected in approximately 55% of the patients. An alternative to conventional

cytogenetic analysis for detection of specific gene rearrangement is fluorescence in situ hybridization (FISH). Finally, molecular analyses should be used to detect specific mutations.

CYTOGENETIC ANALYSIS IN HUMAN AML

Cytogenetic abnormalities are found in approximately 55% of AML patients [11, 15-17], and the karyotype is one of the most powerful independent prognostic parameters in AML [17]. Loss or gain of chromosomes occurs due to unequal segregation of the chromosomes. The first translocation discovered in AML was the balanced translocation between chromosome 8 and 21; t(8;21) [18], and this genotype has distinct biological and clinical characteristics [7]. AML with t(8;21) or the t(16;16)/iv(16) have common characteristics and are often referred to as core binding factor (CBF)-AML. CBF-AML together with the APL variant characterized by t(15;17) have a favorable prognosis. On the other hand, an adverse outcome is especially seen with (i) two or more distinct autosomal chromosome monosomies, (ii) one single autosomal monosomy in the presence of structural abnormalities (referred to as monsomal karyotype) [19], and (iii) complex karyotype with at least three abnormalities [16]. It should also be emphasized that many patients have rare cytogenetic abnormalities, and the prognostic impact of such abnormalities is often uncertain [16]. A classification of cytogenetic abnormalities according to their prognostic impact is given in Table 3 [11].

Table 3. Karyotype and prognosis [11].

Favorable abnormalities
t(15;17), t(8,21), inv(16)/t(16;16)
Intermediate
Normal cytogenetics
Entities not classified as favorable or adverse
Adverse
abn(3q), inv(3)
add(5q), del(5q), –5,
–7, add(7q)/del(7q)
+8
t(6;11), t(10;11)
t(11q23) (excluding t(9;11) and t(11;19)
t(9;22)
–17/abn(17p)
Multiple (>3 abnormalities)
Monosomal karyotype

MOLECULAR GENETICS OF HUMAN AML

Gene mutation and deregulated gene expression allow the description of the genetic diversity within defined cytogenetic groups [20, 21]. This is of particular importance for the large group (45%) with normal cytogenetics [15]. Cytogenetic and molecular genetic abnormalities are not mutually exclusive and often coexist. The mutations can broadly be divided into class I and class II mutation. Class I comprises mutations that activate signal transduction pathways and therefore increase survival and proliferation of the affected cells. In contrast, class II mutations interfere with transcription factors that are important for hematopoietic cell differentiation. Table 4 summarized the most common cytogenetics and molecular genetics aberration in the two classes.

Table 4. Genes involved in class I and class II AML-associated mutations

Class I mutations	Class II mutations
FLT3-ITD FLT3-TKD KIT JAK NRAS KRAS	PML-RARA/t(15;17) AML-ETO/t(8;21) CBFB-MYH11/inv(16)-t(16;16) NPM1 CEBPA MLL RUNX1/AML

The "second hit" hypothesis is based on this classification, postulating that one class I mutation and one class II mutation are necessary for transformation to the complete malignant phenotype. Although this theory does not fit all AML cases, it can explain why a type I mutation often coexist with a type II mutation, e.g. KIT and AML-ETO [7] and FLT3-ITD and NPM1 [15]. The most important mutations will be described below and their characteristics are briefly summarized in Table 5.

ASXL1 mutations

The Additional Sex Comb-Like 1 (ASXL1) gene on chromosome 20q11.1 encodes a protein believed to be involved in chromatin modification and to act as a coactivator for the retinoic acid receptor [22]. The mutations occur in exon 12, with a frequency in AML probably between 5 and 10 % [22, 23]. The

mutation is associated with older age, male sex and secondary AML [22, 23]. The prognostic impact is negative, with inferior complete remission rates [23], and shorter overall survival [22].

BAALC expression level

The Brain And Acute Leukemia, Cytoplasmatic (BAALC) gene is located on chromosome band 8q23 [24]. The function of the BAALC protein is largely unknown, it is highly expressed in hematopoietic precursor cells as well as leukemic blasts and is down-regulated during differentiation [24]. High BAALC expression is a poor prognostic parameter in cytogenetically normal AML [24, 25].

CEBPA mutations

The transcription factor CCAAT enhancer binding protein alpha (CEBPA) is crucial for normal differentiation of granulocytes [26]. The two most important mutations are N-terminal frame-shift mutations and C-terminal in-frame insertions, and together they are observed in roughly 10% of AML patients either in the combination on separate alleles ($CEBPA^{double\text{-}mut}$) or as single mutation ($CEBPA^{single\text{-}mut}$). These patients are often classified as FAB subtype M1 or M2 and have normal cytogenetics. Patient with $CEBPA^{double\text{-}mut}$ seem to have a distinct gene expression profile [27-29], and a more favorable prognosis than patients with $CEBPA^{single\text{-}mut}$ [28, 29]. Both the gene expression profile and the favorable prognostic impact of $CEBPA^{double\text{-}mut}$ indicate that this AML subset is related to the CBF-AMLs [29]. Analysis of CEBPA mutational status has recently been suggested as a part of the routine clinical handling of AML patients [11].

DNMT3A mutations

A recent study described mutations in DNA methyltransferase 3A (DNMT3A) in 22% of AML patients [30]. This was confirmed in two recent studies, reporting a frequence of DNMT3A mutations of 18% [31, 32]. The mutation was absent in APL (t15;17) and CBF-AML, but had an increased frequency in patients with intermediate-risk cytogenetics [30]. The mutation seems to be associated with monocytic features [33] and seems to have an adverse prognostic impact [30-33].

ERG expression level

The ETS-related gene (ERG) is located at chromosome band 21p22 and is involved in regulation of proliferation, apoptosis and differentiation [34, 35]. High ERG expression in AML is associated with upregulation of genes involved in leukemogenesis [34]. Cytogenetically normal AML with high ERG expression has decreased remission rate, higher relapse rate and reduced overall survival [34, 35]. The adverse prognostic impact of high ERG expression is seen especially in patients with NPM1 mutation and normal cytogenetic [35].

FLT3 abnormalities

The FLT3 gene is localized on chromosome 13q12 and encodes a receptor tyrosine kinase (RTK) with an extracellular ligand-binding part and an intracellular catalytic unit [36]. FLT3 ligation leads to complex protein interactions by the intracellular domain [36] that induces a cascade of protein phosphorylation events in downstream targets including mitogen-activated protein (MAP) kinase, signal transducer and activator (STAT) molecules, phosphatidylinositol 3-kinase (PI3K) and AKT. FLT3 interacts with several other cytokines including stem cell factor (SCF), interleukin-3 (IL-3) and granulocyte macrophage colony stimulating factor (GM-CSF) in the regulation of phospholipid metabolism, gene transcription, proliferation and apoptosis of hematopoietic cells.

The most important AML-associated abnormalities of the FLT3 gene are the in frame internal tandem duplications (FLT3-ITD) [37]. These mutations consist of insertions of variable length in the juxtamedullar encoding part of the gene [38], they occur in approximately 25% of the patients [39] and in approximately 30% of patients with normal cytogenetics [40]. FLT3-ITD is associated with an adverse prognosis [39, 40], although this prognostic impact seems to depend on the size of the ITD [38]. Analysis of the FLT3 mutational status is regarded as mandatory in the clinical handling of AML patients [11].

The second type of FLT3 mutations is missense point mutations in the tyrosine kinase domain (TKD), most of them occurring in codon 835 and therefore referred to as D835 mutation. This mutation occurs in approximately 5 % of patients [15]. Although both FLT3-ITD and FLT3-TKD mutations cause constitutive receptor activation, they seem to differ in their downstream signaling events [41] and this is possibly the reason why they do not cluster together in gene expression analyses [20] and differ in their prognostic impact. While FLT3-ITD clearly is associated with a more unfavorable outcome, the

role of FLT3-TKD is controversial. This may have been confounded by the fact that FLT3-TKD often coexist with other mutations, especially the favorable NPM1 mutation [42], whereas patients with FLT3-TKD as the sole abnormality may have a poor outcome [40].

IDH mutations

The IDH1 and IDH2 genes encode two isoforms of isocitrate dehydrogenase (IDH); mutation of this gene was first detected by DNA sequencing of the whole genome of an AML patient [43], and the mutations were thereafter identified in 15 out of 187 patients [44]. Three later studies [45-47] demonstrated that the frequencies of IDH1 and IDH2 mutations were 7.6-9.6% and 3.0-7.0% respectively [46, 47]. Both IDH1 (14% of these patients) and IDH2 (19%) mutations are more frequent in patients with normal cytogenetics [45], and IDH1 mutations were in addition associated with NPM1 mutation [46, 47]. All these studies reported an adverse prognostic impact by both mutations [45-47].

JAK mutations

The janus kinase (JAK) genes encode non-receptor thyrosine kinases that can be divided in the four families JAK1, JAK2, JAK3 and TYK2 [48, 49]. These enzymes are important for activation of STAT molecules that are involved in both normal and leukemic hematopoiesis. The acquired JAK2 V617F mutation is frequently found in myeloproliferative neoplasms, especially polycythemia vera (PV). JAK 1 and JAK2 mutations are relatively uncommon and especially detected in AML secondary to myeloproliferative neoplasms [48, 49], whereas JAK3 mutations are detected especially in the rare variant acute megakaryoblastic leukemia (M7) [50]. The prognostic impact of these mutations is not completely known.

KIT mutations

The KIT-encoding gene is located at chromosome band 4q11 [51, 52]. KIT mutations are observed in approximately 30% of CBF-AML [7, 51, 52], the mutations may then have an adverse prognostic impact [52] and seem to be associated with a distinct gene expression signature and deregulation of the nuclear factor κB (NF-κB) pathway [53]. Specific small-molecule tyrosine kinase inhibitors (TKIs) are considered for the treatment of these patients [54].

RAS mutations

RAS oncogenes (N-RAS, K-RAS and H-RAS) encode a family of membrane-associated proteins that regulate signal transduction and are involved in regulation of proliferation, differentiation and apoptosis [55]. Mutations of RAS genes are predominantly found in codons 12, 13 and 61, and they lead to constitutive activity of the RAS proteins with uncontrolled proliferation and antiapoptotic signaling. Mutations in N-RAS are most frequent and appear in 10-15% of AML patients [15, 55-57], K-RAS mutation occurs in approximately 5% of patients [55] whereas mutated H-RAS is uncommon [55]. Both N-RAS and K-RAS can be associated with inv(16)/t(16;16) [56] and probably represent a "second hit" (see above). RAS mutations do not seem to have any prognostic impact in patients receiving intensive chemotherapy [56, 57].

MLL mutations

Molecular studies of the breakpoint regions of several translocations involving chromosomal band 11q23 identified the Mixed-Lineage Leukemia (MLL) gene [58]. This gene is also called HRX or ALL-1. The MLL protein is a part of a large molecular complex involved in nucleosomal remodeling and histone deacetylation and methylation [58]. MLL mutations occur in approximately 5% of AML cases [58], the frequency being slightly higher in cytogenetically normal AML (7%) [15], and in secondary and therapy-related AML [58]. MLL mutations are considered as an adverse prognostic parameter with reduced remission rates [59]. MLL is a stable genetic marker and may become useful for detection of minimal residual disease (MRD) in human AML [60].

MN1 expression level

The meningioma 1 (MN1) gene is located at chromosome band 22q11 [61, 62], and encodes a protein involved in transcriptional regulation. Overexpression of MN1 is often observed together with NPM1 wild-type and BAALC overexpression [61], high expression is associated with low remission rate and it is an independent adverse prognostic factor [61, 62].

NPM1 mutations

Nucleophosmin1 (NPM1) is a chaperone protein that shuttles between the nucleus and cytoplasm; it predominantly resides in the nucleolus and is involved in the regulation of multiple cellular functions

that possess both oncogenic and tumor-suppressor properties [63]. In 2005 somatic mutations in exon 12 of the NPM1 encoding gene were described; this abnormality leads to aberrant localization of the NPM1 protein in the cytoplasm, thus the designation NPMc+ AML [64]. NPMc+ AML is also characterized by unique global gene expression and microRNA signatures [65, 66].

NPM1 mutations are found in approximately 30-35% of all AML cases and up to 60% of patients with normal cytogenetics [64]. Patients with NPM1 mutations are twice as likely to have FLT3-ITD mutations as those without this abnormality [64], and NPMc+ AML is associated with a favorable prognosis in the absence of a coexisting FLT3-ITD [11, 64].

RUNX1/AML1 mutations

Runt-related transcription factor 1 (RUNX1, also called AML1) was initially identified from the breakpoint region of t(8;21) AML [7]. The encoded protein represents the alpha subunit of CBF and is involved in normal hematopoiesis. Chromosomal translocations involving this gene are well-documented in human leukemia [7]. In addition to translocations, specific mutations in the RUNX1 gene occur in approximately 5% of AML cases [67], and are probably most common in secondary AML [67, 68]. Although an initial study indicated inferior outcoume for patients with RUNX1 mutations [67], the total prognostic impact of these mutations remains to be established.

TET2 mutations

Mutations of the tet oncogene family member 2 (TET2) gene were recently described in different hematological malignancies [69]. The mutations are heterogeneous [69] and are detected in 8-24% of AML patients [69-71]. The biological effects and the prognostic impact of these mutations have to be further evaluated, but the first reports described no significant prognostic impact [71].

TP53 mutations

p53 is important in coordinating cellular responses to a wide range of stress factors. Inactivation of p53 through mutations in the tumor protein 53 (TP53)-encoding gene on chromosome 17p is detected in more than 50% of solid tumors [72]. However, p53 mutations are uncommon in hematologic malignancies and found only in approximately 5% of AML patients [73]. TP53 mutations in AML are associated with complex cytogenetic abnormalities, chemoresistance, high relapse rate and adverse

prognosis [73, 74]. Single TP53 deletion should therefore be regarded as high-risk aberrations in AML [73, 74].

WT1 mutations

The Wilms Tumor 1 (WT1) gene is located on chromosome 11p13, and mutated WT1 gene was first described in AML more than a decade ago [75]. WT1 is probably important in regulation of survival, proliferation and differentiation of hematopoietic cells [76]. The mutations occur in approximately 10% of adult patients and are most frequent in patients with normal cytogenetics [76]. The first report suggested an adverse prognostic impact [75], and this has later been confirmed [77, 78].

Table 5. A summary of the most common molecular genetic abnormalities in human AML.

GENE	DEFECT	APPROXIMATE OCCURRENCE	PROGNOSTIC IMPACT
ASXL1	Mutation	5-10%	Adverse
BAALC	Overexpression	-	Adverse
CEBPA	Double mutation	10%	Favorable
DNMT3A	Mutation	20%	Adverse
ERG	Overexpression	-	Adverse
FLT3	ITD-mutation	25-30%	Adverse
FLT3	TKD-mutation	5%	Adverse
IDH1/2	Mutation	10%	Adverse
JAK	Mutation	5%	Intermediate
KIT	Mutation	10%	Adverse in CBF-AML
K-RAS	Mutation	5%	Intermediate
MLL	Mutation	5%	Adverse
MN1	Overexpression	-	Adverse
NPM1	Insertion	30-35%	Favorable without FLT3-ITD
N-RAS	Mutation	10-15%	Intermediate
RUNX1/AML1	Mutation	5-10%	Intermediate
TET2	Mutation	10%	Intermediate
TP53	Mutation	5%	Adverse
WT1	Frame shift mutation	10%	Adverse

CONVENTIONAL TREATMENT OF AML

The primary objective for the intensive chemotherapy in AML is to induce remission (disease control) through the induction treatment and thereafter to prevent relapse from residual disease (consolidation therapy). The aim of induction therapy is thus to reduce the leukemia cell burden and reach complete hematological remission, i.e. <5% blasts in the bone marrow with absence of Auer rods and extramedullary disease, restoration of normal hematopoiesis with peripheral blood platelet count >100 x 10^9/l, neutrophil count >1,0 x 10^9/l and independence or red cell transfusions [11].

An antracycline, usually daunorubicin, combined with cytarabine is the cornerstone in the induction treatment. Antracycline is given for three days [11], while cytarabine is given as continuous infusion for seven days (100-200 mg/m^2/day), e.g. the 3+7 regimen. Antracyclines work both by inhibiting DNA synthesis and causing DNA damage, and one important side effect is their cardiotoxicity. Cytarabine is an antimetabolite that causes DNA damage and thereby triggering of programmed cell death. High-dose cytarabine in the induction regimen results in excessive toxicity without further therapeutic benefit [79]. Other antracyclines than daunorubicin have been evaluated but seem to be inferior when using equivalent doses [11, 80]. Attempts to improve patient outcome by using additional cytotoxic agents have also failed [80]. Finally, priming of the AML cells to cell-cycle specific agents through the administration of the hematopoietic growth factors G-CSF [81] or GM-CSF [82] has also been tried; some studies have reported promising results especially for subgroups of patients [81, 82], but this treatment is not recommended for routine clinical practice [11].

The consolidation therapy can be either conventional intensive chemotherapy or stem cell transplantation. Firstly, based on a large randomized clinical trial the standard treatment for younger patients has been based on highdose cytarabine (single doses 3 g/m^2) for younger patients below 60 years of age [83]. Secondly, autologous stem cell transplantation (auto-SCT) can also be included in the consolidation treatment. Finally, allogeneic stem cell transplantation (allo-SCT) is the most powerful antileukemic treatment. The stem cells from either a HLA-matched family or unrelated donor are transfused to the patient after initial intensive conditioning therapy. Antileukemic effects are then mediated both by the conditioning therapy and by immune-mediated graft-versus leukemia effects [1, 11, 84, 85]. However, due to the risk of transplant-related mortality patients and donors have to be

carefully selected. A reduced-intensity conditioning (RIC) regimen may offer an advantage for subsets of patients with increased risk of severe transplant-related complications, e.g. elderly patients [86].

Risk stratification is now used as a basis for deciding the consolidation treatment, the most important factors in standardized risk assessments being the response to the first induction cycle together with the cytogenetic analysis and mutation status especially for NPM1 and FLT3 and possibly also KIT and CEBPA [87]. The level of circulating blast cells also seems to be important at least in certain subsets of patients [88]. An example of risk stratification is given in Table 6.

Table 6. Risk assessment of patients with newly diagnosed AML based on cytogenetic analysis and selected gene mutations [11, 80, 87] (wt, wild type; $^{+}$ means mutated, $^{\text{double-mut}}$ means two mutated alleles).

Risk of chemoresistance or AML relapse	Cytogenetic analysis	Cytogenetics combined with molecular genetic analysis
Favorable prognosis	t(8;21) inv(16)/t(16;16) t(15;17)	Normal cytogenetics plus: - $NPM1^{+}$ and FLT3-wt - $CEBPA^{\text{double-mut}}$ and FLT3-wt
Intermediate prognosis	Normal cytogenetics t(9;11), +8, t(3;5) Other cytogenetic abnormalities not classified as favorable or adverse	KIT mutations in the presence of t(8;21) or inv(16)/t(16;16)
Adverse prognosis	Specific abnormalities: Inv(3)/t(3;3), t(6;9), t(v;11), -5, 5q-, -7, 7q- abnl(17p) Monosomal or complex karyotype	Intermediate risk cytogenetics plus $FLT3\text{-}ITD^{+}$

For patients below 60 years of age in the favorable risk group the consolidation therapy will often be repetitive cycles of high-dose cytarabine without auto-SCT or allo-SCT [89]. However, certain subsets of these patients seem to have an inferior prognosis, e.g. t(8;21) AML presenting with high levels of circulating blasts or possibly also with KIT mutations [52, 90]; allo-SCT may be considered for these patients [80, 87]. For the intermediate group consolidation therapy with high-dose cytarabine is still

widely used, but allo-SCT is regarded as superior especially for patient with low transplant risk (e.g. low age and no comorbidity) [91], and for patients harboring FLT3-ITD mutation [15, 92]. Finally, for the adverse group allo-SCT is superior to other regimen [89, 91]. Highdose cytarabine should here only be considered in the consolidation regime if it is impossible to find a matched related or unrelated donor, or if the risk of transplantation is considered too high [80].

SPECIAL SITUATIONS IN THE TREATMENT OF AML

Relapsed AML

Leukemia relapse after achievement of complete remission is usually seen within three years after diagnosis. The possibility to achieve a second remission after intensive conventional induction therapy depends on the time in remission until relapse, age, cytogenetics and whether a previously allo-SCT has been preformed, but the only realistic possibility of cure is an allo-SCT [93]. Clinical studies of intensive chemotherapy are few and often relatively small, and the treatment is therefore often based on local traditions without any consensus. Intermediate or highdose cytarabine is often used, alternative regimen include mitoxantrone or etoposide [11]. Previously allotransplanted patients may be offered a retransplantation if they have a late relapse [94]. If allo-SCT is impossible an auto-SCT may be an alternative [11]. However, for a major part of these patients the best alternative will be optimal palliative or eventually disease-stabilizing treatment.

AML secondary to previous chemotherapy or chronic myeloproliferative disease

AML can develop after earlier chemotherapy and/or radiotherapy, and these patients have an inferior outcome. Therapy-related AML is associated with high frequency of unfavorable cytogenetic abnormalities, the remission rate is relatively low and the risk of relapse is high [95]. According to the WHO classification the term secondary AML includes AML with myelodysplastic changes and AML developing after previous MDS or chronic myeloproliferative neoplasms. The incidence of secondary AML varies between different studies and it is associated with an adverse prognosis due to several factors, including high age, increased comorbidity and high frequencies of high-risk cytogenetic abnormalities. The treatment of secondary AML is in principle the same as for de novo AML, but these

patients are often more difficult to bring into remission, and further treatment should be carefully considered in the delicate balance between benefits and harms for the patient.

Acute promyelocytic leukemia (APL)

APL (FAB classification M3) is a distinct subtype of AML that is characterized by accumulation of promyelocytes in the bone marrow, the presentation is often characterized by rapid progression and disseminated intravascular coagulation (DIC). The leukemic cells usually have a balanced reciprocal translocation between chromosomes 15 and 17 t(15;17) that results in a fusion of the promyelocytic leukemia gene and the retinoic acid receptor α gene with a differentiation-inhibitory fusion protein [9]. Introduction of treatment with the vitamin A-derivative all-trans retinoic acid (ATRA) has dramatically improved the outcome, and APL is now classified as a low-risk AML variant [9]. ATRA therapy should be started immediately if the diagnosis is suspected and should continue until the possibility eventually is excluded [11]. ATRA is currently used in combination with conventional chemotherapy, arsenic trioxide (ATO) may represent a therapeutic alternative [9].

Hyperleukocytosis and tumor lysis syndrome

Hyperleukocytosis is often defined as a peripheral blood blast count exceeding 100 x 10^9/l and is present in 10-20% of newly diagnosed AML [96]. Hyperleukocytosis with leukostatsis; e.g. pulmonary infiltrates and/or retinal or cerebral hemorrhages, is a medical emergency. Tumor lysis syndrome is then a special problem at initiation of the chemotherapy; an alternative may be hydroxyurea at relatively high doses (50-60 mg/kg per day) as an initial cytoreductive therapy. The effect of leukapheresis is controversial because few clinical studies are available [96-98].

Myeloid sarcoma

Patients presenting with an isolated myeloid sarcoma, i.e. an extramedullary leukemic cell tumor, should be considered as having AML. The leukemic cells should be evaluated for genetic and immunophenotypic features to allow classification according to the WHO criteria. Myeloid sarcoma has been associated with the favorable cytogenetic abnormality t(8;21) [7], but whether the extramedullary disease by itself has a prognostic impact is still controversial. Patients should receive standard AML therapy possibly with additional local radiation therapy.

THE BIOLOGY OF HUMAN AML CELLS: NEW THERAPEUTIC APPROACHES TARGETING INTRACELLULAR PATHWAYS

Proliferation, differentiation, invasiveness and apoptosis of leukemic cells are partially regulated by external signals received from cytokines and by interactions with the local microenvironment. The response to these signals is, in turn, transmitted from the cell surface to the nucleus through the extensive series of signal transduction pathways. There is extensive cross-talk and cross-activation between these pathways, so that the activation of one pathway often leads to the activation of others [99]. The disruption of normal signaling through these pathways occurs as a result of either mutations of pathway components or alterations in the external signals received, e.g. from chemokines, cytokines or stroma [100, 101]. Conversely, many of these transduction pathways have emerged as potential target for pharmacological intervention in AML.

The PI3K pathway

PI3Ks are a family of related intracellular signal transducer enzymes involved in diverse cellular functions such as proliferation, differentiation, migration, survival and intracellular trafficking. The PI3Ks phosphorylate 3-hydroxyl groups of the inositol ring of three species of phosphatidylinositol (PtdIns) (Figure 1).

The PI3K family consists of three different classes based on structural features and lipid substrate preferences; class I, II and III, class I is further subdivided into class IA and IB (Table 7). The activation of the different classes of PI3K can be caused through various pathways, but the main activators are RTKs, G-protein coupled receptors (GPCRs), RAS (class IA), various cytokine receptors and integrins (class II). In contrast to the other PI3K the class III enzymes can be directly regulated by nutrients such as amino acids [102]; these enzymes are thereby linked to initiation of autophagy [103] and class III PI3K inhibitors have been considered as inhibitors of autophagy [104]. Class I PI3K frequently have activating mutations in solid tumors, but this is seldom in leukemia [105]. The main product of catalytic class I PI3K activity is PtdIns(3,4,5)P_3 (Table 7) that is rapidly converted to PtdIns(3,4)P_2 and PtdIns(4,5)P_2 by specific phosphatases [102]. These metabolites coordinate the function and localization of several protein kinases, including the central regulator AKT [102].

