Introduction
Cancer: a cellular disease

Normal body cells grow, divide, and die in an orderly fashion. During the early years of a person's life, normal cells divide more rapidly until the person becomes an adult. After that, cells in most parts of the body divide only to replace worn-out or dying cells and to repair injuries.

The origin of the word cancer is credited to the Greek physician Hippocrates (460-370 B.C.), considered the "Father of Medicine." Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors. In Greek this word refers to a crab, most likely applied to the disease because the finger-like spreading projections from a cancer called to mind the shape of a crab. Although there are many kinds of cancer, they all start because of loss of normal growth. Cancer cells grow due to loss of normal growth of cells. Instead of dying, cancer cells outlive normal cells and continue to form new abnormal cells (Figure 1).

Figure 1: Loss of normal growth control

![Loss of Normal Growth Control](Source: National Cancer Institute, www.cancer.gov)

Cancer cells often travel to other parts of the body where they begin to grow and replace normal tissue. This process called metastasis occurs as the cancer cells get into the bloodstream or lymph vessels of our body. Cancer cells develop because of damage to DNA. Most of the time when DNA becomes damaged the body is able to repair it. In cancer cells, the damaged DNA is not repaired. People can inherit
damaged DNA, which accounts for inherited cancers. Different types of cancer can behave very differently.

Cancers in all forms are causing about 12% of deaths throughout the world. In the developing countries cancer ranks third as a cause of death and accounts for 9.5% (3.8 million) of all deaths. Tobacco alcohol, infections and hormones contribute towards occurrence of common cancers all over the world. Cancer has become one of the ten leading causes of death in India. It is estimated that there are nearly 1.5-2 million cancer cases at any given point of time. Over 7 lacs new cancer cases and 3 lacs deaths occur annually due to cancer. Data from population-based registries under National Cancer Registry Programme indicate that the leading sites of cancer are oral cavity, lungs, oesophagus and stomach amongst men and cervix, breast and oral cavity amongst women. Cancer usually occurs in the later years of life and with increase in life expectancy to more than 60 years, an estimate shows that the total cancer burden in India for all sites will increase from 7 lacs new cases per year to 14 lacs by 2026 [http://www.mohfw.nic.in/kk/95/l9/95190e01.htm].

Cancers are classified by the type of cells that resembles the tumor and the tissue presumed to be the origin of the tumor. **Carcinoma**- malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer. **Sarcoma**- malignant tumors derived from connective tissue, or mesenchymal cells. **Mesothelioma**- tumors derived from the mesothelial cells lining the peritoneum and the pleura. **Glioma**- tumors derived from glia, the most common type of brain cell. **Germinoma**- tumors derived from germ cells, normally found in the testicle and ovary. **Choriocarcinoma**: malignant tumors derived from the placenta. **Lymphoma** and **Leukemia**- malignant tumors derived from blood and bone marrow cells [Tannock IF, Hill RP et al., (Eds) 4th ed.2005, Benjamin P and Kleinsmith C LJ 2006].

**Leukemia**: Leukemia is a progressive, malignant disease of the blood-forming organs, marked by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow. There are several types of leukemia and these are classified by how quickly they progress and what cell they affect.
Leukemia is divided into chronic (gets worse slowly) or acute (gets worse quickly) depending on how quickly the disease develops and gets worse. The disease can arise in lymphoid cells or myeloid cells. Leukemia that affects lymphoid cells is called lymphocytic leukemia and that affects myeloid cells is called myeloid leukemia or myelogenous leukemia (Figure 2) [Bain B 1999, Taylor & Francis 1989].

There are four common types of leukemia: (chronic lymphoblastic leukemia, CLL), Chronic myeloid leukemia (CML), Acute lymphocytic leukemia (ALL), Acute Myeloid Leukemia (AML). The most common types of leukemia in adults are AML, with an estimated 12,810 new cases in 2009 [Leukemia & Lymphoma Society - Leukemia Facts & Statistics.mht].

Figure 2: Hematological Malignant lineage of leukemia

Acute Myeloid Leukemia: Acute myeloid leukemia (AML) represents a clinically and biologically heterogeneous group of diseases caused by the malignant transformation of a hematopoietic stem cells or myeloid progenitor cell. The proliferative advantage of the leukemic stem cell, coupled with impairments in differentiation and inhibition of apoptosis, is thought to arise from acquired genetic alterations that lead to accumulation of immature or blast cells in the bone marrow. The blasts eventually suppress normal hematopoiesis and infiltrate other organs and tissues [Wei MC et al., 2008]. AML also known as acute myelogenous leukemia, is a cancer of the myeloid line of white blood cells, characterized by the rapid proliferation of abnormal cells which accumulate in the bone marrow and interfere with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and it is mainly observed in patients with older age. There are 8
different types of AML; depending on the type of WBC they arise. AML is characterized by high levels of abnormal myelocytes or granulocytes that will never fully mature. AML is treated initially with chemotherapy aimed at inducing a remission; some patients may go on to receive a transplant. Areas of active research in AML include further elucidation of the cause of AML, identification of better prognostic indicators, development of new methods of detecting residual disease after treatment, and the development of new drugs and targeted therapies. AML occurs when there is a shortage of mature myeloid cells because of an accumulation of immature or 'baby' myeloid cells. When too many young myeloid cells accumulate in the blood they may occasionally cause blockage of the blood vessels. This type of leukemia may occur in children and adolescents, but is more common in adults.

**Incidence:** AML is the most common form of leukemia. About 15% of childhood leukemia cases are AML. This disease is more likely to affect the older individuals. The average age at diagnosis is 67 years. The incidence of AML is slightly more in men than women. Worldwide, the incidence of AML is highest in the U.S., Australia, and Western Europe [Jemal A et al., 2002]. Annual incidence of AML in Gujarat Cancer & Research Institute, Ahmedabad-Gujarat, India during year 2002-2004 is 183 (27.2%) new cases of total leukemia cases [Agrawal MB 2008].

