GENOMIC MICROARRAYS IN CHROMOSOMAL ANALYSIS OF LEUKEMIA

Anne Tyybäkinoja

Department of Pathology
Haartman Institute and HUSLAB
University of Helsinki and Helsinki University Central Hospital
Finland

Academic Dissertation

To be publicly presented with the permission of the Faculty of Medicine, University of Helsinki, in the small lecture hall of the Haartman Institute, Haartmaninkatu 3, on December 11th, 2009, at 12 noon.

Helsinki 2009

Supervised by:
Professor Sakari Knuutila, PhD
Department of Pathology
Haartman Institute and HUSLAB
University of Helsinki and
Helsinki University Central Hospital
Helsinki, Finland

Reviewed by:
Docent Liisa Volin, MD, PhD
Department of Medicine, Division of Hematology
Helsinki University Central Hospital
Helsinki, Finland

Docent Arto Mannermaa, PhD
Institute of Clinical Medicine
Pathology and Forensic Medicine
University of Kuopio
Kuopio, Finland

Official Opponent:
Docent Kim Vettenranta, MD, PhD
Division of Hematology-Oncology and Stem Cell Transplantation
Hospital for Children and Adolescents
University of Helsinki
Helsinki, Finland

ISBN 978-952-92-6425-4 (paperback)
ISBN 978-952-10-5868-4 (PDF)
http://ethesis.helsinki.fi/

Helsinki 2009
Yliopistopaino

CONTENTS

LIST OF ORIGINAL PUBLICATIONS **5**

ABBREVIATIONS **6**

ABSTRACT **9**

INTRODUCTION **10**

REVIEW OF THE LITERATURE **11**

1. Cancer and genetic alterations 11

2. Chromosomal imbalances in cancer 12
   2.1. Translocations and inversions 13
   2.2. DNA copy number losses 13
   2.3. Amplifications 14
   2.4. Loss of heterozygosity 15
   2.5. Uniparental disomy 16

3. Microarrays in the analysis of chromosomal imbalances 17
   3.1. Array comparative genomic hybridization 17
   3.2. Single-nucleotide polymorphism microarray analysis 19

4. Leukemia 22
   4.1. Acute myeloid leukemia 22
      4.1.1. Clinical characteristics and treatment of acute myeloid leukemia 22
      4.1.2. Genetic alterations in acute myeloid leukemia 24
   4.2. Chronic lymphocytic leukemia 27
      4.2.1 Clinical characteristics and treatment of chronic lymphocytic leukemia 27
      4.2.2. Genetic alterations in chronic lymphocytic leukemia 28

AIMS OF THE STUDY **31**

MATERIALS AND METHODS **32**

1. Specimens (I-IV) 32
   1.1. Acute myeloid leukemia specimens 32
   1.2. Chronic lymphocytic leukemia specimens 32

2. Complementary DNA array comparative genomic hybridization (I) 33

3. Oligonucleotide array comparative genomic hybridization (I-III) 33

4. Fluorescence in situ hybridization (I, II) 34

5. Gene expression (I) 35

6. Mutation analysis (II) 35

7. Loss of heterozygosity analysis with single-nucleotide polymorphism microarray (IV) 35

RESULTS AND DISCUSSION **37**
1. Characterization of 11q amplicons in acute myeloid leukemia (I) 37
2. Cryptic copy number changes in karyotypically normal acute myeloid leukemia (II) 40
3. Array comparative genomic hybridization in chronic lymphocytic leukemia (III) 42
4. Loss of heterozygosity in karyotypically normal acute myeloid leukemia (IV) 44
CONCLUSIONS **47**
ACKNOWLEDGMENTS **50**
REFERENCES **51**

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerials:

I. Tyybäkinoja A, Saarinen-Pihkala U, Elonen E, Knuutila S. Amplified, lost, and fused genes in 11q23-25 amplicon in acute myeloid leukemia, an array-CGH study. Genes Chromosomes Cancer 2006;45:257-264.

II. Tyybäkinoja A, Elonen E, Piippo K, Porkka K, Knuutila S. Oligonucleotide array-CGH reveals cryptic gene copy number alterations in karyotypically normal acute myeloid leukemia. Leukemia 2007;21:571-574 (Letter).

III. Tyybäkinoja A, Vilpo J, Knuutila S. High-resolution oligonucleotide array-CGH pinpoints genes involved in cryptic losses in chronic lymphocytic leukemia. Cytogenet Genome Res 2007;118:8-12.

IV. Tyybäkinoja A, Elonen E, Vauhkonen H, Saarela J, Knuutila S. Single nucleotide polymorphism microarray analysis of karyotypically normal acute myeloid leukemia reveals frequent copy number neutral loss of heterozygosity. Haematologica 2008;93:631-632 (Letter).

These articles have been reprinted with the kind permission of their copyright holders. In addition some unpublished data is presented.

ABBREVIATIONS

ABL1	c-abl oncogene 1, receptor tyrosine kinase
aCGH, array-CGH	array comparative genomic hybridization
ADAMTS8	ADAM metallopeptidase with thrombospondin type 1 motif, 8
AML	acute myeloid leukemia
APLP2	amyloid beta (A4) precursor-like protein 2
ARHGEF12 (LARG)	Rho guanine nucleotide exchange factor (GEF) 12
ATM	ataxia telangiectasia mutated
BAALC	brain and acute leukemia, cytoplasmic
BAC	bacterial artificial chromosome
BARX2	BARX homeobox 2
BCR	breakpoint cluster region
bp	base pair
CBFB	core-binding factor, beta subunit
CBL	Cas-Br-M (murine) ecotropic retroviral transforming sequence
CCDC26	coiled-coil domain containing 26
cCGH	chromosomal comparative genomic hybridization
cDNA	complementary deoxyribonucleic acid
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
CLL	chronic lymphocytic leukemia
CNV	copy number variation
CREBBP	CREB binding protein
CUL5	cullin 5
DEK	DEK oncogene
dmin	double minute chromosome
ERG	v-ets erythroblastosis virus E26 oncogene homolog (avian)
ETS1	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)
ETV6	ets variant 6
EVI1	ecotropic viral integration site 1
FAB	French-American-British
FAM84B	family with sequence similarity 84, member B
FISH	fluorescence *in situ* hybridization
FLI1	Friend leukemia virus integration 1
FLT3	fms-related tyrosine kinase 3
hsr	homogeneously staining region
*Ig*VH	immunoglobulin variable heavy chain
ITD	internal tandem duplication
JAM3	junctional adhesion molecule 3
KCNJ1	potassium inwardly-rectifying channel, subfamily J, member 1
KCNJ5	potassium inwardly-rectifying channel, subfamily J, member 5
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog

KRAS	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
LOH	loss of heterozygosity
MDS	myelodysplastic syndrome
M-FISH	multiplex fluorescence *in situ* hybridization
MIR15A	microRNA 15a
MIR16-1	microRNA 16-1
MIR181	microRNA 181
MIR29	microRNA 29
MLL	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)
MLPA	multiplex ligation-dependent probe amplification
MN1	meningioma (disrupted in balanced translocation) 1
MRD	minimal residual disease
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)
MYCN	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
MYH11	myosin, heavy chain 11, smooth muscle
MYST3	MYST histone acetyltransferase (monocytic leukemia) 3
NCAPD3	non-SMC condensin II complex, subunit D3
NFRKB	nuclear factor related to kappaB binding protein
NGS	next-generation sequencing
NPAT	nuclear protein, ataxia-telangiectasia locus
NPM1	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog
NTM	neurotrimin
NUP214	nucleoporin 214kDa
OPCML	opioid binding protein/cell adhesion molecule-like
p	short chromosome arm
PML	promyelocytic leukemia
PPP2R1B	protein phosphatase 2 (formerly 2A), regulatory subunit A, beta isoform
PVT1	Pvt1 oncogene (non-protein coding)
q	long chromosome arm
qRT-PCR	quantitative real-time polymerase chain reaction
RARA	retinoic acid receptor, alpha
RFLP	restriction fragment length polymorphism
RICS	Rho GTPase-activating protein
RUNX1	runt-related transcription factor 1
RUNX1T1	runt-related transcription factor 1; translocated to, 1 (cyclin D-related)
SKY	spectral karyotyping
SNP	single-nucleotide polymorphism
SNP-array	single-nucleotide polymorphism microarray
SNX19	sorting nexin 19
SRPR	signal recognition particle receptor (docking protein)
ST14	suppression of tumorigenicity 14 (colon carcinoma)
TCL1	T-cell leukemia/lymphoma 1

TP53	tumor protein p53
TRIB1	tribbles homolog 1 (Drosophila)
UPD	uniparental disomy
WGG	whole genome genotyping
WGSA	whole genome sampling assay
WHO	World Health Organization
WT1	Wilms tumor 1
ZAP70	zeta-chain (TCR) associated protein kinase 70kDa

ABSTRACT

Chromosomal alterations in leukemia have been shown to have prognostic and predictive significance and are also important minimal residual disease (MRD) markers in the follow-up of leukemia patients. Although specific oncogenes and tumor suppressors have been discovered in some of the chromosomal alterations, the role and target genes of many alterations in leukemia remain unknown. In addition, a number of leukemia patients have a normal karyotype by standard cytogenetics, but have variability in clinical course and are often molecularly heterogeneous. Cytogenetic methods traditionally used in leukemia analysis and diagnostics; G-banding, various fluorescence *in situ* hybridization (FISH) techniques, and chromosomal comparative genomic hybridization (cCGH), have enormously increased knowledge about the leukemia genome, but have limitations in resolution or in genomic coverage. In the last decade, the development of microarray comparative genomic hybridization (array-CGH, aCGH) for DNA copy number analysis and the SNP microarray (SNP-array) method for simultaneous copy number and loss of heterozygosity (LOH) analysis has enabled investigation of chromosomal and gene alterations genome-wide with high resolution and high throughput.

In these studies, genetic alterations were analyzed in acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL). The aim was to screen and characterize genomic alterations that could play role in leukemia pathogenesis by using aCGH and SNP-arrays. One of the most important goals was to screen cryptic alterations in karyotypically normal leukemia patients. In addition, chromosomal changes were evaluated to narrow the target regions, to find new markers, and to obtain tumor suppressor and oncogene candidates.

The work presented here shows the capability of aCGH to detect submicroscopic copy number alterations in leukemia, with information about breakpoints and genes involved in the alterations, and that genome-wide microarray analyses with aCGH and SNP-array are advantageous methods in the research and diagnosis of leukemia. The most important findings were the cryptic changes detected with aCGH in karyotypically normal AML and CLL, characterization of amplified genes in 11q marker chromosomes, detection of deletion-based mechanisms of *MLL-ARHGEF12* fusion gene formation, and detection of LOH without copy number alteration in karyotypically normal AML. These alterations harbor candidate oncogenes and tumor suppressors for further studies.

INTRODUCTION

The cancer genome is affected by various genetic alterations, including mutations, epigenetic alterations, and such chromosomal alterations as translocations, copy number changes, and loss of heterozygosity (LOH). These alterations can cause inactivation of tumor suppressor genes or activation of oncogenes. Eventually, the expression of genes and proteins is changed, forming the cancer phenotype.

Leukemia is a hematological cancer characterized by malignant clonal growth of blood cells. Cytogenetic and genetic alterations are important prognostic and predictive factors that affect the diagnosis and treatment of leukemia patients (Swerdlow et al. 2008). More detailed analysis of the chromosomal alterations detected in leukemia is important for obtaining knowledge of the pathogenesis of leukemia. In addition, the knowledge about the genes that are targets of chromosomal changes can provide target genes for therapies. More precise classification of the patients to different risk groups can improve the targeting of available treatments to those patients who will benefit from them.

Modern microarray techniques provide possibilities for detailed analysis of the DNA alterations in the cancer genome. Genome-wide analysis with array comparative genomic hybridization (array-CGH, aCGH) and single-nucleotide polymorphism microarrays (SNP-array) can reveal small cryptic copy number changes that are relevant in leukemogenesis. Moreover, the novel alterations detected with these microarray methods may serve as putative minimal residual disease markers. Besides the copy number alterations found with both methods, SNP-array can also reveal copy number neutral allelic loss. SNP-array and aCGH may prove to be useful in leukemia diagnostics as molecular karyotyping techniques that provide additional information to such traditional cytogenetic analyses as G-banding and fluorescence *in situ* hybridization (FISH).

This thesis concentrates on the genetic analyses of two different leukemias, acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL). The main focus is on the analysis of chromosomal alterations in leukemic cells using high-resolution genome-wide analyses with aCGH and SNP-array.

REVIEW OF THE LITERATURE

1. Cancer and genetic alterations

Cancer is a common disease worldwide, often associated with aging. It is detected in various tissues and has a complex genetic background. Most of the genetic alterations in cancer are acquired somatically, although in some cases inherited mutations may also lead to susceptibility. In families with inherited susceptibility, cancer can be detected in many close relatives and specific cancer types can be common. In addition, in these families cancer is often detected in young individuals. Specific mutations can be detected in inherited forms of cancer in the same cancer genes that are often altered in somatic cancer. Some inherited cancers also have inherited defects in repair genes, leading to genetic instability and accumulation of mutations. In many cases, the cause of the cancer is unknown, but such factors as mistakes during DNA replication or exposure to various environmental factors, including carcinogenic chemicals, viruses, and radiation, have been suggested. The main hallmarks of cancer are (1) self-sufficient growth, (2) insensitivity to antigrowth signals, (3) limitless replicative potential, (4) evasion of apoptosis, (5) angiogenesis, and (6) tissue invasion and metastasis (Hanahan & Weinberg 2000). These processes are strictly controlled at various levels in the normal genome. Accumulation of aberrations in these processes and malfunction of normal repair mechanisms can lead to aberrant growth of cells and cancer development.

Clonal proliferation starting from a single progenitor cell is a typical characteristic of cancer. Cancer development is thought to occur in multiple steps, with a minimum of 3-6 mutations (Vogelstein & Kinzler 1993). Multiple mutations in progenitor cells start uncontrolled proliferation, and accumulating alterations induce malignant transformation. Alterations accumulate in the genes involved in cell proliferation, cell differentiation, cell death, and DNA repair (Futreal et al. 2004). The sequence of cancer genes can be altered by point mutations or base pair (bp) level deletions, insertions, and inversions or by chromosomal alterations, including changes in ploidy and individual chromosome numbers, chromosomal deletions, amplifications, and translocations (reviewed by Lengauer et al. 1998). Epigenetic alterations that do not affect the DNA sequence can silence genomic regions involved in cancer pathogenesis by covalent modifications of chromatin components or DNA methylation (reviewed by Jones & Baylin 2002). Of the approximately 25 000 genes in the human genome, over

1% have been reported to be involved in cancer (Futreal et al. 2004). Cancer genes are generally categorized to two types, oncogenes and tumor suppressors.

Proto-oncogenes are the normally functioning genes coding growth factors, growth factor receptors, signaling proteins, and transcription factors. These genes can become oncogenes by gaining an abnormal function. Oncogenes typically have a dominant activity; only one of the alleles needs to be affected. Activation or deregulation of oncogenes can be caused by mechanisms of amplification, mutation, or chromosomal rearrangements such as translocation, inversion, and interstitial deletion.

Genes that normally function in preventing cancer-causing aberrations in the genome and cells are called tumor suppressors. In cancer cells, tumor suppressors can be inactivated by deletion of one or both of the alleles, mutations in the gene sequence, or epigenetic silencing. According to Knudson's (1971) two-hit hypothesis, a minimum of two alterations are needed to inactivate the tumor suppressor. Currently we know that although alterations in both tumor suppressor alleles are usually needed, in cases of haploinsufficiency, dominant-negative mutations, or imprinting, one change can be sufficient (reviewed by Payne & Kemp 2005). Tumor suppressors are divided into three classes: genes that protect the genome from oncogenic mutations and include repair and segregator genes (caretakers), genes that prevent malignant growth by inducing apoptosis or inhibiting growth (gatekeepers), and landscaper genes that affect the microenvironment of cells (Kinzler & Vogelstein 1997, Kinzler & Vogelstein 1998). Loss of caretaker gene functions associated with genomic instability is suggested to be involved in most cancers and is reflected as an increased rate of alterations in the DNA sequence and chromosomes (reviewed by Lengauer et al. 1998, Hoeijmakers 2001).

2. Chromosomal imbalances in cancer

In cancer cells, various chromosomal imbalances changing the chromosome number or rearranging the structure of chromosomes are common. Chromosomal imbalances in cancers have been studied extensively for diagnostic and classification purposes, for discovery of treatment targets, and for elucidation of pathogenic mechanisms.

2.1. Translocations and inversions

Translocations are structural changes that arise when a chromosomal segment is relocated from its normal position in the genome. Various types of translocations are seen in cancer. In balanced translocations, no copy number alterations are detectable in cytogenetic analyses, whereas in unbalanced translocations gains and losses are seen. Simple translocations, which are composed recurrently of the same DNA segments, are common in leukemia and lymphoma, while more random, complex translocations, associated with various chromosomal imbalances, are more common in solid tumors (Lengauer et al. 1998). Inversions are balanced structural changes occurring within one chromosome as a result of two DNA breaks, 180° inversion, and subsequent re-joining. The exact mechanisms of translocations and fusion gene formation remain unknown, but DNA double-strand breaks and subsequent incorrect repair mechanisms have been suggested to be involved (reviewed by Elliott & Jasin 2002, Aplan 2006).