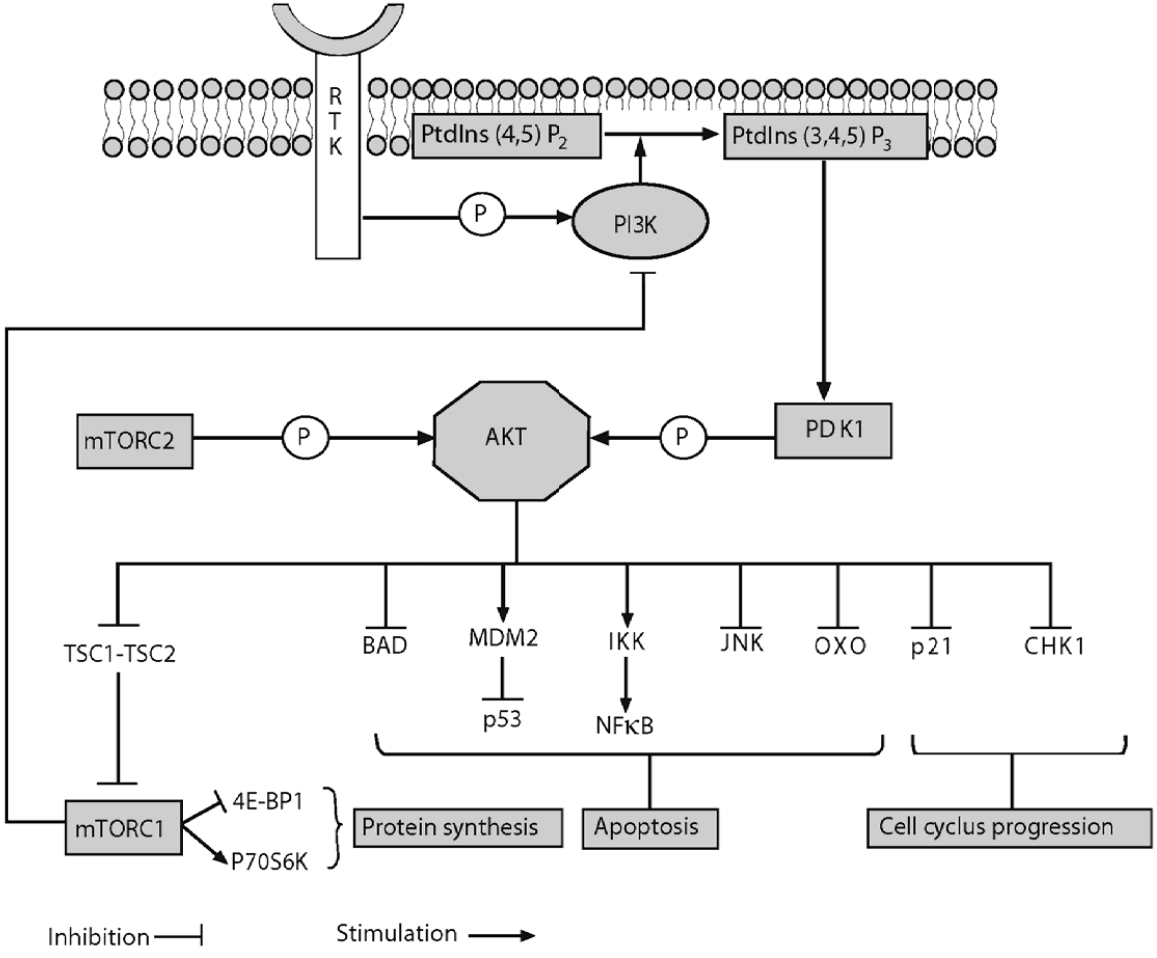
RTK
PtdIns (4,5) P2
PtdIns (3,4,5) P3
P
PI3K
mTORC2
P
AKT
P
PDK1
TSC1-TSC2
BAD
MDM2
IKK
JNK
OXO
p21
CHK1
p53
NFκB
mTORC1
4E-BP1
P70S6K
Protein synthesis
Apoptosis
Cell cyclus progression
Inhibition
Stimulation

Figure 1

The PI3K-Akt-mTOR signaling pathway.

Upstream activation of PI3K involve different mediators, however the common feature seems to be activation of a receptor thyrosin kinase (RTK). PI3Ks consist of three different classes based on their structural features and lipid substrate preferences (classes I-III), and these enzymes phosphorylate the 3-hydroxyl groups of the inositol ring of three species of phosphatidylinositol (PtdIns). The Class I enzymes are most extensively studied and their main product PtdIns(3,4,5)P_3 is rapidly converted to PtdIns(3,4)P_2 and PtdIns(4,5)P_2. These two last mediators stimulate phosphointerdependent kinase 1 (PDK1), and thereby regulate the function and localization of several protein kinases, including AKT (also referred to as Protein kinase B, PKB). AKT is a serine/threonine protein kinase important for regulation of cellular growth and survival. At the plasma membrane AKT is phosphorylated (i) at the catalytic domain (Thr308) by PDK1 and (ii) within the carboxyl terminal hydrophobic domain (Ser473) by the mammalian target of ramapamyin (mTOR) complex 2. Both these phosphorylations are required for maximal AKT activation.

More than 100 AKT substrates have been identified, and these substrates are important for the regulation of apoptosis and cell cycle progression. Among the most important substrates is mTOR, a serine/threonine kinase with a C-terminal homology to PI3K. mTOR exist as two complexes, referred to as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The interactions between PI3K-AKT and mTORC1 are more complex. mTORC1 facilitate an inhibitory effect on PI3K, and inhibition of this negative feedback loop can activate AKT.

Abbreviations: 4E-BP1, Eukaryotic initiation factor 4E-binding protein 1; BAD, Bcl-2 antagonist of cell death; CHK1, Checkpoint kinase 1; FOXO, The Forkhead transcription factors; IKK, Inhibitor of κB kinase; JNK, c-Jun N-terminal kinase; MDM2, Murine double minute 2; mTOR, mammalian target of rapamycin; NF-κB, Nuclear factor κ B; P70S6K, N ribosomal protein S6 kinase; PDK1, phosphoinositidedependent kinase 1; PI3K, Phosphatidylinositol 3-kinase; PtdIns, Phosphatidylinositol; RTK, Receptor thyrosin kinase; TCS, Tuberous Sclerosis Complex.

Table 7. Classification of PI3K isoforms in four families according to activation pathways, structure, lipid substrate preferences, catalytic product and main downstream targets. (PtdIns; Phosphatidylinositol, RTK; receptor tyrosine kinase, GPCR; G-protein coupled receptor).

Classes of PI3Ks	Main activator	Specific catalytic subunit	Regulatory subunit	Substrate	Product	Main downstream activation
Class IA	RTKs GPCRs RAS	p110α, 110β, 110δ	p50α, p55α, p55γ, p85α, p85α	PtdIns(4,5)P_2	PtdIns(3,4,5)P_3	Activation of AKT
Class IB	GPCRs	p110γ	p101	PtdIns(4,5)P_2	PtdIns(3,4,5)P_3	Activation of AKT
Class II	RTKs Cytokine receptors Integrins	PI3KC2α, PI3KC2β, PI3KC2γ		PtdIns PtdIns4P	PtdIns3P PtdIns(3,4)P_2	Mainly unknown
Class III	GPCRs Nutrition (amino acids, glucose)	VPS34		PtdIns	PtdIns3P	mTORC1 Autophagy

PI3K in AML

Deregulation of intracellular signaling together with transcriptional abnormalities contributes to leukemogenesis. The PI3K pathway is one such pathway that is frequently activated in human AML [106, 107]. The mechanisms leading to this PI3K/AKT activation are not completely understood. Mutation in the PI3K encoding gene itself is probably rare in AML [105], but other mechanisms causing activation may involve (i) activating mutation in the FLT3 [108] or KIT receptor [109] and RAS mutations [110]; (ii) autocrine/paracrine release of insulin-like growth factor (IGF-1) [111], vascular endothelial growth factor (VEGF) [112], hepatocyte growth factor (HGF) [113], angiopoietins (Angs) [114] or CXCL12 [115]; or (iii) activation of integrin-linked kinase 1 by neighboring stromal cells [116].

Results from studies on the prognostic impact of PI3K activation in AML are conflicting. A favorable outcome in overall AML-free survival in patients with constitutive PI3K activation has been reported [106], while two other studies found an adverse prognostic impact [99, 117].

Table 8. The main downstream targets of AKT and their most important functional effects; relevance to leukemogenesis in human AML

Important AKT substrates	AKT effect	Main function	Refs.
Bcl-2 antagonist of cell death (BAD)	Inhibition	Phosphorylation by AKT in AML cells inhibits its normal proapoptotic function	[118]
p21	Inhibition	Binds to and inhibits the activity of different cyclin complexes and thereby regulates cell cycle progression	[119]
Murine double minute 2 (MDM2)	Stimulation	Makes a complex with p53, a potential therapeutic target in AML	[120]
Inhibitor of κB kinase (IKK)	Stimulation	Curtail for activation of NF-κB through both the canonical and non-canonical and pathway	[121]
c-Jun N-terminal kinase (JNK)	Inhibition	A regulator of apoptosis, differentiation, proliferation and cytokine production and chemoresistance in AML	[122]
The Forkhead transcription factors (FOXO)	Inhibition	Apoptosis triggers translocation out of the nucleus on phosphorylation by AKT, phosphorylated FOXO3a is an independent adverse prognostic factor in AML	[123]
Checkpoint kinase 1 (CHK1)	Inhibition	CHK1 phosphorylation correlates with cell cycle arrest in AML	[124]
Tuberous Sclerosis Complex (TSC) 1/2	Inhibition	The main inhibitory complex for mTORC1 activation	[125]

AKT/protein kinase B

AKT or protein kinase B (PKB) is a 57 kDa serine/threonine protein kinase that is a critical regulator of many cellular functions including proliferation, viability and metabolism. The AKT kinase family is comprised of three highly homologous isoforms: AKT1 (PKBα), AKT2 (PKBβ) and AKT3 (PKBγ) [126]. The functions of the different isoforms are not completely overlapping and isoform-specific signaling probably contributes to the diversity of AKT activities [126]. AKT is a downstream target of PI3K and it is recruited to sites in the plasma membrane that contain increased PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 produced by PI3K (Figure 1). AKT is then phosphorylated at two distinct sites; (i) at the catalytic domain (Thr308) by phosphoinositide-dependent kinase 1(PDK1), and (ii) within the carboxyl terminal hydrophobic domain (Ser473) by the mammalian target of ramapamyin complex 2 (mTORC2). Both phosphorylation steps are needed for full AKT activation. AKT is one of the most frequently hyperactivated kinases in human cancers with more than 100 identified substrates (see Table 8) [127].

Oncogenic, activating mutations in AKT have been described in solid tumors, especially breast cancer [128], but they are probably very rare in human AML even though they can induce leukemia in an animal model [128]. The most important activation of AKT in AML is mediated downstream from PI3K. Activation of AKT, measured by phosphorylation of Ser473, seems to occur in 50-80 % of AML cases [107, 117, 129] and is associated with an adverse outcome [117, 130]. Due to this prognostic impact AKT is regarded as a possible therapeutic target in human AML.

The role of mTOR in intracellular signaling

mTOR is a serine/threonine kinase that has a C-terminal homology to PI3K and therefore belongs to the PI3K-related kinase family [131]. It has a key role in several signaling pathways both in normal and malignant hematopoiesis. mTOR functions as a sensor to ensure that the cell is in an appropriate nutritional and bioenergetic state, and it thereby supports cell growth by modulating a wide range of processes, including protein synthesis, ribosome activity and autophagy [131].

mTOR exists as two complexes referred to as mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC2 can phosphorylate the Ser473 residue on AKT and is thereby necessary for the maximal activity of AKT. The upstream activation of mTORC2 is mainly unknown, whereas the interactions between PI3K-AKT and mTORC1 are better characterized. Firstly, mTORC1 can be activated by PI3K through the downstream signaling via AKT and the TSC1-TSC2 complex [125] (Figure 1). Secondly, mTORC1 has an inhibitory effect on PI3K and inhibition of this negative feedback loop can thereby activate AKT [132]. Thirdly, mTORC1 can also be activated through signaling in other intracellular pathways [133]. Thus, the interactions between the two mTOR complexes and the AKT pathway are very complex and the final functional effects are difficult to predict.

The two best characterized downstream targets of mTORC1 are the ribosomal protein S6 kinase (P70S6K) and the eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) [132]. P70S6K is phosphorylated and activated by mTORC1 and is involved in ribosomal protein synthesis. In contrast, 4E-BP1 is inhibited by mTORC1. The function of 4E-BP1 is complex, one major effect being a negative regulator of the initiation of protein synthesis. Consequently, the main role of mTORC1 is stimulation of protein synthesis, and mTORC1 has therefore been linked also to autophagy [132].

The concomitant activation of PI3K/AKT and mTOR is frequently observed in several cancers [127], and mTOR activation seems to depend particularly on the PI3K/AKT pathway. PI3K/AKT is activated in 50-80% of AML cases [107, 117, 129], but mTOR is activated in almost all AML cases [134]. This indicates that additional activating pathways proably are involved, supported by the observation that PI3K inhibition does not entirely suppress mTOR activity [134]. One possible pathway is then the Src kinase Lyn that is frequently phosphorylated and activated in AML [135].

Pharmacological targeting of PI3K-AKT-mTOR

Several inhibitors of the PI3K-AKT-mTOR have been developed [136, 137]. Some of these inhibitors are specific for mTOR, PI3K or AKT, respectively. Other inhibitors are dual inhibitors of mTOR and PI3K (Table 9). The mTOR inhibitors everolimus (RAD001) and deforolimus (AP23573) have been evaluated as monotherapy in patient with relapsed or refractory hematological malignancies [138, 139], whereas sirolimus has been evaluated in combination with MEC (mitoxantrone, etoposide and cytarabine) in patients with relapsed and refractory AML [140]. The mTOR inhibitors were then generally well tolerated [137, 139], but the effect in combination-therapy [140] as weel as in mono-therapy seems to be limited [138, 139]. However, the effect may be more important in certain subsets of patients [138, 140]. Future studies are therefore warranted both to define patients eligible for this treatment and to determine the optimal dose and drug combination. This should also include the further evaluation of mTORC2 inhibitors [132].

A possible therapeutic strategy is to use mTOR inhibitors after allo-SCT. Rapamycin as well as other mTOR inhibitors have been used for graft versus host disease (GVHD) prophylaxis, and these drugs may then have an additional direct antileukemic effect. Preliminary clinical results are promising with low non-relapse mortality when combining tacrolimus and sirolimus for GVHD prophylaxis [141]. Microangiopathy is a possible side effect, and for this reason mTOR inhibitors should possibly be used with caution in patients receiving conditioning regimens that cause increased endothelial damage, e.g.busulfan/cyclophosphamide [141]. Finally, so far only specific mTOR inhibitors have been evaluated in clinical trials for AML, but other inhibitors of the PI3K/AKT/mTOR pathway are also considered, including specific PI3K inhibitors, AKT inhibitors and dual PI3K/mTOR inhibitors [142, 143].

Table 9.

Inhibitors of the PI3K-AKT-mTOR pathway currently included in clinical trials.

*) Evaluated or currently under evaluating in AML patients.

PI3K inhibitors		mTOR inhibitors	
BGT226	[144]	Rapamycin/Sirolimus*	[138, 141]
XL147	[145]	Everolimus/RAD001*	[139]
BKM-120*	[146]	Temsirolimus/CCI-779	[147]
GDC-0941	[148]	Deforolimus(AP23573)*	[140]
SF1126	[149]	Ridaforolimus (MK8669)	[150]
LY294002	[151]		
PX-866	[152]		
Dual mTOR/PI3K inhibitors		**AKT inhibitors**	
PI-103	[153, 154]	GSK2141795	[155]
NVP-BEZ235	[142, 156]	SR13668	[157]
PF-04691502	[158]	GSK690693	[159]
XL765(SAR245409)	[160]	MK-2206*	[161]
AZD8055	[162]	Perifosine	[143]

THE BIOLOGY OF HUMAN AML CELLS: FUNCTION AND THERAPEUTIC TARGETING OF THE HEAT SHOCK PROTEIN SYSTEM

Heat shock proteins (HSPs), also called stress proteins, represent a group of ubiquitous proteins that are expressed at low levels under normal physiological conditions, but increased production is triggered by exposure to environmental stress [163]. This is often referred to as the heat shock response [163]. This response can be triggered by malignant transformation, infections, inflammation, exposure to toxins, starvation, oxygen deprivation, nitrogen deficiency or water deprivation [164].

HSPs are usually cytoplasmic proteins, but there is also an increasing interest for a potential extracellular role of HSPs [165, 166]. They act as molecular chaperons together with a group of cochaperones, and play important roles in protein-protein interactions and assisting in proper protein conformation with prevention of unwanted protein aggregation. The HSPs are named according to their molecular weights, e.g. HSP60, HSP70 and HSP90 (Table 10). In contrast to HSP70 that controls folding of all newly synthesized proteins, HSP90 has a more restricted repertoire of client proteins mainly including various protein kinases [167, 168]. HSP90 seems to be important for cellular

proliferation, survival and adaptation to unfavorable microenvironments, and HSP90 inactivation results in inappropriate function and rapid degradation of its client proteins [169].

Table 10.

The most common HSPs and their main physiological functions

Heat shock protein	Main function
HSP27	Protection against protein aggregation, regulation of signaling in the apoptotic pathway, regulation of cell movement.
HSP60	Promotes efficient mitochondrial protein folding, the main function in cytosol is to induce folding of actin and tubulin.
HSP70	Stabilizes proteins prior to complete folding, regulates transport across membranes and proteolysis.
HSP90	Stabilizes kinases prior to complete folding or activation; forms stable complexes with several transcription factors.
HSP110	Dissociates protein aggregates, facilitates proteolysis, essential for thermotolerance.

HSP synthesis is strictly regulated both through (i) activation and nuclear translocation of the transregulatory heat shock transcription factors (HSFs), and (ii) at the level of translation of mRNA into protein [170]. Cells constitutively express HSF proteins, but these proteins do not induce HSP expression genes in the absence of stress reactions. In mammalian cells, HSF1 is maintained in the cytoplasmatic HSP90 complex as a monomer that lacks DNA binding activity [171]. This complex dissociates in response to stress and releases the HSF1 monomer (Figure 2). The monomer then forms a trimer capable of DNA binding [171].

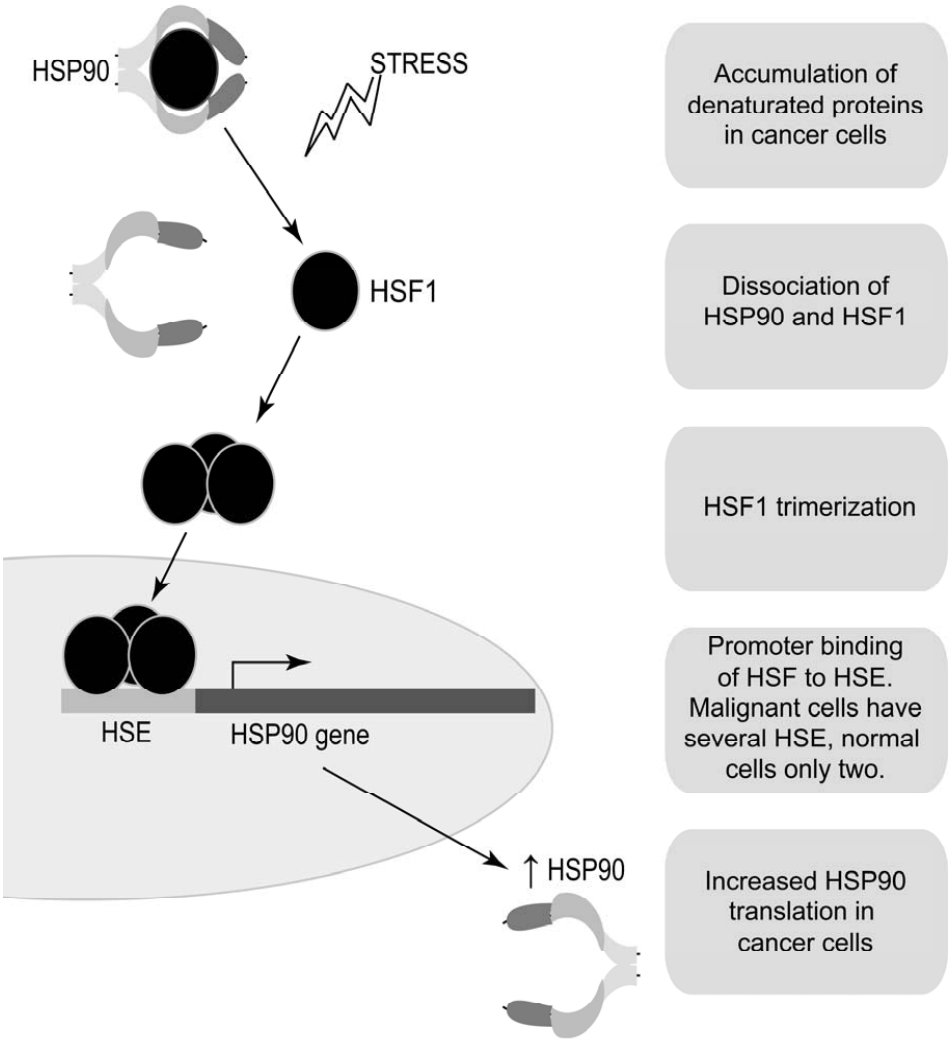

Figure 2

Activation of HSP90 transcription.

HSF1 is maintained in the cytoplasmatic HSP90 complex as a monomer that lacks DNA binding activity. This complex dissociates in response to accumulation of denaturated proteins, and releases the HSF1 monomer. The monomer then forms a trimer that translocates to the nucleus and binds to HSE. Transcription is initiated resulting in increased HSP90 translation in cancer cells.

Abbreviations: HSE, Heat shock element; HSF1, Heat shock transcription factor 1.

Exposure to anticancer agents can induce expression of HSP90 and thereby enhance chemoresistance and cellular recovery after this exposure. Molecular chaperones have been implicated in resistance to anticancer treatments; this is summarized in Table 11. Several HSP90 client proteins have been validated as possible therapeutic targets in cancer treatment, and HSP90 inhibition thereby makes it possible to target several intracellular pathways, including oncoprotein-initiated signaling [169]. Furthermore, HSP90 in malignant cells is often present entirely in multichaperone complexes with high ATPase activity, whereas HSP90 in normal tissues is present in a latent, uncomplexed state [172]. All these characteristics suggest that HSP90 should be regarded as a possible unique target for antileukemic therapy.

HSP90 expression and interaction with important client proteins in AML

The expression of HSPs by leukemic cells varies between patients [173-175]. Complete remission rates and overall survival seem to be higher in patients with generally low HSP expression [174, 176], wheares HSP90 expression has no correlations with white cell counts, morphology (i.e. FAB subclassification) or cytogenetics [176]. Similar observations have been made for patients with preleukemic MDS where high HSP90 expression is seen especially in the high-risk subgroups [177]. FLT3 is a HSP90 client protein and inhibition of HSP90 thereby seems to have an indirect inhibitory effect on FLT3-dependent intracellular signaling [178]. Mutated FLT3 is constitutively activated and probably mediates signaling that is important both for leukemogenesis and chemosensitivity, and the combination of conventional DNA-damaging therapy with agents that mediate direct (TKIs) or indirect (HSP90 inhibitors) FLT3 inhibition should therefore be considered in AML therapy [179]. Other HSP client proteins are probably also involved in leukemogenesis [169], some of the most important being summarized in Table 11. The importance of HSP90 and some of its client proteins for intracellular signaling and intercellular crosstalk will be discussed below. The transcription factor NF-κB controls a wide range of genes regulating proliferation and apoptosis, and this is probably true also for primary human AML cells [121]. HSPs can interact with the function of NF-κB [121] through (i) binding and possibly neutralization of pro-apoptotic or pro-inflammatory NF-κB targets; and (ii) stabilization/binding of both the main NF-κB regulators IκB kinase (IKK) [180] and NF-κB inducing kinase (NIK) [181]. These observations suggest that pharmacological targeting of chaperones in AML should be further

explored [182], and this is further supported by experimental studies suggesting that the role of FLT3 in leukemogenesis involves NF-κB [183].

The two structurally unrelated protein kinases CHK1 and CHK2 are emerging as key mediators in cellular responses to genotoxic stress [184]. Recent studies suggest an important role of both these kinases in the network of genome surveillance pathways that coordinate cell cycle progression with DNA repair and apoptosis regulation. These kinases thus provide a linkage between upstream sensors of cell cycle checkpoints and cell cycle regulation [185]. DNA damage and replication stress will activate the CHK1 signaling pathway, block S phase progression and thereby participate in G2 arrest. Furthermore, AML cells exposed to cytotoxic agents often show cell cycle arrest at the G2/M checkpoint together with development of chemoresistance [186]. CHK1 inhibition may then allow checkpoint exit with induction of chemosensitivity [187]. HSP90 is important for the folding and stabilization of CHK1 [188] and as expected HSP90 inhibition depletes CHK1, disrupts the S-phase checkpoint and enhances the therapeutic effect of cytarabine in primary AML cells [124].

Finally, the matrix metalloproteases (MMPs) are important in the regulation of apoptosis, proliferation and angiogenesis [101]. MMP-2 and MMP-9 are the quantitative most important ones [101], and these proteases can be released from primary AML cells [189]. Both MMP-2 and MMP-9 interact with HSP90 [165, 190], showing that this HSP is also important for the extracellular compartment [191]. HSP inhibition significantly reduces the level of these proteases in *in vitro* models of AML [175].

HSP90 inhibition

HSP90 possesses two binding sites for client proteins located in the N- and C-terminal fragments, respectively. The C-terminal fragment binds partially folded proteins in an ATP-independent way and is potentially regulated by cochaperones. The N-terminal domain contains a peptide binding site that binds preferentially peptides longer than ten amino acids, and peptide dissociation is induced by ATP binding [192, 193]. The majority of HSP90 inhibitors blocks the N-terminal ATPase activity [169]. The benzoquinone amsancines such as geldanamycin were the first HSP90 inhibitors to be developed [194]. Geldanamycin had several pharmacological limitations, including poor solubility, limited in vivo stability and hepatic toxicity and is not ivestigated further in clinical studies [195]. However, more soluble derivates such as 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin), 17-dimethylalaminoethylamino-17-demethoxygeldanamycin (17-DMAG, alvespimycin) and retaspimycin

Table 11. Main client protein of HSP90 in AML.