**Risk Factors:**

**Genetic factors:** Genetics syndromes such as Downs (trisomy of chromosome 21), Klinefelter (XXY and variants) and Patau (trisomy of chromosome 13) have been associated with a higher incidence of AML. Other syndromes like Bloom syndrome, Fanconi anaemia, Kostmann syndrome and Wiskott - Aldrich syndrome or Ataxia telangiectasia syndrome patients are highly prone to develop AML [Pedersen BJ et al., 2002].

**Radiation:** Survivors of the atomic bombs in Japan had an increased incidence of myeloid leukemias that peaked 5 to 7 years after exposure [Pedersen BJ et al., 2002]. Therapeutic radiation increases AML risk, particularly if given with alkylating agents.

**Chemotherapy Drugs:** There are 2 main types of therapy-related AML. The classic alkylating agent (e.g., cyclophosphamide, melphalan, nitrogen mustard) has a latency period of 4 to 8 years and is often associated with abnormalities of
chromosome 5 and/or 7. Exposure to agents that inhibit the DNA repair enzyme topoisomerase II (e.g., etoposide, teniposide) is associated with secondary AML with a shorter latency period (usually 1 to 3 years), chromosome 11q23 at the location of the MLL gene, and M4 or M5 morphologic features [Pedersen BJ et al., 2002, Ayton PM 2003, Armstrong SA et al., 2002]. Drugs such as chloramphenicol, phenylbutazone, chloroquine, and methoxypsoralen can induce marrow damage that may later evolve into AML.

Other factors: Exposure to Benzene, smoking, dyes, herbicides, and pesticides have been implicated as potential risk factors for development of AML [West RR et al., 2000]. In addition, AML may be secondary to progression of a myelodysplastic process or a chronic bone marrow stem cell disorder, such as Polycythemia Vera, CML and primary thrombocytosis.

Symptoms: In AML patients fatigue, bruising or bleeding, fever, infection, and bone marrow failure are common. Only 10% of the patients present with white blood cell (WBC) counts greater than 100X10⁹/L [Appelbaum FR et al., 2006]. These patients are at higher risk of tumor lysis syndrome, central nervous system involvement and leukostasis. Physical findings other than bleeding and infection may include organomegaly, lymphadenopathy, sternal tenderness, retinal haemorrhages and infiltration of gingivae, skin, soft tissues. More common with monocytic variants M4 or M5. Among the forms of AML, APML has distinct biologic and clinical features, as well as unique treatment approaches. Disseminated intravascular coagulopathy with bleeding diathesis are a common presentation in APML [Jabbour EJ et al., 2006].

Diagnosis: The presumptive diagnosis of acute leukemia is usually apparent after examining the patient and reviewing the blood smear and bone marrow smear (Figure 3). Most patients present with pancytopenia and circulating blast forms, which are apparent on the peripheral blood smear. The total white blood count may range from less than 1,000/mm³ to more than 200,000/mm³, with the majority of patients having a total white blood count between 5,000 and 30,000/mm³.
The diagnosis of AML is often demonstrated by an increased number of myeloblasts in the bone marrow or peripheral blood. Unlike other AML subtypes, AML-M3 (APML) subtype is generally characterized at diagnosis by low leukocyte counts and blast infiltration of bone marrow (BM) only. Epidemiologically too, APML differs from the other myeloid leukemias. It occurs most frequently between 15 and 60 years of age. The diagnosis of APML requires the presence of 25% infiltration of the BM with dysplastic appearing promyelocytes. These abnormal promyelocytes are larger (14 to 25 mm) than non-malignant promyelocytes and contain a small amount of purplish – blue cytoplasm following staining with Wright Giemsa reagent. Several pleomorphic nuclei are present as striking hypergranulatity and multiple Auer bodies [Stone RM and Mayer RJ 1990].

After establishing the diagnosis, the blast lineage (myeloid, lymphoid, or undifferentiated) is determined. AML patients are further grouped according to (i) FAB classification system (ii) World Health Organisation (WHO) classification system (iii) Cytogenetic Risk group. The distinction is important and dictates specific therapy. Lineage determination is made using cytochemical stains. If 3% or more blasts stain positive for myeloperoxidase or Sudan black B, the diagnosis is AML. The WHO classification incorporates molecular, cytogenetic, and clinical features (prior hematologic disorder) to the morphologic characteristics to better recognize the diversity of the disease and its response to therapy [Jabbour EJ et al., 2006].
Classification of AML

FAB classification:
In 1976 international group of investigators from France, America and Britain developed a uniform classification system designated as FAB classification [Benett JM et al., 1976], which was subsequently revised in 1985 [Bennett JM et al., 1985]. The classification criteria are based on morphologic and cytochemical features; however for some of the categories, immunophenotyping is necessary [Bennett JM1 et al., 1985, Bennett JM et al., 1991]. It is lineage-based morphological classification that categorizes cases according to the degree of maturation of the leukemic cells and their lineage differentiation. The major advantage of the FAB classification system is its ease of use. The cytological criteria are well defined; they do not require high technology and can be applied in most laboratories throughout the world [McKenna RW 2000]. It is accepted by most of the multi-institutional study groups for management plans and comparison of treatment results between morphologic subtypes for their prognostic significance. AML can be classified into one of eight different sub-types i.e. M0-M7. According to FAB classification main clinical features of the subtypes are as follows.

M0 - Undifferentiated Leukemia -- Characterized by the overproduction of very primitive leukemia cells or "blasts," which are so immature it is sometimes difficult to differentiate AML or ALL cells, it has a poor prognosis and represents less than 5% of AML cases.

M1 - Acute Myeloblastic Leukemia -- This condition is characterized by the overproduction of very primitive white blood cells or "blasts." Bone marrow contains few mature white blood cells and accounts for approximately 15-20% of AML cases, which is associated with an average prognosis.