Translocations and inversions can form oncogenic fusion genes or bring an oncogene into the vicinity of a strong regulator sequence that causes aberrant activation. Oncogenic fusion genes are often transcription factors or tyrosine kinases (Look 1997, Mitelman et al. 2004). The most commonly known example of an oncogenic fusion gene caused by a translocation is the *BCR-ABL1* tyrosine kinase detected in the Philadelphia chromosome in chronic myeloid leukemia (reviewed by Laurent et al. 2001). Currently, over 440 different fusion genes have been detected in cancer (Mitelman et al. 2007, Heim & Mitelman 2008). Based on detection of specific fusion genes, some leukemias and lymphomas can be classified into subtypes (Swerdlow et al. 2008). Although fusion genes can have an important role in developing a specific cancer, fusion genes have also been detected in healthy individuals (Janz et al. 2003), and the formation of cancer is thought to require additional alterations. For example, in AML, a specific fusion gene and an oncogenic mutation are frequently simultaneously present (Dash & Gilliland 2001).

2.2. DNA copy number losses

DNA copy number loss is one of the mechanisms of tumor suppressor inactivation. In Knudson's two-hit hypothesis, one of the tumor suppressor alleles is mutated and the second allele is then lost (Knudson 1971). DNA copy number losses are common in

solid tumors and also recurrent in hematological malignancies. Losses have been found in all chromosome arms in cancer (Knuutila et al. 1999), and they are detected as monosomies, deletions of chromosomal segments, and loss of DNA associated with unbalanced translocations. Defining minimal common regions of loss in particular cancers is a popular strategy in searching for tumor suppressors.

Whole chromosome losses in cancer cells can be caused by mitotic errors, whereas DNA breaks are involved in the formation of structural deletions. One break can cause terminal loss of material from the chromosome, and two breaks can cause interstitial deletion when the DNA between the breaks is lost and the resulting DNA ends are fused. Besides causing the loss of tumor suppression, in some cases interstitial deletion can also form a fusion gene, thus being a mechanism of oncogene activation (Kourlas et al. 2000, Van Vlierberghe et al. 2008).

2.3. Amplifications

Amplification is considered to be a late or secondary event in cancer and is one of the mechanisms that can cause the overexpression of oncogenes (Lengauer et al. 1998). Amplicons are formed of multiple copies of a DNA sequence that is generally under 10 Mb long, whereas copy number gains are generally larger, with fewer copies, and detected as aneuploidy or with unbalanced translocations (reviewed by Myllykangas & Knuutila 2006). All of the genes in the amplicon may not be overexpressed and are called bystanders. An estimated 20-60% of amplified genes show overexpression (Hyman et al. 2002, Pollack et al. 2002, Heidenblad et al. 2005).

Amplifications are detected as homogeneously staining regions (hsrs) within chromosomes or as extrachromosomal structures, including double minute chromosomes (dmins), episomes, and various larger marker chromosomes, e.g. ring chromosomes. Dmins are small circular chromatin structures that are detectable under a light microscope, and episomes are circular DNA particles (<1 Mb) not detectable in standard karyotyping. Amplicons located in chromosomes are more stable than extrachromosomal particles because in cell division they segregate with chromosomes, whereas extrachromosomal structures segregate randomly and may be lost in the process. However, if the particles include genes that have a selective advantage (e.g. oncogenes in cancer tissue), their amount may increase (reviewed by Hahn 1993).

Various models have been suggested for explaining the formation of amplifications such as the breakage-fusion-bridge model, the episome model, and the overreplication model ("onion skin model") (Schimke et al. 1986, Carroll et al. 1988, Von Hoff et al. 1990, reviewed by Myllykangas & Knuutila 2006,). In the overreplication model, replication of DNA is disrupted and overreplication occurs. These overreplicated DNA strands can then ligate together from the ends and recombine to chromosomes or form circular extrachromosomal particles (Schimke et al. 1986). In the episome model, a deletion of a chromosomal segment occurs first, after which the deleted segment forms the episome (Carroll et al. 1988). Episomes can then form dmins by enlarging (Carroll et al. 1988). Episomes and dmins may also integrate to chromosomes and form hsrs (Carroll et al. 1988). Breakage-fusion-bridge cycles can form hsrs, ring chromosomes, and other unspecific chromosomal changes: first, double-strand DNA breakage or telomere erosion occurs and, after replication, the uncapped ends of sister chromatides fuse, then in mitosis an anaphase bridge is formed and a second asymmetric breakage may occur in the bridge, resulting in one daughter cell with a duplicated oncogene and one with a deletion of that gene (Schwab 1999, Gisselsson et al. 2000, Myllykangas & Knuutila 2006).

2.4. Loss of heterozygosity

In loss of heterozygosity (LOH), one of the two heterozygous alleles is lost. As a result of LOH, the heterozygous chromosomal region becomes homozygous or hemizygous. Homozygous LOH is detected when one allele is lost and the other is duplicated. In hemizygous LOH, only one allele remains when the other is deleted. Because LOH analysis detects allelic imbalance, it can reveal copy number changes as well as copy number neutral LOH. LOH can be detected to varying extents, from localized alteration of one allele to whole chromosome alteration. Various mechanisms, including deletion, gene conversion, mitotic recombination, translocation, and nondisjunction, have been shown or suggested to cause LOH (Thiagalingam et al. 2002).

In cancer, LOH has been considered a marker for a tumor suppressor gene based on Knudson's two-hit hypothesis. According to his hypothesis, a recessive tumor suppressor gene is first mutated and the remaining wild-type allele is then lost with a second hit such as LOH (Knudson 1971). Besides mutation, one allele of the recessive gene can also be silenced by epigenetic mechanism (Jones & Baylin 2002), and LOH then causes loss of function of the gene. An additional mechanism associated with LOH

can be haploinsufficiency, when one functional allele is not sufficient to prevent disease (Payne & Kemp 2005). Genomic instability in cancer, which can be caused by malfunction of caretaker and segregator genes, is associated with frequent LOH (Thiagalingam et al. 2002).

2.5. Uniparental disomy

In uniparental disomy (UPD), one of the homologous parental chromosomes is lost and the other is duplicated (Engel 1980). Engel's concept of UPD explained the large homozygous chromosomal segments in abnormal fetuses and suggested the meiotic mechanisms behind constitutional UPD. Since then, the concept of UPD has broadened to also include acquired UPD, which is created by mitotic mechanisms. Two types of UPD exist. In heterodisomy, both homologous chromosomes of one parent are present, while in isodisomy the homologous chromosomes are identical copies from one homolog of one parent. Heterodisomy causes abnormalities only when combined with loss of imprinting and does not affect heterozygosity. Isodisomy, by contrast, causes LOH.

UPD mechanisms in meiosis include gamete complementation, trisomy correction, and monosomy duplication (reviewed by Robinson 2000, Tuna et al. 2009). Contrary to meiotic UPD, which can be seen in each progeny cell, mitotic UPD is present only in a portion of cells. UPD mechanisms proposed to cause acquired UPD include somatic recombination, and mitotic nondisjunction and subsequent duplication of the chromosomes (Robinson 2000, Tuna et al. 2009). Gene conversion has also been suggested to cause small regions of isodisomy (Robinson 2000), but may be too rare an event in humans to be a significant cause (Tuna et al. 2009). Compensatory UPD occurs when a chromosomal aberration or a lost chromosome is replaced by copying the normal homolog (Robinson 2000).

Analysis of UPD-causing mechanisms and elucidation of the importance of UPD in cancer formation are ongoing challenges. Because of the high rate of cell divisions in cancer tissue, UPD can be a common event in cancer. In recent years, acquired UPD has frequently been discovered in various cancers, mostly by using modern SNP-array technologies (Raghavan et al. 2005, Tuna et al. 2009). The degree of UPD detected in cancer includes both whole chromosome UPD, as well as telomeric or interstitial UPDs. UPD in the cancer genome can cause inactivation of tumor suppressors or activation of oncogenes. Possible pathogenic consequences of UPD include homozygosity of a

mutated recessive gene or gene expression changes caused by a combination of UPD and epigenetic mechanisms in regulator sequences (Tuna et al. 2009).

3. Microarrays in the analysis of chromosomal imbalances

G-banding karyotyping is traditionally used to detect structural and numeric chromosomal changes under a light microscope. Molecular cytogenetic methods, such as fluorescence *in situ* hybridization (FISH) (Pinkel et al. 1986, reviewed by Trask 1991), multiplex FISH (M-FISH) (Speicher et al. 1996), spectral karyotyping (SKY) (Schrock et al. 1996), multicolor chromosome banding (Muller et al. 1998, Chudoba et al. 1999), and chromosomal comparative genomic hybridization (cCGH) (Kallioniemi et al. 1992) have improved the resolution of cytogenetic analysis. In addition, molecular genetic analyses, e.g. quantitative real-time PCR (qRT-PCR) (reviewed by Mocellin et al. 2003) or multiplex ligation-dependent probe amplification (MLPA) (Schouten et al. 2002), have been used to study chromosomal changes in detail.

Knowledge of the structures of genomes and chromosomes, gene mapping, and the sequence of the whole human genome has enabled development of DNA microarrays, which are composed of an organized set of DNA probes spotted on a chip. Modern microarrays can contain hundreds of thousands of probes, allowing a genome-wide analysis with a high throughput and high resolution and producing a large number of data in a single experiment. Various DNA microarray analyses with specific platforms have been developed, including gene expression, genotyping, epigenetic, and aCGH microarray analyses (reviewed by Hoheisel 2006).

3.1. Array comparative genomic hybridization

Development of cCGH has enabled study of genome-wide DNA copy number alterations without cell culturing (Kallioniemi et al. 1992). The principle of the cCGH method is that DNA from the test material (e.g. tumor DNA) and reference material are labeled with different colors and hybridized together to the metaphase slides. The relative signal ratio of test and reference is measured with a microscope and image

analysis software and turned into a copy number profile, showing gains and losses of material.

Because resolution of cCGH is limited to a chromosome-band level, a new technique known as aCGH was developed (Solinas-Toldo et al. 1997, Pinkel et al. 1998, Pollack et al. 1999). Array-CGH enables genome-wide analysis of the copy number alterations in a single experiment with a resolution that can detect gene-level or even exon-level changes. A relatively small amount of DNA is needed in an aCGH experiment (200 ng to 1 μg). Furthermore, DNA extracted from paraffin-embedded tissue or produced with whole genome amplification can also be used (Cardoso et al. 2004, Guillaud-Bataille et al. 2004, Lips et al. 2005, Hittelman et al. 2007).

As in cCGH, in an aCGH experiment the sample DNA is compared with reference DNA (Figure 1). The main difference is that instead of metaphase chromosomes the hybridization target is a microarray, usually a glass slide, with DNA spots representing known genomic locations. After the hybridization of sample and reference DNA, microarrays are washed from unbound material and scanned with a laser scanner to form an image file for data analysis. The analysis is done with specific image analysis software that measures the signal intensities of the different colors in each spots and turns them into a signal ratio. Ratios obtained from different data spots are organized and visualized with ordered genomic locations and analyzed with the appropriate software.

Three different types of microarray platforms, classified according to the type of DNA arranged on the microarray, have been used in aCGH applications: bacterial artificial chromosome (BAC), complementary DNA (cDNA), and oligonucleotide-based microarrays (Table 1). The resolution of the different platforms is dependent on the size, number, and uniformity of the genomic distribution of the probes (Coe et al. 2007). Depending on the platform used, the resolution of aCGH ranges from around 1 Mb to a few Kbs. The moving average of signals from adjacent probes is used in cDNA and oligonucleotide analyses to reduce noise, whereas in BAC arrays it is not needed because of the large size of the individual clones. The possibility to design and manufacture specific oligonucleotide probes and the high probe densities in arrays obtained in industrial manufacturing make oligonucleotide arrays the platform with the highest resolution. Due to advances in analysis software development and microarray manufacturing processes, oligonucleotide microarrays are now the most commonly used microarrays. SNP-array, an oligonucleotide-based platform that can be used for the simultaneous analysis of copy number alterations and LOH, is reviewed in the next section.

A limitation of aCGH is that changes that do not affect the relative copy number, such as polyploidy and balanced translocations or inversions, are not detected. It has also been suggested that an alteration has to be present in at least 20-30% of cells in the sample to obtain proper resolution, and thus, aberrations that are present in only a small proportion of cells, e.g. in the case of minimal residual disease or mild mosaicism, are undetectable (Ballif et al. 2006, Gondek et al. 2008).

Array-CGH has been widely used for the detailed analysis of gene copy numbers in cancers, leading to cancer classification proposals as well as the discovery of candidate genes with clinical significance (reviewed by Kallioniemi 2008). Various genome-wide aCGH techniques have also revealed that besides single-nucleotide polymorphisms also large-scale copy number polymorphisms (from 1 kb to 2 Mb in size) are common in the human genome (Iafrate et al. 2004, Sebat et al. 2004, Redon et al. 2006). The common existence of copy number polymorphisms is challenging in aCGH data analysis when polymorphisms and possibly disease-associated alterations need to be distinguished. However, copy number polymorphisms may also be associated with susceptibility to a disease. In fact, a proportion of copy number variations (CNVs) overlap with known disease genes (Redon et al. 2006). The CNV database, which is constantly updated with published CNV data, is available at http://projects.tcag.ca/variation/ and is helpful in interpreting aCGH data.

3.2. Single-nucleotide polymorphism microarray analysis

Various polymorphisms in the human genome, such as restriction fragment length polymorphism (RFLPs), microsatellites, and single-nucleotide polymorphisms (SNPs), have been utilized in the analysis of LOH. SNPs are the most common variation detected in the human genome, with over ten million known SNPs and over 3 million of these genotyped (The International Hapmap Consortium 2007, http://www.ncbi.nlm.nih.gov/snp). SNPs commonly have two alleles; thus, heterozygous and homozygous genotypes can be identified. Because SNPs are densely located in the genome, they provide an excellent basis for high-resolution and genome-wide microarray analysis.

SNP-arrays, comprinsing oligonucleotide probes that indentify specific SNPs, were first designed for large-scale genotyping (Wang et al. 1998). Since then, the SNP-array technology has been shown to be applicable for simultaneous detection of LOH and gene copy number alterations and also to have the ability to reveal UPD, a copy

number neutral LOH (Lindblad-Toh et al. 2000, Bignell et al. 2004, Raghavan et al. 2005).

In SNP-array analysis, only one DNA sample is labeled and hybridized to an array (Figure 1). The signal intensities obtained in the experiment reveal three possible genotypes for each SNP (AA and BB homozygotes, and an AB heterozygote). The copy number and LOH data can be produced in three ways. Firstly, cancerous and normal tissue of the same patient can be hybridized to separate arrays, with the data then being compared with computer analysis tools (Bignell et al. 2004, Zhao et al. 2004). Secondly, the analysis can be done by comparing the sample hybridization to a large set of reference hybridizations by using specific algorithms (Nannya et al. 2005). These reference data can be retrieved from databases, or a set of normal tissue hybridizations can be performed. The third option is to use an analysis tool, which requires no reference hybridizations, but is based on a mathematical model (Beroukhim et al. 2006).

While in the aCGH analysis genomic DNA is successfully used as a sample, in SNP-array analysis the complexity of the human genome has been problematic because of cross-hybridizations and nonspecific signals (Kennedy et al. 2003, Matsuzaki et al. 2004, Gunderson et al. 2005, Steemers et al. 2006). Assays to overcome these problems have been developed such as the whole genome sampling assay (WGSA) (Kennedy et al. 2003, Matsuzaki et al. 2004) and the whole genome genotyping (WGG) assay (Gunderson et al. 2005, Peiffer et al. 2006, Steemers et al. 2006). In WGSA analysis, the genome complexity is reduced to contain only relevant sequences by digestion of DNA with specific enzymes and subsequent selective amplification of the fragments of specific size (Kennedy et al. 2003, Matsuzaki et al. 2004). In the WGG protocol, four basic steps are performed: whole genome amplification, hybridization to capture probes on BeadChip (Illumina) microarray, SNP scoring with allele-specific primer extension, and signal amplification (Gunderson et al. 2005, Steemers et al. 2006).

Developments in microarray technology have enabled production of SNP-arrays with a very high density. Commercial SNP-arrays with around one million SNPs are currently available for genomic analysis (http://www.affymetrix.com, http://www.illumina.com). Furthermore, SNP-arrays have proved to be efficient tools in genomic analysis of cancer since many studies have shown recurrent regions of copy number alterations, LOH, and UPD (Dutt & Beroukhim 2007, Tuna et al. 2009).

Table 1. Different platforms used in aCGH analyses.