Client proteins	Relevance to human AML	Key references
Chaperones and relatives		
Heat shock protein 70 (HSP70)	Cochaperone of HSP90, expression closely correlated to HSP90 expression	[175]
Nuclear distribution C (NudC)	Important for nuclear migration in cell division, high expression in human AML cells	[196]
Transcriptional regulation		
p53	Important for regulation of primary AML cell viability in vitro and in vivo	[197]
Murine double minute 2 (MDM2)	A downstream target of AKT, the p53-MDM2 complex is a possible therapeutic target in AML	[120]
Signal transducer and activator of transcription-3 (STAT3)	Important intracellular mediator downstream to hematopoietic growth factor receptors	[198]
Kinases		
FMS-like tyrosine kinase 3 (FLT3)	Mutated FLT3 is an adverse prognostic factor in human AML, FLT3-ligand is a growth factor for primary human AML cells for most patients	[36]
c-Jun N-terminal kinase (JNK)	A kinase involved in apoptosis, differentiation, proliferation, cytokine production and chemoresistance in AML	[122]
KIT	The second most frequently mutated RTK, often mutated in CBF-AML	[52]
AKT	Important downstream mediators for hematopoietic growth factor receptors in primary human AML cells	[117]
Cyclin B	A cell cycle regulator expressed in cytoplasm and nucleus of primary human AML cells, a possible leukemia-associated antigen	[199]
Checkpoint kinase (CHK1)	A downstream target of AKT, there phosphorylation correlates with cell cycle arrest in AML	[124]
Bcr-Abl	Involved in malignant transformation in CML, can be mutated in AML	[200]
Janus kinase 1 (JAK1)	Can be mutated in primary human AML cells and has a disease-modifying effect	[49]
Insulin-like growth factor 1 receptor (IGF-1 receptor)	Insulin stimulate primary AML cell proliferation	[201]
Inhibitor of κB kinase (IKK)	Downstream target of AKT, activates the NF-κB complex trough both the canonical and non-canonical pathway	[121]
NF-κB-inducing kinase (NIK)	Activates the NF-κB complex through the non-canonical pathway	[121]
Others		
Matrix metalloprotease 2 (MMP-2)	Expressed by AML cells, and has a possible adverse prognostic effect	[189]
Matrix metalloprotease 9 (MMP-9)	Expressed by AML cells, especially with monocytic differentiation	[189]
Survivin	Inhibitor of apoptosis with a possible adverse prognostic impact in AML	[202]

(IPI-504) and 17-allylaminogeldanamycin (17-AG) have entered clinical studies [203]. The natural occurring inhibitor radicicol was also early discovered as a HSP90 inhibitor in laboratory model [169]. This compound lacks in vivo effect, but more potent derivates have been developed and have now entered clinical trials (Table 12). Several synthetic drugs have been obtained by rational design from the crystal structures of the HSP90 binding pocket [204, 205] (Table 12), some of them have shown promising preclinical results have entered clinical trials (Table 12).

The first clinical trials with HSP90 inhibitors have been reported [206-208], including studies in AML [209, 210]. It should be emphasized that the large majority of the patients in these studies had advanced solid tumors [206-208, 211] or relapsed or refractory hematological malignancies [209, 210, 212]. Based on these studies it can be concluded that it is possible to achieve pharmacological HSP90 inhibition with acceptable toxicity in a majority of patients with advanced cancers. However, further studies are warranted to investigate potential effects of combination therapy, the optimal time to start treatment and to define subgroups of patients with special benefit of HSP90 inhibition [213].

Table 12. HSP90 inhibitors in clinical trials. Evaluated or under evaluating in AML

Functional classification	Name of inhibitor	Main administration	Key references
Benzoquinone ansamycins/ Geldanamycin derivates	Tanespimycin, 17-AAG, KOS-953, CNF110	Intravenous	[206-208, 212]
	Alvespimycin, 17-DMAG*	Intravenous	[209]
	Retaspimycin, IPI-504	Intravenous	[211]
	IPI-493, 17-AG*	Oral	[214]
Radicicol derivates	AT13387	Oral	[215]
	NVP-AUY922*	Intravenous	[216]
Synthetic	MPC-3100	Oral	[217]
	BIIB021, CNF2024	Oral	[218]
	Debio 0932,CUDC-305	Oral	[219]
	KW-2478	Intravenous	[220, 221]
	SNX-2112	Oral (by prodrug SNX-5422)	[222]
	STA-9090*	Intravenous	[210]
	XL888	Oral	[223]

THE BIOLOGY OF HUMAN AML CELLS: THERAPEUTIC TARGETING OF NF-κB MEDIATED INTRACELLULAR SIGNALING

The NF-κB family of transcription factors was identified 25 years ago as a nuclear factor that binds the κ light chain enhancer in B-cells [224]. Several studies suggest a role of NF-κB in leukemogenesis including both through its direct effects in AML cells and indirect effects through bi-directional crosstalk between leukemic cells and their neighboring stromal cells [121]. Studies have described constitutive activation with nuclear localization of NF-κB in primary human AML cells [225]. The NF-κB activity could not be detected in normal $CD34^+$ hematopoietic cells, and as expected pharmacological NF-κB inhibition in vitro then induced apoptosis only in AML cells but not in normal $CD34^+$ cells [225]. The NF-κB family consists of the five different transcription factors RelA, RelB, c-Rel, p50 and p52 (Table 13) that bind to their response elements as hetero- or homodimers [121].

Table 13. The most common nomenclature and the encoding genes of the five proteins in the mammalian NF-κB family

Proteins	Alternative names	Genes
RelA	p65, NF-κB3	RELA
RelB		RELB
c-Rel	Rel	REL
p50	p105, NF-κB1	NFKB1
p52	p100, NF-κB2	NFKB2

Under basal conditions this moiety is inactivated in the cytoplasm by the inhibitor IκB, but in response to specific stimulation IκB is degraded and NF-κB is thereby released and can bind other proteins, DNA or RNA. There are two pathways for activation of NF-κB: the canonical and the non-canonical pathway [121]. Inhibitory IκB is then phosphorylated on highly conserved serine residues by an IKK complex formed by the three subunits IKKα, IKKβ and IKKγ; this leads to ubiquitination and proteasomal degradation of IκB. NF-κB can thereby translocate to the nucleus and induce transcription. On the other hand, the non-canonical pathway involves a further activation step through the NIK, which secondarily stimulates IKK-induced phosphorylation (Figure 3).

Once translocated to the nucleus NF-κB promotes cell survival by initiating transcription of genes encoding stress-response enzymes, cell-adhesion molecules, pro-inflammatory cytokines and anti-apoptotic proteins (Figure 3). The pre-existing balance between expression of genes involved in regulation of survival and apoptosis may ultimately determine whether the cell undergoes apoptosis in response to NF-κB activation or inhibition [226]. Under physiological conditions activation of NF-κB triggers expression of antiapoptopic genes [227, 228]. The balance is thereby altered in favor of apoptosis by NF-κB inhibition, and this seems to be true also in human AML where targeting of IKK with the specific inhibitor AS602868 blocks NF-κB activation and thereby leads to apoptosis [229]. In addition to the antiapoptotic effects, NF-κB also has a growth stimulating effect of malignant cells by inducing cell cycle progression and hence increased proliferation of the malignant clone [121]. NF-κB and IKK-controlled pathways are also important functional modulators not only in the malignant cell but also in surrounding stromal cells, and these cells also take part in the leukemic process in human AML (Table 15) [230, 231].

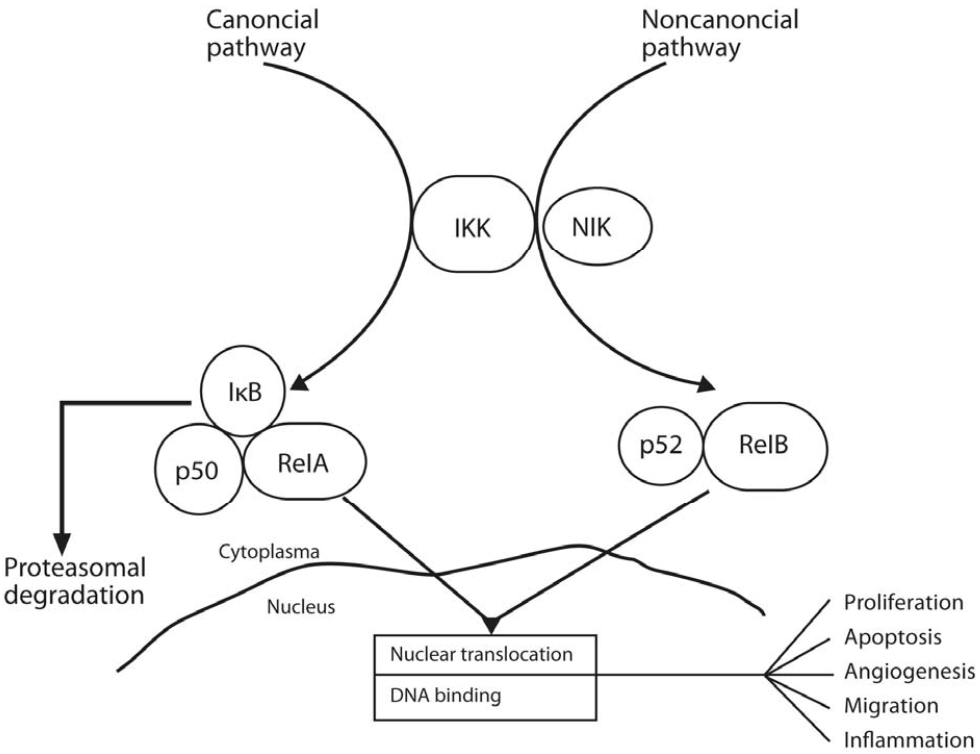

Figure 3

Activation of the NF-κB pathway.

The canonical pathway (left part) activates the IκB kinase (IKK) complex leading to inactivation of inhibitor κB (IκB); the IκB is thereby degraded by the proteasome with loss of NF-κB inhibition and translocation of RelA/p50 to the nucleus. Alternatively, in the noncanonical pathway (right part) NF-κB inducing kinase (NIK) acts via IKK, to phosphorylate p52 that forms a complex with NIK and RelB. In both pathways the released NF-κB dimmer, p50/Rela and p52/Rel B respectively, is thus able to translocate to the nucleus where it binds to specific DNA promoters, initiating transcription of genes that encode stress-response enzymes, cell-adhesion molecules, pro-inflammatory cytokines and anti-apoptotic proteins

Abbreviations: IκB, inhibitor κB; NIK, NF-κB inducing kinase; IKK, IκB kinase.

Cells that display chemokine receptors can respond to chemokine gradients with chemotactic migration due to activation of multiple intracellular signals including NF-κB [232], and malignant transformation associated with constitutive NF-κB activation leads to endogenous expression of chemokines and their receptors [233]. The cytokine release in the bone marrow microenvironment may create autocrine and paracrine loops that keep NF-κB in an activated state. This has been described for human fibroblasts where CXCL8 increased the expression of its receptor CXCR2 that in turn led to higher CXCL8 (IL-8) release in vitro through NF-κB signaling [234]. CXCL8 can thereby promote neoplastic growth through its effect on various cells in the cancer cell microenvironment [235].

NF-κB plays a crucial role in angiogenesis. Several of the angiogenic factors (e.g. VEGF and CXCL8) are regulated by NF-κB, and their constitutive release by malignant cells is suppressed by NF-κB-inhibition [121]. Upregulation of Ang-1 expression in both fibroblasts and endothelial cells occurs via the NF-κB signal transduction pathway [236, 237]. NF-κB also promotes expression of several MMPs, including MMP-2 and MMP-9 [238].

NF-κB inhibition

Numerous natural products and synthetic compounds can inhibit NF-κB, and recently more specific NF-κB inhibitors have been developed [121, 239] (Table 14). The experience from preclinical studies of the specific IKK inhibitors suggests that NF-κB inhibition can eradicate primary human AML cells at the bulk, progenitor and stem cell level [240].

Table 14. Important NF-κB inhibitors

Classification	Important compounds
Modulators of IKK activity	AS602868 MLB120 BMS-345541, BAY11-7085 Thalidomide,lenalidomide DNMT inhibitors, HDAC inhibitors
Proteasome inhibitors	Bortezomib MG262 CEP-18770

Among the most promising drug that also functions as a NF-κB inhibitor in the treatment of hematological malignancies is the proteasome inhibitor bortezomib [241, 242]. The mechanisms of action for proteasome inhibitors are complex and include additional mechanisms and not only NF-κB inhibition [228]. The final step before NF-κB translocation is ubiquitin-dependent degradation of IκB by the 26S proteasome [239], and inhibitors of the ubiquitin-proteasome pathway suppress activation of NF-κB by stabilizing IκB. Thalidomide and its derivate lenalidomide, that lacks the neurotoxic side effects associated with the parent drug, have complex pharmacological effects that can be divided into three major groups; (i) a direct anti-neoplastic effect, (ii) indirect effects mediated via neighboring cells in the neoplastic microenvironment, and (iii) immunomodulation [243]. The drugs attenuate the phosphorylation of IKK as well as the RelA subunit; and all three pharmacological effects are at least partly mediated by NF-κB inhibition. Both drugs are widely used in the treatment of multiple myeloma (MM) [244], and their use in AML therapy is considered, especially of AML secondary to MDS [245]. These studies have provided evidence for antileukemic activity, especially indirect antiangiogenic activity, but the clinical effect of single agent thalidomide in AML seems limited [246]. Lenalidomide seems particularly effective in patient with 5q deletion [247] and trisomy 13 [248].

More specific inhibition of IKK is a very attractive therapeutic strategy. Several IKK inhibitors have been identified; they have not been investigated in clinical studies, but the preclinical results of some agents seem promising. These preclinical studies suggest that the sensitivity to IKK inhibitors correlates with the basal NF-κB activity and the drugs modulate adhesion molecule expression [121], and several agents have also been shown to target even leukemic stem cells (LSCs) [121].

THE BONE MARROW MICROENVIRONMENT: NORMAL AND LEUKEMIC BONE MARROW

The bone marrow organization at the macro- and microscopic level

The bone marrow constitutes 4% of the total body weight in humans. The red marrow consists mainly of hematopoietic tissue and the yellow marrow mainly of fat cells. At birth all the bone marrow is red, whereas with increasing age this is reduced and in adults approximately half of the marrow is red, i.e. mainly the marrow in the pelvic bones, skull, sternum, vertebrae, costa and the proximal extremity bones (femur and humerus). The non-hematopoietic cells of the marrow are referred to as the stroma (Table 15), and these cells provide a microenvironment that facilitates hematopoiesis.

Table 15. Bone marrow stromal cells

Cell type	Main Function
Fibroblasts	Synthesis of collagen, glycosaminoglycans and glycoproteins; the main component of the extracellular matrix
Osteoblasts	Responsible for bone formation and mineralization, supporting the hematopoietic cells, forming endosteal stem cell niches
Osteoclasts	Involved in bone resorption by removing mineralized bone
Endothelial cells	Surrounding the vascular system, acting as a barrier between the vessel lumen and surrounding tissue, supporting hematopoiesis and forming the vascular stem cell niches
Adipocytes	Cells specialized in storing energy

The main function for the red bone marrow is to produce mature blood cells. These cells originate from the hematopoietic stem cell (HSC). The definition of a stem cell is under debate, but there is a general agreement that these cells (i) show self-renewal, i.e. have the ability to go through numerous

cell divisions while maintaining the undifferentiated state; and (ii) have the potential to differentiate into specialized cells (potency). This potency can be classified as described in Table 16.

Table 16. Stem cell potency.

Potency	Definition	Example
Totipotency	Cells with the ability to divide and produce all the differentiated cells in an organism, including extraembryonic tissues	Zygote
Pluripotency	Cells that have the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm	Embryonic stem cell
Multipotency	Cells that have the potential to give rise to cells from several, however limited number of lineages	Hematopoietic stem cell
Oligopotency	Cells with the ability to differentiate into a few cell types	Common myeloid progenitor cell
Unipotency	Cells that have the capacity to develop/differentiate into only one distinct cell type	Erythroblast

The HSCs are multipotent stem cells with the capacity of self-renewal, giving rice to oligopotent myeloid or lymphoid progenitors. The bone marrow stroma represents a supportive network of the HSCs [249] that are thought to reside within specific niches (Figure 4). These niches are specialized microenvironments created by supportive cells that facilitate stem cell survival and self renewal through expression of membrane molecules and release of soluble mediators. HSCs tend to reside near the endosteal surface [250], and studies of HSCs homing after transplantation in animal models have documented redistribution of HSCs to the endosteal region [251]. This is currently referred to as the osteoblastic or endosteal niche located at some distance from the blood supply in a relatively hypoxic microenvironment [252]. The hypoxia is probably important for maintenance of pluripotency and self-renewal capacity [252]. The major molecular response to the hypoxia is increase in the transcription factor hypoxia-inducible factor-1 (HIF-1). HIF-1 binds to responsive target regulatory sequences of multiple genes involved in energy metabolism, myelopoiesies and angiogenesis [252], several of these probably being important for HSC maintenance. One well-characterized HIF-1 target involved in HSC maintenance is VEGF, which is important for survival and repopulation of HSCs [253]. However, several other molecules are also involved in direct crosstalk or paracrine interactions supporting maintenance, including the Notch [254], and Ang-Tie2 systems [255] . Pharmacological targeting of both these systems is currently considered in hematological malignancies [230, 254].

Although the endosteal niche is believed to be the main facilitator of HSCs maintenance and quiescence, a large part of the HSCs are found adjacent to sinusoids in the so called vascular niches (Figure 4) [249]. This is possibly an explanation why HSCs can rapidly be recruited to the peripheral circulation during clinical stem cell mobilization. Whether endothelial cells contribute to maintenance of HSCs or whether HSCs only transiently migrate trough the vascular niche is not known [249].

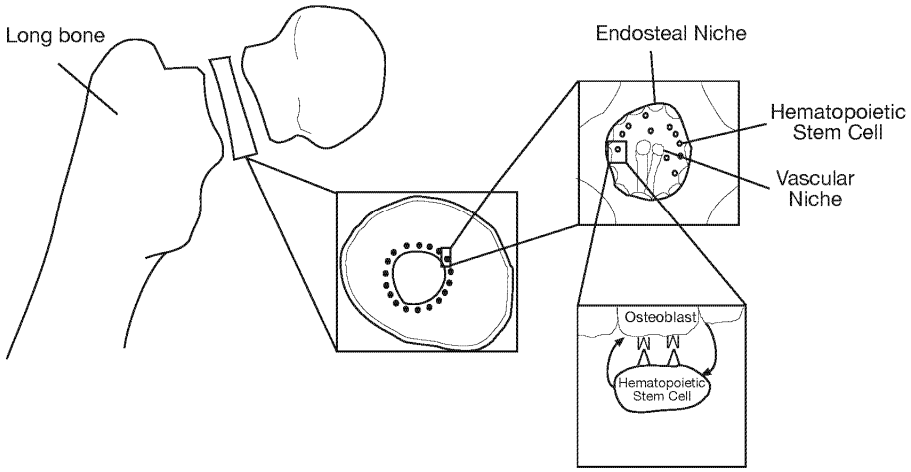

Figure 4

Hematopoietic stem cells niches.

Trabercular long bone is richly vascularised, giving rise to organization of distinct stem cell niches; e.g. the endosteal niche and the vascular niche. Hematopoietic stem cells, and probably also leukemic stem cells, can reside to both these niches, although hematopoietic stem cells tend to localize near the endostial surface. Osteoblasts and other supporting cells are involved in direct crosstalk or paracrine interactions that support the maintenance of the hematopoietic stem cells. Hematopoietic stem cells are also found adjacent to sinusoids in the vascular niche, explaining why hematopoietic stem cells can rapidly be recruited to the peripheral circulation. Whether endothelial cells contribute to maintenance of hematopoietic stem cells or whether they only transiently migrate trough the vascular niche, is mainly unknown.

Leukemic stem cells

The theory that most cancers contain a minor population of functionally distinct cancer stem cell has been postulated for several years. The theory is controversial [256], but in the case of AML there is growing support for existence of such LSC [257]. These fractions of cells probably count for one per million of the whole leukemic blast population [257]. The cells are usually located within the $CD34^+/CD38^-$ compartment [257] and are able to recapitulate the disease in mouse xenograft models [257]. However, LSC can probably also rest in other compartments, especially the $CD34^+/CD38^+$ compartment [258]. Thus, LSCs show both self-renewal and a capacity to produce leukemic progenitors with proliferative but not self-renewal capacity [256], and similar to their normal counterparts they are dependent of support from the bone marrow microenvironment [259]. LSCs also engraft and reside in the endosteal niche in murine models [260], most of them are in the G_o phase and are relatively resistant to cytarabine treatment [259, 260]. Selectively inhibition of LSCs has now emerged as a possible therapeutic strategy [256].

THE BONE MARROW MICROENVIRONMENT: ANGIOGENESIS AND THE ANGIOREGULATORY NETWORK IN AML

Angiogenesis is regulated by angiogenic activators and inhibitors, and the shift in the normal homeostatic balance in favor of angiogenesis is often referred to as the angiogenic switch [261]. Cancer-associate angiogenesis was originally described in solid tumors [262], but more recent studies have brought attention to angiogenesis as important for development, progression and prognosis in hematological malignancies:

- Bone marrow microvessel density (MVD) is often increased in hematological malignancies, especially in patients with advanced stage disease [263].
- Antiangiogenic therapy causes vascular disruption and has antileukemic effect [264].
- The crosstalk between leukemic and microvascular endothelial cells can increase the proliferation of both the endothelial [265] and leukemic cells [266].
- The use of magnetic resonance techniques can provide functional imaging of bone marrow vascularity that can possibly be used for prognostication in AML [267].

Several mediators have been postulated to have pro- or antiangiogenic effects, and some of the most important angioregulators in human AML will be described below.

VEGF

There are five members of the mammalian VEGF family: VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor. VEGFA (hereafter referred to as VEGF) is a growth and survival factor for vascular endothelial cells, and it is well characterized for its role in developmental and pathological angiogenesis [268]. VEGF is highly expressed in several human cancers, this is at least partly caused by its transcriptional upregulation by hypoxia and by various oncogenes [268]. VEGF can be released both by malignant cells, including AML blasts, and by surrounding stromal cells [269]. It binds and activates the two related RTKs of VEGF receptor 1 (VEGFR1) and VEGF receptor 2 (VEGFR2). Most of the activities attributed to VEGF are mediated by signaling through VEGFR2, including its mitogenic as well as survival and motility enhancing effects. The role of VEGFR1 in angiogenesis is controversial and less well understood. VEGFR2 is predominantly found in endothelial cells where its activation is critical for blood vessel formation, migration, differentiation and vascular permeability. Several strategies have been tried to inhibit these effects [268], including (i) neutralizing antibodies to VEGF or VEGFRs; (ii) soluble VEGFRs or chimeric receptors that trap circulating VEGF and (iii) -VEGFR TKIs. VEGF-targeted agents in clinical use in Norway include the VEGF-specific monoclonal antibody bevacizumab, and the TKIs sorafenib, sunitinib and pazopanib.

HGF

HGF binds to its receptor mesenchymal-epithelial transition factor (MET); MET is expressed by cells of epithelial origin whereas HGF is produced by mesenchymal cells, and HGF is the only known MET ligand [113]. HGF can be produced both by bone marrow stromal cells [270] and by AML blasts [231], and high serum levels are associated with an adverse prognosis in AML [271, 272]. HGF binding to its receptor activates the RTK activity, initiates intracellular protein phosphorylation and thereby several intracellular signaling cascades; the two most important being the PI3K and STAT pathways [113]. MET activation then leads to a proliferative, invasive, antiapoptotic and angiogenic phenotype of endothelial cells. HGF/MET has emerged as potential therapeutic target in cancer therapy, and these inhibitors can be divided in two main subclasses; (i) biological HGF/MET antagonists and (ii) synthetic MET kinase inhibitors [273]. The biological antagonists include truncated or uncleavable HGF forms

that antagonize full-length HGF binding and neutralizing monoclonal antibodies directed against HGF or MET [113, 273]. The MET kinase inhibitors competitively antagonize the intracellular ATP binding site, thereby preventing the RTK-mediated intracellular phosphorylation cascade [113, 273]. Compounds in both classes have now entered early clinical trials [273].

Chemokines

Chemokines are small *chemo*tactic cyto*kines* with a molecular weight of 8-10 kDa. They are divided into four subgroups (C, CC, CXC, CX_3C) based on the presence of amino (N)-terminal cysteine residues; the largest are the CC and the CXC chemokines [100]. The chemokines have a complex angioregulatory role [274], and they can be classified as proangiogenic or angiostatic, e.g. facilitators or inhibitors of angiogenesis, respectively. The most important angiogenic and angiostatic chemokines are briefly described in Table 17 [100, 274, 275].

Table 17. Angiogenic and angiostatic chemokines; the most important chemokines (corresponding receptors) are given in the upper part and their main functions in the lower.

Angiogenic chemokines	**Angiostatic chemokines**
CCL1 (CCR8) CCL2 (CCR2) CCL3 (CCR5) CXCL1 (CXCR2) CXCL8 (CXCR2)	CXCL4 (CXCR3B) CXCL9 (CXCR3B) CXCL10 (CXCR3B) CXCL11 (CXCR3B)
Main angiogenic function	**Main angiostatic function**
Recruitment of angiogenic hematopoietic cells Activation of endothelial cell chemotaxis and tubular formation Stimulating angiogenic growth factor signaling Direct stimulating and activation of RTKs	Recruitment of T-cells which induce expression of angiostatic factors Activation of endothelial cell apoptosis and regression Inhibition of angiogenic growth factors Direct binding and inhibition of RTKs

Primary human AML cells generally show constitutive release of several angioregulatory chemokines [233], the highest levels are usually seen for proangiogenic CXCL8 [233]. Antiangiogenic chemokines, e.g. CXCL9-11, can also be released but usually at lower levels than CXCL8 [233]. The only chemokine yet to be a pharmacological target is CXCR4, and the specific inhibitor plerixafor is

now used for stem cell mobilization [276]. However, this drug is also investigated as a possible antileukemic agent and is tried in the treatment of AML [276].