M2 - Acute Myeloblastic Leukemia with Maturation -- This condition is characterized by the overproduction of primitive white blood cells or "blasts," where the bone marrow contains mature white blood cells as well as blast. It accounts for approximately 20-30 % of AML cases and t(8;21) is characteristic translocation and suggests a better prognosis.

M3 - Acute Promyelocytic Leukemia (APML) -- Characterized by the presence of atypical promyelocytes in the BM and blood, it can be associated with severe
bleeding. This condition represents approximately 10-15% of AML cases and t(15;17) is characteristic translocation and suggests a better prognosis.

**M4 - Acute Myelomonocytic Leukemia** -- Characterized by the overproduction of monocytes and myelocytes, which are white blood cells that battle infectious agents throughout the body, it represents approximately 20-25% of AML cases. And t(16;16) or inv(16) is frequently occurs. Prognosis is similar to the average prognosis for all AML cases.

**M4 Eos: Acute Myelomonocytic Leukemia with Eosinophilia.** In addition to a higher than normal proportion of monocytes and promonocytes, this M4 variant also involves the presence of abnormal eosinophils in the bone marrow. It account for up to 5% of adult AML cases, and the prognosis for patients with this type of AML is better than average.

**M5 - Acute Monocytic Leukemia** -- Characterized by the overproduction of white blood cells, monocytes and monoblasts, which are white blood cells that battle infectious agents throughout the body, this type of leukemia represents ~5-10% of AML cases, prognosis for this patients is average when compared with that of all adult patients with AML.

**M6 - Acute Erythroblastic Leukemia** -- Characterized by the overproduction of primitive red blood cells, this leukemia has a poor prognosis and often evolves from a disorder called myelodysplasia. It represents less than 5% of AML cases.

**M7 - Acute Megakaryoblastic Leukemia** -- Characterized by the overproduction of primitive megakaryocytes (the cells that give rise to platelets), this is a rare form of AML with an extremely poor prognosis [Cancer Medicine 6th Ed.].

Morphological pictures of BM cells in different FAB subtypes were shown in **figure 4**.
WHO Classification:
The WHO classification of AML is also clinically useful and produces more information that is relevant to prognosis. The new WHO classification of AML uses cytogenetic abnormalities as a major criterion. Therefore parameters are needed to classify this disease into biologic entities to understand its pathogenesis and development of specific treatment approaches [Schoch C et al., 2002]. The WHO subtypes of AML are as follows;

AML with characteristic genetic abnormalities: This includes AML with translocations between chromosome 8 and 21 [t(8;21)], inversions in chromosome 16 [inv(16)], or translocations between chromosome 15 and 17 [t(15;17)]. Patients with AML in this category generally have a high rate of remission and a better prognosis compared to other types of AML.
AML with multilineage dysplasia: This category includes patients who have had a prior myelodysplastic syndrome (MDS) or myeloproliferative disease (MPD) that transforms into AML. This category of AML occurs most often in elderly patients and often has a worse prognosis.

AML and MDS, therapy-related: This category includes patients who have had prior chemotherapy and/or radiation and subsequently develop AML or MDS. These leukemias may be characterized by specific chromosomal abnormalities, and often carry a worse prognosis.

Acute leukemias of ambiguous lineage: Acute leukemias of ambiguous lineage (also known as mixed phenotype or biphenotypic acute leukemia) occur when the leukemic cells cannot be classified as either myeloid or lymphoid cells, or where both types of cells are present.

Treatment: The contemporary strategies for the treatment of AML are outlined in table 1 with the exception of APML. APML is treated with all-trans retinoic acid.

**Table-1: General treatment strategies for adults with AML.**

<table>
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<th>Induction Therapy</th>
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<td>Chemotherapy including anthracycline plus cytarabine</td>
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<th>Post remission therapy</th>
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<th>Maintenance chemotherapy</th>
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<td>Low dose chemotherapy in older adults</td>
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Induction Therapy is the initial phase of specific treatment. In most cases an anthracyline antibiotic (e.g. daunorubicin, doxorubicin, or idarubicin) is combined
with cytarabine (syn. cytosine arabinoside, Ar a-C). The anthracycline antibiotic is given, usually, in the first three days of treatment. Cytarabine is started at the same time but is given for seven to 10 days of treatment. The goal of induction therapy is to rid the blood and marrow of visible leukemic blast cells. If blast cells are still evident, a second course of chemotherapy may be required to rid the marrow of blasts. Usually the same drugs are used for each course [Cripe LD and Tallman MS].

Treatment for childhood AML consists of remission induction chemotherapy followed by post remission chemotherapy with or without bone marrow transplantation. The AML Berlin-Frankfurt-Munster (BFM)-93 protocols with induction-consolidation-maintenance chemotherapy for 2 years has been reported to result in a 6-year event-free survival (EFS) and event-free interval (EFI) of 49% and 61% respectively. The improvement seen after 1993 was related to a reduction in induction deaths for that period and probably reflected increased capability and familiarity to cope with the demands of the AML-BFM-93 protocol and accompanying complications in the treatment of AML [Chan LL et al., 2004].

Treatment of AML-M3 with ATRA is the first example of differentiation induction therapy of human cancer. In PML-RARα positive APML about 70% of patients are expected to be cured with a combination of ATRA and anthracycline-based chemotherapy. However, relapse remains a major problem. The identification of patients at high risk of relapse and the development of risk-adapted treatment schedules are therefore clearly the most challenging tasks in the treatment of APML. Recent studies have shown that preventive chemotherapy at the time of molecular relapse improves survival compared to treatment at the point of hematological relapse [Reiter A et al., 2004].