Platform	Probe size	Special characteristics
BAC	80-200 kb	Sensitive to relatively large alterations
cDNA	~300 bp	Represents coding sequence
Oligonucleotide	25-85 bp	Industrial synthesis
Oligonucleotide SNP	21-85 bp	Industrial synthesis, also reveals copy number neutral LOH

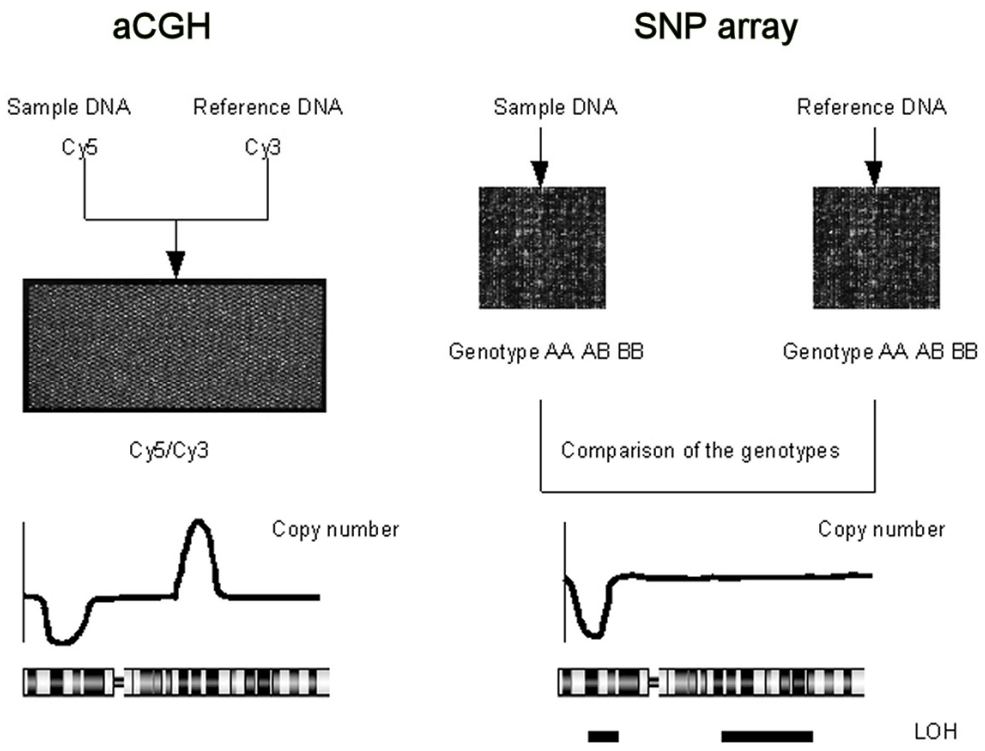


Figure 1. Comparison of aCGH and SNP-array methods. In the aCGH experiment, sample and reference are labeled differently and hybridized together on one array. The ratio of signal intensities then shows the copy number profile. In the SNP-array experiment, sample and reference DNA are both similarly labeled and hybridized on separate arrays. The signal intensities of each SNP produce specific genotypic call (AA, AB, or BB). Sample and reference data are then compared with computer analysis tools and both copy number profile, and LOH regions can be detected.

4. Leukemia

Leukemia is a disease characterized by clonal expansion of malignant blood cells. Leukemia is divided into acute and chronic types based on the maturity of the leukemic cells. Chronic leukemia can be asymptomatic or symptoms can be mild and slowly worsen. In acute leukemia, the symptoms appear rapidly and are more severe. Leukemias are also divided into myeloid and lymphoid types according to the malignant cell lineage. Leukemia can cause various symptoms that are common in many diseases, including fever, night sweats, frequent infections, weakness, fatigue, headache, pains in joints, bruising and bleeding, enlarged spleen, and swollen lymph nodes. Therefore, various tests are done to confirm the diagnosis. Distinguishing between different hematological malignancies is based on cell morphology, immunophenotyping, and cyto- and molecular genetic alterations (Swerdlow et al. 2008). Two different types of leukemia, AML and CLL, the two most common types in adults, were studied here and are described in more detail.

4.1. Acute myeloid leukemia

4.1.1. Clinical characteristics and treatment of acute myeloid leukemia

In AML, myeloid blasts are clonally expanded in bone marrow and blood, and increased proliferation, reduced apoptosis, and lack of differentiation characterize the malignant cells (Lowenberg et al. 1999). AML is the most common acute leukemia in adults with a median age of 60-70 years and it is slightly more common in males than in females (Estey & Dohner 2006). Overall annual incidence of AML is approximately 3.8/100 000 in people under 65 years of age and 17.9/100 000 in older populations (Estey & Dohner 2006). In Finland, approximately 120 AML cases are detected every year in adults (Elonen 2007).

Etiology of AML is largely unknown, but associated factors include ionizing radiation, earlier cytotoxic chemotherapy, benzene exposure, and smoking (Smith et al. 2004, Estey & Dohner 2006). Familial AML is very rare and little is known of the genetic background, although germline *CEBPA* mutations have been detected in some cases (reviewed by Owen et al. 2008, Pabst et al. 2008). Additionally, risk of AML is

increased in such conditions as Down syndrome, Fancony anemia, ataxia-telangiectasia, and Bloom syndrome because of increased genomic instability (Smith et al. 2004). A variable scale of symptoms mainly caused by burden of leukemic cells, abnormal marrow function, and tissue infiltration is detected in AML patients, including fever, fatigue, anemia, infections, and bleeding (Lowenberg et al. 1999, Smith et al 2004, Estey & Dohner 2006).

At least 20-30% of blasts in marrow or blood are detected typically in the diagnosis of AML (Lowenberg et al. 1999, Smith et al 2004, Estey & Dohner 2006). The AML blasts are immature cells with round to oval nuclei and finely granular chromatin (Smith et al. 2004). *Auer bodies*, the rod-like structures that are formed of aggregates of normal granules are a specific finding in cytoplasm of blasts in one-third of cases (Smith et al. 2004). Two different classification systems categorize AML cases into subtypes. The French-American-British (FAB) classification is based on cell morphology and maturity and classifies AML into eight subtypes (M0 through to M7) (Bennett et al. 1976). A more novel system currently in use, the World Health Organization (WHO) system, divides AML into subtypes based on genetic and clinical factors (Table 2, Swerdlow et al. 2008). Immunophenotypic markers, such as CD45, CD34, CD117, and cytoplasmic myeloperoxidase, are analyzed in diagnosis of AML to identify the myeloid origin of blasts, and some markers (e.g. CD14, CD11b, glycophorin A, CD41, CD42, and CD 61) are also associated with specific lineage differentiation (Smith et al. 2004).

AML is a genetically and clinically heterogeneous malignancy. Prognostic factors of AML include age, response to therapy, cytogenetics, and bone marrow blast count (Estey & Dohner 2006). Treatment of AML is commonly based on multidrug chemotherapy and allogeneic bone marrow stem cell transplantation (Lowenberg et al. 1999, Estey & Dohner 2006). Although initial remission is often obtained, relapse is common. The five-year survival rate of young AML patients is 40-65% and elderly patients 10-15% depending on age and other prognostic factors (Derolf et al. 2009). The treatment of elderly patients has been problematic because chemotherapy causes severe side-effects and mortality (Estey & Dohner 2006, Kantarjian et al. 2008).

Table 2. WHO classification of acute myeloid leukemia.

Categories	Common associated chromosomal alterations
AML with recurrent genetic aberrations	t(8;21)(q22;q22), inv(16)(p13q22), t(16;16)(p13;q22), t(15;17)(q22q12), t(9;11)(p22;q23)
AML with myelodysplasia-related alterations	complex karyotype, -7/del(7q), -5/(del5q)
Therapy-related myeloid neoplasms	complex karyotype, -7/del(7q), -5/(del5q), balanced translocations
AML, not otherwise categorized	no unique alterations

Data retrieved from Swerdlow et al. (2008).

4.1.2. Genetic alterations in acute myeloid leukemia

Approximately 200 recurrent chromosomal aberrations have been detected in cytogenetic analyses of AML (Mrozek et al. 2004). The most common cytogenetic changes are trisomy 8, t(8;21), t(15;17), inv(16), t(6;9), and t(8;16) (Table 3). Balanced reciprocal translocations typical for AML usually cause alterations in the function or transcription factors involved in myeloid differentiation or in tyrosine kinases (Dash & Gilliland 2001, Steffen et al. 2005). Despite the common existence of translocations in AML, they are seen only in a small proportion (20%) of patients (Heim & Mitelman 2009). Other characteristic chromosome changes of AML, detected as a sole alteration or in association with other changes, include +4, +8, +11, +13, +19, +21, -5, and -7 deletions in 5q, 7q, and 9q and various alterations involving 11q23 (Heim & Mitelman 2009). Of the relatively rarely detected amplifications in AML, the most common regions and genes include *MYC* in 8q24 (Alitalo et al. 1985, Thomas et al. 2004) and *MLL* in 11q23 (Allen et al. 1998, Ariyama et al. 1998, Poppe et al. 2004). Complex karyotype with three or more alterations, in the absence of the common translocations, is detected in 10% of AML patients. Furthermore, in standard cytogenetic analysis, 40-50% of AML remain karyotypically normal (Grimwade et al. 1998, Byrd et al. 2002, Heim & Mitelman 2009). The molecular pathogenesis of the complex karyotype group of patients as well as karyotypically normal patients is largely unknown. Cytogenetic and genetic alterations are considered important prognostic factors in AML. Based on cytogenetic alterations, AML patients can be divided into three risk groups; poor, intermediate, and favorable (Grimwade et al. 1998, Slovak et al. 2000, Grimwade et al. 2001, Byrd et al. 2002, Table 4).

A number of mutated genes have been described in the pathogenesis of AML (Table 5). Many of the commonly mutated genes in AML are involved in cell proliferation, myeloid differentiation, cell cycle regulation, and apoptosis (Renneville et al. 2008). Since the fusion genes detected commonly in AML cannot alone cause the

pathogenesis, some of these mutated genes have been suggested to be the second hit needed. For example, mutations of the tyrosine kinase gene *KIT* are often associated with inv(16) or t(8;21) (Beghini et al. 1998, Gari et al. 1999, Wang et al. 2005).

The large normal karyotype group of AML is a heterogeneous entity at the molecular level, and although the prognosis is intermediate compared with other cytogenetic groups, there is also variation in the clinical course (Mrozek et al. 2007). Although part of the heterogeneity is explained by frequently detected gene mutations, the mutations detected to date have not been shown to cause the leukemogenesis solely (Mrozek et al. 2007). *FLT3* and *NPM1* mutations are detected most often in karyotypically normal AML cases. *FLT3* ITD mutations are detected in 28-33%, *FLT3* tyrosine kinase domain point mutations in 5-10%, and *NPM1* mutations in 40-60% of cases (Falini et al. 2005, Thiede et al. 2006, Mrozek et al. 2007). Besides mutations, altered gene expression of *BAALC*, *ERG*, and *MN1* has been associated with prognosis in karyotypically normal AML patients (Baldus et al. 2007, Mrozek et al. 2007). Cryptic gene copy number alterations might explain some of the heterogeneity, but in karyotypically normal AML, FISH and cCGH have uncovered cryptic alterations only occasionally (Dalley et al. 2002, Frohling et al. 2002).

Microarray methods have in recent years been used to analyze the AML genome. Array-CGH studies with BAC arrays have revealed cryptic alterations in AML patients with known karyotypic alterations (Baldus et al. 2004, Tchinda et al. 2004, Paulsson et al. 2006, Rucker et al. 2006), oligonucleotide aCGH was in one study able to reveal cryptic alterations in 60% of karyotypically normal AML cases (Suela et al. 2007), and SNP-array analyses have shown UPD in 10-20% of karyotypically normal AML (Gorletta et al. 2005, Raghavan et al. 2005). Recently, frequent UPD and cryptic copy number alterations have been detected in larger patient cohorts of MDS and AML using 250K SNP-array (Dunbar et al. 2008, Gondek et al. 2008). In addition, acquired UPD regions have been found to harbor homozygous mutations of *FLT3*, *WT1*, *CEBPA*, *RUNX1*, and *CBL* (Fitzgibbon et al. 2005, Griffiths et al. 2005, Dunbar et al. 2008). Improvements in prognostic classification based on gene expression profiling have also been suggested (Bullinger et al. 2004, Valk et al. 2004).

Although many recurrent chromosomal alterations in AML are known, many of the target genes remain obscure. More than one genetic alteration is needed in AML pathogenesis since many alterations are insufficient alone to cause leukemia, reflecting the multistep nature of cancer. Classification of AML according to genetic changes to the prognostic subgroups and development of targeted therapies to various genetic subgroups continue to be important research goals.

Table 3. Common cytogenetic alterations in acute myeloid leukemia.

Alteration	Frequency (%) among cytognetically aberrant cases[a]	Target genes
trisomy 8	15	Not known
t(8;21)	7	*RUNX1-RUNX1T1*
t(15;17)	5	*PML-RARA*
inv(16), t(16;16)	4	*CBFB-MYH11*
11q23 alterations	7-8	*MLL*
t(6;9)	0.5	*DEK- NUP214*
inv(3), t(3;3)	1	*EVI1*
t(8;16)	0.5	*MYST3-CREBBP*

[a]frequencies obtained from Heim & Mitelman (2009).

Table 4. Prognostic risk groups in acute myeloid leukemia based on cytogenetics.

Risk group	Cytogenetic alterations
Favorable	t(8;21), inv(16), t(15,17)
Intermediate	Normal karyotype, +8, +21,+22, del(7q), del(9q), 11q23 alterations
Poor	Complex karyotype, -5,-7, del(5q)

Data based on Grimwade et al. (1998).

Table 5. Common mutated genes in acute myeloid leukemia.

Gene	Location	References
FLT3	13q12	Nakao et al. (1996), Yamamoto et al. (2001), Thiede et al. (2002)
NPM1	5q35	Falini et al. (2005), Thiede et al. (2006)
MLL	11q23	Schichman et al. (1994), Dohner et al. (2002)
NRAS	1p13.2	Janssen et al. (1987), Bowen et al. (2005)
KRAS	12p12.1	Bowen et al. (2005)
KIT	4q11-q12	Beghini et al. (1998), Gari et al. (1999)
CEBPA	19q13.1	Pabst et al. (2001), Preudhomme et al. (2002)
RUNX1	21q22.3	Osato et al. (1999), Preudhomme et al. (2000)
WT1	11p13	King-Underwood et al. (1996), King-Underwood & Pritchard-Jones (1998)
TP53	17p13	Fenaux et al. (1991), Slingerland et al. (1991)

4.2. Chronic lymphocytic leukemia

4.2.1. Clinical characteristics and treatment of chronic lymphocytic leukemia

CLL is characterized by clonal proliferation and accumulation of mature B-lymphocytes in the blood, bone marrow, and lymphoid tissue. CLL cells have experienced antigen stimulation and the malignancy has been proposed to originate from memory B-cells, naïve B-cells, or marginal-zone B-cells (reviewed by Chiorazzi et al. 2005, Kuppers 2005). CLL is the most common adult leukemia in developed countries, having a median age of onset of 65 years. It is more common in males, with a 2:1 male female ratio (Jaffe et al. 2001). The annual incidence of CLL is 3.5/100 000 in the US and 1.3-13.7/100 000 in Europe (reviewed by Dighiero & Hamblin 2008). In Finland, slightly more than 100 CLL cases are diagnosed every year (Ruutu 2007).

The etiology of CLL remains unknown, with no associations reported with viral infections, chemicals, or radiation (reviewed by Chen & McMillan 2008). Age-related events, such as an increase of DNA damage by reactive oxygen species, a decline in B-cell production associated with defective B-cell receptor signaling, decrease in T-cell immunity causing defects in elimination of aberrant B-cells, and chronic stimulation by an antigen, are also suggested to affect CLL pathogenesis (Chen & McMillan 2008). In subset of patients, inherited susceptibility may also play a role in CLL development. Family studies have revealed that genetic susceptibility is detected in approximately 5-10% of CLL patients (Houlston et al. 2002, Rawstron et al. 2002, reviewed by Goldin & Caporaso 2007). However, the exact CLL susceptibility genes remain unknown.

Generally, CLL progresses slowly, but the disease type can vary greatly from asymptomatic to a more serious form with various symptoms. Symptoms of CLL include lymphadenopathy, fatigue, night sweats, weight loss, anemia, and infections (Hallek et al. 2008). However, approximately 70-80% of CLL cases are found incidentally in a routine full blood count (Dighiero & Hamblin 2008) Diagnosis of CLL is based on detection of characteristic small and mature lymphocyte morphology, characteristic immunophenotype, and a blood lymphocyte count of over $5x10^9$/L (or $3x10^9$/L if the cell morphology is mature) (Hallek et al. 2008). Although there is no specific immunophenotypic marker for CLL, a combination of markers is characteristic for CLL. Typical markers for CLL include low-density surface membrane immunoglobulin, CD5+, CD19+, CD20+, CD23+ FMC7 -/+, and CD22-/+ (Hallek et al. 2008).

WHO classification groups CLL with mature B-cell neoplasms together with small lymphocytic lymphoma (Swerdlow et al. 2008). Differentiation diagnostics of CLL from other lymphoproliferative diseases is based on morphology, immunophenotype, and recently also genetic markers (Swerdlow et al. 2008). Traditionally, Rai (Rai et al. 1975) and Binet (Binet et al. 1977, Binet et al. 1981) staging systems, which are based on clinical and cytological findings, have been used to predict prognosis of CLL patients; these classification systems classify CLL cases as early, intermediate, or advanced stage.

Various prognostic factors are considered when making treatment choices for CLL patients. The prognostic factors that are currently considered important in CLL include age, stage, gender, performance, tumor burden, disease activity, specific serum parameters, and genetic aberrations (reviewed by Dohner & Stilgenbauer 2006, Seiler 2006). Generally, asymptomatic patients survive without treatment, while chemo- and immunological therapies are used in advanced or more aggressive disease (Auer et al. 2007, Dighiero & Hamblin 2008, Hallek et al. 2008). In young patients with aggressive disease, curation can be achieved with allogeneic transplantation (Gribben 2008). Although remission is often obtained with the current therapies, most of the patients relapse; thus, the treatment of CLL is rarely curative (Auer et al. 2007, Dighiero & Hamblin 2008). Since treatment of early and stable disease is not currently beneficial, markers that would help to reveal the cases that will develop into more aggressive forms of CLL are needed.