Ang and Tie system

Tie1 and Tie2 constitute a distinct family of RTKs expressed mainly by endothelial cells. Their naturally occurring agonists are the Angs that constitute four different classes (Ang1-4). Ang1 and Ang2 are the most important agonists, and Tie2 is the most relevant receptor in angiogenesis. Ang1 is a Tie2 agonist, whereas the role of Ang2 is more complex. Ang2 can be a competitive Tie2 antagonist for Ang1 in endothelial cells [277], but more recent studies have demonstrated that Ang2 is a context-dependent partial agonist/antagonist of Tie2 signaling [278]. The function of Ang2 then seems to depend on cell and tissue localization, developmental stage of the cells, Ang2 concentration and duration of exposure [279]. Furthermore, Ang1 ligation of Tie2 strengthens the interactions between endothelial and periendothelial supportive cells; whereas Ang2 primarily disrupts these interactions resulting in vessel destabilization [280]. The final effect of Ang2 will then depend on the presence of other angioregulatory cytokines, the effect being either an angiogenic response or vessel regression (Table 18).

Table 18. The Ang/Tie2 system in angiogenesis.

	Main source	Receptor	Tie2 effect	Main effect on endothelial cells
Ang1	Non-endothelial cells AML cells	Tie1 Tie2	Agonist	Stabilizing and maturation of neovasculature Important for cell-cell interaction Antiapoptotic effect
Ang2	Endothelial cells AML cells	Tie2	Partial agonist/ antagonist	Context-dependent effects Destabilizing and regression of neovasculature Angiogenic response in the presence of VEGF or absence of Ang1

Several studies suggest that the Ang/Tie2 system is important both for leukemogenesis and chemosensitivity in AML [231, 281], and these effects may then be mediated through a bidirectional crosstalk between AML cells with constitutive Ang release and their neighboring endothelial cells. However, the effect of Ang/Tie2, and especially Ang2 expression, in leukemogenesis and on

chemosensitivity is still controversial. High pretreatment levels of Ang2, investigated both by mRNA expression levels in peripheral blood mononuclear cells [281], and by semi quantitative immunohistochemistry in bone marrow biopsies [282], have been associated with chemosensitivity and favorable outcome [281, 282]. In contrast, high levels of Ang2 in bone marrow blast detected by real time polymerase chain reaction (PCR) [283], or high plasma levels of Ang2 [284], have also been associated with an unfavorable prognosis [283, 284]. The latest is also in concordance with a study demonstrating that high levels of circulating Ang2 were associated with an unfavorable outcome after allo-SCT among AML or high risk MDS patients [285]. Taken together, these conflicting results demonstrated that both the origin of Ang2, e.g. leukemic cells or stromal cells [230], and the context, e.g. agonistic/antagonistic interactions in the presence of Ang1 and other cytokines [279], probably also is clinical relevant.

Therapeutic targeting of the Ang/Tie2 system is possible by administration of selective Ang1/2-neutralizing or Tie2 blocking antibodies [230]. In experimental models this strategy decreases endothelial cell proliferation and thereby inhibits angiogenesis, causes tumor necrosis and reduces tumor growth [230, 286]. Ang1/2 neutralization can also inhibit angiogenesis driven by other proangiogenic cytokines [287], e.g. the VEGF-dependent angiogenesis that seems to have a prognostic impact in AML [288]. Furthermore, experimental studies suggest that combined antiangiogenic targeting is more effective than targeting VEGF alone [289]. Both VEGF and Ang2 have prognostic impact in AML [231, 289], and combined targeting is then of particular interest [290].

MMPs

MMP expression is upregulated in several malignancies, including AML. These proteases were originally thought to be responsible for degradation and turnover of the extracellular matrix, but they are in addition involved in angiogenesis, inflammation and cell migration [101]. The MMPs are strictly regulated by their endogenous inhibitors called Tissue Inhibitor of MMPs (TIMPs). The TIMPs are broad-spectrum inhibitors of MMPs, although there are some differences in their affinities for various MMPs. An imbalance between MMPs and TIMPs is believed to promote AML progression [101]. Importantly, the TIMP molecules should not only be regarded as MMP antagonists; they have complex functions and both growth-enhancing and antiapoptotic effects have been described [291]. A total of

23 different human MMPs and four different TIMPS have been identified; those that are important in AML are summarized in Table 19.

Table 19. MMPs and TIMPs in AML (expression frequencies according to [189]).

Name	AML expression	Characteristics	Refs
MMP-2	49%	Both secreted and membrane bound in AML cells, up regulation is associated with drug resistance and adverse prognostic outcome in AML	[189, 292-295]
MMP-7	21%	Can generate soluble FasLigand which further increases apoptosis in surrounding cells through the activation of Fas receptor. Associated with resistance to chemotherapeutics	[296]
MMP-9	47%	Both secreted and membrane bound in AML cells, higher expressed in more mature AML (FAB M4/M5) and associated with an invasive phenotype	[189, 295, 297, 298]
MMP-10	100%	Demonstrated to induce growth both in lymphoma and solid tumors	[299]
TIMP-1	60%	Antiapoptotic effects in malignant cells, including malignant hematopoietic cells.	[291]
TIMP-2	94%	Growth-regulatory effects through specific binding to intergrins	[291]

MMPs are considered promising targets for cancer therapy. Several MMP inhibitors (MMPIs) have been devoloped and can be broadly divided into peptidomimetic, non-peptidomimetic, tetracycline derivatives and naturally occurring inhibitors (Table 20). The results from testing in animal models and in cell cultures were compelling, including studies in AML cell lines [300]. These results generate a great expectation [301, 302]. However, results from clinical trials have been disappointing with several studies demonstrating none or only marginal effects [301, 302]. The reasons for these failures are assumed to be multifactorial. Firstly, results from animal models may not predict the outcome for human tumors [303]. Secondarily, most clinical studied were performed in advanced tumors, while MMPIs may be more effective in the early stages due to their cytostatic rather than cytotoxic effect [303]. Thirdly, the MMPIs used in clinical studies target a broad spectrum of MMPs, and improved target specificity may be more beneficial [303]. Finally, MMPs and TIMPs may have protective roles in certain cancers [304].

Although no MMPIs have been ivestigated in clinical trials for AML, MMPs still represent an potential interesting therapeutic target in these patients [101]. However, a better understanding of the role of different MMPs in leukemogenesis is needed before optimal clinical studies can be designed.

Table 20. Classification, nomenclature and pharmacological characteristics for MMP inhibitors used in clinical trials [101].

Classification	Central inhibitors	Characteristics
Peptidomimetic	Marimastat / BB-2516 Batimastat / BB-94 ABT-518	Low water solubility and/or bioavailability. Not for further clinical development.
Non-peptidomimetic	Prinomastat / AG3340 Tanomastat / BAY12-9566 BMS-275291 CGS27023A / MMI270	Good oral bioavailability. Musculoskeletal symptoms are the main side effects.
Tetracycline derivates	Metastat/COL-3/ SC-683551	Good oral bioavailability. Main side effects include malaise and photosensitivity.
Naturally occurring inhibitors	Neovastat/AE-941	Good oral bioavailability. An additional effect is inhibition of VEGF and induction of apoptosis in endothelial cells.

Endocan

Endocan, previously called endothelial cell specific molecule-1, is a soluble proteoglycan with a molecular weight of 50 kDa [305]. Experimental evidence suggests that endocan act as a key player in the regulation of cell adhesion, local inflammation and tumor progression. Endocan serum levels are elevated in patients with various malignancies [306], and in patients with severe sepsis [306]. Endocan is produced by endothelial cells but not by AML cells [265]. Pro-angiogenic growth factors (e.g. VEGF and HGF) increase endocan expression by human endothelial cells [305], whereas Ang2 seems to have an opposite effect [230]. The possible role of endocan in leukemogenesis and its possible use as a biomarker in human AML require further studies [305].

AIMS OF THE THESIS

AML is a heterogenic disease and the response to chemotherapy differs between patients. The AML genotype is an important prognostic parameter in AML. Chromosomal abnormalities, gene mutations and gene expression abnormalities all affect critical protein levels and functions in primary human AML cells and may exert profound effects on chemosensitivity. Pharmacological targeting of these protein abnormalities has emerged as a possible therapeutic strategy in human malignancies, including AML. However, given the heterogeneity of AML one would expect differences between patients in their responses to such targeted therapies. Furthermore, the interactions between the AML cells and their neighboring bone marrow stromal elements are important in leukemogenesis and will probably also be important for chemosensitivity. Bone marrow angiogenesis with increased MVD is observed in human AML, and the endothelial cells will then be important both for the supply of nutritients to the leukemic cells and as directly leukemia-supporting stromal cells.

In this context the aims of this thesis were:

- characterization of intracellular and extracellular signaling events that affect primary human AML cell proliferation, viability and constitutive release of angioregulatory cytokines;
- based on the studies described above, to describe the biological heterogeneity in human AML;
- to investigate how pharmacological targeting of intracellular molecules (HSP90, NF-κB, the PI3K-mTOR pathway) and Tie-2 receptor signaling affects the functional characteristics of primary human AML;
- to investigate differences in systemic cytokine levels, including hematopoietic growth factors and angioregulatory cytokines, between acute leukemia patients after they have achieved complete hematological remission.

MATERIAL AND METHODOLOGICAL CONSIDERATIONS

PREPARATION AND CULTURE OF PRIMARY HUMAN AML CELLS

By definition the bone marrow of AML patients contains at least 20% leukemic blasts [11]. In our studies we used AML blast derived from peripheral blood, and we then selected patients with high peripheral blood blast counts so that enriched AML cell populations could be prepared by density gradient separation alone [307]. Thus, our results may be representative only for this subset of patients. This methodological approach was used because the AML cell populations then contained 95% AML cells, and more extensive separation procedures can alter gen expression and/or the constitutive release for soluble mediators [307]. Cells were stored in liquid nitrogen until used. The advantages of this approach are the possibility to use well-characterized AML cells, additional experiment are possible for the same patient and it is possible to study larger patient populations within reasonable time.

Addition of serum to cultures represents a non-standardized factor and in general our experiments were performed in serum-free medium. However, serum supplementation is often necessary when culturing non-myeloid cell, e.g. in stromal cell experiments and coculture experiments [266, 270].

Spontaneous apoptosis will always occur during in vitro culture of AML cells [307]. The fraction of cell that stays viable during culture varies between patients [230]. Thus, the effect of pharmacological intervention always occurs in the context of ongoing spontaneous apoptosis as can be seen from the control cultures that were included in all experiments.

Several cytokines can function as growth factors in AML [307]. The optimal cytokine combination for AML cell proliferation varies between patients [307]. In our studies of cytokine dependent proliferation we usually added FLT3-L, SCF and GM-CSF. Although detectable proliferation is not observed in all patients, a majority of AML blasts will proliferative in the presence of these cytokines [36].

In our studies we usually included consecutive or unselected patients diagnosed with AML at our hospital. As discussed in detail previously [36], our patients are probably representative for the general AML patient population with regard to clinically relevant characteristics (i.e. prognosis). The patient age is relatively high, in contrast to many clinical studies that mainly include younger patients, e.g.

<60-65 years of age. Such clinical studies are not representative for the AML patient population as a whole. The disadvantages are that our patients are heterogeneous with regard to treatment and survival analyses are therefore difficult, and our populations included an expected low number of patients with good-prognosis genetic abnormalities.

Table 21. Comparison between AML patients included in article III and a general AML population. The table compares age/gender and biological AML cell characteristics [1, 11, 16, 308].

Demographic data and disease history		**Study paper III**	**General AML population**
Gender	Male/female	53%/47%	60%/40%
Age (median)	Years	64	70 (often lower in clinical trials)
AML cell differentiation			
FAB classification	M0-1	41%	20-25%
	M2	21%	25-30%
	M3	0%	5-10%
	M4-5	38%	35-40%
	M6	0%	3-5%
	M7	0%	3-5%
Genetic abnormalities			
Cytogenetic	Favorable	5%	15-20%
	Unfavorable	28%	15-20%
	Intermediate	8%	10-15%
	Normal	58%	45%
FLT3	ITD	41%	25-30%
	TKD	3%	5%
NPM1	Mutated	31%	30-35%

We did not included patients with APL (FAB-M3), and the uncommon erythroleukemia (FAB-M6) and megakaryocytic leukemia (FAB-M7) were not detected among our patients. We also have few patient with favorable cytogenetic, e.g. t(8;21), inv(16)/t(16;16). These abnormalities are uncommon in older patients and these patients often have lower peripheral blood blast counts, the low frequency is therefore expected [309].

SYSTEM BIOLOGY AND BIOINFORMATICS APPROACHES

The term system biology has been widely used in a variety of contexts the last decade. The term describe a biology based inter disciplinary study field that focuses on complex interactions in biological system, trying to use a holistic instead of a reduction perspective [310]. System biology can be divided into minor groups where the most important are given in Table 22.

Table 22. System biology approaches in AML. The table lists the most common system biology approaches together with their main characteristics and key references for the use in AML are given.

Approaches	Characteristics	AML examples
Genomics	DNA sequencing	[43]
Transriptomics	Global gene expression profiling by DNA microarray analyses	[20, 21]
Interferomics	Studying of transcription correcting factors, i.e. micro RNA	[311, 312]
Epigenomics	Epigenetic regulation of transcription, e.g. DNA methylation and histone acetylation	[313, 314]
Proteomics	Proteomics is large-scale study of proteins or peptides, particularly their expressions, structures and functions	[315]
Phosphoproteomics	A branch of proteomic that identifies, classifies, and characterizes protein phosphorylation	[198]
Metabolimics	Characterization of cell metabolism	[316]

System biology requires several graphical and staticall methods that will be further discuss. Cluster analysis is the assignment of a set of observations into subsets (referred to as clusters), observations in the same cluster then show similarities [317]. There are different types of clustering that can be used. Hierarchical clustering is a method that seeks to build a hierarchy, e.g. groups, of clusters. The algorithms begin with each element as a group and merge them into successively larger clusters. An important step clustering analysis is to select a distance measure, which will determine how the similarity of two elements is calculated. There are different methods to calculate this distance that can influence the results from the clustering, although the differences between methods are in general

small [317]. A more simplified method to describe correlations between different patients and mediators present this as correlation maps, this approach can give an overview regarding association of different samples [189].

Hierarchical clustering creates groups of clusters which often are presented in a tree structure called a denogram. The root of the tree consists of a single cluster containing all observations, and the leaves correspond to individual observations. Algorithms for hierarchical clustering are generally agglomerative, in which one starts at the leaves and successively joins samples or subclusters together [317]. The hierarchical cluster analysis can be performed by unsupervised or supervised analysis. In the first one the results of experiments do not take external factors such as clinical or biological features into account. In contrast, a supervised clustering can take these parameters into account in the final analysis [20].

Interpretation of data obtained by unsupervised hierarchical cluster analysis is frequently performed by visualization as heatmaps. A heatmap is a graphical presentation of data where the values taken by a variable in a two-dimensional map are represented as colors [317]. The mosaic of a heatmap illustrates expression level by color intensities. Heatmap is often combined with hierarchal clustering where the rows and columns of the heatmap are organized based on results from the hierarchal clustering. Heat maps originated in two dimension displays of the values in a data matrix, low or high values are often given the most intense colors.

Hierarchical clustering and heatmaps are often followed by a hierarchal clustering with distance matrix, e.g. pairwise correlation between samples using Parson's Correlation [20]. This gives a visualization of the similarities/differences between samples. The strongest colors, e.g. deep red or deep green, represent one hundred percent negative or positive correlation. A hundred percent positive correlation between two samples indicates that high expression level in one sample is always followed by high expression in the corresponding sample and vise versa [20].

The advantages in the use this approach in AML is to visualize heterogeneity between individual patients [310]. In our experiments we have used this approach to classify effects of pharmacological targeting; this approach will then have the potential to define patient subgroups with regard to pharmacological effects.

SUMMARY OF THE RESULTS

Paper I

Primary human acute myelogenous leukemia cells release matrix metalloproteases and their inhibitors: release profile and pharmacological modulation.

Reikvam H, Hatfield KJ, Oyan AM, Kalland KH, Kittang AO, Bruserud Ø.

European Journal of Haematology 2010 Mar; 84(3): 239-251

Background: Angiogenesis seems important both for leukemogenesis and chemosensitivity in AML. Angiogenesis is regulated by the balance between pro- and antiangiogenic cytokines and probably also by MMPs and their natural inhibitors, TIMPs, in angiogenesis. We investigated the constitutive release of MMPs and TIMPs for a large group of consecutive AML patients.

Material and methods: AML cells were cultured *in vitro* either alone or together with microvascular endothelial cells, and the levels of MMPs and TIMPs were determined in culture supernatants. The effects of various emerging pharmacological agents on this release were evaluated.

Results: AML cells showed constitutive release of several MMPs and TIMPs. For all patients detectable MMP-10 release was observed, and most patients showed detectable release of at least one additional MMP, usually MMP-9 or MMP-2. A significant correlation was found between MMP-9 and TIMP-1 release and the release of several CCL and CXCL chemokines. MMP-9 release was higher for AML cells with monocytic differentiation corresponding to the FAB-subtype M4/M5 AML; it was mainly released in its inactive form, but endogenous active MMP-9 could be detected even in the presence of the constitutively released TIMP-1/2. Endothelial cells released relatively high levels of MMP-10, and these levels were further increased by coculture with AML cells. Heterogeneous responses to different pharmacological agents were observed. Patients achieving complete hematological remission after only one induction cycle often had undetectable MMP-2 levels, indicating a prognostic role of this MMP in AML.

Conclusion: Primary human AML cells show constitutive release of both MMPs and TIMPs, and this release may be important for leukemogenesis and possibly also for chemosensitivity.

Paper II

Targeting the angiopoietin (Ang)/Tie-2 pathway in the crosstalk between acute myeloid leukaemia and endothelial cells: studies of Tie-2 blocking antibodies, exogenous Ang-2 and inhibition of constitutive agonistic Ang-1 release.

Reikvam H, Hatfield KJ, Lassalle P, Kittang AO, Ersvaer E, Bruserud Ø.

Expert Opin Investig Drugs 2010 Feb; 19(2): 169-183.

Background: The Tie-2 receptor can bind its agonistic ligand Ang-1 and the potential antagonist Ang-2. Tie-2 can be expressed both by primary human AML cells and endothelial cells, and Tie-2-blocking antibodies are now being evaluated in clinical trials for cancer treatment.

Material and methods: We investigated the effects of Tie-2-blocking antibodies, exogenous Ang-2 and pharmacological targeting of various intracellular signaling pathways on AML cell proliferation and the constitutive release of angioregulatory mediators.

Results: Tie-2-blocking antibodies had a growth inhibitory effect on human AML cells cocultured with microvascular endothelial cells, but this inhibition was not observed when leukemic cells were cocultured with fibroblasts or osteoblasts. AML cell viability in cocultures was not altered by anti-Tie-2. Furthermore, anti-Tie-2 decreased HGF levels and increased CXCL8 levels in cocultures, whereas the levels of endocan (a proteoglycan released by endothelial cells) were not altered. The only significant effects of exogenous Ang-2 were decreased levels of HGF and endocan. Constitutive AML cell release of agonistic Ang-1 was decreased by the proteasomal inhibitor bortezomib and the specific IκB-kinase/NFκB inhibitor BMS-345541.

Conclusion: Various strategies for modulation of Tie-2 mediated signaling should be considered in AML therapy, possibly in combination with other antiangiogenic strategies.

Paper III

Heat shock proteins expression profile for AML patients revels a distinct signature strongly associated with FLT3 mutation status - consequences and potentials for pharmacological intervention

Reikvam H, Ersvær E, Skavalnd J, Hovland R, Petersen K, Hatfield K, Bruserud Ø.

British Journal of Haematology 2012 Feb; 156(4): 468-80

Background: HSPs act as molecular chaperones that prevent the formation protein aggregates and assist proteins in their folding to native structures. Malignant cells can express different HSPs and use them to avoid cellular differentiation and apoptosis. Therefore HSPs have emerged as therapeutic targets, and special inhibition of HSP90 chaperoning activity seems to be a promising approach. The aim of the study was to compare HSP levels for a large cohort of AML patients, and to investigate the effect of the HSP90 inhibitor 17-DMAG.

Material and methods: Primary human AML cells derived from 75 consecutive patients were lysated and intracellular HSP levels measured. We used standardized bioinformatical tools to subclassify patients based on their HSP expression. Furthermore we also investigate the *in vitro* effect of HSP90 inhibition on proliferation, apoptosis and angioregulators of primary human AML cells.

Results: Intracellular expression of HSP27, HSP40, HSP60, HSP70 and HSP90α were detected for all patients. Hierarchical clustering identified two major subsets, broadly divided into low and high expression of HSPs. HSP70 and HSP90 levels showed a strong correlation demonstrating a highly coordinated expression. Patients harboring FLT3 mutations generally showed high HSP levels, indicating a strong dependence of especially HSP90 in stabilizing oncogenic mutated FLT3. HSP90 inhibition had a stronger proapoptotic effect in FLT3-ITD positive patients. Furthermore, HSP90 inhibition had a strong antiproliferative and antiangiogenic effect regardless of other patient characteristics.

Conclusions: HSP expression levels can be used to subclassify AMP patients, and the levels seem to depend on FLT3 mutation status. HSP90 inhibition has antiproliferative, antiangiogenic and proapoptotic effect, the later is especially strong in patients harboring FLT3-ITD mutations. This study supports the further investigation of HSP90 inhibitors in preclinical and clinical AML studies.

Paper IV:

Angiogenic signature for AML patients and the possible effects of different pharmacological agents acting on the PI3K-mTOR pathway

Reikvam H, Hatfield K, Tamburini J, Poulain J, Ersvær E, Ryningen A, Bruserud Ø.

Manuscript

Background: AML is a heterogeneous hematologic malignancy, with an overall AML-free survival of only 40-50% even after intensive therapy. Angiogenesis seems important both for leukemogenesis and chemosensitivity in AML and has emerged as a potential therapeutic target. It is regulated by the balance between pro- and antiangiogenic factors. The aims of the study were to investigate the effect on primary human AML cells of new therapeutic agents targeting the mTOR and the PI3K pathways, and especially the effects on angioregulatory mechanisms.

Material and methods: AML cells were cultured under highly standard *in vitro* condition, and different pharmacological effects on proliferation, apoptosis and cytokine release were evaluated. Bioinformatical tools were used to subclassify patients according to the effects of the different agents.

Results: The effect of two mTOR inhibitors (rapamycin and temsirolimus), and two PI3K inhibitors (GDC-0941 and 3-methyladenin (3-MA)) were evaluated. All agents showed a general antiproliferative effect but only modest proapoptotic effect, demonstrating a cytostatic rather than a cytotoxic effect. The effects on proliferation varied considerably among patients, but all four drugs often had similar effects in the same patient. From the constitutive release profile of angiogenic factors we where able to identified two major subset based on the clustering of angioregulatory mediators, and further two major patient clusters. The effect of the agents differed between patients, but the drugs often had similar effects on the various angioregulatory mediators in the same patients.

Conclusion: Pharmacological targeting of the intracellular signaling pathway PI3K-mTOR has direct antileukemic effects for most patients, but for a minimal subset the proliferation is increased. The drugs may also have indirect antileukemic effects through altered regulation of bone marrow angiogenesis.

Paper V:

The pretransplant serum cytokine profile in allogeneic stem cell recipients differ from healthy individuals and different profiles are associated with different risk of posttransplant complications

Reikvam H, Mosevoll KA, Melve G, Günther CC, Sjo M, Bentsen PT, Bruserud Ø

Biol Blood Marrow Transplant 2012 Feb; 18(2): 190-9.

Background: Cytokines play a key role in regulation of normal and malignant hematopoiesis, angiogenesis and inflammation. Serum levels of several cytokines are altered in patients with hematologic malignancies, and certain pretransplant cytokine levels may have a prognostic impact in patients treated with allogeneic stem cell transplantation (allo-SCT). However, the cytokine system constitutes an interacting functional network, and it may therefore be more relevant examine at serum cytokine profiles rather than the serum levels of single cytokines in allotransplanted patients.

Material and methods: We therefore investigated the pretransplant serum levels of 35 cytokines in a group of 44 consecutive allo-SCT patients, mainly with a primary diagnosis of acute leukemia. Serum samples were collected before start of myeloablative conditioning therapy when all patients were in complete hematological remission. Bioinformatic approaches were used to identify patient groups/subsets.

Results: Unsupervised hierarchical clustering analysis identified three major patient groups/subsets. These groups differed especially in the levels of hepatocyte growth factor (HGF) and granulocyte-colony-stimulating factor (G-CSF), and one of the groups was characterized by low early treatment-related morbidity and high levels of HGF and G-CSF. The degree of weight gain/fluid retention after conditioning therapy did not differ between the patient subsets, but fluid retention showed a significant correlation with pretransplant serum levels of basic Fibroblast growth factor (bFGF).

Conclusion: We conclude that the pretransplant serum cytokine profile shows a considerable variation even between patients in complete hematological remission. These differences are clinically relevant in allo-SCT recipients, and they may also be relevant for patients who can not receive allo-SCT and instead receive consolidation or maintenance treatment with new target therapy.

GENERAL DISCUSSION

AML HETEROGENEITY

Cytogenetic analysis allows a prognostic subclassification of patients receiving intensive chemotherapy for non-APL variants of AML [19]. Distinct mutations involved in leukemogenesis have additional prognostic impact, e.g. patients with FLT3-ITD have an adverse prognosis whereas patients with NPM1 mutation have a good prognosis in the absence of FLT3-ITD [15]. Global gene expression profiling in AML has revealed that major prognostic subgroups based on these genetic markers are recapitulated in global gene expression patterns and have identified specific signatures for patients with distinct cytogenetic abnormalities and gene mutations [20, 21, 318]; their prediction can then be made with almost 100% specificity and sensitivity [319].

DNA methylation and histone modulation are important epigenetic mechanisms involved in leukemogenesis, and different cytogenetic subgroups are characterized by distinct epigenetic patterns [313, 314, 320]. However, clustering based on methylation patterns seems less pronounced than the gene expression profiles [313]. It has in addition been demonstrated that AML patient subsets express specific signatures of microRNA, i.e. a class of small noncoding RNAs involved in regulation of protein-encoding mRNA [311, 312].