Response criteria for AML:
For hematologic response, morphologic leukemia free state is defined as bone marrow blast<5% in an aspirate with spicules. There should be no blasts with Auer rods or persistence of extramedullary disease. Complete remission is defined as morphologic complete remission—patient independent of transfusions, absolute neutrophill count>1000/µL. No residual evidence of extra medullary disease. 50% blast decreased to 5-25% in bone marrow and the normalization of blood counts,
same as complete remission (CR). Patients failing to achieve a complete response are considered treatment failures. Reappearance of leukemic blasts in the peripheral blood or the finding of more than 5% blasts in the bone marrow, not attributable to another cause is called as relapse. Cytogenetic complete remission is defined as disappearance of abnormal cytogenetic clone which was previously observed. Molecular remission can be defined as, negative with molecular studies. [NCCN clinical practice guidelines in oncology 2009].

**PROGNOSIS:**
A number of clinical and biologic features that reflect the heterogeneity of AML are used to predict the likelihood that a patient will have a response to treatment. Adverse prognostic factors include an age over 60 years, a poor performance score before treatment, AML resulting from prior chemotherapy or an antecedent hematologic disorder such as MDS, and a white cell count of more than 20000/cmm or an elevated serum lactate dehadyrogenease level at presentation. There are correlations between certain FAB subtypes and cytogenetic abnormalities, such as between FAB subtype AML-M3 and the t(15;17) translocation, the abnormalities themselves appear to be the more important prognostic factor. There may be subtle differences in the criteria used to define these groups; the prognostic discrimination made possible by the presence of various cytogenetic abnormalities has become more important as the efficacy of treatment for AML has improved.

The favorable prognosis subgroup, which includes approximately 20 percent of cases among patients who are 60 years of age or younger is defined by the presence of leukemic blasts with the t(15;17), t(8;21), or inv(16) abnormalities. The other end of the spectrum is the unfavorable prognostic subgroup, which includes approximately 15 percent of the cases among patients who are 15 to 60 years of age. These unfavorable cases are defined by the presence of leukemic blasts with cytogenetic abnormalities of chromosome 5 or 7, deletion of the del(5)(q), abnormalities of the chromosome 3. These abnormalities are more frequent in older patients and in patients with secondary AML, but even younger patients; the survival rate is less than 20 percent five years. They represent a considerable therapeutic challenge for which no current treatment approach including transplantation is
satisfactory. Between these two groups, the patients are characterized as having a standard (or intermediate) risk of relapse. The leukemic blasts of these patients have either a normal karyotype or cytogenetic abnormalities that are not included in the definition of the other subgroups. In some series this includes patients with cytogenetic abnormalities of 11q23, where as in others these patients are included in the unfavorable prognostic subgroup.

Patients who are older than 60 years generally have a poor prognosis; with a probability of survival at five years of less than 10%. The prognosis for AML is highly dependent upon the subtype. Age is a very strong predictor of survival in AML, predominantly due to the different subtypes of AML seen at different ages. Nevertheless, survival is more dependent upon the biological subtype (usually defined by the chromosome change or karyotype), than age alone. Patients with a complex karyotype at any age have a very poor prognosis with only 5-10% alive after 5 years. In general, study of cytogenetics has a major role in diagnosis, management and therapeutic advances in AML [Lowenberg B et al., 1999, Jabbour EJ et al., 2006].

**History of Cytogenetics:**

Cytogenetics is the study of the structure and properties of chromosomes, their behaviour during somatic cell division during growth and development (mitosis), and germ cell division during reproduction (meiosis), as well as their influence on phenotype. Cytogenetics also includes the study of factors that cause chromosomal changes. Initially, it was difficult to determine the diploid number of mammalian species because the chromosomes were crowded in metaphase. In the 1950s, several technical improvements, such as the addition of colchicines to arrest cells in metaphase and the use of hypotonic solution to obtain better chromosome spreads, were made. In 1956, the diploid number of chromosomes in man was established as 46 and the peripheral leucocyte culture method of Moorehead et al was adopted by many cytogeneticists. It was then possible to describe correctly the normal human chromosome number and chromosome abnormalities. The metaphase chromosomes were classified into seven groups based on the Denver classification [1960], with revisions at the London Conference [1963] and the Chicago Conference [1966]. Jau-
hong Kao et al described a chromosome classification based on the band profile similarity along the approximate medial axis [Thirumulu PK and Alwi ZB 2009].

**Short history of chromosome staining**

In 1970, T. Caspersson discovered the nucleobase-specific fluorochrome quinacrin. When stained with quinacrin, each chromosome will show a specific pattern of bands, the so-called Q-bands. These bands allowed for the first time the identification of all 22 human autosomal pairs and the two human sex chromosomes. In 1971, Sumner, Schned and Seabright discovered additional stains and preparation methods, which brought forth new banding types: G, R, T and C Bands. Dark and light bands show regions of close-packed and loose-packed DNA respectively.

**Types of banding:**

Cytogenetics employs several techniques to visualize different aspects of chromosome. **C-banding:** Giemsa binds to constitutive heterochromatin and thus stains centromeric part of the chromosome. **R-banding:** Ions tend to be heterochromatic, late-replicating and AT rich. The light regions tend to be euchromatic, early-replicating and GC rich. The dark the pattern of bands is very similar to that seen in **G-banding.** **T-banding:** visualize telomeres. **Q-banding:** A fluorescent stain Quinacrine used to find out unknown origin and a subtle or complex rearrangement. Giemsa is used to stain bands on the chromosomes. **G-banding:** G-banding is obtained with stain following digestion of chromosomes with trypsin. It yields a series of lightly and dark stained bands. The dark bands tend to be heterochromatic, late replicating and AT rich. The light regions tend to be euchromatic, early replicating and GC rich.

**Chromosome Structure:**

Depending on the staining technique, the dark bands can be rich in AT base pairs, and the white bands rich in GC base pairs. Other stains can produce the exact opposite pattern. Each chromosome has been arranged in pairs and in order of size. At one point along their length, each chromosome has a constriction, called the **centromere.** The centromere divides the chromosomes into two ‘arms’: a long arm ‘q’ and a short arm ‘p’ (**Figure 5**). The centromere is where mitotic spindles attach to
pull homologous chromosomes apart during mitosis. This form of the chromosomes is highly compact and only occurs during cell division. The ends of each chromosome are capped with a structure called a telomere, which is a repeating base sequence that stabilizes the ends.