4.2.2. Genetic alterations in chronic lymphocytic leukemia

CLL cells have low mitotic activity *in vitro*. Before optimized mitogen stimulation was developed for karyotyping of CLL, obtaining mitoses of good quality and, more importantly, obtaining mitoses representing malignant cells was problematic (Knuutila et al. 1986). With karyotyping methodology using mitogen stimulation, chromosomal alterations are detected in 40-50% of CLL cases (Juliusson et al. 1990). The development of other cytogenetic techniques has increased the number of alterations detected. Especially interphase FISH, targeted to the most commonly altered regions, has proved to be useful in diagnostic analyses of CLL and can reveal alterations in 80% of cases (Dohner et al. 2000). Also cCGH has revealed additional new aberrations in CLL (Bentz et al. 1995, Bea et al. 2002). In contrast to other hematological malignancies, translocations are rare in CLL (Stilgenbauer et al. 2002, Guipaud et al.

2003). The prognostically significant copy number alterations in CLL include the unfavorable deletions of 11q22-23 and 17p and the favorable deletion of 13q14.3 (Dohner et al. 2000, Table 6). Trisomy 12 is among the common chromosomal alterations of CLL, but its prognostic significance has not yet been clarified (Krober et al. 2002, Oscier et al. 2002, Oscier et al. 2004, Table 6).

Despite extensive studies, only a few target genes with mutations in the frequently altered chromosomal regions have been suggested, such as *TP53* in 17p (Gaidano et al. 1991, Fenaux et al. 1992), *ATM* in 11q (Stankovic et al. 1999), and micro-RNA genes *MIR15A* and *MIR16-1* in 13q14.3 (Calin et al. 2002). Other recurrent cytogenetic changes in CLL include deletion of 6q21 and gains of 12q, 8q, and 3q (Heim & Mitelman 2009). Target genes of these regions and many other chromosomal alterations in CLL remain unknown.

The *TCL1* gene located in 14q31.2 may play an important role in CLL pathogenesis. It is overexpressed in aggressive forms of CLL (Pekarsky et al. 2006, reviewed by Calin et al. 2007). MicroRNAs *MIR29* and *MIR181* have also been discovered to inhibit *TCL1* expression and to often be downregulated in patients with high *TCL1* expression (Pekarsky et al. 2006, reviewed by Calin et al. 2007). In addition, mutations have been found in *TCL1* in CLL (Pekarsky et al. 2008).

Besides chromosomal alterations, immunoglobulin variable heavy chain (*IgVH*) gene mutation status is considered to be an important prognostic marker. *IgVH* gene hypermutation is detected in approximately half of CLL patients and indicates favorable outcome, while patients with unmutated status have worse prognosis (Damle et al. 1999, Hamblin et al. 1999). Expression of *ZAP70* and *CD38* were suggested to be surrogate markers for VH mutation status (Chen et al. 2002), but have in later studies provided controversial results (Stilgenbauer et al. 2002, Seiler et al. 2006).

Microarray analyses have also been used to characterize the CLL genome. Although CLL is a heterogeneous disease, gene expression microarray profiling studies have shown that CLL has a characteristic expression signature that is different from other lymphoid malignancies (Klein et al. 2001, Rosenwald et al. 2001). Based on gene expression, CLL cases can also be clustered into subgroups with specific genetic alterations (Haslinger et al. 2004, Kienle et al. 2005). With aCGH and SNP-array methods, common CLL specific alterations as well as novel imbalances have been detected with high resolution, suggesting that these techniques could be used in clinical diagnostics of CLL (Schwaenen et al. 2004, Pfeifer et al. 2007).

Table 6. The most common chromosomal alterations in chronic lymphocytic leukemia.

Aberration	Prognosis	Suggested target genes
-13q14	favorable	*MIR15A* , *MIR16-1*
-11q22-23	unfavorable	*ATM*
trisomy 12	controversial	-
-17p13	unfavorable	*TP53*

AIMS OF THE STUDY

The aim of this study was to analyze chromosomal imbalances in AML and CLL using recent microarray technologies, including aCGH and SNP-array analyses, and to characterize the genomic alterations that potentially have a role in leukemogenesis. Specific objectives were the following:

- To characterize marker chromosomes and 11q amplicons in AML.
- To screen cryptic copy number alterations in karyotypically normal AML and CLL.
- To characterize known chromosomal alterations with aCGH to uncover minimal altered genomic regions and altered genes.
- To screen LOH, including UPD, with SNP-arrays from karyotypically normal AML.

MATERIALS AND METHODS

1. Specimens (I-IV)

1.1. Acute myeloid leukemia specimens

Specimens from AML patients that were diagnosed and treated in Helsinki University Central Hospital were analyzed in Studies I, II, and IV (Table 7.). DNA was extracted from bone marrow samples (Studies I, II, and IV) or blood samples (Study I) with standard methods. For the FISH analysis, fixed bone marrow cells or archival G-banding slides were used. RNA extracted from bone marrow mononuclear cells was used in Study I. Samples were collected with informed consent from patients and approval from the HUS Ethics Committee, Department of Medicine, Helsinki, Finland.

1.2. Chronic lymphocytic leukemia specimens

CLL patients in Study III were diagnosed and treated at Tampere University Hospital. Clinical details of these patients have previously been published (Koski et al. 2000, Karhu et al. 2003). Archival DNA samples extracted from CLL cells were used for the analyses (Table 7). Samples were collected with informed consent from patients and approval from the Tampere University Hospital Ethics Committee.

Table 7. Specimens and studies.

Study	n	Age*/Median age (range)**	Female/male	Leukemia type	Microarrays used
I	2	14 & 38 *	0/2	AML	cDNA, oligonucleotide
II	26	49 (21-75) **	10/16	AML	oligonucleotide
III	20	67 (48-79) **	7/13	CLL	oligonucleotide
IV	19	49 (21-74) **	7/12	AML	SNP

2. Complementary DNA array comparative genomic hybridization (I)

Analysis with cDNA aCGH was used in Study I in two cases of AML. Agilent human 1 cDNA microarray (Agilent Technologies, Palo Alto, CA, USA) containing 13 000 cDNA features genome-wide was used in the experiments. DNA extracted from pooled peripheral blood of healthy males was used as a reference. Digestion, labeling of sample, and reference genomic DNA with separate colors, hybridization, and washing of slides were done as described by Atiye et al. (2005), with minor modifications. Briefly, a digested *(AluI* and *RsaI*, Sigma, St. Louis, MO, USA) and phenol-chloroform-purified DNA sample (6 μg) was labeled by random labeling with the RadPrime DNA labeling system (Gibco BRL, Gaithersburg, MD, USA) using Cy5-dUTP for sample DNA and Cy3-dUTP for reference DNA (Amersham Pharmacia, Piscataway, NJ, USA). Hybridization was performed at 65˚C for 16-17 h, and slides were washed for 3-5 min at room temperature in each of three different solutions (0.1% SDS, 0.5xSSC/0.01% SDS, and 0.06xSSC). Slides were scanned with the Agilent microarray scanner G2565AA (Agilent Technologies) and microarray image analysis was done with Feature Extraction software version 7.5 (Agilent Technologies) using Lowess normalization. Data analysis was then performed with Microsoft Excel. Poor-quality features, flagged by Feature Extraction software were removed. The Log2 ratio calculated from the green and red signal intensities was then plotted with chromosomal bp locations (April 2003 freeze, University of California-Santa Cruz genome browser; http://genome.ucsc.edu/) to create the copy number profiles.

3. Oligonucleotide array comparative genomic hybridization (I-III)

Chromosomal alterations in AML were analyzed in Studies I and II and in CLL in Study III by using oligonucleotide aCGH. Agilent 44K CGH oligonucleotide microarray with ~44 000 60-mer oligonucleotide probes (Agilent Technologies, Santa Clara, CA, USA) was used in the experiments. Reference DNA from pooled blood of healthy males or females was used in hybridizations. DNA samples were digested, labeled, hybridized, and washed according to Agilent's protocols, and slides were

scanned with Agilent's microarray scanner G2565AA (Agilent Technologies). Image analysis was performed with Agilent's Feature Extraction 7.5 (Study I) or 8.1 (Studies II and III) software with linear and Lowess normalizations and background corrections. Data were analyzed and visualized with CGH Analytics software (Agilent Technologies), with the genomic locations retrieved from NCBI build 35, May 2004 (hg 17).

4. Fluorescence in situ hybridization (I, II)

Some of the aCGH findings were further characterized and validated in AML in Studies I and II using fluorescence *in situ* hybridization (FISH). Commercial probes from Vysis Inc. (Vysis, Downers Grove, IL, USA) were used in all of the experiments. The LSI MLL dual color probe was used in Study I to analyze amplifications in 11q, the LSI MYC dual color break-apart probe in Study II to analyze amplifications detected in 8q24.13-24.21, and the LSI TEL-AML1 ES dual color translocation probe in Study II to analyze *ETV6* (*TEL*) loss in 12p13.2. If available, methanol-acetic acid fixed cells were used to prepare the metaphase slides for FISH analysis. When fixed cells were unavailable, archival G-banding slides were used. The protocol according to Skacel et al. (2001) was applied. In brief, cover slips were removed by soaking the slides several days in xylene. Slides were then washed with fresh xylene and two descending ethanol series (100% - 95% - 80%). Finally, 0.5% HCL-70% ethanol wash was used to remove the old staining. Vysis instructions for locus-specific FISH were followed. Briefly, after formamid denaturation and cold alcohol washes, the hybridization was done by incubation overnight at 37˚C. Post-hybridization washes with 0.4xSSC/0.3% Igepal (2 min) and 2xSSC/0.1% Igepal (5 s-1 min) were performed. Analysis of the results was done with an Axioplan 2 microscope (Carl Zeiss, Oberkochen, Germany). Images were captured with Isis software (Metasystems, Altlussheim, Germany). Analysis was based on ~50 metaphases, and if insufficient proper metaphases were available, interphases (~400) were analyzed.

5. Gene expression (I)

In Study I, gene expression of amplified genes in one AML case was analyzed using Agilent human 1 cDNA microarray (Agilent Technologies, Palo Alto, CA, USA). Patient RNA extracted from mononuclear cells of bone marrow was hybridized with reference RNA from pooled cancer cell lines. Labeling, hybridization, and washing were done according to the manufacturer's instructions. Scanning was performed with Agilent's microarray scanner G2565AA (Agilent Technologies) and image analysis with Feature Extraction 7.5 using Lowess normalization. Poor-quality data was filtered out, and the log2 ratio was calculated from the signal intensities. A threshold of log2 ratio over 1.00 was selected for overexpression.

6. Mutation analysis (II)

In Study II, FLT3 and NPM1 mutations were analyzed in AML. Two types of FLT3 mutations, internal tandem duplication (ITD) in the juxtamembrane domain and D835 point mutation in the tyrosine kinase domain activation loop, were analyzed with the FLT3 Mutation Assay kit for gel detection (*In Vivo*Scribe Technologies, San Diego, CA, USA), following the manufacturer's instructions. Exon 12 mutations of *NPM1* were analyzed by PCR and sequencing using the following primers: NPMseqF, 5'-gttaactctctggtggtagaatgaa-3', and NPMseqR, 5'- aaaaggacagccagatatcaac-3', and standard protocols. Sequencing was done using the BigDyeTerminator kit (Applied Biosystems Inc, Foster City, CA, USA) and ABI Prism 3130xl Genetic Analyzer (Applied Biosystems Inc).

7. Loss of heterozygosity analysis with single-nucleotide polymorphism microarray (IV)

In Study IV, SNP-array experiments were performed in AML cases using GeneChip Human Mapping 50K Array Xba 240 (Affymetrix, Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. Washing and staining of arrays were done

using the Affymetrix Fluidics station 450, and scanning was performed with the Affymetrix GeneChip Scanner 3000. Images were analyzed with GeneChip operating software GCOS (Affymetrix, Sacramento, CA, USA), and genotype data were obtained with GTYPE 4.1 software (Affymetrix) using the dynamic model algorithm. Reference data, obtained from the international HapMap project's homepage (http://www.hapmap.org/), comprised data of 46 HapMap CEPH females. LOH and DNA copy numbers were analyzed with the CNAT4 tool (Affymetrix) and CNAG2.0 software (Nannya et al. 2005). The log2 copy number ratios were also imported to CGH analytics software (Agilent Technologies, Palo Alto, CA, USA) for visualization and comparison with oligonucleotide aCGH data of the same patients.

RESULTS AND DISCUSSION

1. Characterization of 11q amplicons in acute myeloid leukemia (I)

Sometimes unidentified fragments of chromosomal material are detected with karyotyping. These fragments are called marker chromosomes in the cytogenetic nomenclature. Marker chromosomes may be composed of specific amplified segments from one chromosome, but can also have complicated structures that are formed from material of several chromosomes. Our two studies (I and II) demonstrated the value of aCGH in combination with other cytogenetic techniques in defining the marker chromosomes and amplified candidate oncogenes. Because aCGH shows only a relative copy number change compared with a normal genome and does not show the structural form of the change, it can not be used as the sole basis for an analysis of extrachromosomal markers. It reveals, for example, that certain genes are amplified, but it fails to show whether it is an hsr inside the chromosome or a dmin. However, aCGH does identify the unknown amplified genes with high resolution, and their location and chromosomal structures can then be visualized using locus-specific FISH or M-FISH, which shows each chromosome with a specific color.

Alterations in 11q are relatively common in AML. The best clarified gene in 11q is the *MLL*, which is known to form fusion genes with over 70 different partners (Harper & Aplan 2008). Additionally, many different breakpoints and losses and gains of copy number have been detected in 11q in AML (Tanaka et al. 2001). Characterization of the amplicons in two AML cases with marker chromosomes by various methods in Study I revealed two different types of 11q amplicons.

We characterized a complex amplicon pattern in one case, associated with the *MLL-ARHGEF12 (MLL-LARG)* fusion gene, in 11q23.3-q25 (Figure 2). The amplicon was composed of two distinct amplicons (1.2 Mb and 13.3 Mb) and a small deleted sequence (1.9 Mb) in between. The proximal breakpoint could be defined to be in the middle of *MLL* and the distal breakpoint in the middle of *ARHGEF12.* With MLL dual color FISH probe (Vysis), which marks with red signal the distal part and with green signal the proximal part of the MLL, normal red and green signals were detected in one homologous chromosome 11. The other homologous chromosome lacked the red signal, and only green signals were detected in marker chromosomes. These results confirmed

deletion of the distal part and amplification of the proximal part of the MLL. In addition, the case II also had a small distinct gain in 11q22. With the high-resolution oligonucleotide aCGH, the sizes of the altered regions and genes involved in the amplified and deleted regions in 11q could be identified. Although exact mechanisms involved in the formation of amplicons and fusion genes remain obscure, breakages in fragile sites have been suggested to be involved in initiating these events (reviewed by Myllykangas & Knuutila 2006). Since fragile sites in 11q22.3 are known (reviewed by Popescu 2003), they might have been also involved in the mechanisms that led to the formation of the complex amplicon and *MLL-ARHGEF12* fusion in our study case. Several genes in 11q besides *MLL* could be involved in the pathogenesis. Besides the *MLL-ARHGEF12* oncogene, the deletion leading to the fusion gene formation might also have contained a tumor suppressor, one potential candidate being *CBL*. Additionally, other oncogenes could be located in the amplified regions.

In the other patient, an additional ring chromosome, which consisted of chromosome 11 material, was seen with M-FISH. The amplicon did not include the *MLL* gene, and aCGH was done to detect the amplified region in detail. CDNA-based aCGH analysis showed continuous 5.5-Mb high-level amplification in 11q24.3-q25 telomeric to *MLL*. Furthermore, the amplicons of the two cases analyzed in the Study I were overlapping, and we were able to define a minimal common region with additional candidate genes in 11q besides MLL. The common region contained 14 genes (*FLI1*, *KCNJ1*, *KCNJ5*, *RICS*, *BARX2*, *NFRKB*, *APLP2*, *ST14*, *ADAMTS8*, *SNX19*, *NTM*, *OPCML*, *JAM3*, and *NCAPD3*). Based on gene expression analysis, the most probable candidates of these were *FLI1*, *NFRKB* and *SNX19* since they were highly overexpressed. In previous studies 11q amplifications not involving *MLL* have been detected (Nacheva et al. 1993, Rossbach et al. 1998, Crossen et al. 1999, Sait et al. 2002). The amplified candidate genes in 11q aberrations telomeric to MLL include *ETS1* (Rovigatti et al. 1986, Crossen et al. 1999, Yoshida et al. 1999, Sait et al. 2002), *FLI1*, *SRPR*, *NFRKB*, and *KCNJ5* (Crossen et al. 1999), some of the previous studies thus supporting our target gene suggestions.