In our present studies the majority of patients were characterized by cytogenetic analyses and in addition analysis of FLT3 and NPM1 mutations. Cytogenetic analyses were not available for a subset of patients. For some patients no mitosis were available for culture for chromosomal analyses, for other patients the samples for cytogenetic analyses were not collected at the time of diagnosis and we did not performed cytogenetic analyses on cryopreserved cells because previous reports suggest that these results will be unreliable [321].

"ONE SIZE FITS ALL" – AML THERAPY REVISITED

The standard induction regimen in AML has for many years been an antracycline plus cytarabine, and this is followed by repetitive cycles of intensive consolidation therapy usually including high-dose cytarabine. This treatment is curative for most patients with favorable and for a large subset of patients

with intermediate risk disease, but only for a small minority with unfavorable risk profile. Knowing the biological heterogeneity of AML, an important question is now whether one still should recommend the same treatment to all patients. However, the most important improvements in AML therapy during the last two decades have not been the introduction of new therapeutic agents, but rather a more optimal use of well-known antileukemic drugs combined with a better supportive care [85]. Further studies based on specific targeted therapy will hopefully improve patient survival, even though the results from clinical studies of gemetuzumab ozogamacin [322], FLT3 inhibitors [323], farnesyltransferase inhibitors [324], histone deactylase (HDAC)-inhibitors [325] and bortezomib [326] so far have been rather disappointing. Our present experimental observations in article III suggest that therapeutic targeting of HSP90 will be most effective in FLT3-ITD patients, wears based on observations in article IV indicate that targeting of PI3K-mTOR may be less effective in a minority of patients

CURATIVE VERSUS DISEASE STABILIZING AML-THERAPY

The overall AML relapse risk after conventional therapy is still 40-50% for younger patients [85], and the large group of patients above >65-70 years of age has an inferior prognosis [327] due to (i) patient related factors like general comorbidity, low performance status and unacceptable toxicity when using high-dose cytarabine [309]; and (ii) disease-related factors with a high frequency of high-risk cytogenetics and secondary AML [327]. The therapeutic decisions in elderly patients should be individualized and be based on the possibility to achieve complete remission versus the risk of treatment-related morbidity or mortality [309]. Combination of ATRA, valproic acid or another HDAC-inhibitors and eventually theophylline or low-toxicity chemotherapy may be an alternative with low toxicity, and this treatment can induce disease stabilization for a subset of patients but only for a limited time [328-330]. The pharmacological agents investigated in the present thesis would therefore be considered for (i) combination with induction and/or consolidation therapy to increase AML-free survival; (ii) to be used alone or in combination with other drugs of palliative or disease stabilizing therapy; or (iii) as maintenance therapy in patients in complete hematological remission to eradicate residual disease and thereby decrease the relapse risk. Maintenance therapy is not common in AML, but results from recent studies suggest that this strategy may be effective and reduce the relapse risk [331, 332].

ANTIANGIOGENIC THERAPY IN HUMAN AML

The biological and clinical background for antiangiogenic therapy

Several antiangiogenic agents have a relatively low toxicity compared with conventional chemotherapy and are used for disease stabilization in other malignancies [244]. Crosstalk between AML cells and neighboring cells is probably important for cancer cell survival and proliferation, as well as for disease progression and chemosensitivity [265, 266]. Firstly, the bone marrow MVD can be evaluated either in biopsies [333], or by magnetic resonance examination, indicating that high density is associated with shorter overall and AML-free survival [267]. Secondly, a semiquantitative examination of specific angioregulators can be done by histochemistry of bone marrow biopsies, and high Ang2 levels then have a prognostic impact [281]. Secondly, high levels of intracellular or serum angiogenic factors are also associated with an adverse prognosis [284, 288, 294]. Finally, *in vitro* studies have demonstrated that the cytokine crosstalk between leukemic and endothelial cells can increase AML cell growth [189, 231]. These observations support the hypothesis that angiogenesis and endothelial cells are important for AML cell proliferation, survival and chemosensitivity.

Direct targeting of angiogenic factors

VEGF is probably the best characterized target for antiangiogenic therapy. Bevacizumab is a recombinant monoclonal antibody specific for VEGF-A. In two studies bevacizumab was administered to patients with refractory AML and resulted in modest clinical benefit [334, 335]. Suntinib is a VEGF RTK inhibitor that in addition inhibits KIT and FLT3 initiated signaling, and based on the available results in AML the drug seems to have a limited clinical effect with complete or partial remissions of only short duration for a subgroup of patients [336, 337]. HGF/MET inhibitors [273] and targeting of the Ang/Tie2 system [338] have not yet been investigated in human AML. MMPs were previously considered as possible targets in antiangiogenic therapy [101]; they have not been evaluated in hematological malignancies, but results from initial clinical studies in solid tumors have been disappointing [101]. Thus, antiangiogenic therapy alone seems to have limited anticancer or antileukemic effects, however the clinical use of these strategies should be further evaluated. The

results from the present thesis clearly suggest that targeting of angioregulatory mechanisms is a part of the antileukemic effect of several targeted therapies that now are considered for AML therapy.

Targeting of endothelial cells - vascular disrupting agents

An alternative approach is to destabilize the cancer-induced microvessel network through selective targeting of proliferating endothelial cells [339]. Vascular disrupting agents (VDAs) are a new class of agents that target blood vessels by direct binding to microtubules in endothelial cells. The main goals for VDAs are to generate a rapid and selective vascular shutdown in the malign microenvironment and thereby induce secondary cancer cell death due to ischemia [339]. The effects of VDAs have not yet been completely elucidated, but probably involve inhibitory effects of the tubulin skeleton [339]. The question of possible systemic toxicity due to general endothelial cell damage or endothelial dysfunction has not been completely answered [290]. The best studied of these drugs is fosbretabulin/CA4P [339], and this drug and its analogue OXi4503 have shown promising preclinical results [264, 340]

Inhibition of angiogenesis through targeting of intracellular signaling pathways

An alternative strategy is to target intracellular signaling pathways and thereby inhibit malignant cells as well as the stromal cells, including endothelial cells [244]. Although we do not have detailed knowledge about the molecular mechanism of thalidomide, lenaladomide and bortezomib, these drugs seem to inhibit angiogenesis possibly through NF-κB inhibition when used for the treatment of MM [121]. Similar effects may also be seen in AML.

HSP90 inhibition probably targets multiple proangiogenic regulators and may thus have direct inhibitory effects on leukemic cells and in addition have indirect antileukemic effects through inhibition of angiogenesis by targeting a wide range of client proteins [175, 341]. Firstly, one important pathway that is usually upregulated during angiogenesis is the HIF/VEGF signaling axis [342]. Several key mediators of this pathway, including the HIF and VEGF receptor, are HSP90 client proteins [341]. Secondly, HSP90 is also important for stabilization of AKT; this kinase mediates phosphorylation and activation of NO synthase (NOS) that is important for stimulation and activation of endothelial cells [341]. Thirdly, HSP90 inhibition can reduce the constitutive release of several angiogenic factors by AML cells [175]. Finally, the HSP90 inhibitor SNX-2112 suppresses capillary tube formation in human umbilical vein endothelial cells through inhibition of the AKT/NOS pathway [222]. Clinical trials of

HSP90 inhibitors in AML are in progress [209] and it will then be important to evaluate both direct and indirect antileukemic effects [213].

The PI3K-mTOR pathway also seems to have a crucial role in angiogenesis. PI3K activation and phosphorylation is probably important for activation of the VEGF receptor [343]. Furthermore, Ang1 can phosphorylate and thereby activate Tie2 in a PI3K-dependent manner and thereby induce survival and migration of endothelial cells [343]. mTOR also seems to be important in angiogenesis, possibly by acting as a switch in endothelial cell metabolism that supports their proliferation [133]. mTOR inhibition thereby seems to have an antiangiogenic effect [344], which is also observed in AML [345].

Targeted therapy after remission induction – the possible importance of an altered cytokine network

Targeted therapy to AML patients in complete hematological remission is now considered (see above), and our observation described in article V show that this treatment will be given in a specific biological context, i.e. an altered cytokine network. Our present results suggest that the pretherapy status of the cytokine network has an impact of the post therapy clinical course in allo-SCT patients. Future studies have to clarify whether the altered cytokine network also will influence the efficiency of the new target therapy, e.g. HSP90 and PI3K-mTOR targeting treatment.

CONCLUDING REMARKS

New targeting therapies with antiangiogenic effects are currently considered in human AML. Although the clinical experience so far is limited, several of these therapies alone appear to have limited antileukemic effects. However, the scientific basis for simultaneous inhibition of several intracellular signaling systems in cancer treatment is emerging and this strategy may be more effective. Antiangiogenic effects may then be a part of such strategies. Given the heterogeneity of AML, further research is warranted to try to identify patient subsets that are likely to benefit from these approaches.

REFERENCES

1 Estey E, Dohner H: Acute myeloid leukaemia. Lancet. 2006;368: 1894-907.
2 Little MP: Cancer and non-cancer effects in Japanese atomic bomb survivors. J Radiol Prot. 2009;29: A43-59.
3 Gundestrup M, Storm HH: Radiation-induced acute myeloid leukaemia and other cancers in commercial jet cockpit crew: a population-based cohort study. Lancet. 1999;354: 2029-31.
4 Khalade A, Jaakkola MS, Pukkala E, Jaakkola JJ: Exposure to benzene at work and the risk of leukemia: a systematic review and meta-analysis. Environ Health. 2010;9: 31.
5 Kane EV, Roman E, Cartwright R, Parker J, Morgan G: Tobacco and the risk of acute leukaemia in adults. Br J Cancer. 1999;81: 1228-33.
6 Larson RA: Is secondary leukemia an independent poor prognostic factor in acute myeloid leukemia? Best Pract Res Clin Haematol. 2007;20: 29-37.
7 Reikvam H, Hatfield KJ, Kittang AO, Hovland R, Bruserud O: Acute Myeloid Leukemia with the t(8;21) Translocation: Clinical Consequences and Biological Implications. J Biomed Biotechnol. 2011;2011: 104631.
8 WHO: World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissues, 4th Edition. (Lyon: international Agency for Cancer). 2008.
9 Wang ZY, Chen Z: Acute promyelocytic leukemia: from highly fatal to highly curable. Blood. 2008;111: 2505-15.
10 Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C: Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33: 451-8.
11 Döhner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Lowenberg B, Bloomfield CD: Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115: 453-74.
12 Craig FE, Foon KA: Flow cytometric immunophenotyping for hematologic neoplasms. Blood. 2008;111: 3941-67.
13 Tsykunova G, Reikvam H, Hovland R, Bruserud Ø: The surface molecule signature of primary human acute myeloid leukemia (AML) cells is highly associated with NPM1 mutation status. Leukemia. 2011;In press.
14 Oyan AM, Bo TH, Jonassen I, Ulvestad E, Gjertsen BT, Kalland KH, Bruserud O: CD34 expression in native human acute myelogenous leukemia blasts: differences in CD34 membrane molecule expression are associated with different gene expression profiles. Cytometry B Clin Cytom. 2005;64: 18-27.
15 Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, Habdank M, Spath D, Morgan M, Benner A, Schlegelberger B, Heil G, Ganser A, Dohner H: Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. The New England journal of medicine. 2008;358: 1909-18.
16 Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, Wheatley K, Harrison CJ, Burnett AK, on behalf of the National Cancer Research Institute Adult Leukaemia Working G: Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116: 354-65.
17 Mrozek K, Heerema NA, Bloomfield CD: Cytogenetics in acute leukemia. Blood Rev. 2004;18: 115-36.
18 Rowley JD: Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann Genet. 1973;16: 109-12.
19 Breems DA, Van Putten WL, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KB, Mellink CH, Nieuwint A, Jotterand M, Hagemeijer A, Beverloo HB, Lowenberg B: Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol. 2008;26: 4791-7.
20 Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani S, Boer JM, Beverloo HB, Moorhouse MJ, van der Spek PJ, Lowenberg B, Delwel

R: Prognostically useful gene-expression profiles in acute myeloid leukemia. The New England journal of medicine. 2004;350: 1617-28.
21 Bullinger L, Dohner K, Bair E, Frohling S, Schlenk RF, Tibshirani R, Dohner H, Pollack JR: Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. The New England journal of medicine. 2004;350: 1605-16.
22 Chou W-C, Huang H-H, Hou H-A, Chen C-Y, Tang J-L, Yao M, Tsay W, Ko B-S, Wu S-J, Huang S-Y, Hsu S-C, Chen Y-C, Huang Y-N, Chang Y-C, Lee F-Y, Liu M-C, Liu C-W, Tseng M-H, Huang C-F, Tien H-F: Distinct clinical and biological features of de novo acute myeloid leukemia with additional sex comb-like 1 (ASXL1) mutations. Blood. 2010;116: 4086-94.
23 Paschka P, Schlenk R, Aulitzky T, Gaidzik V, Habdank M, Corbacioglu A, Bullinger L, Späth D, Köhne C, Kündgen A, von Lilienfeld-Toal M, Helld G, Horst HA, Rummel M, Wilhelm S, Döhner H, Döhner K: ASXL1 mutations in acute myeloid leukemia: Results on 799 patients within the AML studt group (AMSLG). Haematologica. 2011;96(s2): Abstract no 1017.
24 Baldus CD, Tanner SM, Ruppert AS, Whitman SP, Archer KJ, Marcucci G, Caligiuri MA, Carroll AJ, Vardiman JW, Powell BL, Allen SL, Moore JO, Larson RA, Kolitz JE, de la Chapelle A, Bloomfield CD: BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: a Cancer and Leukemia Group B Study. Blood. 2003;102: 1613-8.
25 Baldus CD, Thiede C, Soucek S, Bloomfield CD, Thiel E, Ehninger G: BAALC Expression and FLT3 Internal Tandem Duplication Mutations in Acute Myeloid Leukemia Patients With Normal Cytogenetics: Prognostic Implications. Journal of Clinical Oncology. 2006;24: 790-7.
26 Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, Behre G, Hiddemann W, Tenen DG: Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet. 2001;27: 263-70.
27 Fuchs O, Provaznikova D, Kocova M, Kostecka A, Cvekova P, Neuwirtova R, Kobylka P, Cermak J, Brezinova J, Schwarz J, Markova J, Salaj P, Klamova H, Maaloufova J, Lemez P, Novakova L, Benesova K: CEBPA polymorphisms and mutations in patients with acute myeloid leukemia, myelodysplastic syndrome, multiple myeloma and non-Hodgkin's lymphoma. Blood Cells Mol Dis. 2008;40: 401-5.
28 Wouters BJ, Lowenberg B, Erpelinck-Verschueren CAJ, van Putten WLJ, Valk PJM, Delwel R: Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood. 2009;113: 3088-91.
29 Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, van der Poel-van de Luytgaarde SC, Damm F, Krauter J, Ganser A, Schlenk RF, Lowenberg B, Delwel R, Dohner H, Valk PJ, Dohner K: Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood. 2011;117: 2469-75.
30 Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, Harris CC, Lichti CF, Townsend RR, Fulton RS, Dooling DJ, Koboldt DC, Schmidt H, Zhang Q, Osborne JR, Lin L, O'Laughlin M, McMichael JF, Delehaunty KD, McGrath SD, Fulton LA, Magrini VJ, Vickery TL, Hundal J, Cook LL, Conyers JJ, Swift GW, Reed JP, Alldredge PA, Wylie T, Walker J, Kalicki J, Watson MA, Heath S, Shannon WD, Varghese N, Nagarajan R, Westervelt P, Tomasson MH, Link DC, Graubert TA, DiPersio JF, Mardis ER, Wilson RK: DNMT3A mutations in acute myeloid leukemia. The New England journal of medicine. 2010;363: 2424-33.
31 Gaidzik V, Schlenk R, Paschka P, Stoelzle A, Corbacioglu A, Mergenthaler H, Goetze K, Salwender H, Nachbaur D, Koller E, Haase D, Spaeth D, Bullinger L, Döhner H, Döhner K: DNMT3A mutations in acute myeloid leukemia: Results on 687 patients treated within the AML HD98A study of teh AML study group (AMSLG). Haematologica. 2011;96(s2): Abstract no 0541.
32 Thol F, Damm F, Lüdeking A, Winschel C, Wagner K, Morgan M, Göhring G, Schlegelberger B, Hoelzer D, Lübbert M, Kanz L, Fiedler W, Kirchner H, Heil G, Krauter J, Ganser A, Heuser M: DNMT3A mutations in acute myeloid leukemia: Frequency and prognostic impact. Haematologica. 2011;96(s2): Abstract no 0472.
33 Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, Shi JY, Zhu YM, Tang L, Zhang XW, Liang WX, Mi JQ, Song HD, Li KQ, Chen Z, Chen SJ: Exome sequencing identifies somatic mutations of

DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43: 309-15.
34 Marcucci G, Baldus CD, Ruppert AS, Radmacher MD, Mrozek K, Whitman SP, Kolitz JE, Edwards CG, Vardiman JW, Powell BL, Baer MR, Moore JO, Perrotti D, Caligiuri MA, Carroll AJ, Larson RA, de la Chapelle A, Bloomfield CD: Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. J Clin Oncol. 2005;23: 9234-42.
35 Marcucci G, Maharry K, Whitman SP, Vukosavljevic T, Paschka P, Langer C, Mrozek K, Baldus CD, Carroll AJ, Powell BL, Kolitz JE, Larson RA, Bloomfield CD: High expression levels of the ETS-related gene, ERG, predict adverse outcome and improve molecular risk-based classification of cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B Study. J Clin Oncol. 2007;25: 3337-43.
36 Bruserud O, Hovland R, Wergeland L, Huang TS, Gjertsen BT: Flt3-mediated signaling in human acute myelogenous leukemia (AML) blasts: a functional characterization of Flt3-ligand effects in AML cell populations with and without genetic Flt3 abnormalities. Haematologica. 2003;88: 416-28.
37 Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T, Misawa S: Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia. 1996;10: 1911-8.
38 Stirewalt DL, Kopecky KJ, Meshinchi S, Engel JH, Pogosova-Agadjanyan EL, Linsley J, Slovak ML, Willman CL, Radich JP: Size of FLT3 internal tandem duplication has prognostic significance in patients with acute myeloid leukemia. Blood. 2006;107: 3724-6.
39 Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, Asou N, Kuriyama K, Jinnai I, Shimazaki C, Akiyama H, Saito K, Oh H, Motoji T, Omoto E, Saito H, Ohno R, Ueda R: Prognostic Implication of FLT3 and N-RAS Gene Mutations in Acute Myeloid Leukemia. Blood. 1999;93: 3074-80.
40 Schlenk RF, Pasquini MC, Perez WS, Zhang MJ, Krauter J, Antin JH, Bashey A, Bolwell BJ, Buchner T, Cahn JY, Cairo MS, Copelan EA, Cutler CS, Dohner H, Gale RP, Ilhan O, Lazarus HM, Liesveld JL, Litzow MR, Marks DI, Maziarz RT, McCarthy PL, Nimer SD, Sierra J, Tallman MS, Weisdorf DJ, Horowitz MM, Ganser A: HLA-identical sibling allogeneic transplants versus chemotherapy in acute myelogenous leukemia with t(8;21) in first complete remission: collaborative study between the German AML Intergroup and CIBMTR. Biol Blood Marrow Transplant. 2008;14: 187-96.
41 Choudhary C, Schwable J, Brandts C, Tickenbrock L, Sargin B, Kindler T, Fischer T, Berdel WE, Muller-Tidow C, Serve H: AML-associated Flt3 kinase domain mutations show signal transduction differences compared with Flt3 ITD mutations. Blood. 2005;106: 265-73.
42 Mead AJ, Linch DC, Hills RK, Wheatley K, Burnett AK, Gale RE: FLT3 tyrosine kinase domain mutations are biologically distinct from and have a significantly more favorable prognosis than FLT3 internal tandem duplications in patients with acute myeloid leukemia. Blood. 2007;110: 1262-70.
43 Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, Dooling D, Dunford-Shore BH, McGrath S, Hickenbotham M, Cook L, Abbott R, Larson DE, Koboldt DC, Pohl C, Smith S, Hawkins A, Abbott S, Locke D, Hillier LW, Miner T, Fulton L, Magrini V, Wylie T, Glasscock J, Conyers J, Sander N, Shi X, Osborne JR, Minx P, Gordon D, Chinwalla A, Zhao Y, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson M, Baty J, Ivanovich J, Heath S, Shannon WD, Nagarajan R, Walter MJ, Link DC, Graubert TA, DiPersio JF, Wilson RK: DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature. 2008;456: 66-72.
44 Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, Koboldt DC, Fulton RS, Delehaunty KD, McGrath SD, Fulton LA, Locke DP, Magrini VJ, Abbott RM, Vickery TL, Reed JS, Robinson JS, Wylie T, Smith SM, Carmichael L, Eldred JM, Harris CC, Walker J, Peck JB, Du F, Dukes AF, Sanderson GE, Brummett AM, Clark E, McMichael JF, Meyer RJ, Schindler JK, Pohl CS, Wallis JW, Shi X, Lin L, Schmidt H, Tang Y, Haipek C, Wiechert ME, Ivy JV, Kalicki J, Elliott G, Ries RE, Payton JE, Westervelt P, Tomasson MH, Watson MA, Baty J, Heath S, Shannon WD, Nagarajan R, Link DC, Walter MJ, Graubert TA, DiPersio JF, Wilson RK, Ley TJ: Recurring mutations found by sequencing an acute myeloid leukemia genome. The New England journal of medicine. 2009;361: 1058-66.
45 Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrozek K, Margeson D, Holland KB, Whitman SP, Becker H, Schwind S, Metzeler KH, Powell BL, Carter TH, Kolitz JE, Wetzler M, Carroll AJ, Baer MR, Caligiuri MA, Larson RA, Bloomfield CD: IDH1 and IDH2 gene mutations

identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28: 2348-55.
46 Boissel N, Nibourel O, Renneville A, Gardin C, Reman O, Contentin N, Bordessoule D, Pautas C, de Revel T, Quesnel B, Huchette P, Philippe N, Geffroy S, Terre C, Thomas X, Castaigne S, Dombret H, Preudhomme C: Prognostic impact of isocitrate dehydrogenase enzyme isoforms 1 and 2 mutations in acute myeloid leukemia: a study by the Acute Leukemia French Association group. J Clin Oncol. 2010;28: 3717-23.
47 Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L, Spath D, Kayser S, Zucknick M, Gotze K, Horst HA, Germing U, Dohner H, Dohner K: IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol. 2010;28: 3636-43.
48 Steensma DP, McClure RF, Karp JE, Tefferi A, Lasho TL, Powell HL, DeWald GW, Kaufmann SH: JAK2 V617F is a rare finding in de novo acute myeloid leukemia, but STAT3 activation is common and remains unexplained. Leukemia. 2006;20: 971-8.
49 Xiang Z, Zhao Y, Mitaksov V, Fremont DH, Kasai Y, Molitoris A, Ries RE, Miner TL, McLellan MD, DiPersio JF, Link DC, Payton JE, Graubert TA, Watson M, Shannon W, Heath SE, Nagarajan R, Mardis ER, Wilson RK, Ley TJ, Tomasson MH: Identification of somatic JAK1 mutations in patients with acute myeloid leukemia. Blood. 2008;111: 4809-12.
50 Walters DK, Mercher T, Gu T-L, O'Hare T, Tyner JW, Loriaux M, Goss VL, Lee KA, Eide CA, Wong MJ, Stoffregen EP, McGreevey L, Nardone J, Moore SA, Crispino J, Boggon TJ, Heinrich MC, Deininger MW, Polakiewicz RD, Gilliland DG, Druker BJ: Activating alleles of JAK3 in acute megakaryoblastic leukemia. Cancer Cell. 2006;10: 65-75.
51 Wang YY, Zhou GB, Yin T, Chen B, Shi JY, Liang WX, Jin XL, You JH, Yang G, Shen ZX, Chen J, Xiong SM, Chen GQ, Xu F, Liu YW, Chen Z, Chen SJ: AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec. Proc Natl Acad Sci U S A. 2005;102: 1104-9.
52 Paschka P, Marcucci G, Ruppert AS, Mrozek K, Chen H, Kittles RA, Vukosavljevic T, Perrotti D, Vardiman JW, Carroll AJ, Kolitz JE, Larson RA, Bloomfield CD: Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. J Clin Oncol. 2006;24: 3904-11.
53 Lück SC, Russ AC, Du J, Gaidzik V, Schlenk RF, Pollack JR, Dohner K, Dohner H, Bullinger L: KIT mutations confer a distinct gene expression signature in core binding factor leukaemia. Br J Haematol. 2010;148: 925-37.
54 Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL: Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. The New England journal of medicine. 2001;344: 1031-7.
55 Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ, Langabeer SE, Kottaridis PD, Moorman AV, Burnett AK, Linch DC: RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. Blood. 2005;106: 2113-9.
56 Boissel N, Leroy H, Brethon B, Philippe N, de Botton S, Auvrignon A, Raffoux E, Leblanc T, Thomas X, Hermine O, Quesnel B, Baruchel A, Leverger G, Dombret H, Preudhomme C: Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia. 2006;20: 965-70.
57 Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S: Implications of NRAS mutations in AML: a study of 2502 patients. Blood. 2006;107: 3847-53.
58 De Braekeleer M, Morel F, Le Bris MJ, Herry A, Douet-Guilbert N: The MLL gene and translocations involving chromosomal band 11q23 in acute leukemia. Anticancer Res. 2005;25: 1931-44.
59 Döhner K, Tobis K, Ulrich R, Fröhling S, Benner A, Schlenk RF, Döhner H: Prognostic Significance of Partial Tandem Duplications of the MLL Gene in Adult Patients 16 to 60 Years Old With Acute Myeloid Leukemia and Normal Cytogenetics: A Study of the Acute Myeloid Leukemia Study Group Ulm. Journal of Clinical Oncology. 2002;20: 3254-61.
60 Weisser M, Kern W, Schoch C, Hiddemann W, Haferlach T, Schnittger S: Risk assessment by monitoring expression levels of partial tandem duplications in the MLL gene in acute myeloid leukemia during therapy. Haematologica. 2005;90: 881-9.
61 Langer C, Marcucci G, Holland KB, Radmacher MD, Maharry K, Paschka P, Whitman SP, MrÃ³zek K, Baldus CD, Vij R, Powell BL, Carroll AJ, Kolitz JE, Caligiuri MA, Larson RA,