Figure 5: Schematic diagram of chromosome showing chromosome arms and centromere.

A karyotype is an organized profile of a person's chromosomes. In a karyotype, chromosomes are arranged and numbered by size, from largest to smallest. Karyotype can be used for many purposes (i) To study chromosomal aberrations (ii) To study cellular function, taxonomic relationships, (iii) To gather information about past evolutionary events etc. Normal male karyotype is 46,XY (Figure 6) and normal female karyotype is 46,XX (Figure 6). Karyotype result can be described as; total number of chromosome including sex chromosomes, followed by a comma (,), the sex chromosomes are given next e.g. 46,XY, number of metaphase plates analysed are written in [], If there is an abnormality of autosomes, that is specified next e.g. 46,XY,t(9;22). If there is sex chromosomal aberration, it is written first. In addition if autosomal abnormalities are noted they are written next in numerical order e.g. 47,X,t(X;13)(q27;q12). Loss of sex chromosome (Turners syndrome) - 45,X-. Gain of chr.21 in Downs syndrome- 47,XX,+21. In a mosaic cell-line, both the cell lines are separated by a slash(/) e.g. 46,XY,t(9;22) /46,XY. In addition, symbols are used in rearrangements. Symbol is placed ahead of the chromosome involved and the
involved chromosome is written in the parenthesis e.g. 46,XX,r(20) means a female karyotype with a ring form of chromosome 20 [Shaffer LS et al., 2009].

**Figure 6:** *G banded karyotype of normal male  G banded karyotype of normal female*

<table>
<thead>
<tr>
<th>Chromosome aberrations:</th>
<th>Chromosome aberration origin may be either paternal, maternal or may be from combination of both chromosomes. Mechanism of chromosome aberrations may be non disjunction or rearrangement during meiotic, premeiotic, post meiotic stage. Chromosomal aberrations are grouped as structural abnormalities and numerical abnormalities they are briefly summarized below (Figure 7);</th>
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<td>19 20 21 22 X Y</td>
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Translocation: Translocation is one of the most significant structural chromosome rearrangements; which occur when chromosomes break and the fragments rejoin to other chromosomes. It is denoted as t( ; ).

Robertsonian translocation: This translocation involves the reciprocal transfer of the long arms of two of the acrocentric chromosomes; 13, 14, 15, 21 or 22. A reciprocal transfer of the whole long or short arms close to the centromere. A relatively common in meiosis, a trivalent is formed.

Insertions: One portion of a chromosome is inserted into another chromosome. Here genetic material is not swapped. It is just moved to another chromosome. It is denoted as ins.

Inversions: Inversion involves only one chromosome in which two breaks occur and in the process of repair, the intervening segment is rejoined in an inverted or
opposite manner. There is neither loss nor gain of chromosomal material, so inversion carriers are normal. It is denoted as *inv*. An inversion is *paracentric* if the inverted segment is on the long arm or the short arm and does not include the centromere. The inversion is *pericentric* if breaks occur on both the short arm and the long arm and the inverted segment contains the centromere.

**Deletions:** Deletion refers to the loss of a segment of a chromosome. This can be terminal (close to the end of the chromosome on the long arm or the short arm), or it can be interstitial (within the long arm or the short arm). Deletions have been described on all chromosomes. Deletions are expected to be more clinically severe than their counterpart, duplications. It is denoted as *del*.

**Duplications:** Duplication refers to an extra chromosomal segment within the same homologous chromosome or an extra chromosomal segment on another nonhomologous chromosome. The clinical findings are highly variable depending upon the chromosomal segments involved. It is denoted as *dup*.

**Other structural abnormalities:** There are other rarer forms of structural chromosome abnormalities such as rings, insertions, isochromosomes and markers. In some cases these abnormalities lead to duplication of chromosome material. In other cases, such as ring chromosome a deletion occurs.

**Ring chromosome:** A ring chromosome is a chromosome whose arms have fused together to form a ring. Normally, the ends of a chromosome are lost, enabling the arms to fuse together. Ring formation can also occur with only one end being lost. It is denoted as *r*.

**Isochromosome:** One of the arms is deleted and the other arm is duplicated, denoted as *i*.

**Complex Translocation:** If more than two chromosomes involved in translocation it forms complex translocation.

**Marker chromosome:** A marker chromosome is abnormal, is morphologically different from the known chromosomes of the genome. It must have a centromere.
and it is derived from breakage of a chromosome with loss of the acentric fragment and nondisjunction from its homolog during meiosis. Markers can also be isochromosomes or normal chromosomes with unusual heteromorphic variants such as inversions or extra heterochromatin. It is denoted as mar.

**Numerical abnormalities:** Numerical abnormalities are one type of chromosome abnormality. These types of birth defects occur when there is a different number of a chromosome in the cells of the body than is usually found. It is called as constitutional abnormality. So, instead of the usual 46 chromosomes in each cell of the body, there may be 45 or 47 chromosomes.

**Trisomy:** The term "trisomy" is used to describe the presence of three chromosomes, rather than the usual pair of chromosomes.

**Monosomies:** The term "monosomy" is used to describe the absence of one member of a pair of chromosomes, as a result there is a total of 45 chromosomes in each cell of the body, rather than 46 [http://www.healthsystem.virginia.edu/uvahealth/peds_genetics/numeric.cfm].

**Ploidy aberrations:** When cells carry complete extra sets of chromosomes, this is called polyploidy. When there are one extra complete set and sixty-nine chromosomes in total, then this is known as triploidy. Two extra sets of chromosomes and ninety-two chromosomes in total called tetraploidy. When individual whole chromosomes are missing or extra, this is called aneuploidy. This can happen with any of the autosomal chromosomes (1 to 22) or the sex chromosome (X or Y) [http://www.cafamily.org.uk/medicalinformation].