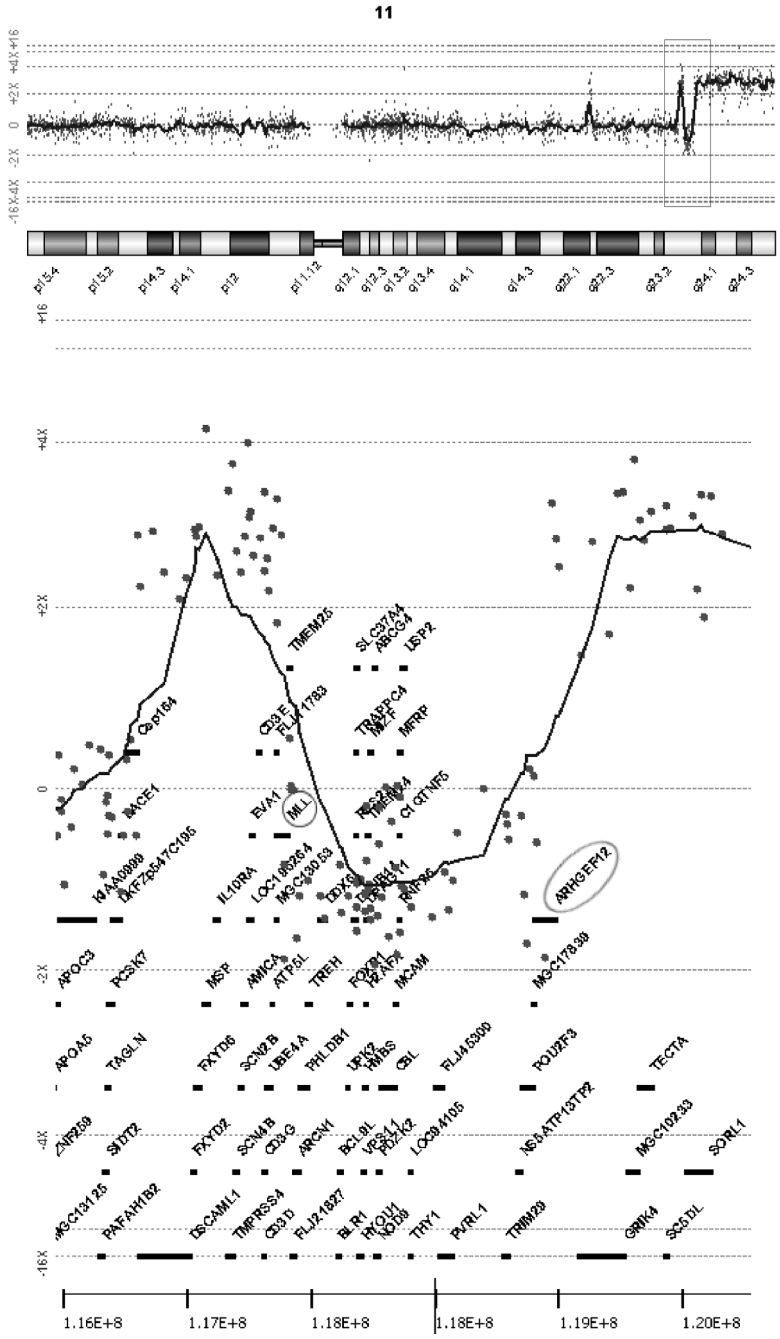


Figure 2. Array-CGH profile of chromosome 11 (upper panel) showing the structure of the amplicon and a loss within. In the lower panel, the marked region in a zoomed view shows the genes in the breakpoint of the deletion (circled), the *MLL* and *ARHGEF12* (*LARG*), which form the fusion gene.

2. Cryptic copy number changes in karyotypically normal acute myeloid leukemia (II)

It has been estimated that only alterations larger than 5 Mb can be detected in standard G-banding (Salman et al. 2004). In Study II, oligonucleotide (44K) aCGH showed cryptic copy number alterations in 4/26 of karyotypically normal acute myeloid leukemia cases (15%). The detected cryptic alterations were 0.4-4.1 Mb in size, showing the improved resolution of aCGH relative to G-banding. Three cases each harbored one loss. These losses were located in 8q24.11, 12p12.3, and 12p13.2. One patient had a more complex pattern of alterations, including two losses (1q41 and 18q21.32), one gain (3p21.3), and one high-level amplification (8q24.13-q24.21, Figure 3). Besides novel candidate genes, some known cancer genes detected previously in AML, namely *ETV6* (reviewed by Bohlander 2005) and *MYC* (Alitalo et al. 1985, Thomas et al. 2004), were also detected and confirmed with FISH analysis.

When the case with the 8q24.13-q24.21 amplicon was further analyzed with FISH, it was shown that the *MYC* oncogene was amplified extrachromosomally (Figure 3). The re-evaluation of the G-banding slides showed very small, hardly detectable particles in some of the metaphases that had escaped detection in the diagnostic analysis. These particles, according to their size (4.1 Mb) that could be deduced from the aCGH results, were small episome-like, double minute chromosomes. In the amplicon, the following potential target genes besides MYC were also detected: *TRIB1*, *FAM84B*, *PVT1*, and *CCDC26*. A similar minimal common region has previously been detected with FISH in AML (Storlazzi et al. 2004, Storlazzi et al. 2006), and it has been suggested that *MYC* might not be the target gene because it has not been highly overexpressed (Storlazzi et al. 2004, Rucker et al. 2006, Storlazzi et al. 2006). The episome model of amplification, which explains the episome formation through a deletion mechanism, has been proposed to be involved in *MYC* amplification in AML (Storlazzi et al. 2006). Episomes have also been speculated to turn into dmins by growing and then possibly integrating with chromosomes to form hsrs (Carroll et al. 1988, Von Hoff et al. 1988, Von Hoff et al. 1990). Because of the very small-sized dmins, our case might illustrate events in the episomal amplification mechanism where episomes have started to become dmins.

Small extrachromosomal markers are relatively rarely detected in karyotypic analysis and require a skillful eye to detect them. The advantage of aCGH is that it can discover these types of amplifications without prior knowledge of their existence. Submicroscopic amplifications could be more common in leukemia than previously

thought and can be increasingly detected with high-resolution techniques such as aCGH. Besides the case in our study, an other example of the cryptic amplicon detected in leukemia with FISH and aCGH but not with karyotyping is the 500-kb episomal *NUP214-ABL1* amplification found in acute lymphoblastic leukemia (Graux et al. 2004).

Mutations of *FLT3* and *NPM1* were also analyzed since they are relatively frequent alterations in karyotypically normal AML and also have prognostic significance (reviewed by Mrozek et al. 2007). *FLT3* internal tandem duplications (ITD) were detected in 33%, tyrosine kinase domain activation loop point mutations in 21%, and *NPM1* mutations in 57% of analyzed cases. Only one of the four cases with cryptic alterations detected with aCGH had mutations in *FLT3* or *NPM1*. Mutations can be used as MRD markers in follow-up of karyotypically normal AML patients, but if they are not detected, the information of cryptic copy number alterations could be valuable when MRD markers are needed.

After the publication of our results of AML, an other aCGH study of a larger cohort of patients from all cytogenetic subgroups described cryptic changes in 60% of karyotypically normal AML cases (Suela et al. 2007). Together with our study, these results show that although no specific recurrent and cryptic copy number alteration has been detected that would characterize the karyotypically normal group of patients, cryptic copy number alterations are relatively common in karyotypically normal AML.

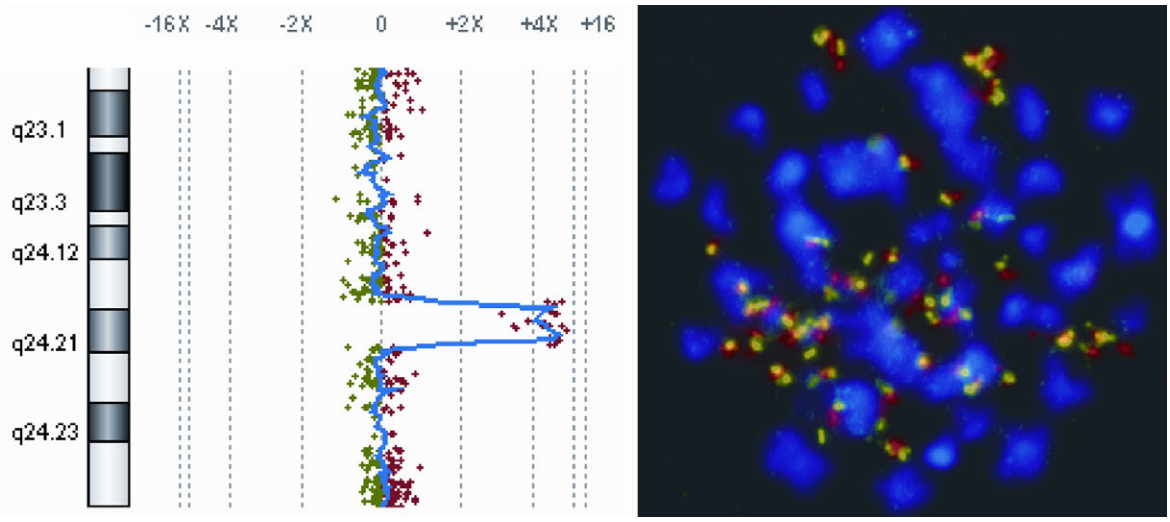


Figure 3. The left panel shows the aCGH profile of the amplification in 8q24.13-q24.21 (4.1 Mb in size), and the right panel the extrachromosomal amplification of *MYC* detected with FISH in the same patient.

3. Array comparative genomic hybridization in chronic lymphocytic leukemia (III)

Despite the numerous known chromosomal alterations in CLL, only a few tumor suppressor or oncogenes have been described. Array-CGH is an effective tool for screening candidate oncogenes and tumor suppressors. In Study III, we analyzed 20 CLL cases by using Agilent 44K oligonucleotide CGH microarray and detected new cryptic alterations not visible with standard karyotyping in three cases. We also analyzed the breakpoints and genes involved in known chromosomal alterations detected previously with other cytogenetic methods. Losses are the most commonly detected chromosomal alterations in CLL (Caporaso et al. 2007, Dighiero & Hamblin 2008), and the alterations detected in our study followed the same trend.

Cryptic changes were detected in both karyotypically normal and aberrant cases. The novel changes were located in 1q23.2-q23.3, 3p21.31, 16p13.3-pter, 17p13.2-p13.3, 17q25.3-qter, and 22q11.22, providing new candidate genes. One of the most interesting cryptic findings was the 17p alteration telomeric to the *TP53* gene, which was found in one case, suggesting other possible target genes beside *TP53* in 17p deletions.

The 13q14 deletion was the most common alteration, detected with aCGH in half of the cases (Figure 4). Sizes of the 13q deletions varied between 0.79 Mb and 29.33 Mb. In six cases, losses in 13q14 were not detectable with G-banding and cCGH, but could be detected with aCGH, showing the superior resolution of this method. The exact breakpoints could be determined and a minimal common region of 158 kb harboring the suggested target microRNA genes *MIR15A* and *MIR16-1* (Calin et al. 2002) was identified. Homozygous deletions were detected in the minimal common region in some cases, thus possibly inactivating both alleles of the target genes. In the pathogenesis of CLL, microRNA genes have been proposed to have an important role (reviewed by Caligaris-Cappio et al. 2008).

The second most common finding was losses in 11q (Figure 4); the losses were relatively large in size, 7.44 Mb being the smallest. This region also included one of the few suggested target genes in CLL, the *ATM*, which is a tyrosine kinase gene possibly involved in pathogenesis by participating in regulation of *TP53* (reviewed by Dighiero & Hamblin 2008). However, several other genes in 11q22-23 can be involved in the pathogenesis such as *NPAT*, *CUL5*, and *PPP2R1B* (Kalla et al. 2007).

Aberrations were detected with aCGH in our study in 70% of cases. The analyzed patient cohort included a large number of patients with normal karyotype by G-banding and cCGH (11 cases). With aCGH, we detected most of the known copy

number changes detected with G-banding or cCGH, and, furthermore, discovered some novel cryptic changes. Previously, chromosomal changes have been found in 80% of CLL cases by using FISH (Dohner et al. 2000). The percentage of the detected alterations is lower in our study, probably because we had in sample selection preferred normal karyotypic cases that would most likely have important cryptic alterations. In addition, with more high-resolution platforms such as Agilent 244k (Agilent technologies, http://www.agilent.com), even more alterations would likely have been revealed. For example, one of the CLL cases that had 13q loss detected by FISH but showed normal karyotype in 44k aCGH analysis was hybridized to the Agilent 244k array and a small 13q deletion was detected (unpublished data).

Array-CGH has shown its ability to detect alterations previously detected only by FISH and also overcomes the problems that may arise with CLL in cell culturing and mitosis-dependent G-banding analysis. Array-CGH has therefore been suggested for diagnostic analysis of chromosome imbalances in CLL (Schwaenen et al. 2004, Pfeifer et al. 2007). In addition, some novel alterations have been detected with aCGH, such as a gain of *MYCN* in 2p24 (Schwaenen et al. 2004), and the new cryptic deletions in our study.

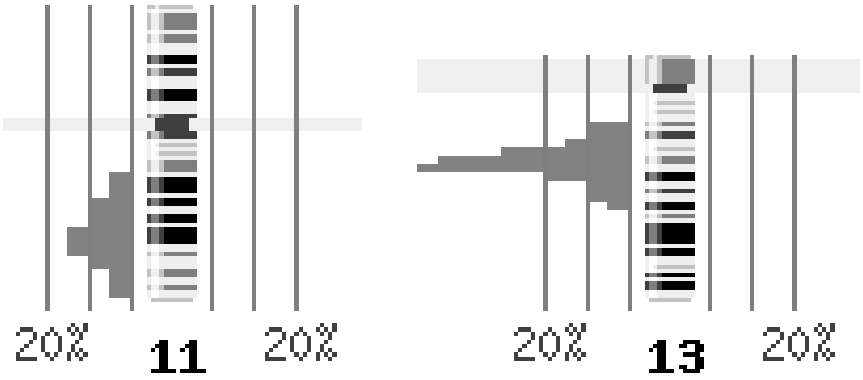


Figure 4. Most recurrent deletions in chromosomes 11 and 13, detected using aCGH (figures prepared by using progenetix software, www.progenetix.net).

4. Loss of heterozygosity in karyotypically normal acute myeloid leukemia (IV)

UPD has been detected in 20-30% of karyotypically normal AML (Gorletta et al. 2005, Raghavan et al. 2005) and in various cancers as a potential pathogenic mechanism (reviewed by Tuna et al. 2009). Tumor suppressors or oncogenes can be detected in regions of UPD that might be affected by either a mutation that can become homozygous with the UPD mechanism or epigenetic silencing. Homozygous mutations have been found in some of the genes suggested to be involved in AML pathogenesis such as *FLT3*, *WT1*, *CEBPA*, *RUNX1*, and *CBL* (Fitzgibbon et al. 2005, Griffiths et al. 2005, Dunbar et al. 2008). Homozygous FLT3 mutations have also been suggested to have an adverse prognosis (Whitman et al. 2001). In Study IV, AML cases (n=19) were investigated using Affymetrix GeneChip Human Mapping 50K (*XbaI*) SNP-array for detection of cryptic copy number alterations and LOH.

We detected LOH and potential UPD in several chromosomal regions. LOH was found in 12/19 cases (63%) (Table 8). Potential regions of UPD were detected in 10/19 cases (53%). Recurrent LOH in our study was detected in 7q and 13q, suggesting that important genes in these chromosomes can be involved in karyotypically normal AML. In one case, the whole chromosome 13 showed UPD, which was confirmed by detecting the increased ITD-FLT3 mutant allele dosage from the agarose gel (lane 3, Figure 5). *FLT3* is one of the known target genes of chromosome 13 in AML (Nakao et al. 1996, Yamamoto et al. 2001, Thiede et al. 2002). Two cases were detected with other potential regions of UPD than *FLT3* region, suggesting also other possible target genes.

The same AML cases were evaluated with aCGH using an Agilent 60K oligonucleotide array (Study II) and with SNP-array analysis using an Affymetrix 50k mapping array (Study IV). Thus, SNP-array results could be compared with aCGH data of the same cases. SNP-array analysis did not reveal additional copy number changes compared with Study II, but showed a 4.1-Mb amplification in 8q24.13-q24.21 and a 3.6-Mb loss in 12p12.3. The smaller alterations (0.4-1.7) that were detected with aCGH were not visible with SNP-array. 8q24.13-q24.21 showed besides copy number alteration also LOH, suggesting preferential amplification of one allele. The differences between aCGH and SNP-array results could be explained by different spatial resolutions. Spatial resolution can in some cases be problematic in SNP-array analysis since the SNPs are not uniformly distributed throughout the genome (Coe et al. 2007). More densely arrayed SNP probes are required to achieve a resolution similar to that of oligonucleotide aCGH. There might also have been lower quality in SNP-array results

in our study compared with aCGH results since more steps, including PCR-based genome complexity reduction, are performed in the Affymetrix protocol.

The aCGH experiment was faster to perform than the SNP-array experiment, and the protocol was less demanding, making aCGH a more convenient choice for diagnostic work. On the other hand, for research purposes, genome-wide SNP-array analysis might be the better option since more information is obtained in a single analysis. The availability of SNP-arrays with higher densities and the development of algorithms as well as usage of methodologies where genome complexity reduction is not needed can make SNP-array the most beneficial platform in the future.

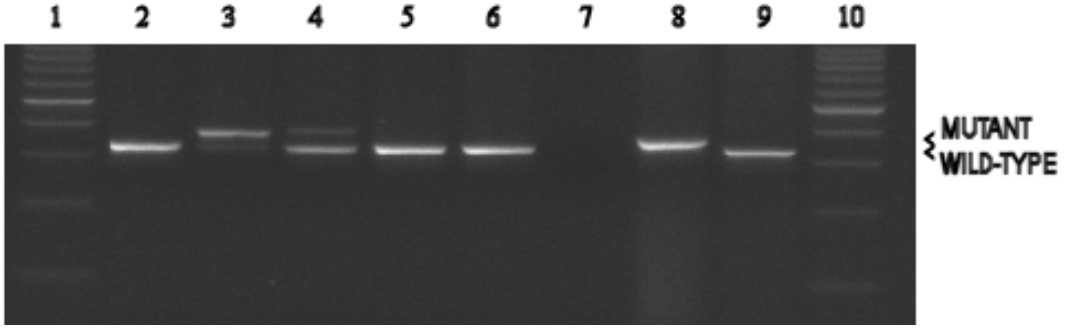


Figure 5. FLT3-ITD mutation test. Agarose gel illustrating the results of FLT3 mutation analysis. Lanes 1 and 10 show the DNA size standards. The arrows in the right side show the locations of mutant and wild-type bands. Lanes 2, 5, and 6 show patient samples with negative FLT3-ITD mutation status. Lane 3 shows a patient sample with ITD mutation and a more intense upper band reflecting the increased dosage of the mutant allele. Lane 4 shows a patient sample with an ITD mutation, but a more intense wild-type band. Lane 7 displays the PCR-negative control (H_2O), lane 8 the positive control, and lane 9 the wild-type control.