Bloomfield CD: Prognostic Importance of MN1 Transcript Levels, and Biologic Insights From MN1-Associated Gene and MicroRNA Expression Signatures in Cytogenetically Normal Acute Myeloid Leukemia: A Cancer and Leukemia Group B Study. Journal of Clinical Oncology. 2009;27: 3198-204.
62 Heuser M, Beutel G, Krauter J, Dohner K, von Neuhoff N, Schlegelberger B, Ganser A: High meningioma 1 (MN1) expression as a predictor for poor outcome in acute myeloid leukemia with normal cytogenetics. Blood. 2006;108: 3898-905.
63 Falini B, Bolli N, Liso A, Martelli MP, Mannucci R, Pileri S, Nicoletti I: Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. Leukemia. 2009;23: 1731-43.
64 Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, La Starza R, Diverio D, Colombo E, Santucci A, Bigerna B, Pacini R, Pucciarini A, Liso A, Vignetti M, Fazi P, Meani N, Pettirossi V, Saglio G, Mandelli F, Lo-Coco F, Pelicci PG, Martelli MF: Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. The New England journal of medicine. 2005;352: 254-66.
65 Alcalay M, Tiacci E, Bergomas R, Bigerna B, Venturini E, Minardi SP, Meani N, Diverio D, Bernard L, Tizzoni L, Volorio S, Luzi L, Colombo E, Lo Coco F, Mecucci C, Falini B, Pelicci PG, for the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto Acute Leukemia Working P: Acute myeloid leukemia bearing cytoplasmic nucleophosmin (NPMc+ AML) shows a distinct gene expression profile characterized by up-regulation of genes involved in stem-cell maintenance. Blood. 2005;106: 899-902.
66 Garzon R, Garofalo M, Martelli MP, Briesewitz R, Wang L, Fernandez-Cymering C, Volinia S, Liu C-G, Schnittger S, Haferlach T, Liso A, Diverio D, Mancini M, Meloni G, Foa R, Martelli MF, Mecucci C, Croce CM, Falini B: Distinctive microRNA signature of acute myeloid leukemia bearing cytoplasmic mutated nucleophosmin. Proceedings of the National Academy of Sciences. 2008;105: 3945-50.
67 Gaidzik VI, Bullinger L, Schlenk RF, Zimmermann AS, Rock J, Paschka P, Corbacioglu A, Krauter J, Schlegelberger B, Ganser A, Spath D, Kundgen A, Schmidt-Wolf IG, Gotze K, Nachbaur D, Pfreundschuh M, Horst HA, Dohner H, Dohner K: RUNX1 Mutations in Acute Myeloid Leukemia: Results From a Comprehensive Genetic and Clinical Analysis From the AML Study Group. J Clin Oncol. 2011;29: 1364-72.
68 Tang JL, Hou HA, Chen CY, Liu CY, Chou WC, Tseng MH, Huang CF, Lee FY, Liu MC, Yao M, Huang SY, Ko BS, Hsu SC, Wu SJ, Tsay W, Chen YC, Lin LI, Tien HF: AML1/RUNX1 mutations in 470 adult patients with de novo acute myeloid leukemia: prognostic implication and interaction with other gene alterations. Blood. 2009;114: 5352-61.
69 Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, Kosmider O, Le Couedic JP, Robert F, Alberdi A, Lecluse Y, Plo I, Dreyfus FJ, Marzac C, Casadevall N, Lacombe C, Romana SP, Dessen P, Soulier J, Viguie F, Fontenay M, Vainchenker W, Bernard OA: Mutation in TET2 in myeloid cancers. The New England journal of medicine. 2009;360: 2289-301.
70 Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, Malinge S, Yao J, Kilpivaara O, Bhat R, Huberman K, Thomas S, Dolgalev I, Heguy A, Paietta E, Le Beau MM, Beran M, Tallman MS, Ebert BL, Kantarjian HM, Stone RM, Gilliland DG, Crispino JD, Levine RL: Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009;114: 144-7.
71 Gaidzik VI, Schlenk RF, Paschka P, Kohne C-H, Held G, Habdank M, Gohlke J, Mergenthaler H-G, Salwender HJ, Bullinger L, Dohner H, Dohner K: TET2 Mutations In Acute Myeloid Leukemia (AML): Results on 783 Patients Treated within the AML HD98A Study of the AML Study Group (AMLSG). ASH Annual Meeting Abstracts. 2010;116: Abstract no 97.
72 Cheok CF, Verma CS, Baselga J, Lane DP: Translating p53 into the clinic. Nat Rev Clin Oncol. 2011;8: 25-37.
73 Seifert H, Mohr B, Thiede C, Oelschlagel U, Schakel U, Illmer T, Soucek S, Ehninger G, Schaich M: The prognostic impact of 17p (p53) deletion in 2272 adults with acute myeloid leukemia. Leukemia. 2009;23: 656-63.
74 Ishikawa Y, Kiyoi H, Tsujimura A, Miyawaki S, Miyazaki Y, Kuriyama K, Tomonaga M, Naoe T: Comprehensive analysis of cooperative gene mutations between class I and class II in de novo acute myeloid leukemia. Eur J Haematol. 2009;83: 90-8.
75 King-Underwood L, Pritchard-Jones K: Wilms' Tumor (WT1) Gene Mutations Occur Mainly in Acute Myeloid Leukemia and May Confer Drug Resistance. Blood. 1998;91: 2961-8.

76 Owen C, Fitzgibbon J, Paschka P: The clinical relevance of Wilms Tumour 1 (WT1) gene mutations in acute leukaemia. Hematol Oncol. 2010;28: 13-9.
77 Virappane P, Gale R, Hills R, Kakkas I, Summers K, Stevens J, Allen C, Green C, Quentmeier H, Drexler H, Burnett A, Linch D, Bonnet D, Lister TA, Fitzgibbon J: Mutation of the Wilms' tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical Research Council Adult Leukaemia Working Party. J Clin Oncol. 2008;26: 5429-35.
78 Paschka P, Marcucci G, Ruppert AS, Whitman SP, Mrozek K, Maharry K, Langer C, Baldus CD, Zhao W, Powell BL, Baer MR, Carroll AJ, Caligiuri MA, Kolitz JE, Larson RA, Bloomfield CD: Wilms' tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol. 2008;26: 4595-602.
79 Lowenberg B, Pabst T, Vellenga E, van Putten W, Schouten HC, Graux C, Ferrant A, Sonneveld P, Biemond BJ, Gratwohl A, de Greef GE, Verdonck LF, Schaafsma MR, Gregor M, Theobald M, Schanz U, Maertens J, Ossenkoppele GJ: Cytarabine dose for acute myeloid leukemia. The New England journal of medicine. 2011;364: 1027-36.
80 Burnett A, Wetzler M, Lowenberg B: Therapeutic advances in acute myeloid leukemia. J Clin Oncol. 2011;29: 487-94.
81 Lowenberg B, van Putten W, Theobald M, Gmur J, Verdonck L, Sonneveld P, Fey M, Schouten H, de Greef G, Ferrant A, Kovacsovics T, Gratwohl A, Daenen S, Huijgens P, Boogaerts M: Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. The New England journal of medicine. 2003;349: 743-52.
82 Thomas X, Raffoux E, Botton S, Pautas C, Arnaud P, de Revel T, Reman O, Terre C, Corront B, Gardin C, Le QH, Quesnel B, Cordonnier C, Bourhis JH, Elhamri M, Fenaux P, Preudhomme C, Michallet M, Castaigne S, Dombret H: Effect of priming with granulocyte-macrophage colony-stimulating factor in younger adults with newly diagnosed acute myeloid leukemia: a trial by the Acute Leukemia French Association (ALFA) Group. Leukemia. 2007;21: 453-61.
83 Mayer RJ, Davis RB, Schiffer CA, Berg DT, Powell BL, Schulman P, Omura GA, Moore JO, McIntyre OR, Frei E, 3rd: Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. The New England journal of medicine. 1994;331: 896-903.
84 Lowenberg B: Acute myeloid leukemia: the challenge of capturing disease variety. Hematology Am Soc Hematol Educ Program. 2008: 1-11.
85 Stapnes C, Gjertsen BT, Reikvam H, Bruserud O: Targeted therapy in acute myeloid leukaemia: current status and future directions. Expert Opin Investig Drugs. 2009;18: 433-55.
86 McClune BL, Weisdorf DJ, Pedersen TL, Tunes da Silva G, Tallman MS, Sierra J, Dipersio J, Keating A, Gale RP, George B, Gupta V, Hahn T, Isola L, Jagasia M, Lazarus H, Marks D, Maziarz R, Waller EK, Bredeson C, Giralt S: Effect of age on outcome of reduced-intensity hematopoietic cell transplantation for older patients with acute myeloid leukemia in first complete remission or with myelodysplastic syndrome. J Clin Oncol. 2010;28: 1878-87.
87 Foran JM: New Prognostic Markers in Acute Myeloid Leukemia: Perspective from the Clinic. Hematology Am Soc Hematol Educ Program. 2010;2010: 47-55.
88 Wheatley K, Burnett AK, Goldstone AH, Gray RG, Hann IM, Harrison CJ, Rees JK, Stevens RF, Walker H: A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Br J Haematol. 1999;107: 69-79.
89 Koreth J, Schlenk R, Kopecky KJ, Honda S, Sierra J, Djulbegovic BJ, Wadleigh M, DeAngelo DJ, Stone RM, Sakamaki H, Appelbaum FR, Dohner H, Antin JH, Soiffer RJ, Cutler C: Allogeneic Stem Cell Transplantation for Acute Myeloid Leukemia in First Complete Remission: Systematic Review and Meta-analysis of Prospective Clinical Trials. JAMA. 2009;301: 2349-61.
90 Nguyen S, Leblanc T, Fenaux P, Witz F, Blaise D, Pigneux A, Thomas X, Rigal-Huguet F, Lioure B, Auvrignon A, Fiere D, Reiffers J, Castaigne S, Leverger G, Harousseau J-L, Socie G, Dombret H: A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia (AML): a survey of 161 cases from the French AML Intergroup. Blood. 2002;99: 3517-23.

91 Cornelissen JJ, van Putten WLJ, Verdonck LF, Theobald M, Jacky E, Daenen SMG, van Marwijk Kooy M, Wijermans P, Schouten H, Huijgens PC, van der Lelie H, Fey M, Ferrant A, Maertens J, Gratwohl A, Lowenberg B: Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? Blood. 2007;109: 3658-66.
92 Borthakur G, de Lima M, Kantarjian H, Xiao L, de Padua Silva L, Garcia-Manero G, Giralt S, Ravandi-Kashani F, Pierce S, Champlin R: Stem Cell Transplantation in Remission Improves Survival in Acute Myelogenous Leukemia Associated with FLT3 Mutations. ASH Annual Meeting Abstracts. 2008;112: Abstract no 3302.
93 Breems DA, Van Putten WLJ, Huijgens PC, Ossenkoppele GJ, Verhoef GEG, Verdonck LF, Vellenga E, De Greef GE, Jacky E, Van der Lelie J, Boogaerts MA, LÃ¶wenberg B: Prognostic Index for Adult Patients With Acute Myeloid Leukemia in First Relapse. Journal of Clinical Oncology. 2005;23: 1969-78.
94 Eapen M, Giralt SA, Horowitz MM, Klein JP, Wagner JE, Zhang MJ, Tallman MS, Marks DI, Camitta BM, Champlin RE, Ringden O, Bredeson CN, Martino R, Gale RP, Cairo MS, Litzow MR, deLima M: Second transplant for acute and chronic leukemia relapsing after first HLA-identical sibling transplant. Bone Marrow Transplant. 2004;34: 721-7.
95 Kern W, Haferlach T, Schnittger S, Hiddemann W, Schoch C: Prognosis in Therapy-Related Acute Myeloid Leukemia and Impact of Karyotype. Journal of Clinical Oncology. 2004;22: 2510-1.
96 Marbello L, Ricci F, Nosari AM, Turrini M, Nador G, Nichelatti M, Tedeschi A, Vismara E, Morra E: Outcome of hyperleukocytic adult acute myeloid leukaemia: a single-center retrospective study and review of literature. Leuk Res. 2008;32: 1221-7.
97 Bug G, Anargyrou K, Tonn T, Bialleck H, Seifried E, Hoelzer D, Ottmann OG: Impact of leukapheresis on early death rate in adult acute myeloid leukemia presenting with hyperleukocytosis. Transfusion. 2007;47: 1843-50.
98 Chang MC, Chen TY, Tang JL, Lan YJ, Chao TY, Chiu CF, Ho HT: Leukapheresis and cranial irradiation in patients with hyperleukocytic acute myeloid leukemia: no impact on early mortality and intracranial hemorrhage. Am J Hematol. 2007;82: 976-80.
99 Kornblau SM, Womble M, Qiu YH, Jackson CE, Chen W, Konopleva M, Estey EH, Andreeff M: Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. Blood. 2006;108: 2358-65.
100 Kittang AO, Hatfield K, Sand K, Reikvam H, Bruserud O: The chemokine network in acute myelogenous leukemia: molecular mechanisms involved in leukemogenesis and therapeutic implications. Curr Top Microbiol Immunol. 2010;341: 149-72.
101 Hatfield KJ, Reikvam H, Bruserud O: The crosstalk between the matrix metalloprotease system and the chemokine network in acute myeloid leukemia. Curr Med Chem. 2010;17: 4448-61.
102 Vanhaesebroeck B, Guillermet-Guibert J, Graupera M, Bilanges B: The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol. 2010;11: 329-41.
103 Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT: Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol. 2008;182: 685-701.
104 Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ: Potential therapeutic applications of autophagy. Nat Rev Drug Discov. 2007;6: 304-12.
105 Bousquet M, Recher C, Queleen C, Demur C, Payrastre B, Brousset P: Assessment of somatic mutations in phosphatidylinositol 3-kinase gene in human lymphoma and acute leukaemia. Br J Haematol. 2005;131: 411-3.
106 Tamburini J, Elie C, Bardet V, Chapuis N, Park S, Broet P, Cornillet-Lefebvre P, Lioure B, Ugo V, Blanchet O, Ifrah N, Witz F, Dreyfus F, Mayeux P, Lacombe C, Bouscary D: Constitutive phosphoinositide 3-kinase/Akt activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. Blood. 2007;110: 1025-8.
107 Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M: Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood. 2003;102: 972-80.
108 Muranyi AL, Dedhar S, Hogge DE: Combined inhibition of integrin linked kinase and FMS-like tyrosine kinase 3 is cytotoxic to acute myeloid leukemia progenitor cells. Experimental Hematology. 2009;37: 450-60.

109 Faderl S, Pal A, Bornmann W, Albitar M, Maxwell D, Van Q, Peng Z, Harris D, Liu Z, Hazan-Halevy I, Kantarjian HM, Estrov Z: Kit Inhibitor APcK110 Induces Apoptosis and Inhibits Proliferation of Acute Myeloid Leukemia Cells. Cancer Research. 2009;69: 3910-7.
110 Birkenkamp KU, Geugien M, Schepers H, Westra J, Lemmink HH, Vellenga E: Constitutive NF-[kappa]B DNA-binding activity in AML is frequently mediated by a Ras//PI3-K//PKB-dependent pathway. Leukemia. 2003;18: 103-12.
111 Doepfner KT, Spertini O, Arcaro A: Autocrine insulin-like growth factor-I signaling promotes growth and survival of human acute myeloid leukemia cells via the phosphoinositide 3-kinase//Akt pathway. Leukemia. 2007;21: 1921-30.
112 Imai N, Miwa H, Shikami M, Suganuma K, Gotoh M, Hiramatsu A, Wakabayashi M, Watarai M, Hanamura I, Imamura A, Mihara H, Shitara K, Shibuya M, Nitta M: Growth inhibition of AML cells with specific chromosome abnormalities by monoclonal antibodies to receptors for vascular endothelial growth factor. Leukemia Research. 2009;33: 1650-7.
113 Naran S, Zhang X, Hughes SJ: Inhibition of HGF/MET as therapy for malignancy. Expert Opin Ther Targets. 2009;13: 569-81.
114 Wakabayashi M, Miwa H, Shikami M, Hiramatsu A, Ikai T, Tajima E, Yamamoto H, Miura K, Satoh A, Itoh M, Imamura A, Mihara H, Katoh Y, Nitta M: Autocrine pathway of angiopoietins-Tie2 system in AML cells: association with phosphatidyl-inositol 3 kinase. Hematol J. 2004;5: 353-60.
115 Zeng Z, Xi Shi Y, Samudio IJ, Wang R-Y, Ling X, Frolova O, Levis M, Rubin JB, Negrin RR, Estey EH, Konoplev S, Andreeff M, Konopleva M: Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood. 2009;113: 6215-24.
116 Tabe Y, Jin L, Tsutsumi-Ishii Y, Xu Y, McQueen T, Priebe W, Mills GB, Ohsaka A, Nagaoka I, Andreeff M, Konopleva M: Activation of Integrin-Linked Kinase Is a Critical Prosurvival Pathway Induced in Leukemic Cells by Bone Marrowâ€“Derived Stromal Cells. Cancer Research. 2007;67: 684-94.
117 Min YH, Eom JI, Cheong JW, Maeng HO, Kim JY, Jeung HK, Lee ST, Lee MH, Hahn JS, Ko YW: Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. Leukemia. 2003;17: 995-7.
118 Zhao S, Konopleva M, Cabreira-Hansen M, Xie Z, Hu W, Milella M, Estrov Z, Mills GB, Andreeff M: Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias. Leukemia. 2003;18: 267-75.
119 Peterson LF, Yan M, Zhang DE: The p21Waf1 pathway is involved in blocking leukemogenesis by the t(8;21) fusion protein AML1-ETO. Blood. 2007;109: 4392-8.
120 McCormack E, Haaland I, Venas G, Forthun RB, Bruserud O, Gjertsen BT: Evaluation of Combinational Therapy of MDM2-Antagonist Nutlin-3 and HDAC-Inhibitor Valproic Acid in Acute Myeloid Leukemia in Vitro and in Vivo. ASH Annual Meeting Abstracts. 2008;112: Abstract no 2981.
121 Reikvam H, Olsnes AM, Gjertsen BT, Ersvar E, Bruserud O: Nuclear factor-kappaB signaling: a contributor in leukemogenesis and a target for pharmacological intervention in human acute myelogenous leukemia. Crit Rev Oncog. 2009;15: 1-41.
122 Lagadinou ED, Ziros PG, Tsopra OA, Dimas K, Kokkinou D, Thanopoulou E, Karakantza M, Pantazis P, Spyridonidis A, Zoumbos NC: c-Jun N-terminal kinase activation failure is a new mechanism of anthracycline resistance in acute myeloid leukemia. Leukemia. 2008;22: 1899-908.
123 Kornblau SM, Singh N, Qiu Y, Chen W, Zhang N, Coombes KR: Highly phosphorylated FOXO3A is an adverse prognostic factor in acute myeloid leukemia. Clin Cancer Res. 2010;16: 1865-74.
124 Mesa RA, Loegering D, Powell HL, Flatten K, Arlander SJ, Dai NT, Heldebrant MP, Vroman BT, Smith BD, Karp JE, Eyck CJ, Erlichman C, Kaufmann SH, Karnitz LM: Heat shock protein 90 inhibition sensitizes acute myelogenous leukemia cells to cytarabine. Blood. 2005;106: 318-27.
125 Zhang H, Cicchetti G, Onda H, Koon HB, Asrican K, Bajraszewski N, Vazquez F, Carpenter CL, Kwiatkowski DJ: Loss of Tsc1/Tsc2 activates mTOR and disrupts PI3K-Akt signaling through downregulation of PDGFR. J Clin Invest. 2003;112: 1223-33.
126 Gonzalez E, McGraw TE: The Akt kinases: isoform specificity in metabolism and cancer. Cell Cycle. 2009;8: 2502-8.
127 Manning BD, Cantley LC: AKT/PKB signaling: navigating downstream. Cell. 2007;129: 1261-74.

128 Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, Hostetter G, Boguslawski S, Moses TY, Savage S, Uhlik M, Lin A, Du J, Qian YW, Zeckner DJ, Tucker-Kellogg G, Touchman J, Patel K, Mousses S, Bittner M, Schevitz R, Lai MH, Blanchard KL, Thomas JE: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007;448: 439-44.
129 Grandage VL, Gale RE, Linch DC, Khwaja A: PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways. Leukemia. 2005;19: 586-94.
130 Kornblau SM, McCue D, Singh N, Chen W, Estrov Z, Coombes KR: Recurrent expression signatures of cytokines and chemokines are present and are independently prognostic in acute myelogenous leukemia and myelodysplasia. Blood. 2010;116: 4251-61.
131 Wullschleger S, Loewith R, Hall MN: TOR signaling in growth and metabolism. Cell. 2006;124: 471-84.
132 Chapuis N, Tamburini J, Green AS, Willems L, Bardet V, Park S, Lacombe C, Mayeux P, Bouscary D: Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. Leukemia. 2010;24: 1686-99.
133 Green AS, Chapuis N, Maciel TT, Willems L, Lambert M, Arnoult C, Boyer O, Bardet V, Park S, Foretz M, Viollet B, Ifrah N, Dreyfus F, Hermine O, Moura IC, Lacombe C, Mayeux P, Bouscary D, Tamburini J: The LKB1/AMPK signaling pathway has tumor suppressor activity in acute myeloid leukemia through the repression of mTOR-dependent oncogenic mRNA translation. Blood. 2010;116: 4262-73.
134 Tamburini J, Green AS, Bardet V, Chapuis N, Park S, Willems L, Uzunov M, Ifrah N, Dreyfus F, Lacombe C, Mayeux P, Bouscary D: Protein synthesis is resistant to rapamycin and constitutes a promising therapeutic target in acute myeloid leukemia. Blood. 2009;114: 1618-27.
135 Dos Santos C, Demur C, Bardet V, Prade-Houdellier N, Payrastre B, Recher C: A critical role for Lyn in acute myeloid leukemia. Blood. 2008;111: 2269-79.
136 Kong D, Yamori T: Advances in development of phosphatidylinositol 3-kinase inhibitors. Curr Med Chem. 2009;16: 2839-54.
137 Teachey DT, Grupp SA, Brown VI: Mammalian target of rapamycin inhibitors and their potential role in therapy in leukaemia and other haematological malignancies. Br J Haematol. 2009;145: 569-80.
138 Perl AE, Kasner MT, Tsai DE, Vogl DT, Loren AW, Schuster SJ, Porter DL, Stadtmauer EA, Goldstein SC, Frey NV, Nasta SD, Hexner EO, Dierov JK, Swider CR, Bagg A, Gewirtz AM, Carroll M, Luger SM: A phase I study of the mammalian target of rapamycin inhibitor sirolimus and MEC chemotherapy in relapsed and refractory acute myelogenous leukemia. Clin Cancer Res. 2009;15: 6732-9.
139 Yee KW, Zeng Z, Konopleva M, Verstovsek S, Ravandi F, Ferrajoli A, Thomas D, Wierda W, Apostolidou E, Albitar M, O'Brien S, Andreeff M, Giles FJ: Phase I/II study of the mammalian target of rapamycin inhibitor everolimus (RAD001) in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res. 2006;12: 5165-73.
140 Rizzieri DA, Feldman E, Dipersio JF, Gabrail N, Stock W, Strair R, Rivera VM, Albitar M, Bedrosian CL, Giles FJ: A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. Clin Cancer Res. 2008;14: 2756-62.
141 Rodriguez R, Nakamura R, Palmer JM, Parker P, Shayani S, Nademanee A, Snyder D, Pullarkat V, Kogut N, Rosenthal J, Smith E, Karanes C, O'Donnell M, Krishnan AY, Senitzer D, Forman SJ: A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. Blood. 2010;115: 1098-105.
142 Chapuis N, Tamburini J, Green AS, Vignon C, Bardet V, Neyret A, Pannetier M, Willems L, Park S, Macone A, Maira SM, Ifrah N, Dreyfus F, Herault O, Lacombe C, Mayeux P, Bouscary D: Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. Clin Cancer Res. 2010;16: 5424-35.
143 Tazzari PL, Tabellini G, Ricci F, Papa V, Bortul R, Chiarini F, Evangelisti C, Martinelli G, Bontadini A, Cocco L, McCubrey JA, Martelli AM: Synergistic proapoptotic activity of recombinant TRAIL plus the Akt inhibitor Perifosine in acute myelogenous leukemia cells. Cancer Res. 2008;68: 9394-403.