**Clonal Evolution and Chromosome Evolution:** According to the International System for Chromosomal Nomenclature (ISCN 2009) a clone is recognized if three or more cells have the same missing chromosome or two or more cells with the same additional chromosome or structural abnormality. The reason for the difference in number of cells needed to define a clone with a missing chromosome (monosomy) versus one with trisomy relates to the possibility in cytogenetic preparation of overspreading of chromosomes and hence artifactual "missing" chromosomes. If one
of the first 20 cells showed a particular aberration likely related to the cancer, a count of additional cells can be initiated or molecular cytogenetic methods used to confirm the presence of a clone. In any case, once a clone is identified this can provide a baseline for diagnosis and prognosis and also for follow-up study of relapse.

**Secondary changes:** The aberrations which are present in addition to specific primary abnormalities within the same clone, or in a second related clone, are called secondary changes and are observed in ~45% of all AML patients. These aberrations may already be present at diagnosis or may appear for the first time at relapse. In general, they are not restricted to leukemia of the myeloid lineage or even to haematopoietic malignancy.

**Molecular cytogenetics: FISH and M-FISH:**
Chromosome banding is still the gold standard for all routine techniques of clinical and tumor cytogenetics; however there are several technical restrictions. As chromosomes morphology combined with a black and white banding pattern are the only two parameters to be evaluated, changes exclusively within the normal pattern such as size variations in a chromosomal band or the chromosome itself and changes of the centromere index can be detected. Thus the origin of additional material in a structurally altered chromosome often remains questionable. To overcome such limitations fluorescence in situ hybridizations (FISH) approaches were introduced into cytogenetics in the 1980s and the new field of molecular cytogenetics was established. In recent years many progress of this area has been the invention of multicolor FISH (M-FISH). M FISH assays are nowadays indispensible for a precise description of complex chromosomal rearrangements and research. Since then different approaches for chromosomal different ion based on M—FISH assays have been described. Predominantly they were established to characterize marker chromosomes, which have high clinical impact and is a requisite condition for further molecular investigations and aimed at the identification of disease related genes [Karney L 2006].
Fluorescence In Situ Hybridization: *In Situ* Hybridization (ISH) was first introduced in 1969 independently by Gall and Pardue [1969], Nardelli B and Amaldi [1969], and John et al. [1969]. Radioactively labeled RNA was used to detect DNA *in vivo*. Tritium was incorporated into proliferating cells in order to label the newly synthesized nucleic acids. While the labeling techniques and probe design have changed slightly, the basic technique of ISH remains the same today.

A large number of cells can be studied by FISH as interphase nuclei can also be analyzed. This helps in the detection of minimal residual disease, assessment of the rate of cytogenetic remission and detection of disease recurrence [Amare et al., 2001]. Another advantage of FISH is that it can detect subtle translocations like t(15;17) which cannot always be detected by conventional cytogenetics. The FISH test is thus rapidly gaining importance as a useful diagnostic tool in oncology, as more probes are now available for chromosome abnormalities [Madon P et al., 2003]. The identification of inv(16)/t(16;16) can be difficult by conventional cytogenetics, especially on suboptimal G-banded chromosomes and in cases with a masked inv(16) by translocation. The FISH technique has been proved helpful in identifying such subtle abnormalities [Aventin A et al., 2002].

Conventional cytogenetic analysis is not always informative, especially in cases of cryptic translocations leading to *AML1/ETO, CBFB/MYH11* and *MLL* fusion transcript. In cases of normal or failed cytogenetics, FISH for *MLL* fusion transcript is recommended. FISH for monosomy 7 detection is also recommended in pediatric patients, due to the prognostic value of this aberration [Morra E et al., 2009].

**Multicolor FISH:** The development of molecular cytogenetic techniques based around FISH heralded a significant advance in the accuracy of cytogenetic analysis and has provided many tools for research in both clinical and tumor cytogenetics [Speicher and carter 2005]. The achievement of 24 color FISH based karyotyping (MFISH, SKY, Cobra) [Schrock et al., 1996; Speicher et al., 1996; Tanke et al., 1999], was the culmination of further technological advances in this technology and has been one of the great success of molecular cytogenetics in the past decade. This technology has progressed even further with chromosome arm specific [Speicher et

Complex chromosomal aberrations (CCA) can be detected in a substantial proportion of MDS and AML, which are associated with very poor prognosis. Conventional cytogenetics cannot accurately define the specific alterations in CCA. Multiplex fluorescence in situ hybridization (M-FISH) allows the comprehensive identification of CCA. M-FISH could refine CCA, find or correct the missed or misidentified aberrations by Conventional cytogenetics analysis [Xu W et al., 2010]. Till date, more than 600 cases of AML and MDS have been analyzed by M-FISH technique [Tchinda J et al., 2003].

**Significance of cytogenetic studies in AML:** The significance of cytogenetic studies of AML is twofold. First, cytogenetics has significantly increased our knowledge of the basic genetic mechanisms involved in leukaemogenesis thus contributing to our understanding of the remarkable histopathological, immunophenotypic and clinical heterogeneity of AML. The discovery of several recurring chromosome aberrations has not only supported the notion that leukaemia is a genetic disease at the cellular level but also has led to mapping and cloning of genes whose disruption and/or deregulation contribute to the neoplastic process. Secondly, chromosome aberrations, irrespective of whether they have been characterized molecularly or not, have been shown to constitute tumour markers of diagnostic and prognostic value. In the new WHO classification of haematological malignancies, specific cytogenetic abnormalities have been used to help define distinct disease entities among myeloid disorders. Furthermore, cytogenetic results have been repetitively shown to be among the most important, independent prognostic factors in AML.