Table 8. Loss of heterozygosity regions detected with single nucleotide polymorphism microarray.

Case no.	Cytogenetic location	bp location	Size of alteration (MB)
1(2)	4q31.1-q31.21	139996583-145144092	5,1
	4q32.1-q34.1	157994837-175609159	17,6
	16q13-q21	56562885-61294302	4,7
2 (3)	12p12.1-p11.22	24989341-30592175	5,6
	13q	18300000-114142980	95,8
	14q22.1-q22.3	50533072-55620173	5,1
3(4)	normal		
4(5)	2q21.2-q22.1	134253668-138626175	4,4
	7q21.11	81656156-85659633	4
	7q21.12-q21.2	87329855-90967737	3,6
5(6)	normal		
6(7)	13q22.1-q31.1	73284990-79304088	6
7(8)	normal		
8(9)	18q12.1-q12.2	27637275-31998764	4,4
9(11)	normal		
10(12)	3q12.1-q13.3	99474101-115623059	16,1
	4q12-q13.1	55998070-64460188	8,5
	4q21.1-q21.21	76653600-79616778	3
	8p11.21-q11.23	40312067-54281947	14
	12q14.1-q14.2	57581720-61962984	4,4
11(13)	2q14.2-q14.3	119648809-124972819	5,3
12(14)	17q12-q21.32	34176036-43615673	9,4
13(15)	normal		
14(16)	1p21.2-p13.3	101650684-107327698	5,7
	1q23.2-q23.3	156963203-161324123	4,4
	7q21.11-q21.12	83598401-87661101	4,1
	8q24.13-q24.22	126984731-131660463	4,7
15(17)	normal		
16(18)	13q21.1-q21.31	57989044-62966508	5
17(24)	normal		
18(25)	1p32.2-p31.3	56290829-61321550	5
	4q28.2-q28.3	129890398-135011057	5,1
19(26)	1q22-q23.2	151606871-156963203	5,4
	10p11.21-q11.22	35976759-49313232	13,3

CONCLUSIONS

Chromosomal alterations are considered important predictive and prognostic factors in leukemia, and many chromosomal imbalances are known; however, many target genes of these alterations remain obscure. Heterogeneity among molecular and clinical traits in AML and CLL has been described, especially in cases with normal karyotype. Genetic alterations are thought to explain some of this heterogeneity.

Microarray methods aCGH and SNP-array are used to study chromosomal imbalances with high resolution enabling genome-wide molecular analysis of chromosomes without cell culturing. The detection of gene-level alterations and breakpoint detection in a single experiment without prior knowledge of the alterations are other benefits provided by the high resolution. New mutated genes are likely to be found in the regions with recurrent deletions, copy neutral LOH, or amplifications. Furthermore, the defining of minimal altered regions of the recurrent alterations can lead to identification of probable candidate genes involved in leukemia pathogenesis.

The aim of this thesis was to detect novel chromosomal alterations and to characterize previously known alterations with high resolution among CLL and AML patients by using genomic microarray methods. In all studies, cryptic alterations were revealed with aCGH or SNP-array, and in Studies I and III known alterations were characterized at the gene level.

In Study I, our aim was to characterize marker chromosomes with 11q amplicons by using aCGH in combination with other cytogenetic methods. A cryptic deletion causing the *MLL-ARHGEF12* fusion gene in 11q was detected in AML. Amplified genes could be also identified in marker chromosomes composed of chromosomal material from 11q.

Normal karyotype with standard G-banding is detected in 40-50% of AML cases and in 20% of CLL cases. One specific interest was to screen cryptic copy number alterations in karyotypically normal AML and CLL using aCGH. In addition, we analyzed LOH and UPD in karyotypically normal AML in Study IV. Array-CGH and SNP-array analyses were able to reveal novel aberrations that were undetectable with G-banding or cCGH. In karyotypically normal AML, we found cryptic copy number alterations with oligonucleotide aCGH in 15% of cases and regions of LOH, including potential UPD, with SNP-array in 63% of cases. In CLL, novel cryptic copy number alterations were detected in 15% of cases.

We aimed also to define minimal common regions in case where recurrent alterations were detected. Searches for target oncogenes and tumor suppressors from

common cytogenetically altered regions have previously focused on mapping the minimal regions by FISH or LOH analyses. Array-CGH can reveal the minimal regions less laboriously than these methods. In this thesis, minimal regions with putative candidate genes could be defined in 13q14.3 and 11q22.3-q23.2 in CLL and in 11q24-25 in AML.

SNP-array and aCGH methods are effective screening methods that can reveal novel molecular alterations in cancer research. However, they can also be useful for some specific clinical diagnostic purposes. In diagnostics, the advantages of microarray methods would be in the potential discovery of small alterations in cases with cancer or constitutional disorders buth with normal karyotype, the identification of genes located in unknown marker chromosomes or in small deletions and amplifications, and the detection of small deletions and amplifications associated with apparently balanced chromosomal translocations. Microarray methods also enable analysis of genome-wide gene copy number alterations in tumors that are difficult to grow in cell cultures. In leukemia diagnosis, the discovery of potential MRD markers with microarray methods can be advantageous. When analyzing the MRD in leukemia patients after treatment, MRD markers are needed. The patients with a normal karyotype in diagnosis and no other potential MRD markers such as a specific mutation, can benefit from aCGH analysis of the diagnostic sample because aCGH might reveal a submicroscopic copy number alteration that can later be used as a MRD marker and be analyzed with more sensitive method such as FISH or RT-PCR. Array-CGH findings can be informative also in cases where a cryptic alteration with prognostic significance is detected, possibly changing the prognostic group to which the patient is classified. Furthermore, detected cryptic alterations provide candidate oncogenes and tumor suppressors for future studies.

Some limitations also exist in current aCGH analyses. The high-resolution analyses might in some cases have problems distinguishing cancer-associated changes from copy number variation. Some copy number polymorphisms were overlapping, with some of the smallest cryptic alterations detected in Studies II and III. However, the detected alterations were mostly larger in size than the polymorphisms. Another limitation is that one clone produced from one progenitor cell with many alterations cannot be distinguished from several different clones originating from different progenitors. In addition, the proportion of aberrant cells needed in samples has been suggested to range between 20% and 30% for reliable detection with aCGH (Ballif et al. 2006, Gondek et al. 2008). For example, in three CLL cases in Study III, alterations detected in only two cells with G-banding were not visible with aCGH. Since balanced

ploidies, translocations, or inversions are not visible in genomic microarray analyses, standard cytogenetic techniques are still needed in diagnostic laboratories.

Array-CGH and SNP-array analyses are nevertheless useful methods for obtaining the ”molecular karyotype”. These methods allow the chromosomal alterations to be viewed at the gene-level resolution in a single experiment, providing a fast and precise analysis. The studies of this thesis together with previous literature indicate that aCGH can be a valuable addition to the existing technology. Conventional methods, such as G-banding karyotyping and FISH, are still needed for comprehensive diagnostics of leukemia samples, but the new array technologies have proved their power in high-resolution, genome-wide, and cost-effective analyses.

Besides current microarray techniques, future cancer research and diagnostics will likely be strongly affected by next-generation sequencing (NGS) techniques (reviewed by Ansorge 2009). With these techniques, a large number of sequences can be produced considerably faster and more cost-effectively than with traditional sequencing. Either whole genome or targeted regions can be analyzed. In cancer research, these techniques have already started to be utilized. For example, novel mutated genes were found in karyotypically normal AML patient with whole genome NGS (Ley et al. 2008). Besides mutation analysis, NGS has various potential applications, including genome-wide analysis of epigenetic alterations, transcription factor regions, or gene expression (reviewed by Ansorge 2009). Thus, NGS techniques may someday replace some of the current microarray applications. At present, these techniques are limited by their high costs, time-consuming data analysis, and data storage requirements, as well as by some technical challenges. However, they are among the most promising future genome analysis technologies.

ACKNOWLEDGMENTS

This work was carried out at the Department of Pathology in Haartman Institute, University of Helsinki, during 2004-2009 and was supported financially by the Finnish Cancer Society and Helsinki University Central Hospital research funds. I warmly thank everyone who made this work possible. My sincere gratitude is especially due to the following persons:

My supervisor, Professor Sakari Knuutila, for introducing me to science and microarray technology and for providing me with the opportunity to learn so many things. I'm grateful for his enthusiastic attitude and supportive guidance over the years.

The official reviewers, Docent Arto Mannermaa and Docent Liisa Volin, for constructive criticism and suggestions for improving my thesis. Carol Ann Pelli, my author-editor, for revising the language of this manuscript. Docent Kim Vettenranta for accepting the task of being my opponent.

The coauthors of the studies, Erkki Elonen, Ulla Saarinen-Pihkala, Juhani Vilpo, Kimmo Porkka, Hanna Vauhkonen, Janna Saarela, and Kirsi Piippo, for their collaboration and for making this work possible.

Pirjo Pennanen for the language revision of my articles and for practical support. Tarja Nieminen for help with various everyday matters.

My colleagues, the former and present CMG group members, Anna, Anu, Fabricio, Ioana, Ilari, Kowan, Linda, Mohamed, Pamela, Shinsuke, Sippy, Suvi, Tarja, Tuija, and many others for creating such a friendly working atmosphere.

Finally, my family, relatives, and friends. I'm especially grateful to my brother Juha and my parents Leena and Jorma for their continuous support, and to my husband Jarno and daughter Emilia, to whom I dedicate this work.

Helsinki, December 2009
Anne Tyybäkinoja

REFERENCES

Alitalo K, Saksela K, Winqvist R, Alitalo R, Keski-Oja J, Laiho M, Ilvonen M, Knuutila S, de la Chapelle A. 1985. Acute myelogenous leukaemia with c-myc amplification and double minute chromosomes. Lancet 2:1035-1039.

Allen RJ, Smith SD, Moldwin RL, Lu MM, Giordano L, Vignon C, Suto Y, Harden A, Tomek R, Veldman T, Ried T, Larson RA, Le Beau MM, Rowley JD, Zeleznik-Le N. 1998. Establishment and characterization of a megakaryoblast cell line with amplification of MLL. Leukemia 12:1119-1127.

Ansorge WJ. 2009. Next-generation DNA sequencing techniques. N Biotechnol 25:195-203.

Aplan PD. 2006. Causes of oncogenic chromosomal translocation. Trends Genet 22:46-55.

Ariyama Y, Fukuda Y, Okuno Y, Seto M, Date K, Abe T, Nakamura Y, Inazawa J. 1998. Amplification on double-minute chromosomes and partial-tandem duplication of the MLL gene in leukemic cells of a patient with acute myelogenous leukemia. Genes Chromosomes Cancer 23:267-272.

Atiye J, Wolf M, Kaur S, Monni O, Bohling T, Kivioja A, Tas E, Serra M, Tarkkanen M, Knuutila S. 2005. Gene amplifications in osteosarcoma-CGH microarray analysis. Genes Chromosomes Cancer 42:158-163.

Auer RL, Gribben J, Cotter FE. 2007. Emerging therapy for chronic lymphocytic leukaemia. Br J Haematol 139:635-644.

Baldus CD, Liyanarachchi S, Mrozek K, Auer H, Tanner SM, Guimond M, Ruppert AS, Mohamed N, Davuluri RV, Caligiuri MA, Bloomfield CD, de la Chapelle A. 2004. Acute myeloid leukemia with complex karyotypes and abnormal chromosome 21: Amplification discloses overexpression of APP, ETS2, and ERG genes. Proc Natl Acad Sci U S A 101:3915-3920.

Baldus CD, Mrozek K, Marcucci G, Bloomfield CD. 2007. Clinical outcome of de novo acute myeloid leukaemia patients with normal cytogenetics is affected by molecular genetic alterations: a concise review. Br J Haematol 137:387-400.

Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, Coppinger J, Kashork CD, Shaffer LG, Bejjani BA. 2006. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. Am J Med Genet A 140:2757-2767.

Bea S, Lopez-Guillermo A, Ribas M, Puig X, Pinyol M, Carrio A, Zamora L, Soler F, Bosch F, Stilgenbauer S, Colomer D, Miro R, Montserrat E, Campo E. 2002. Genetic imbalances in progressed B-cell chronic lymphocytic leukemia and transformed large-cell lymphoma (Richter's syndrome). Am J Pathol 161:957-968.

Beghini A, Larizza L, Cairoli R, Morra E. 1998. C-Kit Activating Mutations and Mast Cell Proliferation in Human Leukemia. Blood 92:701-702.

Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, Sultan C. 1976. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 33:451-458.

Bentz M, Huck K, du Manoir S, Joos S, Werner CA, Fischer K, Dohner H, Lichter P. 1995. Comparative genomic hybridization in chronic B-cell leukemias shows a high incidence of chromosomal gains and losses. Blood 85:3610-3618.

Beroukhim R, Lin M, Park Y, Hao K, Zhao X, Garraway LA, Fox EA, Hochberg EP, Mellinghoff IK, Hofer MD, Descazeaud A, Rubin MA, Meyerson M, Wong WH, Sellers WR, Li C. 2006.

Inferring loss-of-heterozygosity from unpaired tumors using high-density oligonucleotide SNP arrays. PLoS Comput Biol 2:e41.

Bignell GR, Huang J, Greshock J, Watt S, Butler A, West S, Grigorova M, Jones KW, Wei W, Stratton MR, Futreal PA, Weber B, Shapero MH, Wooster R. 2004. High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res 14:287-295.

Binet JL, Lepoprier M, Dighiero G, Charron D, D'Athis P, Vaugier G, Beral HM, Natali JC, Raphael M, Nizet B, Follezou JY. 1977. A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 40:855-864.

Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, Vaugier G, Potron G, Colona P, Oberling F, Thomas M, Tchernia G, Jacquillat C, Boivin P, Lesty C, Duault MT, Monconduit M, Belabbes S, Gremy F. 1981. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 48:198-206.

Bohlander SK. 2005. ETV6: a versatile player in leukemogenesis. Semin Cancer Biol 15:162-174.

Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ, Langabeer SE, Kottaridis PD, Moorman AV, Burnett AK, Linch DC. 2005. RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. Blood 106:2113-2119.

Bullinger L, Dohner K, Bair E, Frohling S, Schlenk RF, Tibshirani R, Dohner H, Pollack JR. 2004. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. N Engl J Med 350:1605-1616.

Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, Pettenati MJ, Patil SR, Rao KW, Watson MS, Koduru PR, Moore JO, Stone RM, Mayer RJ, Feldman EJ, Davey FR, Schiffer CA, Larson RA, Bloomfield CD, Cancer and Leukemia Group B (CALGB 8461). 2002. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood 100:4325-4336.

Caligaris-Cappio F and Ghia P. 2008. Novel insights in chronic lymphocytic leukemia: are we getting closer to understanding the pathogenesis of the disease? J Clin Oncol 26:4497-4503.

Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. 2002. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 99:15524-15529.

Calin GA, Pekarsky Y, Croce CM. 2007. The role of microRNA and other non-coding RNA in the pathogenesis of chronic lymphocytic leukemia. Best Pract Res Clin Haematol 20:425-437.

Caporaso N, Goldin L, Plass C, Calin G, Marti G, Bauer S, Raveche E, McMaster ML, Ng D, Landgren O, Slager S. 2007. Chronic lymphocytic leukaemia genetics overview. Br J Haematol 139:630-634.

Cardoso J, Molenaar L, de Menezes RX, Rosenberg C, Morreau H, Moslein G, Fodde R, Boer JM. 2004. Genomic profiling by DNA amplification of laser capture microdissected tissues and array CGH. Nucleic Acids Res 32:e146.

Carroll SM, DeRose ML, Gaudray P, Moore CM, Needham-Vandevanter DR, Von Hoff DD, Wahl GM. 1988. Double minute chromosomes can be produced from precursors derived from a chromosomal deletion. Mol Cell Biol 8:1525-1533.

Chen J and McMillan NA. 2008. Molecular basis of pathogenesis, prognosis and therapy in chronic lymphocytic leukaemia. Cancer Biol Ther 7:174-179.

Chen L, Widhopf G, Huynh L, Rassenti L, Rai KR, Weiss A, Kipps TJ. 2002. Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. Blood 100:4609-4614.

Chiorazzi N, Rai KR, Ferrarini M. 2005. Chronic lymphocytic leukemia. N Engl J Med 352:804-815.

Chudoba I, Plesch A, Lorch T, Lemke J, Claussen U, Senger G. 1999. High resolution multicolor-banding: a new technique for refined FISH analysis of human chromosomes. Cytogenet Cell Genet 84:156-160.

Coe BP, Ylstra B, Carvalho B, Meijer GA, Macaulay C, Lam WL. 2007. Resolving the resolution of array CGH. Genomics 89:647-653.

Crossen PE, Morrison MJ, Rodley P, Cochrane J, Morris CM. 1999. Identification of amplified genes in a patient with acute myeloid leukemia and double minute chromosomes. Cancer Genet Cytogenet 113:126-133.