144 Glienke W: The effect of dual PI3K/mTOR inhibitor NVP-BGT226 on cell cycle and survivin and STAT3 gene expression in human pancreatic cancer cell lines. ASCO Meeting Abstracts. 2011;29: 15s: Abstract no 14523.
145 Shapiro G, Kwak E, Baselga J, Rodon J, Scheffold C, Laird AD, Bedell C, G. E: Phase I dose-escalation study of XL147, a PI3K inhibitor administered orally to patients with solid tumors. J Clin Oncol 2009;27:15s: Abstract no 3500.
146 Baselga J, De Jonge MJ, Rodon J, Burris III HA, Birle DC, De Buck SS, Demanse D, Ru QC, Goldbrunner M, Bendell JC: A first-in-human phase I study of BKM120, an oral pan-class I PI3K inhibitor, in patients (pts) with advanced solid tumors. J Clin Oncol. 2010;28:15s, : Abstract no 3003
147 Amadori S, Venditti A, Ammatuna E, Martelli AM, Meloni G, Pane F, Martinelli G, Lunghi M, Pagano L, Cilloni D, Rizzoli V, Di Raimondo F, Fozza C, Annino L, Piciocchi A, La Sala E, Fazi P, Vignetti M: Temsirolimus, An mTOR Inhibitor, In Combination with Low-Dose Clofarabine in Older Patients with Advanced Acute Myeloid Leukemia: Results of a Phase 2 GIMEMA Study (AML-1107). ASH Annual Meeting Abstracts. 2010;116: Abstract no 510.
148 Folkes AJ, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, Friedman LS, Hayes A, Hancox TC, Kugendradas A, Lensun L, Moore P, Olivero AG, Pang J, Patel S, Pergl-Wilson GH, Raynaud FI, Robson A, Saghir N, Salphati L, Sohal S, Ultsch MH, Valenti M, Wallweber HJ, Wan NC, Wiesmann C, Workman P, Zhyvoloup A, Zvelebil MJ, Shuttleworth SJ: The identification of 2-(1H-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin -4-yl-thieno[3,2-d]pyrimidine (GDC-0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. J Med Chem. 2008;51: 5522-32.
149 Schwertschlag US, Chiorean EG, Anthony SP, Sweeney CJ, Borad MJ, Von Hoff DD, Garlich JR, Shelton CF, Ramanathan RK: Phase 1 pharmacokinetic (PK) and pharmacodynamic(PD) evaluation of SF1126 a vascular targeted pan phosphoinositide 3- kinase (PI3K) inhibitor in patients with solid tumors. J Clin Oncol. 2008;26: Abstract no 14532.
150 Perotti A, Locatelli A, Sessa C, Hess D, Vigano L, Capri G, Maur M, Cerny T, Cresta S, Rojo F, Albanell J, Marsoni S, Corradino I, Berk L, Rivera VM, Haluska F, Gianni L: Phase IB Study of the mTOR Inhibitor Ridaforolimus With Capecitabine. J Clin Oncol. 2010;28: 4554-61.
151 Vlahos CJ, Matter WF, Hui KY, Brown RF: A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J Biol Chem. 1994;269: 5241-8.
152 Ihle NT, Williams R, Chow S, Chew W, Berggren MI, Paine-Murrieta G, Minion DJ, Halter RJ, Wipf P, Abraham R, Kirkpatrick L, Powis G: Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. Mol Cancer Ther. 2004;3: 763-72.
153 Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, Knight ZA, Shokat KM, Azar N, Viguie F, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D: PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leukemia. 2008;22: 1698-706.
154 Kojima K, Shimanuki M, Shikami M, Samudio IJ, Ruvolo V, Corn P, Hanaoka N, Konopleva M, Andreeff M, Nakakuma H: The dual PI3 kinase/mTOR inhibitor PI-103 prevents p53 induction by Mdm2 inhibition but enhances p53-mediated mitochondrial apoptosis in p53 wild-type AML. Leukemia. 2008;22: 1728-36.
155 Burris HA, Siu LL, Infante JR, Wheler JJ, Kurkjian C, Opalinska J, Smith DA, Antal JM, Gauvin JL, Gonzalez T, Adams LM, Bedard P, Gerecitano JF, Kurzrock R, Moore KN, Morris SR, Aghajanian C: Safety, pharmacokinetics (PK), pharmacodynamics (PD), and clinical activity of the oral AKT inhibitor GSK2141795 (GSK795) in a phase I first-in-human study. ASCO Meeting Abstracts. 2011;29: 15s: Abstract no 3003.
156 Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia-Echeverria C: Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. Mol Cancer Ther. 2008;7: 1851-63.
157 Jong L, Chao W-R, Amin K, Yean D: SR13668: An orally active inhibitor of phospho-Akt potently suppresses tumor growth and synergizes with chemotherapeutics both in vitro and in vivo. AACR Meeting Abstracts. 2005;2005: Abstract no 401.

158 Cheng H, Bagrodia S, Bailey S, Edwards M, Hoffman J, Hu Q, Kania R, Knighton DR, Marx MA, Ninkovic S, Sun S, Zhang E: Discovery of the highly potent PI3K/mTOR dual inhibitor PF-04691502 through structure based drug design. MedChemComm. 2010;1: 139-44.
159 Levy DS, Kahana JA, Kumar R: AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines. Blood. 2009;113: 1723-9.
160 Nghiemphu PL, Omuro AM, Cloughesy T, Mellinghoff IK, Norden AD, Nguyen LT, Rajangam K, Wen PY: A phase I safety and pharmacokinetic study of XL765 (SAR245409), a novel PI3K/TORC1/TORC2 inhibitor, in combination with temozolomide (TMZ) in patients (pts) with newly diagnosed malignant glioma. J Clin Oncol. 2009;28: 15s: Abstract no 3085.
161 Tolcher AW, A. YT, Fearen I, Taylor A, Carpenter. C., Brunetto A, Beeram TM, Papadopoulos K, Yan L, de Bono J: A phase I study of MK-2206, an oral potent allosteric Akt inhibitor (Akti), in patients (pts) with advanced solid tumor (ST). J Clin Oncol. 2009 27:15s: Abstract no 3503.
162 Chresta CM, Davies BR, Hickson I, Harding T, Cosulich S, Critchlow SE, Vincent JP, Ellston R, Jones D, Sini P, James D, Howard Z, Dudley P, Hughes G, Smith L, Maguire S, Hummersone M, Malagu K, Menear K, Jenkins R, Jacobsen M, Smith GC, Guichard S, Pass M: AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity. Cancer Res. 2010;70: 288-98.
163 Burdon RH: Heat shock and the heat shock proteins. Biochem J. 1986;240: 313-24.
164 Shamovsky I, Nudler E: New insights into the mechanism of heat shock response activation. Cell Mol Life Sci. 2008;65: 855-61.
165 Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G, Scroggins BT, Neckers L, Ilag LL, Jay DG: Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. Nat Cell Biol. 2004;6: 507-14.
166 Bruserud O, Hatfield KJ, Reikvam H: Heat shock protein 90 (HSP90) inhibition in acute myeloid leukemia-Targeting of disease heterogeneity through direct and indirect antileukemic effects. Leukemia Research. 2011;35: 1156-8.
167 Dezwaan DC, Freeman BC: HSP90: the Rosetta stone for cellular protein dynamics? Cell Cycle. 2008;7: 1006-12.
168 Caplan AJ, Mandal AK, Theodoraki MA: Molecular chaperones and protein kinase quality control. Trends Cell Biol. 2007;17: 87-92.
169 Reikvam H, Ersvaer E, Bruserud O: Heat shock protein 90 - a potential target in the treatment of human acute myelogenous leukemia. Curr Cancer Drug Targets. 2009;9: 761-76.
170 Lindquist S, Craig EA: The heat-shock proteins. Annu Rev Genet. 1988;22: 631-77.
171 Zou J, Guo Y, Guettouche T, Smith DF, Voellmy R: Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. Cell. 1998;94: 471-80.
172 Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, Burrows FJ: A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature. 2003;425: 407-10.
173 Chant ID, Rose PE, Morris AG: Analysis of heat-shock protein expression in myeloid leukaemia cells by flow cytometry. Br J Haematol. 1995;90: 163-8.
174 Thomas X, Campos L, Mounier C, Cornillon J, Flandrin P, Le QH, Piselli S, Guyotat D: Expression of heat-shock proteins is associated with major adverse prognostic factors in acute myeloid leukemia. Leuk Res. 2005;29: 1049-58.
175 Reikvam H, Ersvaer E, Hovland R, Skavland J, Petersen K, Hatfield K, Bruserud Ø: Heat shock proteins expression profile for AML patients reveals a distinct signature strongly associated with FLT3 mutation status- consequences and potentials for pharmacological intervention. Haematologica. 2011;96(s2): Abstract no. 0039.
176 Flandrin P, Guyotat D, Duval A, Cornillon J, Tavernier E, Nadal N, Campos L: Significance of heat-shock protein (HSP:) 90 expression in acute myeloid leukemia cells. Cell Stress Chaperones. 2008;13: 357-64.
177 Duval A, Olaru D, Campos L, Flandrin P, Nadal N, Guyotat D: Expression and prognostic significance of heat-shock proteins in myelodysplastic syndromes. Haematologica. 2006;91: 713-4.
178 Al Shaer L, Walsby E, Gilkes A, Tonks A, Walsh V, Mills K, Burnett A, Rowntree C: Heat shock protein 90 inhibition is cytotoxic to primary AML cells expressing mutant FLT3 and results in altered downstream signalling. Br J Haematol. 2008;141: 483-93.

179 Yao Q, Weigel B, Kersey J: Synergism between etoposide and 17-AAG in leukemia cells: critical roles for Hsp90, FLT3, topoisomerase II, Chk1, and Rad51. Clin Cancer Res. 2007;13: 1591-600.
180 Chen G, Cao P, Goeddel DV: TNF-induced recruitment and activation of the IKK complex require Cdc37 and Hsp90. Mol Cell. 2002;9: 401-10.
181 Qing G, Yan P, Qu Z, Liu H, Xiao G: Hsp90 regulates processing of NF-kappa B2 p100 involving protection of NF-kappa B-inducing kinase (NIK) from autophagy-mediated degradation. Cell research. 2007;17: 520-30.
182 Citri A, Harari D, Shohat G, Ramakrishnan P, Gan J, Lavi S, Eisenstein M, Kimchi A, Wallach D, Pietrokovski S, Yarden Y: Hsp90 recognizes a common surface on client kinases. J Biol Chem. 2006;281: 14361-9.
183 Grosjean-Raillard J, Ades L, Boehrer S, Tailler M, Fabre C, Braun T, De Botton S, Israel A, Fenaux P, Kroemer G: Flt3 receptor inhibition reduces constitutive NFkappaB activation in high-risk myelodysplastic syndrome and acute myeloid leukemia. Apoptosis. 2008;13: 1148-61.
184 Shimada M, Nakanishi M: DNA damage checkpoints and cancer. J Mol Histol. 2006;37: 253-60.
185 Chen Y, Poon RY: The multiple checkpoint functions of CHK1 and CHK2 in maintenance of genome stability. Front Biosci. 2008;13: 5016-29.
186 Amico D, Barbui AM, Erba E, Rambaldi A, Introna M, Golay J: Differential response of human acute myeloid leukemia cells to gemtuzumab ozogamicin in vitro: role of Chk1 and Chk2 phosphorylation and caspase 3. Blood. 2003;101: 4589-97.
187 Didier C, Cavelier C, Quaranta M, Galcera MO, Demur C, Laurent G, Manenti S, Ducommun B: G2/M checkpoint stringency is a key parameter in the sensitivity of AML cells to genotoxic stress. Oncogene. 2008;27: 3811-20.
188 Arlander SJ, Eapen AK, Vroman BT, McDonald RJ, Toft DO, Karnitz LM: Hsp90 inhibition depletes Chk1 and sensitizes tumor cells to replication stress. J Biol Chem. 2003;278: 52572-7.
189 Reikvam H, Hatfield KJ, Oyan AM, Kalland KH, Kittang AO, Bruserud O: Primary human acute myelogenous leukemia cells release matrix metalloproteases and their inhibitors: release profile and pharmacological modulation. Eur J Haematol. 2010;84: 239-51.
190 Stellas D, El Hamidieh A, Patsavoudi E: Monoclonal antibody 4C5 prevents activation of MMP2 and MMP9 by disrupting their interaction with extracellular HSP90 and inhibits formation of metastatic breast cancer cell deposits. BMC Cell Biol. 2010;11: 51.
191 Eustace BK, Jay DG: Extracellular roles for the molecular chaperone, hsp90. Cell Cycle. 2004;3: 1098-100.
192 Scheibel T, Weikl T, Buchner J: Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence. Proc Natl Acad Sci U S A. 1998;95: 1495-9.
193 Workman P, Burrows F, Neckers L, Rosen N: Drugging the cancer chaperone HSP90: combinatorial therapeutic exploitation of oncogene addiction and tumor stress. Ann N Y Acad Sci. 2007;1113: 202-16.
194 Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM: Inhibition of heat shock protein HSP90-pp60v-src heteroprotein complex formation by benzoquinone ansamycins: essential role for stress proteins in oncogenic transformation. Proc Natl Acad Sci U S A. 1994;91: 8324-8.
195 Solit DB, Chiosis G: Development and application of Hsp90 inhibitors. Drug Discov Today. 2008;13: 38-43.
196 Gocke CD, Reaman GH, Stine C, Zhang MY, Osmani SA, Miller BA: The nuclear migration gene NudC and human hematopoiesis. Leuk Lymphoma. 2000;39: 447-54.
197 Irish JM, Anensen N, Hovland R, Skavland J, Borresen-Dale AL, Bruserud O, Nolan GP, Gjertsen BT: Flt3 Y591 duplication and Bcl-2 overexpression are detected in acute myeloid leukemia cells with high levels of phosphorylated wild-type p53. Blood. 2007;109: 2589-96.
198 Irish JM, Hovland R, Krutzik PO, Perez OD, Bruserud O, Gjertsen BT, Nolan GP: Single cell profiling of potentiated phospho-protein networks in cancer cells. Cell. 2004;118: 217-28.
199 Ersvaer E, Skavland J, Ulvestad E, Gjertsen BT, Bruserud O: Effects of interferon gamma on native human acute myelogenous leukaemia cells. Cancer Immunol Immunother. 2007;56: 13-24.
200 Soupir CP, Vergilio JA, Dal Cin P, Muzikansky A, Kantarjian H, Jones D, Hasserjian RP: Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with

clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007;127: 642-50.
201 Frostad S, Bruserud O: In vitro effects of insulin-like growth factor-1 (IGF-1) on proliferation and constitutive cytokine secretion by acute myelogenous leukemia blasts. Eur J Haematol. 1999;62: 191-8.
202 Adida C, Recher C, Raffoux E, Daniel MT, Taksin AL, Rousselot P, Sigaux F, Degos L, Altieri DC, Dombret H: Expression and prognostic significance of survivin in de novo acute myeloid leukaemia. Br J Haematol. 2000;111: 196-203.
203 Pearl LH, Prodromou C, Workman P: The Hsp90 molecular chaperone: an open and shut case for treatment. Biochem J. 2008;410: 439-53.
204 Taldone T, Gozman A, Maharaj R, Chiosis G: Targeting Hsp90: small-molecule inhibitors and their clinical development. Curr Opin Pharmacol. 2008;8: 370-4.
205 Drysdale MJ, Brough PA: Medicinal chemistry of Hsp90 inhibitors. Curr Top Med Chem. 2008;8: 859-68.
206 Solit DB, Osman I, Polsky D, Panageas KS, Daud A, Goydos JS, Teitcher J, Wolchok JD, Germino FJ, Krown SE, Coit D, Rosen N, Chapman PB: Phase II trial of 17-allylamino-17-demethoxygeldanamycin in patients with metastatic melanoma. Clin Cancer Res. 2008;14: 8302-7.
207 Modi S, Stopeck AT, Gordon MS, Mendelson D, Solit DB, Bagatell R, Ma W, Wheler J, Rosen N, Norton L, Cropp GF, Johnson RG, Hannah AL, Hudis CA: Combination of trastuzumab and tanespimycin (17-AAG, KOS-953) is safe and active in trastuzumab-refractory HER-2 overexpressing breast cancer: a phase I dose-escalation study. J Clin Oncol. 2007;25: 5410-7.
208 Ramanathan RK, Egorin MJ, Eiseman JL, Ramalingam S, Friedland D, Agarwala SS, Ivy SP, Potter DM, Chatta G, Zuhowski EG, Stoller RG, Naret C, Guo J, Belani CP: Phase I and pharmacodynamic study of 17-(allylamino)-17-demethoxygeldanamycin in adult patients with refractory advanced cancers. Clin Cancer Res. 2007;13: 1769-74.
209 Lancet JE, Gojo I, Burton M, Quinn M, Tighe SM, Kersey K, Zhong Z, Albitar MX, Bhalla K, Hannah AL, Baer MR: Phase I study of the heat shock protein 90 inhibitor alvespimycin (KOS-1022, 17-DMAG) administered intravenously twice weekly to patients with acute myeloid leukemia. Leukemia. 2010;24: 699-705.
210 Lancet JE, Smith BD, Bradley R, Komrokji RS, Teofilovici F, Rizzieri DA: A Phase I/II Trial of the Potent Hsp90 Inhibitor STA-9090 Administered Once Weekly In Patients with Advanced Hematologic Malignancies. ASH Annual Meeting Abstracts. 2010;116: Abstract no 3294.
211 Sequist LV, Gettinger S, Senzer NN, Martins RG, Janne PA, Lilenbaum R, Gray JE, Iafrate AJ, Katayama R, Hafeez N, Sweeney J, Walker JR, Fritz C, Ross RW, Grayzel D, Engelman JA, Borger DR, Paez G, Natale R: Activity of IPI-504, a novel heat-shock protein 90 inhibitor, in patients with molecularly defined non-small-cell lung cancer. J Clin Oncol. 2010;28: 4953-60.
212 Richardson PG, Chanan-Khan AA, Alsina M, Albitar M, Berman D, Messina M, Mitsiades CS, Anderson KC: Tanespimycin monotherapy in relapsed multiple myeloma: results of a phase 1 dose-escalation study. Br J Haematol. 2010;150: 438-45.
213 Bruserud O, Reikvam H: Heat shock protein 90 (HSP90) inhibition--from experimental to clinical studies. Leuk Res. 2010;34: 1422-3.
214 Lee J, Greiner L, Holson E, Slocum K, Ge J, Normant E, Hoyt J, Cushing J, Sydor J, Wright J: IPI-493, a potent bioaviable Hsp90 inhibitor of the ansamycins class. Eur J Cancer. 2008;supl 6: Abstract no 153.
215 Woodhead AJ, Angove H, Carr MG, Chessari G, Congreve M, Coyle JE, Cosme J, Graham B, Day PJ, Downham R, Fazal L, Feltell R, Figueroa E, Frederickson M, Lewis J, McMenamin R, Murray CW, O'Brien MA, Parra L, Patel S, Phillips T, Rees DC, Rich S, Smith DM, Trewartha G, Vinkovic M, Williams B, Woolford AJ: Discovery of (2,4-dihydroxy-5-isopropylphenyl)-[5-(4-methylpiperazin-1-ylmethyl)-1,3-di hydroisoindol-2-yl]methanone (AT13387), a novel inhibitor of the molecular chaperone Hsp90 by fragment based drug design. J Med Chem. 2010;53: 5956-69.
216 Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung K-M, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P: NVP-AUY922: A Novel Heat Shock

Protein 90 Inhibitor Active against Xenograft Tumor Growth, Angiogenesis, and Metastasis. Cancer Research. 2008;68: 2850-60.
217 Wettstein D, Baichwal V, Papac D, Cimbora D, McKinnon R, Bajji A, Kim SH, Tangallapally R, Markovitz B, Trovato R: MPC-3100: A non-natural product Hsp90 inhibitor witj anti-tumor activity in pre-clinical models. Eur J Cancer. 2008;supl 6: Abstract no 150.
218 Lundgren K, Zhang H, Brekken J, Huser N, Powell RE, Timple N, Busch DJ, Neely L, Sensintaffar JL, Yang YC, McKenzie A, Friedman J, Scannevin R, Kamal A, Hong K, Kasibhatla SR, Boehm MF, Burrows FJ: BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. Mol Cancer Ther. 2009;8: 921-9.
219 Bao R, Lai CJ, Qu H, Wang D, Yin L, Zifcak B, Atoyan R, Wang J, Samson M, Forrester J, DellaRocca S, Xu GX, Tao X, Zhai HX, Cai X, Qian C: CUDC-305, a novel synthetic HSP90 inhibitor with unique pharmacologic properties for cancer therapy. Clin Cancer Res. 2009;15: 4046-57.
220 Juliger S, Nakashima T, Maharaj L, Ishii T, Nakagawa H, Kanda Y, Okakervee H, Cavenagh J, Akinaga S, Shiotsu Y, Joel SP: A Novel Heat Shock Protein (HSP) 90 Inhibitor KW-2478 shows Activity in B-Cell Malignancies in Vitro and in Vivo ASH Annual Meeting Abstracts. 2008;112: Abstract no 1625.
221 Nakashima T, Ishii T, Tagaya H, Seike T, Nakagawa H, Kanda Y, Akinaga S, Soga S, Shiotsu Y: New molecular and biological mechanism of antitumor activities of KW-2478, a novel nonansamycin heat shock protein 90 inhibitor, in multiple myeloma cells. Clin Cancer Res. 2010;16: 2792-802.
222 Okawa Y, Hideshima T, Steed P, Vallet S, Hall S, Huang K, Rice J, Barabasz A, Foley B, Ikeda H, Raje N, Kiziltepe T, Yasui H, Enatsu S, Anderson KC: SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. Blood. 2009;113: 846-55.
223 Nicoll M: XL888, a novel, synthetic, orally bioaviable inhibitor of Hsp90. Eur J Cancer. 2008;supl 6: Abstract no 144.
224 Sen R, Baltimore D: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell. 1986;46: 705-16.
225 Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, Luger SM, Jordan CT: Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood. 2001;98: 2301-7.
226 Aggarwal BB: Apoptosis and nuclear factor-kappa B: a tale of association and dissociation. Biochem Pharmacol. 2000;60: 1033-9.
227 Baldwin AS, Jr.: Series introduction: the transcription factor NF-kappaB and human disease. J Clin Invest. 2001;107: 3-6.
228 Bruserud O, Reikvam H: Therapeutic targeting of NF-kappaB in myelodysplastic syndromes and acute myeloid leukaemia - the biological heterogeneity. Expert Opin Ther Targets. 2010;14: 1139-42.
229 Frelin C, Imbert V, Griessinger E, Peyron AC, Rochet N, Philip P, Dageville C, Sirvent A, Hummelsberger M, Berard E, Dreano M, Sirvent N, Peyron JF: Targeting NF-kappaB activation via pharmacologic inhibition of IKK2-induced apoptosis of human acute myeloid leukemia cells. Blood. 2005;105: 804-11.
230 Reikvam H, Hatfield KJ, Lassalle P, Kittang AO, Ersvaer E, Bruserud O: Targeting the angiopoietin (Ang)/Tie-2 pathway in the crosstalk between acute myeloid leukaemia and endothelial cells: studies of Tie-2 blocking antibodies, exogenous Ang-2 and inhibition of constitutive agonistic Ang-1 release. Expert Opin Investig Drugs. 2010;19: 169-83.
231 Hatfield KJ, Hovland R, Oyan AM, Kalland KH, Ryningen A, Gjertsen BT, Bruserud O: Release of angiopoietin-1 by primary human acute myelogenous leukemia cells is associated with mutations of nucleophosmin, increased by bone marrow stromal cells and possibly antagonized by high systemic angiopoietin-2 levels. Leukemia. 2008;22: 287-93.
232 Breuil V, Schmid-Antomarchi H, Schmid-Alliana A, Rezzonico R, Euller-Ziegler L, Rossi B: The receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) is a new chemotactic factor for human monocytes. FASEB J. 2003;17: 1751-3.
233 Bruserud O, Ryningen A, Olsnes AM, Stordrange L, Oyan AM, Kalland KH, Gjertsen BT: Subclassification of patients with acute myelogenous leukemia based on chemokine responsiveness and constitutive chemokine release by their leukemic cells. Haematologica. 2007;92: 332-41.

234 Acosta JC, O'Loghlen A, Banito A, Guijarro MV, Augert A, Raguz S, Fumagalli M, Da Costa M, Brown C, Popov N, Takatsu Y, Melamed J, d'Adda di Fagagna F, Bernard D, Hernando E, Gil J: Chemokine signaling via the CXCR2 receptor reinforces senescence. Cell. 2008;133: 1006-18.
235 Sparmann A, Bar-Sagi D: Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell. 2004;6: 447-58.
236 Scott BB, Zaratin PF, Gilmartin AG, Hansbury MJ, Colombo A, Belpasso C, Winkler JD, Jackson JR: TNF-alpha modulates angiopoietin-1 expression in rheumatoid synovial fibroblasts via the NF-kappa B signalling pathway. Biochem Biophys Res Commun. 2005;328: 409-14.
237 Mitola S, Moroni E, Ravelli C, Andres G, Belleri M, Presta M: Angiopoietin-1 mediates the proangiogenic activity of the bone morphogenic protein antagonist Drm. Blood. 2008;112: 1154-7.
238 Tabruyn SP, Griffioen AW: NF-kappa B: a new player in angiostatic therapy. Angiogenesis. 2008;11: 101-6.
239 Gilmore TD, Herscovitch M: Inhibitors of NF-kappaB signaling: 785 and counting. Oncogene. 2006;25: 6887-99.
240 Hassane DC, Guzman ML, Corbett C, Li X, Abboud R, Young F, Liesveld JL, Carroll M, Jordan CT: Discovery of agents that eradicate leukemia stem cells using an in silico screen of public gene expression data. Blood. 2008;111: 5654-62.
241 Attar EC, De Angelo DJ, Supko JG, D'Amato F, Zahrieh D, Sirulnik A, Wadleigh M, Ballen KK, McAfee S, Miller KB, Levine J, Galinsky I, Trehu EG, Schenkein D, Neuberg D, Stone RM, Amrein PC: Phase I and pharmacokinetic study of bortezomib in combination with idarubicin and cytarabine in patients with acute myelogenous leukemia. Clin Cancer Res. 2008;14: 1446-54.
242 Stapnes C, Doskeland AP, Hatfield K, Ersvaer E, Ryningen A, Lorens JB, Gjertsen BT, Bruserud O: The proteasome inhibitors bortezomib and PR-171 have antiproliferative and proapoptotic effects on primary human acute myeloid leukaemia cells. Br J Haematol. 2007;136: 814-28.
243 Vallet S, Palumbo A, Raje N, Boccadoro M, Anderson KC: Thalidomide and lenalidomide: Mechanism-based potential drug combinations. Leuk Lymphoma. 2008;49: 1238-45.
244 Palumbo A, Rajkumar SV: Treatment of newly diagnosed myeloma. Leukemia. 2009;23: 449-56.
245 Raza A, Mehdi M, Mumtaz M, Ali F, Lascher S, Galili N: Combination of 5-azacytidine and thalidomide for the treatment of myelodysplastic syndromes and acute myeloid leukemia. Cancer. 2008;113: 1596-604.
246 Thomas DA, Estey E, Giles FJ, Faderl S, Cortes J, Keating M, O'Brien S, Albitar M, Kantarjian H: Single agent thalidomide in patients with relapsed or refractory acute myeloid leukaemia. Br J Haematol. 2003;123: 436-41.
247 Mesa RA, Tefferi A, Li CY, Steensma DP: Hematologic and cytogenetic response to lenalidomide monotherapy in acute myeloid leukemia arising from JAK2(V617F) positive, del(5)(q13q33) myelodysplastic syndrome. Leukemia. 2006;20: 2063-4.
248 Fehniger TA, Byrd JC, Marcucci G, Abboud CN, Kefauver C, Payton JE, Vij R, Blum W: Single-agent lenalidomide induces complete remission of acute myeloid leukemia in patients with isolated trisomy 13. Blood. 2009;113: 1002-5.
249 Kiel MJ, Morrison SJ: Uncertainty in the niches that maintain haematopoietic stem cells. Nat Rev Immunol. 2008;8: 290-301.
250 Gong JK: Endosteal marrow: a rich source of hematopoietic stem cells. Science. 1978;199: 1443-5.
251 Nilsson SK, Johnston HM, Coverdale JA: Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. Blood. 2001;97: 2293-9.
252 Eliasson P, Jonsson JI: The hematopoietic stem cell niche: low in oxygen but a nice place to be. J Cell Physiol. 2010;222: 17-22.
253 Gerber HP, Malik AK, Solar GP, Sherman D, Liang XH, Meng G, Hong K, Marsters JC, Ferrara N: VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. Nature. 2002;417: 954-8.
254 Ersvaer E, Hatfield K, Reikvam H, Bruserud O: Future perspectives: Therapeutic targeting of NOTCH signalling may become a strategy in patients receiving stem cell transplantation for hematologic malignancies. Bone Marrow Research. 2011;2011: 570796.