In clinics the diagnostic cytogenetics provides one of the most valuable prognostic indicators in AML. Multiple recurring chromosomal aberrations have been identified, and in many instances, genes altered by these aberrations have been mapped and cloned. Further characterization of the genes rearranged by AML-associated translocations and inversions has provided insights into the mechanisms of leukemogenesis and will likely facilitate designing of novel therapeutic strategies that
target particular genetic abnormalities in leukemic blasts. In addition, acquired
cytogenetic abnormalities, whether characterized at the molecular level or not, have
been shown to represent tumor markers of diagnostic and prognostic importance.
Many recurrent aberrations have been correlated with presenting hematologic and
morphologic parameters. Selected chromosomal aberrations, and their molecular
equivalents, are now being used to help define distinct disease categories within AML
in the new WHO classification of hematologic malignancies. Moreover, karyotypic
findings at diagnosis have been repeatedly shown to be among the most significant
independent prognostic factors regarding AML [Mrozek K 2000].

When chromosome abnormalities occur in a single cells they can be ignored, as
random changes as occasionally arise in all types of cells, but if it is found in a single
cell which is known to be associated with a particular disease type (for example
t(15;17)) in a case of suspected or known APML. If abnormalities remain as single
cell findings, they should be reported with an explanation on the report, but will
require confirmation before being considered as a genuine abnormal clone. Prof.
T.C.HSU quoted that;“Each competently analyzed chromosomal aberration in cancer
cell should be placed on record to contribute to information pool”.

Cytogenetic risk groups: Recent large collaborative studies have proposed
cytogenetic-risk systems classifying patients into favorable, intermediate and adverse
risk groups (Table-2) according to the specific karyotype findings at diagnosis
[Grimwade D et al., 2009].

Table- 2: Cytogenetic Risk group.

<table>
<thead>
<tr>
<th>Category</th>
<th>Abnormality</th>
</tr>
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<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21), t(15;17), inv(16) with or without other abnormalities</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Normal, +6, +8, +21, +22-Y, del(9q), abn(11q) t(9;22), other numerical and structural abnormalities</td>
</tr>
<tr>
<td>Adverse</td>
<td>complex karyotype, -5/del(5q), -7/Del(7q), abn(3q) t(6;9), 20q or 21q, abn(17p),</td>
</tr>
</tbody>
</table>
1st Cytogenetic Risk group-Favorable group

This group includes t(8;21), t(15;17), inv(16) with or without other abnormalities. In this type of chromosomal abnormalities patients are considered to be having favorable prognosis or low risk. For conveniences of presentation and analysis t(8;21) is considered as 1st chromosomal abnormality in favorable risk group, t(15;17) as 2nd chromosomal abnormality in favorable risk group and inv(16) as 3rd chromosomal abnormality.

Chromosomal anomaly in favorable risk group:

(i) t(8;21)(q22;q22): The translocation t(8;21)(q22;q22) was first described by Rowley in 1973. The t(8;21) is relatively easy to detect, even if the quality of chromosome preparations is suboptimal (Figure 8). The annual incidence of t(8;21) is 1of 10^6. It comprises 10% of AML and is a recurrent aberration, preferentially appearing in the FAB M2 in about 40% of cases and is one of the most common examples of association between a non-random reciprocal translocation and a specific subtype of AML, and less frequently in M1 or M4. In childhood AML, this aberration is the most prevalent karyotypic anomaly, with an incidence of about 12% [Yoo SJ et al., 2005, Gamerdinger U et al., 2003]. The t(8;21) occurs in approximately 15% of AML in adult patients [McNeil S et al., 1999]. Relapse occurs in about 35% of patients, especially in the first two years of remission. Furthermore; the relapse rate in older patients is up to 84% [Yoo SJ et al., 2005].

Figure 8: Representative G banded partial karyotype of t(8;21) and Ideogramatic explanation

![Figure 8: Representative G banded partial karyotype of t(8;21) and Ideogramatic explanation](image)

The translocation leads to the fusion of the AML1 (RUNX1) and ETO (CBFA2T1) genes on the derivative chromosome 8 i.e. der(8), and patients positive for the
chimeric gene are known to have a favorable prognosis [Grimwade D. 2001]. Therefore, the translocation is highly important for diagnosis and therapy [Gamerdinger U et al., 2003]. In most instances, leukemogenesis in AML results from gene fusion, when segments from two different genes are fused together to give rise to a chimeric structure consisting of the 5' end of one gene and the 3' end of another [Caligiuri MA et al., 1997].

**AML1:** *AML1* is located on 21q22.3 also known as *RUNX1* (runt-related transcription factor). This gene spans a region of more than 120kb. Transcription is from telomere to centromere. It is widely expressed including hematopoietic cells at various stages of differentiation and role in haematopoiesis. It functions as transcription factor for various hematopoietic specific genes [URL: http://AtlasGeneticsOncology.org/Genes/AML1.html].

**ETO:** *ETO* gene is also known as *RUNX1T1* (runt-related transcription factor 1 and as *MTG8* (myeloid translocation gene on 8). It is situated on 8q22. It is mainly expressed in the brain, and not in the hematopoietic cells. It functions as a putative transcription factor in agreement with the fact that both genes are transcribed from telomere to centromere 5' AML1-ETO [URL: http://AtlasGeneticsOncology.org/Genes/ETO.html].

**AML- ETO fusion:** The *AML1-ETO* fusion gene is a distinct type of AML generally associated with a favourable prognosis. AML with the t(8;21)(q22;q22) conventional cytogenetics may overlook the rearrangement in patients with hidden aberrations. By routine karyotyping, the t(8;21) translocation is detected in 6-8% of all AML cases and in 20-30% of AML-M2.

**Secondary changes with t(8;21):** Additional anomalies in favorable risk group include numerical in 2/3, and structural anomalies in 1/3 of patients. Loss of Y or X found in 50% of cases, (1X must be present), del (9q) or -7 +8, del(9)(q) are found in 10% of each cases. t(8;21)(q24;q22) that is fusion of *AML1-ETO* found mainly in AML-M2 and complete remission obtained in most of the patients, median survival is (1.5-2 yrs). Additional anomalies which frequently found are loss of Y (39.3%) or X (16.3%) chromosome, del(7q)/-7, del(9)(q) (12.7%); complex t(8;21;var) are known and have revealed that the crucial event lies on der(8) [Bakshi SR et al., 2004].