Dalley CD, Neat MJ, Foot NJ, Burridge M, Byrne L, Amess JA, Rohatiner AZ, Lister A, Young BD, Lillington DM. 2002. Comparative genomic hybridization and multiplex-fluorescence in situ hybridization: an appraisal in elderly patients with acute myelogenous leukemia. Hematol J 3:290-298.

Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N. 1999. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 94:1840-1847.

Dash A and Gilliland DG. 2001. Molecular genetics of acute myeloid leukaemia. Best Pract Res Clin Haematol 14:49-64.

Derolf AR, Kristinsson SY, Andersson TM, Landgren O, Dickman PW, Bjorkholm M. 2009. Improved patient survival for acute myeloid leukemia: a population-based study of 9729 patients diagnosed in Sweden between 1973 and 2005. Blood 113:3666-3672.

Dighiero G and Hamblin TJ. 2008. Chronic lymphocytic leukaemia. Lancet 371:1017-1029.

Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P. 2000. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343:1910-1916.

Dohner K, Tobis K, Ulrich R, Frohling S, Benner A, Schlenk RF, Dohner H. 2002. 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. J Clin Oncol 20:3254-3261.

Dunbar AJ, Gondek LP, O'Keefe CL, Makishima H, Rataul MS, Szpurka H, Sekeres MA, Wang XF, McDevitt MA, Maciejewski JP. 2008. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. Cancer Res 68:10349-10357.

Dutt A and Beroukhim R. 2007. Single nucleotide polymorphism array analysis of cancer. Curr Opin Oncol 19:43-49.

Elliott B and Jasin M. 2002. Double-strand breaks and translocations in cancer. Cell Mol Life Sci 59:373-385.

Elonen E. Akuutit leukemiat. In book: Ruutu T, Rajamäki A, Lassila R, Porkka K (eds). 2007. Veritaudit. Helsinki, Finland: Kustannus Oy Duodecim 285-309.

Engel E. 1980. A new genetic concept: uniparental disomy and its potential effect, isodisomy. Am J Med Genet 6:137-143.

Estey E and Dohner H. 2006. Acute myeloid leukaemia. Lancet 368:1894-1907.

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, GIMEMA Acute Leukemia Working Party. 2005. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 352:254-266.

Fenaux P, Jonveaux P, Quiquandon I, Lai JL, Pignon JM, Loucheux-Lefebvre MH, Bauters F, Berger R, Kerckaert JP. 1991. P53 gene mutations in acute myeloid leukemia with 17p monosomy. Blood 78:1652-1657.

Fenaux P, Preudhomme C, Lai JL, Quiquandon I, Jonveaux P, Vanrumbeke M, Sartiaux C, Morel P, Loucheux-Lefebvre MH, Bauters F. 1992. Mutations of the p53 gene in B-cell chronic lymphocytic leukemia: a report on 39 cases with cytogenetic analysis. Leukemia 6:246-250.

Fitzgibbon J, Smith LL, Raghavan M, Smith ML, Debernardi S, Skoulakis S, Lillington D, Lister TA, Young BD. 2005. Association between acquired uniparental disomy and homozygous gene mutation in acute myeloid leukemias. Cancer Res 65:9152-9154.

Frohling S, Skelin S, Liebisch C, Scholl C, Schlenk RF, Dohner H, Dohner K, Acute Myeloid Leukemia Study Group, Ulm. 2002. Comparison of cytogenetic and molecular cytogenetic detection of chromosome abnormalities in 240 consecutive adult patients with acute myeloid leukemia. J Clin Oncol 20:2480-2485.

Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR. 2004. A census of human cancer genes. Nat Rev Cancer 4:177-183.

Gaidano G, Ballerini P, Gong JZ, Inghirami G, Neri A, Newcomb EW, Magrath IT, Knowles DM, Dalla-Favera R. 1991. p53 mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 88:5413-5417.

Gari M, Goodeve A, Wilson G, Winship P, Langabeer S, Linch D, Vandenberghe E, Peake I, Reilly J. 1999. C-Kit Proto-Oncogene Exon 8 In-Frame Deletion Plus Insertion Mutations in Acute Myeloid Leukaemia. Br J Haematol 105:894-900.

Gisselsson D, Pettersson L, Hoglund M, Heidenblad M, Gorunova L, Wiegant J, Mertens F, Dal Cin P, Mitelman F, Mandahl N. 2000. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. Proc Natl Acad Sci U S A 97:5357-5362.

Goldin LR and Caporaso NE. 2007. Family studies in chronic lymphocytic leukaemia and other lymphoproliferative tumours. Br J Haematol 139:774-779.

Gondek LP, Tiu R, O'Keefe CL, Sekeres MA, Theil KS, Maciejewski JP. 2008. Chromosomal lesions and uniparental disomy detected by SNP arrays in MDS, MDS/MPD, and MDS-derived AML. Blood 111:1534-1542.

Gorletta TA, Gasparini P, D'Elios MM, Trubia M, Pelicci PG, Di Fiore PP. 2005. Frequent loss of heterozygosity without loss of genetic material in acute myeloid leukemia with a normal karyotype. Genes Chromosomes Cancer 44:334-337.

Graux C, Cools J, Melotte C, Quentmeier H, Ferrando A, Levine R, Vermeesch JR, Stul M, Dutta B, Boeckx N, Bosly A, Heimann P, Uyttebroeck A, Mentens N, Somers R, MacLeod RA, Drexler

HG, Look AT, Gilliland DG, Michaux L, Vandenberghe P, Wlodarska I, Marynen P, Hagemeijer A. 2004. Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia. Nat Genet 36:1084-1089.

Gribben JG. 2008. Stem cell transplantation in chronic lymphocytic leukemia. Biol Blood Marrow Transplant 15:53-58.

Griffiths M, Mason J, Rindl M, Akiki S, McMullan D, Stinton V, Powell H, Curtis A, Bown N, Craddock C. 2005. Acquired isodisomy for chromosome 13 is common in AML, and associated with FLT3-itd mutations. Leukemia 19:2355-2358.

Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, Rees J, Hann I, Stevens R, Burnett A, Goldstone A. 1998. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood 92:2322-2333.

Grimwade D, Walker H, Harrison G, Oliver F, Chatters S, Harrison CJ, Wheatley K, Burnett AK, Goldstone AH, Medical Research Council Adult Leukemia Working Party. 2001. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 98:1312-1320.

Guillaud-Bataille M, Valent A, Soularue P, Perot C, Inda MM, Receveur A, Smaili S, Roest Crollius H, Benard J, Bernheim A, Gidrol X, Danglot G. 2004. Detecting single DNA copy number variations in complex genomes using one nanogram of starting DNA and BAC-array CGH. Nucleic Acids Res 32:e112.

Guipaud O, Deriano L, Salin H, Vallat L, Sabatier L, Merle-Beral H, Delic J. 2003. B-cell chronic lymphocytic leukaemia: a polymorphic family unified by genomic features. Lancet Oncol 4:505-514.

Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS. 2005. A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37:549-554.

Hahn PJ. 1993. Molecular biology of double-minute chromosomes. Bioessays 15:477-484.

Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ, International Workshop on Chronic Lymphocytic Leukemia. 2008. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 111:5446-5456.

Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. 1999. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 94:1848-1854.

Hanahan D and Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57-70.

Harper DP and Aplan PD. 2008. Chromosomal rearrangements leading to MLL gene fusions: clinical and biological aspects. Cancer Res 68:10024-10027.

Haslinger C, Schweifer N, Stilgenbauer S, Dohner H, Lichter P, Kraut N, Stratowa C, Abseher R. 2004. Microarray gene expression profiling of B-cell chronic lymphocytic leukemia subgroups defined by genomic aberrations and VH mutation status. J Clin Oncol 22:3937-3949.

Heidenblad M, Lindgren D, Veltman JA, Jonson T, Mahlamaki EH, Gorunova L, van Kessel AG, Schoenmakers EF, Hoglund M. 2005. Microarray analyses reveal strong influence of DNA copy number alterations on the transcriptional patterns in pancreatic cancer: implications for the interpretation of genomic amplifications. Oncogene 24:1794-1801.

Heim S, Mitelman F. 2008. Molecular screening for new fusion genes in cancer. Nat Genet 40:685-686.

Heim S, Mitelman F (Eds). 2009. Cancer cytogenetics, Chromosomal and molecular genetic aberrations of tumor cells. thrd ed. John Wiley & Sons Inc., Hoboken New Jersey USA 730pp.

Hittelman A, Sridharan S, Roy R, Fridlyand J, Loda M, Collins C, Paris PL. 2007. Evaluation of whole genome amplification protocols for array and oligonucleotide CGH. Diagn Mol Pathol 16:198-206.

Hoeijmakers JH. 2001. Genome maintenance mechanisms for preventing cancer. Nature 411:366-374.

Hoheisel JD. 2006. Microarray technology: beyond transcript profiling and genotype analysis. Nat Rev Genet 7:200-210.

Houlston RS, Catovsky D, Yuille MR. 2002. Genetic susceptibility to chronic lymphocytic leukemia. Leukemia 16:1008-1014.

Hyman E, Kauraniemi P, Hautaniemi S, Wolf M, Mousses S, Rozenblum E, Ringner M, Sauter G, Monni O, Elkahloun A, Kallioniemi OP, Kallioniemi A. 2002. Impact of DNA amplification on gene expression patterns in breast cancer. Cancer Res 62:6240-6245.

Iafrate AJ, Feuk L, Rivera MN, Listewnik ML, Donahoe PK, Qi Y, Scherer SW, Lee C. 2004. Detection of large-scale variation in the human genome. Nat Genet 36:949-951.

Jaffe ES, Harris NL, Stein H, Vardiman JW, (eds). 2001. World health organization classification of tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. Lyon, France: International Agency for Research on cancer (IARC).

Janssen JW, Steenvoorden AC, Lyons J, Anger B, Bohlke JU, Bos JL, Seliger H, Bartram CR. 1987. RAS gene mutations in acute and chronic myelocytic leukemias, chronic myeloproliferative disorders, and myelodysplastic syndromes. Proc Natl Acad Sci U S A 84:9228-9232.

Janz S, Potter M, Rabkin CS. 2003. Lymphoma- and leukemia-associated chromosomal translocations in healthy individuals. Genes Chromosomes Cancer 36:211-223.

Jones PA and Baylin SB. 2002. The fundamental role of epigenetic events in cancer. Nat Rev Genet 3:415-428.

Juliusson G, Oscier DG, Fitchett M, Ross FM, Stockdill G, Mackie MJ, Parker AC, Castoldi GL, Guneo A, Knuutila S. 1990. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. N Engl J Med 323:720-724.

Kalla C, Scheuermann MO, Kube I, Schlotter M, Mertens D, Dohner H, Stilgenbauer S, Lichter P. 2007. Analysis of 11q22-q23 deletion target genes in B-cell chronic lymphocytic leukaemia: evidence for a pathogenic role of NPAT, CUL5, and PPP2R1B. Eur J Cancer 43:1328-1335.

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. 1992. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258:818-821.

Kallioniemi A. 2008. CGH microarrays and cancer. Curr Opin Biotechnol 19:36-40.

Kantarjian H, O'Brien S, Cortes J, Wierda W, Faderl S, Garcia-Manero G, Issa JP, Estey E, Keating M, Freireich EJ. 2008. Therapeutic advances in leukemia and myelodysplastic syndrome over the past 40 years. Cancer 113:1933-1952.

Karhu R, Tobin G, Thunberg U, Vilpo L, Sundstrom C, Knuutila S, Rosenquist R, Vilpo J. 2003. More extensive genetic alterations in unmutated than in hypermutated cases of chronic lymphocytic leukemia. Genes Chromosomes Cancer 37:417-420.

Kennedy GC, Matsuzaki H, Dong S, Liu WM, Huang J, Liu G, Su X, Cao M, Chen W, Zhang J, Liu W, Yang G, Di X, Ryder T, He Z, Surti U, Phillips MS, Boyce-Jacino MT, Fodor SP, Jones KW. 2003. Large-scale genotyping of complex DNA. Nat Biotechnol 21:1233-1237.

Kienle DL, Korz C, Hosch B, Benner A, Mertens D, Habermann A, Krober A, Jager U, Lichter P, Dohner H, Stilgenbauer S. 2005. Evidence for distinct pathomechanisms in genetic subgroups of chronic lymphocytic leukemia revealed by quantitative expression analysis of cell cycle, activation, and apoptosis-associated genes. J Clin Oncol 23:3780-3792.

King-Underwood L, Renshaw J, Pritchard-Jones K. 1996. Mutations in the Wilms' tumor gene WT1 in leukemias. Blood 87:2171-2179.

King-Underwood L and Pritchard-Jones K. 1998. Wilms' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance. Blood 91:2961-2968.

Kinzler KW and Vogelstein B. 1997. Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 386:761, 763.

Kinzler KW and Vogelstein B. 1998. Landscaping the cancer terrain. Science 280:1036-1037.

Klein U, Tu Y, Stolovitzky GA, Mattioli M, Cattoretti G, Husson H, Freedman A, Inghirami G, Cro L, Baldini L, Neri A, Califano A, Dalla-Favera R. 2001. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 194:1625-1638.

Knudson AG,Jr. 1971. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68:820-823.

Knuutila S, Elonen E, Teerenhovi L, Rossi L, Leskinen R, Bloomfield CD, de la Chapelle A. 1986. Trisomy 12 in B cells of patients with B-cell chronic lymphocytic leukemia. N Engl J Med 314:865-869.

Knuutila S, Aalto Y, Autio K, Bjorkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, Kiuru-Kuhlefelt S, Larramendy ML, Lushnikova T, Monni O, Pere H, Tapper J, Tarkkanen M, Varis A, Wasenius VM, Wolf M, Zhu Y. 1999. DNA copy number losses in human neoplasms. Am J Pathol 155:683-694.

Koski T, Karhu R, Visakorpi T, Vilpo L, Knuutila S, Vilpo J. 2000. Complex chromosomal aberrations in chronic lymphocytic leukemia are associated with cellular drug and irradiation resistance. Eur J Haematol 65:32-39.

Kourlas PJ, Strout MP, Becknell B, Veronese ML, Croce CM, Theil KS, Krahe R, Ruutu T, Knuutila S, Bloomfield CD, Caligiuri MA. 2000. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: evidence for its fusion with MLL in acute myeloid leukemia. Proc Natl Acad Sci U S A 97:2145-2150.

Krober A, Seiler T, Benner A, Bullinger L, Bruckle E, Lichter P, Dohner H, Stilgenbauer S. 2002. V(H) mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. Blood 100:1410-1416.

Kuppers R. 2005. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer 5:251-262.

Laurent E, Talpaz M, Kantarjian H, Kurzrock R. 2001. The BCR gene and philadelphia chromosome-positive leukemogenesis. Cancer Res 61:2343-2355.

Lengauer C, Kinzler KW, Vogelstein B. 1998. Genetic instabilities in human cancers. Nature 396:643-649.

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. 2008. DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature 456:66-72.

Lindblad-Toh K, Tanenbaum DM, Daly MJ, Winchester E, Lui WO, Villapakkam A, Stanton SE, Larsson C, Hudson TJ, Johnson BE, Lander ES, Meyerson M. 2000. Loss-of-heterozygosity analysis of small-cell lung carcinomas using single-nucleotide polymorphism arrays. Nat Biotechnol 18:1001-1005.

Lips EH, Dierssen JW, van Eijk R, Oosting J, Eilers PH, Tollenaar RA, de Graaf EJ, van't Slot R, Wijmenga C, Morreau H, van Wezel T. 2005. Reliable high-throughput genotyping and loss-of-heterozygosity detection in formalin-fixed, paraffin-embedded tumors using single nucleotide polymorphism arrays. Cancer Res 65:10188-10191.

Look AT. 1997. Oncogenic transcription factors in the human acute leukemias. Science 278:1059-1064.

Lowenberg B, Downing JR, Burnett A. 1999. Acute myeloid leukemia. N Engl J Med 341:1051-1062.

Matsuzaki H, Dong S, Loi H, Di X, Liu G, Hubbell E, Law J, Berntsen T, Chadha M, Hui H, Yang G, Kennedy GC, Webster TA, Cawley S, Walsh PS, Jones KW, Fodor SP, Mei R. 2004. Genotyping over 100,000 SNPs on a pair of oligonucleotide arrays. Nat Methods 1:109-111.

Mitelman F, Johansson B, Mertens F. 2004. Fusion genes and rearranged genes as a linear function of chromosome aberrations in cancer. Nat Genet 36:331-334.

Mitelman F, Johansson B, Mertens F. 2007. The impact of translocations and gene fusions on cancer causation. Nat Rev Cancer 7:233-245.

Mocellin S, Rossi CR, Pilati P, Nitti D, Marincola FM. 2003. Quantitative real-time PCR: a powerful ally in cancer research. Trends Mol Med 9:189-195.

Mrozek K, Heerema NA, Bloomfield CD. 2004. Cytogenetics in acute leukemia. Blood Rev 18:115-136.

Mrozek K, Marcucci G, Paschka P, Whitman SP, Bloomfield CD. 2007. Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: are we ready for a prognostically prioritized molecular classification? Blood 109:431-448.

Muller S, O'Brien PC, Ferguson-Smith MA, Wienberg J. 1998. Cross-species colour segmenting: a novel tool in human karyotype analysis. Cytometry 33:445-452.

Myllykangas S and Knuutila S. 2006. Manifestation, mechanisms and mysteries of gene amplifications. Cancer Lett 232:79-89.

Nacheva E, Kearney L, Bower M, Chaplin T, Douek E, Das S, Young BD. 1993. In situ hybridisation analysis of a homogeneously staining region at 11q23-24 in an acute myeloid leukaemia (M5) using yeast artificial chromosomes. Genes Chromosomes Cancer 7:123-127.

Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, Sonoda Y, Fujimoto T, Misawa S. 1996. Internal tandem duplication of the flt3 gene found in acute myeloid leukemia. Leukemia 10:1911-1918.

Nannya Y, Sanada M, Nakazaki K, Hosoya N, Wang L, Hangaishi A, Kurokawa M, Chiba S, Bailey DK, Kennedy GC, Ogawa S. 2005. A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. Cancer Res 65:6071-6079.

Osato M, Asou N, Abdalla E, Hoshino K, Yamasaki H, Okubo T, Suzushima H, Takatsuki K, Kanno T, Shigesada K, Ito Y. 1999. Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alphaB gene associated with myeloblastic leukemias. Blood 93:1817-1824.

Oscier DG, Gardiner AC, Mould SJ, Glide S, Davis ZA, Ibbotson RE, Corcoran MM, Chapman RM, Thomas PW, Copplestone JA, Orchard JA, Hamblin TJ. 2002. Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. Blood 100:1177-1184.

Oscier D, Fegan C, Hillmen P, Illidge T, Johnson S, Maguire P, Matutes E, Milligan D, Guidelines Working Group of the UK CLL Forum. British Committee for Standards in Haematology. 2004. Guidelines on the diagnosis and management of chronic lymphocytic leukaemia. Br J Haematol 125:294-317.

Owen C, Barnett M, Fitzgibbon J. 2008. Familial myelodysplasia and acute myeloid leukaemia--a review. Br J Haematol 140:123-132.

Pabst T, Mueller BU, Zhang P, Radomska HS, Narravula S, Schnittger S, Behre G, Hiddemann W, Tenen DG. 2001. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. Nat Genet 27:263-270.

Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. 2008. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. J Clin Oncol 26:5088-5093.

Paulsson K, Heidenblad M, Strombeck B, Staaf J, Jonsson G, Borg A, Fioretos T, Johansson B. 2006. High-resolution genome-wide array-based comparative genome hybridization reveals cryptic chromosome changes in AML and MDS cases with trisomy 8 as the sole cytogenetic aberration. Leukemia .

Payne SR and Kemp CJ. 2005. Tumor suppressor genetics. Carcinogenesis 26:2031-2045.

Peiffer DA, Le JM, Steemers FJ, Chang W, Jenniges T, Garcia F, Haden K, Li J, Shaw CA, Belmont J, Cheung SW, Shen RM, Barker DL, Gunderson KL. 2006. High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. Genome Res 16:1136-1148.

Pekarsky Y, Santanam U, Cimmino A, Palamarchuk A, Efanov A, Maximov V, Volinia S, Alder H, Liu CG, Rassenti L, Calin GA, Hagan JP, Kipps T, Croce CM. 2006. Tcl1 expression in chronic lymphocytic leukemia is regulated by miR-29 and miR-181. Cancer Res 66:11590-11593.

Pekarsky Y, Palamarchuk A, Maximov V, Efanov A, Nazaryan N, Santanam U, Rassenti L, Kipps T, Croce CM. 2008. Tcl1 functions as a transcriptional regulator and is directly involved in the pathogenesis of CLL. Proc Natl Acad Sci U S A 105:19643-19648.

Pfeifer D, Pantic M, Skatulla I, Rawluk J, Kreutz C, Martens UM, Fisch P, Timmer J, Veelken H. 2007. Genome-wide analysis of DNA copy number changes and LOH in CLL using high-density SNP arrays. Blood 109:1202-1210.

Pinkel D, Straume T, Gray JW. 1986. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci U S A 83:2934-2938.

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG. 1998. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20:207-211.

Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. 1999. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23:41-46.

Pollack JR, Sorlie T, Perou CM, Rees CA, Jeffrey SS, Lonning PE, Tibshirani R, Botstein D, Borresen-Dale AL, Brown PO. 2002. Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci U S A 99:12963-12968.

Popescu NC. 2003. Genetic alterations in cancer as a result of breakage at fragile sites. Cancer Lett 192:1-17.

Poppe B, Vandesompele J, Schoch C, Lindvall C, Mrozek K, Bloomfield CD, Beverloo HB, Michaux L, Dastugue N, Herens C, Yigit N, De Paepe A, Hagemeijer A, Speleman F. 2004. Expression analyses identify MLL as a prominent target of 11q23 amplification and support an etiologic role for MLL gain of function in myeloid malignancies. Blood 103:229-235.

Preudhomme C, Warot-Loze D, Roumier C, Grardel-Duflos N, Garand R, Lai JL, Dastugue N, Macintyre E, Denis C, Bauters F, Kerckaert JP, Cosson A, Fenaux P. 2000. High incidence of biallelic point mutations in the Runt domain of the AML1/PEBP2 alpha B gene in Mo acute myeloid leukemia and in myeloid malignancies with acquired trisomy 21. Blood 96:2862-2869.

Preudhomme C, Sagot C, Boissel N, Cayuela JM, Tigaud I, de Botton S, Thomas X, Raffoux E, Lamandin C, Castaigne S, Fenaux P, Dombret H, ALFA Group. 2002. Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood 100:2717-2723.

Raghavan M, Lillington DM, Skoulakis S, Debernardi S, Chaplin T, Foot NJ, Lister TA, Young BD. 2005. Genome-wide single nucleotide polymorphism analysis reveals frequent partial uniparental disomy due to somatic recombination in acute myeloid leukemias. Cancer Res 65:375-378.

Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. 1975. Clinical staging of chronic lymphocytic leukemia. Blood 46:219-234.

Rawstron AC, Yuille MR, Fuller J, Cullen M, Kennedy B, Richards SJ, Jack AS, Matutes E, Catovsky D, Hillmen P, Houlston RS. 2002. Inherited predisposition to CLL is detectable as subclinical monoclonal B-lymphocyte expansion. Blood 100:2289-2290.

Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, Gonzalez JR, Gratacos M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. 2006. Global variation in copy number in the human genome. Nature 444:444-454.

Renneville A, Roumier C, Biggio V, Nibourel O, Boissel N, Fenaux P, Preudhomme C. 2008. Cooperating gene mutations in acute myeloid leukemia: a review of the literature. Leukemia 22:915-931.

Robinson WP. 2000. Mechanisms leading to uniparental disomy and their clinical consequences. Bioessays 22:452-459.

Rosenwald A, Alizadeh AA, Widhopf G, Simon R, Davis RE, Yu X, Yang L, Pickeral OK, Rassenti LZ, Powell J, Botstein D, Byrd JC, Grever MR, Cheson BD, Chiorazzi N, Wilson WH, Kipps TJ, Brown PO, Staudt LM. 2001. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J Exp Med 194:1639-1647.

Rossbach HC, Sutcliffe MJ, Chamizo W, Haag MM, Grana NH, Washington KR, Barbosa JL. 1998. Pre-B acute lymphoblastic leukemia in a 3-year-old boy with pre-acute myelogenous leukemia myelodysplastic syndrome: cytogenetic evidence of common early progenitor cell ontogeny. J Pediatr Hematol Oncol 20:347-352.

Rovigatti U, Watson DK, Yunis JJ. 1986. Amplification and rearrangement of Hu-ets-1 in leukemia and lymphoma with involvement of 11q23. Science 232:398-400.

Rucker FG, Bullinger L, Schwaenen C, Lipka DB, Wessendorf S, Frohling S, Bentz M, Miller S, Scholl C, Schlenk RF, Radlwimmer B, Kestler HA, Pollack JR, Lichter P, Dohner K, Dohner H. 2006. Disclosure of candidate genes in acute myeloid leukemia with complex karyotypes using microarray-based molecular characterization. J Clin Oncol 24:3887-3894.

Ruutu T. Leukemiat, myelodysplastiset oireyhtymät ja myeloproliferatiiviset tilat. In book: Joensuu H, Roberts PJ, Teppo L, Tenhunen M, (eds). 2007. Syöpätaudit. Helsinki, Finland: Kustannus Oy Duodecim 651-679.

Sait SN, Qadir MU, Conroy JM, Matsui S, Nowak NJ, Baer MR. 2002. Double minute chromosomes in acute myeloid leukemia and myelodysplastic syndrome: identification of new amplification regions by fluorescence in situ hybridization and spectral karyotyping. Genes Chromosomes Cancer 34:42-47.

Salman M, Jhanwar SC, Ostrer H. 2004. Will the new cytogenetics replace the old cytogenetics? Clin Genet 66:265-275.

Schichman SA, Caligiuri MA, Gu Y, Strout MP, Canaani E, Bloomfield CD, Croce CM. 1994. ALL-1 partial duplication in acute leukemia. Proc Natl Acad Sci U S A 91:6236-6239.

Schimke RT, Sherwood SW, Hill AB, Johnston RN. 1986. Overreplication and recombination of DNA in higher eukaryotes: potential consequences and biological implications. Proc Natl Acad Sci U S A 83:2157-2161.

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. 2002. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 30:e57.

Schrock E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T. 1996. Multicolor spectral karyotyping of human chromosomes. Science 273:494-497.

Schwab M. 1999. Oncogene amplification in solid tumors. Semin Cancer Biol 9:319-325.

Schwaenen C, Nessling M, Wessendorf S, Salvi T, Wrobel G, Radlwimmer B, Kestler HA, Haslinger C, Stilgenbauer S, Dohner H, Bentz M, Lichter P. 2004. Automated array-based genomic profiling in chronic lymphocytic leukemia: development of a clinical tool and discovery of recurrent genomic alterations. Proc Natl Acad Sci U S A 101:1039-1044.

Sebat J, Lakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, Navin N, Lucito R, Healy J, Hicks J, Ye K, Reiner A, Gilliam TC, Trask B, Patterson N, Zetterberg A, Wigler M. 2004. Large-scale copy number polymorphism in the human genome. Science 305:525-528.

Seiler T, Dohner H, Stilgenbauer S. 2006. Risk stratification in chronic lymphocytic leukemia. Semin Oncol 33:186-194.

Skacel M, Pettay JD, Tsiftsakis EK, Procop GW, Biscotti CV, Tubbs RR. 2001. Validation of a multicolor interphase fluorescence in situ hybridization assay for detection of transitional cell

carcinoma on fresh and archival thin-layer, liquid-based cytology slides. Anal Quant Cytol Histol 23:381-387.

Slingerland JM, Minden MD, Benchimol S. 1991. Mutation of the p53 gene in human acute myelogenous leukemia. Blood 77:1500-1507.

Slovak ML, Kopecky KJ, Cassileth PA, Harrington DH, Theil KS, Mohamed A, Paietta E, Willman CL, Head DR, Rowe JM, Forman SJ, Appelbaum FR. 2000. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood 96:4075-4083.

Smith M, Barnett M, Bassan R, Gatta G, Tondini C, Kern W. 2004. Adult acute myeloid leukaemia. Crit Rev Oncol Hematol 50:197-222.

Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, Cremer T, Lichter P. 1997. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer 20:399-407.

Speicher MR, Gwyn Ballard S, Ward DC. 1996. Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12:368-375.

Stankovic T, Weber P, Stewart G, Bedenham T, Murray J, Byrd PJ, Moss PA, Taylor AM. 1999. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukaemia. Lancet 353:26-29.

Steemers FJ, Chang W, Lee G, Barker DL, Shen R, Gunderson KL. 2006. Whole-genome genotyping with the single-base extension assay. Nat Methods 3:31-33.

Steffen B, Muller-Tidow C, Schwable J, Berdel WE, Serve H. 2005. The molecular pathogenesis of acute myeloid leukemia. Crit Rev Oncol Hematol 56:195-221.

Stilgenbauer S, Bullinger L, Lichter P, Dohner H, German CLL Study Group (GCLLSG). Chronic lymphocytic leukemia. 2002. Genetics of chronic lymphocytic leukemia: genomic aberrations and V(H) gene mutation status in pathogenesis and clinical course. Leukemia 16:993-1007.

Storlazzi CT, Fioretos T, Paulsson K, Strombeck B, Lassen C, Ahlgren T, Juliusson G, Mitelman F, Rocchi M, Johansson B. 2004. Identification of a commonly amplified 4.3 Mb region with overexpression of C8FW, but not MYC in MYC-containing double minutes in myeloid malignancies. Hum Mol Genet 13:1479-1485.

Storlazzi CT, Fioretos T, Surace C, Lonoce A, Mastrorilli A, Strombeck B, D'Addabbo P, Iacovelli F, Minervini C, Aventin A, Dastugue N, Fonatsch C, Hagemeijer A, Jotterand M, Muhlematter D, Lafage-Pochitaloff M, Nguyen-Khac F, Schoch C, Slovak ML, Smith A, Sole F, Van Roy N, Johansson B, Rocchi M. 2006. MYC-containing double minutes in hematologic malignancies: evidence in favor of the episome model and exclusion of MYC as the target gene. Hum Mol Genet 15:933-942.

Suela J, Alvarez S, Cifuentes F, Largo C, Ferreira BI, Blesa D, Ardanaz M, Garcia R, Marquez JA, Odero MD, Calasanz MJ, Cigudosa JC. 2007. DNA profiling analysis of 100 consecutive de novo acute myeloid leukemia cases reveals patterns of genomic instability that affect all cytogenetic risk groups. Leukemia 21:1224-1231.

Swerdlow SH, Cambo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, (eds). 2008. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th edn. Lyon, France: International Agency for Research on cancer (IARC).

Tanaka K, Eguchi M, Eguchi-Ishimae M, Hasegawa A, Ohgami A, Kikuchi M, Kyo T, Asaoku H, Dohy H, Kamada N. 2001. Restricted chromosome breakpoint sites on 11q22-q23.1 and 11q25 in

various hematological malignancies without MLL/ALL-1 gene rearrangement. Cancer Genet Cytogenet 124:27-35.

Tchinda J, Dijkhuizen T, Vlies Pv P, Kok K, Horst J. 2004. Translocations involving 6p22 in acute myeloid leukaemia at relapse: breakpoint characterization using microarray-based comparative genomic hybridization. Br J Haematol 126:495-500.

The International HapMap Consortium. 2003. The International HapMap Project. Nature 426:789-796.

Thiagalingam S, Foy RL, Cheng KH, Lee HJ, Thiagalingam A, Ponte JF. 2002. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. Curr Opin Oncol 14:65-72.

Thiede C, Steudel C, Mohr B, Schaich M, Schakel U, Platzbecker U, Wermke M, Bornhauser M, Ritter M, Neubauer A, Ehninger G, Illmer T. 2002. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood 99:4326-4335.

Thiede C, Koch S, Creutzig E, Steudel C, Illmer T, Schaich M, Ehninger G. 2006. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood 107:4011-4020.

Thomas L, Stamberg J, Gojo I, Ning Y, Rapoport AP. 2004. Double minute chromosomes in monoblastic (M5) and myeloblastic (M2) acute myeloid leukemia: two case reports and a review of literature. Am J Hematol 77:55-61.

Trask BJ. 1991. Fluorescence in situ hybridization: applications in cytogenetics and gene mapping. Trends Genet 7:149-154.

Tuna M, Knuutila S, Mills GB. 2009. Uniparental disomy in cancer. Trends Mol Med 15:120-128.

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. 2004. Prognostically useful gene-expression profiles in acute myeloid leukemia. N Engl J Med 350:1617-1628.

Van Vlierberghe P, van Grotel M, Tchinda J, Lee C, Beverloo HB, van der Spek PJ, Stubbs A, Cools J, Nagata K, Fornerod M, Buijs-Gladdines J, Horstmann M, van Wering ER, Soulier J, Pieters R, Meijerink JP. 2008. The recurrent SET-NUP214 fusion as a new HOXA activation mechanism in pediatric T-cell acute lymphoblastic leukemia. Blood 111:4668-4680.

Vogelstein B and Kinzler KW. 1993. The multistep nature of cancer. Trends Genet 9:138-141.

Von Hoff DD, Needham-VanDevanter DR, Yucel J, Windle BE, Wahl GM. 1988. Amplified human MYC oncogenes localized to replicating submicroscopic circular DNA molecules. Proc Natl Acad Sci U S A 85:4804-4808.

Von Hoff DD, Forseth B, Clare CN, Hansen KL, VanDevanter D. 1990. Double minutes arise from circular extrachromosomal DNA intermediates which integrate into chromosomal sites in human HL-60 leukemia cells. J Clin Invest 85:1887-1895.

Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES. 1998. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280:1077-1082.

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. 2005. 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 102:1104-1109.

Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, Carroll AJ, Mrozek K, Vardiman JW, George SL, Kolitz JE, Larson RA, Bloomfield CD, Caligiuri MA. 2001. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res 61:7233-7239.

Yamamoto Y, Kiyoi H, Nakano Y, Suzuki R, Kodera Y, Miyawaki S, Asou N, Kuriyama K, Yagasaki F, Shimazaki C, Akiyama H, Saito K, Nishimura M, Motoji T, Shinagawa K, Takeshita A, Saito H, Ueda R, Ohno R, Naoe T. 2001. Activating mutation of D835 within the activation loop of FLT3 in human hematologic malignancies. Blood 97:2434-2439.

Yoshida T, Kimura N, Akiyoshi T, Ohshima K, Nagano M, Morioka E, Hisano S, Ohyashiki K, Kamada N, Tamura K. 1999. Jumping translocation of homogeneously staining region and tetraploidy with double minutes in acute myelomonocytic leukemia. Cancer Genet Cytogenet 109:40-44.

Zhao X, Li C, Paez JG, Chin K, Janne PA, Chen TH, Girard L, Minna J, Christiani D, Leo C, Gray JW, Sellers WR, Meyerson M. 2004. An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. Cancer Res 64:3060-3071.