255 Zhang CC, Kaba M, Ge G, Xie K, Tong W, Hug C, Lodish HF: Angiopoietin-like proteins stimulate ex vivo expansion of hematopoietic stem cells. Nat Med. 2006;12: 240-5.
256 Huntly BJ, Gilliland DG: Leukaemia stem cells and the evolution of cancer-stem-cell research. Nat Rev Cancer. 2005;5: 311-21.
257 Bonnet D, Dick JE: Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3: 730-7.
258 Taussig DC, Miraki-Moud F, Anjos-Afonso F, Pearce DJ, Allen K, Ridler C, Lillington D, Oakervee H, Cavenagh J, Agrawal SG, Lister TA, Gribben JG, Bonnet D: Anti-CD38 antibody-mediated clearance of human repopulating cells masks the heterogeneity of leukemia-initiating cells. Blood. 2008;112: 568-75.
259 Lane SW, Scadden DT, Gilliland DG: The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009;114: 1150-7.
260 Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, Nakamura R, Tanaka T, Tomiyama H, Saito N, Fukata M, Miyamoto T, Lyons B, Ohshima K, Uchida N, Taniguchi S, Ohara O, Akashi K, Harada M, Shultz LD: Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25: 1315-21.
261 Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A: The history of the angiogenic switch concept. Leukemia. 2007;21: 44-52.
262 Folkman J: Tumor angiogenesis: therapeutic implications. The New England journal of medicine. 1971;285: 1182-6.
263 Negaard HF, Iversen N, Bowitz-Lothe IM, Sandset PM, Steinsvik B, Ostenstad B, Iversen PO: Increased bone marrow microvascular density in haematological malignancies is associated with differential regulation of angiogenic factors. Leukemia. 2009;23: 162-9.
264 Madlambayan GJ, Meacham AM, Hosaka K, Mir S, Jorgensen M, Scott EW, Siemann DW, Cogle CR: Leukemia regression by vascular disruption and antiangiogenic therapy. Blood. 2010;116: 1539-47.
265 Hatfield K, Oyan AM, Ersvaer E, Kalland KH, Lassalle P, Gjertsen BT, Bruserud O: Primary human acute myeloid leukaemia cells increase the proliferation of microvascular endothelial cells through the release of soluble mediators. Br J Haematol. 2009;144: 53-68.
266 Hatfield K, Ryningen A, Corbascio M, Bruserud O: Microvascular endothelial cells increase proliferation and inhibit apoptosis of native human acute myelogenous leukemia blasts. Int J Cancer. 2006;119: 2313-21.
267 Shih TT, Hou HA, Liu CY, Chen BB, Tang JL, Chen HY, Wei SY, Yao M, Huang SY, Chou WC, Hsu SC, Tsay W, Yu CW, Hsu CY, Tien HF, Yang PC: Bone marrow angiogenesis magnetic resonance imaging in patients with acute myeloid leukemia: peak enhancement ratio is an independent predictor for overall survival. Blood. 2009;113: 3161-7.
268 Kessler T, Fehrmann F, Bieker R, Berdel WE, Mesters RM: Vascular endothelial growth factor and its receptor as drug targets in hematological malignancies. Current drug targets. 2007;8: 257-68.
269 Hatfield KJ, Olsnes AM, Gjertsen BT, Bruserud O: Antiangiogenic therapy in acute myelogenous leukemia: targeting of vascular endothelial growth factor and interleukin 8 as possible antileukemic strategies. Curr Cancer Drug Targets. 2005;5: 229-48.
270 Bruserud O, Tronstad KJ, Berge R: In vitro culture of human osteosarcoma cell lines: a comparison of functional characteristics for cell lines cultured in medium without and with fetal calf serum. J Cancer Res Clin Oncol. 2005;131: 377-84.
271 Hjorth-Hansen H, Seidel C, Lamvik J, Borset M, Sundan A, Waage A: Elevated serum concentrations of hepatocyte growth factor in acute myelocytic leukaemia. Eur J Haematol. 1999;62: 129-34.
272 Kim JG, Sohn SK, Kim DH, Baek JH, Lee NY, Suh JS, Chae SC, Lee KS, Lee KB: Clinical implications of angiogenic factors in patients with acute or chronic leukemia: hepatocyte growth factor levels have prognostic impact, especially in patients with acute myeloid leukemia. Leuk Lymphoma. 2005;46: 885-91.
273 Cecchi F, Rabe DC, Bottaro DP: Targeting the HGF/Met signalling pathway in cancer. Eur J Cancer. 2010;46: 1260-70.
274 Dimberg A: Chemokines in angiogenesis. Curr Top Microbiol Immunol. 2010;341: 59-80.
275 Bruserud O, Kittang AO: The chemokine system in experimental and clinical hematology. Curr Top Microbiol Immunol. 2010;341: 3-12.
276 Calandra G, Bridger G, Fricker S: CXCR4 in clinical hematology. Curr Top Microbiol Immunol. 2010;341: 173-91.

277 Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD: Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science. 1997;277: 55-60.
278 Yuan HT, Khankin EV, Karumanchi SA, Parikh SM: Angiopoietin 2 is a partial agonist/antagonist of Tie2 signaling in the endothelium. Mol Cell Biol. 2009;29: 2011-22.
279 Eklund L, Olsen BR: Tie receptors and their angiopoietin ligands are context-dependent regulators of vascular remodeling. Exp Cell Res. 2006;312: 630-41.
280 Shim WS, Ho IA, Wong PE: Angiopoietin: a TIE(d) balance in tumor angiogenesis. Mol Cancer Res. 2007;5: 655-65.
281 Loges S, Heil G, Bruweleit M, Schoder V, Butzal M, Fischer U, Gehling UM, Schuch G, Hossfeld DK, Fiedler W: Analysis of concerted expression of angiogenic growth factors in acute myeloid leukemia: expression of angiopoietin-2 represents an independent prognostic factor for overall survival. J Clin Oncol. 2005;23: 1109-17.
282 Schliemann C, Bieker R, Padro T, Kessler T, Hintelmann H, Buchner T, Berdel WE, Mesters RM: Expression of angiopoietins and their receptor Tie2 in the bone marrow of patients with acute myeloid leukemia. Haematologica. 2006;91: 1203-11.
283 Hou HA, Chou WC, Lin LI, Tang JL, Tseng MH, Huang CF, Yao M, Chen CY, Tsay W, Tien HF: Expression of angiopoietins and vascular endothelial growth factors and their clinical significance in acute myeloid leukemia. Leuk Res. 2008;32: 904-12.
284 Schliemann C, Bieker R, Thoennissen N, Gerss J, Liersch R, Kessler T, Buchner T, Berdel WE, Mesters RM: Circulating angiopoietin-2 is a strong prognostic factor in acute myeloid leukemia. Leukemia. 2007;21: 1901-6.
285 Kümpers P, Koenecke C, Hecker H, Hellpap J, Horn R, Verhagen W, Buchholz S, Hertenstein B, Krauter J, Eder M, David S, Gohring G, Haller H, Ganser A: Angiopoietin-2 predicts disease-free survival after allogeneic stem cell transplantation in patients with high-risk myeloid malignancies. Blood. 2008;112: 2139-48.
286 Popkov M, Jendreyko N, McGavern DB, Rader C, Barbas CF, 3rd: Targeting tumor angiogenesis with adenovirus-delivered anti-Tie-2 intrabody. Cancer Res. 2005;65: 972-81.
287 Oliner J, Min H, Leal J, Yu D, Rao S, You E, Tang X, Kim H, Meyer S, Han SJ, Hawkins N, Rosenfeld R, Davy E, Graham K, Jacobsen F, Stevenson S, Ho J, Chen Q, Hartmann T, Michaels M, Kelley M, Li L, Sitney K, Martin F, Sun JR, Zhang N, Lu J, Estrada J, Kumar R, Coxon A, Kaufman S, Pretorius J, Scully S, Cattley R, Payton M, Coats S, Nguyen L, Desilva B, Ndifor A, Hayward I, Radinsky R, Boone T, Kendall R: Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell. 2004;6: 507-16.
288 Aguayo A, Estey E, Kantarjian H, Mansouri T, Gidel C, Keating M, Giles F, Estrov Z, Barlogie B, Albitar M: Cellular vascular endothelial growth factor is a predictor of outcome in patients with acute myeloid leukemia. Blood. 1999;94: 3717-21.
289 Jendreyko N, Popkov M, Rader C, Barbas CF, 3rd: Phenotypic knockout of VEGF-R2 and Tie-2 with an intradiabody reduces tumor growth and angiogenesis in vivo. Proc Natl Acad Sci U S A. 2005;102: 8293-8.
290 Bruserud O, Hatfield K: Antivascular combo therapy: up-and-coming. Blood. 2010;116: 1389-90.
291 Stetler-Stevenson WG: Tissue inhibitors of metalloproteinases in cell signaling: metalloproteinase-independent biological activities. Sci Signal. 2008;1: re6.
292 Song JH, Kim SH, Cho D, Lee IK, Kim HJ, Kim TS: Enhanced invasiveness of drug-resistant acute myeloid leukemia cells through increased expression of matrix metalloproteinase-2. Int J Cancer. 2009;125: 1074-81.
293 Sawicki G, Matsuzaki A, Janowska-Wieczorek A: Expression of the active form of MMP-2 on the surface of leukemic cells accounts for their in vitro invasion. J Cancer Res Clin Oncol. 1998;124: 245-52.
294 Aref S, Osman E, Mansy S, Omer N, Azmy E, Goda T, El-Sherbiny M: Prognostic relevance of circulating matrix metalloproteinase-2 in acute myeloid leukaemia patients. Hematol Oncol. 2007;25: 121-6.
295 Feng S, Cen J, Huang Y, Shen H, Yao L, Wang Y, Chen Z: Matrix metalloproteinase-2 and -9 secreted by leukemic cells increase the permeability of blood-brain barrier by disrupting tight junction proteins. PLoS One. 2011;6: e20599.
296 Mitsiades N, Yu WH, Poulaki V, Tsokos M, Stamenkovic I: Matrix metalloproteinase-7-mediated cleavage of Fas ligand protects tumor cells from chemotherapeutic drug cytotoxicity. Cancer Res. 2001;61: 577-81.

297 Paupert J, Mansat-De Mas V, Demur C, Salles B, Muller C: Cell-surface MMP-9 regulates the invasive capacity of leukemia blast cells with monocytic features. Cell Cycle. 2008;7: 1047-53.
298 Stefanidakis M, Karjalainen K, Jaalouk DE, Gahmberg CG, O'Brien S, Pasqualini R, Arap W, Koivunen E: Role of leukemia cell invadosome in extramedullary infiltration. Blood. 2009;114: 3008-17.
299 Van Themsche C, Alain T, Kossakowska AE, Urbanski S, Potworowski EF, St-Pierre Y: Stromelysin-2 (matrix metalloproteinase 10) is inducible in lymphoma cells and accelerates the growth of lymphoid tumors in vivo. J Immunol. 2004;173: 3605-11.
300 Nakamura Y, Sato K, Wakimoto N, Kimura F, Okuyama A, Motoyoshi K: A new matrix metalloproteinase inhibitor SI-27 induces apoptosis in several human myeloid leukemia cell lines and enhances sensitivity to TNF alpha-induced apoptosis. Leukemia. 2001;15: 1217-24.
301 Coussens LM, Fingleton B, Matrisian LM: Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science. 2002;295: 2387-92.
302 Tu G, Xu W, Huang H, Li S: Progress in the development of matrix metalloproteinase inhibitors. Curr Med Chem. 2008;15: 1388-95.
303 Fingleton B: MMPs as therapeutic targets--still a viable option? Semin Cell Dev Biol. 2008;19: 61-8.
304 Martin MD, Matrisian LM: The other side of MMPs: protective roles in tumor progression. Cancer Metastasis Rev. 2007;26: 717-24.
305 Sarrazin S, Adam E, Lyon M, Depontieu F, Motte V, Landolfi C, Lortat-Jacob H, Bechard D, Lassalle P, Delehedde M: Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. Biochim Biophys Acta. 2006;1765: 25-37.
306 Hatfield K, Lassalle P, Leiva RA, Lindås R, Wendelbo Ø, Bruserud Ø: Serum levels of endothelium-derived endocan are increased in patients with untreated acute myeloid leukemia. Hematology. 2011;In press.
307 Bruserud O, Gjertsen BT, Foss B, Huang TS: New strategies in the treatment of acute myelogenous leukemia (AML): in vitro culture of aml cells--the present use in experimental studies and the possible importance for future therapeutic approaches. Stem Cells. 2001;19: 1-11.
308 Lowenberg B, Downing JR, Burnett A: Acute myeloid leukemia. The New England journal of medicine. 1999;341: 1051-62.
309 Kantarjian H, Ravandi F, O'Brien S, Cortes J, Faderl S, Garcia-Manero G, Jabbour E, Wierda W, Kadia T, Pierce S, Shan J, Keating M, Freireich EJ: Intensive chemotherapy does not benefit most older patients (age 70 years or older) with acute myeloid leukemia. Blood. 2010;116: 4422-9.
310 Jorgensen KM, Hjelle SM, Oye OK, Puntervoll P, Reikvam H, Skavland J, Anderssen E, Bruserud O, Gjertsen BT: Untangling the intracellular signalling network in cancer - A strategy for data integration in acute myeloid leukaemia. J Proteomics. 2010;74: 269-81.
311 Li Z, Lu J, Sun M, Mi S, Zhang H, Luo RT, Chen P, Wang Y, Yan M, Qian Z, Neilly MB, Jin J, Zhang Y, Bohlander SK, Zhang DE, Larson RA, Le Beau MM, Thirman MJ, Golub TR, Rowley JD, Chen J: Distinct microRNA expression profiles in acute myeloid leukemia with common translocations. Proc Natl Acad Sci U S A. 2008;105: 15535-40.
312 Jongen-Lavrencic M, Sun SM, Dijkstra MK, Valk PJ, Lowenberg B: MicroRNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. Blood. 2008;111: 5078-85.
313 Bullinger L, Ehrich M, Dohner K, Schlenk RF, Dohner H, Nelson MR, van den Boom D: Quantitative DNA methylation predicts survival in adult acute myeloid leukemia. Blood. 2010;115: 636-42.
314 Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, Schifano E, Booth J, van Putten W, Skrabanek L, Campagne F, Mazumdar M, Greally JM, Valk PJ, Lowenberg B, Delwel R, Melnick A: DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell. 2010;17: 13-27.
315 Kornblau SM, Tibes R, Qiu YH, Chen W, Kantarjian HM, Andreeff M, Coombes KR, Mills GB: Functional proteomic profiling of AML predicts response and survival. Blood. 2009;113: 154-64.
316 Tiziani S, Lodi A, Khanim FL, Viant MR, Bunce CM, Gunther UL: Metabolomic profiling of drug responses in acute myeloid leukaemia cell lines. PLoS One. 2009;4: e4251.
317 Bergkvist A, Rusnakova V, Sindelka R, Garda JM, Sjogreen B, Lindh D, Forootan A, Kubista M: Gene expression profiling--Clusters of possibilities. Methods. 2010;50: 323-35.

318 Miller BG, Stamatoyannopoulos JA: Integrative meta-analysis of differential gene expression in acute myeloid leukemia. PLoS One. 2010;5: e9466.
319 Verhaak RG, Wouters BJ, Erpelinck CA, Abbas S, Beverloo HB, Lugthart S, Lowenberg B, Delwel R, Valk PJ: Prediction of molecular subtypes in acute myeloid leukemia based on gene expression profiling. Haematologica. 2009;94: 131-4.
320 Alvarez S, Suela J, Valencia A, Fernandez A, Wunderlich M, Agirre X, Prosper F, Martin-Subero JI, Maiques A, Acquadro F, Rodriguez Perales S, Calasanz MJ, Roman-Gomez J, Siebert R, Mulloy JC, Cervera J, Sanz MA, Esteller M, Cigudosa JC: DNA methylation profiles and their relationship with cytogenetic status in adult acute myeloid leukemia. PLoS One. 2010;5.
321 Gjertsen BT, Oyan AM, Marzolf B, Hovland R, Gausdal G, Doskeland SO, Dimitrov K, Golden A, Kalland KH, Hood L, Bruserud O: Analysis of acute myelogenous leukemia: preparation of samples for genomic and proteomic analyses. J Hematother Stem Cell Res. 2002;11: 469-81.
322 Lowenberg B, Beck J, Graux C, van Putten W, Schouten HC, Verdonck LF, Ferrant A, Sonneveld P, Jongen-Lavrencic M, von Lilienfeld-Toal M, Biemond BJ, Vellenga E, Breems D, de Muijnck H, Schaafsma R, Verhoef G, Dohner H, Gratwohl A, Pabst T, Ossenkoppele GJ, Maertens J, for the Dutch-Belgian Hemato-Oncology Cooperative G, German Austrian AMLSG, Swiss Group for Clinical Cancer Research Collaborative G: Gemtuzumab ozogamicin as postremission treatment in AML at 60 years of age or more: results of a multicenter phase 3 study. Blood. 2010;115: 2586-91.
323 Serve H, Wagner R, Sauerland C, Brunnberg U, Krug U, Schaich M, Ottmann OG, Duyster J, Wandt H, Herr W, Giagounidis AAN, Neubauer A, Reichle A, Aulitzky WE, Noppeney R, Blau IW, Kunzmann V, Schmitz N, Kreuzer K-A, Kramer A, Brandts C, Steffen B, Heinecke A, Thiede C, Muller-Tidow C, Ehninger G, Berdel WE: Sorafenib In Combination with Standard Induction and Consolidation Therapy In Elderly AML Patients: Results From a Randomized, Placebo-Controlled Phase II Trial. ASH Annual Meeting Abstracts. 2010;116: Abstract no 333.
324 Jabbour E, Kantarjian H, Ravandi F, Garcia-Manero G, Estrov Z, Verstovsek S, O'Brien S, Faderl S, Thomas DA, Wright JJ, Cortes J: A phase 1-2 study of a farnesyltransferase inhibitor, tipifarnib, combined with idarubicin and cytarabine for patients with newly diagnosed acute myeloid leukemia and high-risk myelodysplastic syndrome. Cancer. 2011;117: 1236-44.
325 Quintas-Cardama A, Santos FP, Garcia-Manero G: Histone deacetylase inhibitors for the treatment of myelodysplastic syndrome and acute myeloid leukemia. Leukemia. 2011;25: 226-35.
326 Attar EC, Donohue KA, Amrein PC, Wadleigh M, DeAngelo DJ, Kolitz JE, Powell BL, Voorhees PM, Wang ES, Blum W, Booth A, Stone RM, Moser BK, Larson R: Phase II Study of Bortezomib Added to Standard Daunorubicin and Cytarabine Induction and Dose Escalation of Bortezomib with Intermediate-Dose Cytarabine Consolidation Therapy for Patients with Previously Untreated Acute Myeloid Leukemia Age 60-75 Years: Cancer and Leukemia Group B (CALGB) Study 10502. ASH Annual Meeting Abstracts. 2010;116: Abstract no 331.
327 Latagliata R, Bongarzoni V, Carmosino I, Mengarelli A, Breccia M, Borza PA, D'Andrea M, D'Elia GM, Mecarocci S, Morano SG, Petti MC, Mandelli F, Alimena G: Acute myelogenous leukemia in elderly patients not eligible for intensive chemotherapy: the dark side of the moon. Ann Oncol. 2006;17: 281-5.
328 Fredly H, Stapnes Bjornsen C, Gjertsen BT, Bruserud O: Combination of the histone deacetylase inhibitor valproic acid with oral hydroxyurea or 6-mercaptopurin can be safe and effective in patients with advanced acute myeloid leukaemia--a report of five cases. Hematology. 2010;15: 338-43.
329 Fredly H, Ersvaer E, Stapnes C, Gjertsen BT, Bruserud Ø: The Combination of Conventional Chemotherapy with New Targeted Therapy in Hematologic Malignancies: The Safety and Efficiency of Low- Dose Cytarabine Supports its Combination with New Therapeutic Agents in Early Clinical Trials. Current Cancer Therapy Reviews. 2009;5: 243-55.
330 Bruserud O, Stapnes C, Ersvaer E, Gjertsen BT, Ryningen A: Histone deacetylase inhibitors in cancer treatment: a review of the clinical toxicity and the modulation of gene expression in cancer cell. Curr Pharm Biotechnol. 2007;8: 388-400.
331 Brune M, Castaigne S, Catalano J, Gehlsen K, Ho AD, Hofmann WK, Hogge DE, Nilsson B, Or R, Romero AI, Rowe JM, Simonsson B, Spearing R, Stadtmauer EA, Szer J, Wallhult E, Hellstrand K: Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. Blood. 2006;108: 88-96.

332 Romero AI, Thoren FB, Aurelius J, Askarieh G, Brune M, Hellstrand K: Post-consolidation immunotherapy with histamine dihydrochloride and interleukin-2 in AML. Scand J Immunol. 2009;70: 194-205.
333 Padro T, Ruiz S, Bieker R, Burger H, Steins M, Kienast J, Buchner T, Berdel WE, Mesters RM: Increased angiogenesis in the bone marrow of patients with acute myeloid leukemia. Blood. 2000;95: 2637-44.
334 Karp JE, Gojo I, Pili R, Gocke CD, Greer J, Guo C, Qian D, Morris L, Tidwell M, Chen H, Zwiebel J: Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-beta-d-arabinofuranosylcytosine, mitoxantrone, and bevacizumab. Clin Cancer Res. 2004;10: 3577-85.
335 Zahiragic L, Schliemann C, Bieker R, Thoennissen NH, Burow K, Kramer C, Zuhlsdorf M, Berdel WE, Mesters RM: Bevacizumab reduces VEGF expression in patients with relapsed and refractory acute myeloid leukemia without clinical antileukemic activity. Leukemia. 2007;21: 1310-2.
336 Fiedler W, Serve H, Dohner H, Schwittay M, Ottmann OG, O'Farrell AM, Bello CL, Allred R, Manning WC, Cherrington JM, Louie SG, Hong W, Brega NM, Massimini G, Scigalla P, Berdel WE, Hossfeld DK: A phase 1 study of SU11248 in the treatment of patients with refractory or resistant acute myeloid leukemia (AML) or not amenable to conventional therapy for the disease. Blood. 2005;105: 986-93.
337 Fiedler W, Krauter J, Gotze K, Salih HR, Bokemeyer C, Spaeth D, Dohner K, Dohner H, Schlenk RF: A Phase I/II Study Combining Sunitinib with Standard Ara-C/Daunorubicin Chemotherapy In Patients 60 Years or Older with FLT3 Mutated AML. ASH Annual Meeting Abstracts. 2010;116: Abstract no 3285.
338 Herbst RS, Hong D, Chap L, Kurzrock R, Jackson E, Silverman JM, Rasmussen E, Sun YN, Zhong D, Hwang YC, Evelhoch JL, Oliner JD, Le N, Rosen LS: Safety, pharmacokinetics, and antitumor activity of AMG 386, a selective angiopoietin inhibitor, in adult patients with advanced solid tumors. J Clin Oncol. 2009;27: 3557-65.
339 Siemann DW, Chaplin DJ, Walicke PA: A review and update of the current status of the vasculature-disabling agent combretastatin-A4 phosphate (CA4P). Expert Opin Investig Drugs. 2009;18: 189-97.
340 Petit I, Karajannis MA, Vincent L, Young L, Butler J, Hooper AT, Shido K, Steller H, Chaplin DJ, Feldman E, Rafii S: The microtubule-targeting agent CA4P regresses leukemic xenografts by disrupting interaction with vascular cells and mitochondrial-dependent cell death. Blood. 2008;111: 1951-61.
341 Bohonowych JE, Gopal U, Isaacs JS: Hsp90 as a gatekeeper of tumor angiogenesis: clinical promise and potential pitfalls. J Oncol. 2010;2010: 412985.
342 Hatfield KJ, Bedringsaas SL, Ryningen A, Gjertsen BT, Bruserud O: Hypoxia increases HIF-1alpha expression and constitutive cytokine release by primary human acute myeloid leukaemia cells. Eur Cytokine Netw. 2010;21: 154-64.
343 Jiang BH, Liu LZ: PI3K/PTEN signaling in angiogenesis and tumorigenesis. Adv Cancer Res. 2009;102: 19-65.
344 Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK: Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med. 2002;8: 128-35.
345 Bohm A, Aichberger KJ, Mayerhofer M, Herrmann H, Florian S, Krauth MT, Derdak S, Samorapoompichit P, Sonneck K, Vales A, Gleixner KV, Pickl WF, Sperr WR, Valent P: Targeting of mTOR is associated with decreased growth and decreased VEGF expression in acute myeloid leukaemia cells. Eur J Clin Invest. 2009;39: 395-405.