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**Loss of Sex chromosome:** t(8;21) is often accompanied by the loss of a sex chromosome. This association is noteworthy because sex chromosome abnormalities are otherwise rarely observed in AML. Loss of sex chromosome in association with the t(8;21) was initially considered to denote a poorer prognosis [Diane Roulston and Michelle MLe Beau]. Approximately 30–50% of the t(8;21) cases are accompanied by the loss of a sex chromosome [Gamerdinger U et al., 2003]. 70% of the males with the t(8;21) were missing the Y chromosome and 60% of female were missing X chromosome [Fourth International workshop of Association of cytogenetic technologist].

**Del 9q:** Deletion of 9q is the most frequent structural additional abnormality in patients with AML associated with t(8;21) and is found in nearly 10% of the cases. The outcome reported in the literature for AML patients with t(8;21) and del(9q) is controversial. Schoch and co-workers [Schoch C et al., 1996] showed a significantly shorter median overall survival in patients with t(8;21) and del(9q) compared with patients without this abnormality. In contrast, Rege and co-workers, demonstrated a slightly better outcome in patients with t(8;21) and del(9q), measured by the probability of event-free survival [Huang L et al., 2006].

**Variants of t(8;21):** Variants of the t(8;21)(q22;q22) involving chromosome 8, 21, and other chromosomes account for about 3% of all t(8;21)(q22;q22) in AML patients [Vundinti BR et al., 2008]. Complex t(8;21;Var) involving a (variable) third chromosome, part from chromosome 21 goes on der(8), part of the 8 on der(var), and part of var on der(21); therefore, the crucial event lies on der(8) [Udayakumar AM et al., 2008]. It was estimated that variant translocations have a much lower frequency than the classical t(8;21) and was about 6–10%. The clinicopathologic features of AML with variant t(8;21) are less well characterized, and their clinical significance is controversial. Some investigators have reported a favorable outcome for patients with AML with variant t(8;21) [Hagemeijer A et al 1984, Ishii Y et al 2005], whereas others have reported that patients who have AML with variant t(8;21) have a worse prognosis [Huang L et al., 2006].

There are different partners of *AML1* gene, in which t(8;21) and t(3;21), *AML1* is interrupted by the chromosomal breakpoint, and the 5' section of *AML1*, is fused to a
partner gene. The partner gene is \textit{ETO}, in the t(8;21), and in the t(3;21) it can be \textit{EAP, MDSI,} or \textit{EVII}. The complex fusion event in the t(3;21) is an important component of the leukemogenesis process is supported by the detection of multiple fusions in all of the t(3;21) patients studied [Nucifora G and Rowley JD 1995].

\textbf{(ii) t(15;17)(q22;q22)}: Second cytogenetic abnormalities mainly observed in AML is t(15;17) in APML. The cytogenetic hallmark of the vast majority of APML is defined by the reciprocal translocation of t(15;17)(q22;q22) (\textbf{Figure 9}). The promyelocytic leukemia (\textit{PML}) gene present on chromosome 15q22 and the retinoic acid receptor \(\alpha\) (\textit{RAR}\(\alpha\)) gene present on chromosome 17q22 with a resultant \textit{PML-RAR}\(\alpha\) fusion gene. This fusion gene is detected in more than 70\% of APML cases. t(15;17) is never found in other subtypes of AML, except in rare blastic promyelocytic leukemic transformation of a CML [Scolnik MP et al., 1998]. Diagnosis of APML requires demonstrating the t(15;17) with conventional karyotype, FISH, and/or detection of the \textit{PML-RAR}\(\alpha\) transcript by reverse transcriptase–polymerase chain reaction (RT-PCR) [Wang HY et al., 2009].

Approximately 10\% of cases with morphologically typical APML have other cytogenetic abnormalities or a normal karyotype. In majority of such cases a cryptic rearrangement of \textit{PML} and \textit{RAR}\(\alpha\) are reported as result from insertion events or more complex rearrangements [Reitera A et al., 2004]. With cytogenetic analysis results can be obtained in 48 hours approximately, but only 70-90\% of cases display the t(15;17) translocation. This is probably due either to interpretation problems in evaluating poor quality metaphases, or to the possible occurrence of microtranslocations. In some cases t(15;17) may be accompanied by other karyotypic changes such as trisomies [Berger R et al., 1991].

\textit{Figure 9: Representative partial karyotype of t(15;17)(q22;q22) and ideogramatic explanation}
**PML-RARA fusion:** In the t(15;17)(q22;q22) the breakpoint on chromosome 17q is always located in the second intron of RARa. Due to heterogeneous breakpoints on chromosome 15, different PML portions are retained in the 5' region of PML-RARa, and this produces different fusion gene products in which the 3' RARa portion is always constant [Pandolfi PP, 1992]. These PML-RARa fusion proteins disrupt the function of RARa which blocks the normal maturation of granulocytes. The chromosomal translocation involving RARa is believed to be the initiating event; additional mutations are required for the development of leukemia. PML gene is situated at 15q22. It founds in approximately 98% of all cases of APML [Grignani F et al., 1994]. It is express in a wide variety of tissues. In hematopoietic tissue, expression apparently restricted to myeloid precursors. Its function is unknown to date; putative transcription factor; in conjunction with other proteins included in the PML oncogenic domains, it would play a role as tumor suppressor and in apoptosis.

The reciprocal RARa-PML fusion generated in t(15;17) is present in 70% to 80% of APML cases. There is no difference in ATRA sensitivity or clinical outcomes of patients who do or do not harbor the RARa-PML fusion. Patients with prolonged remissions of APML may express the RARa-PML fusion and not the PML-RARa fusion suggesting that the small number of cells that harbor these genes may not have leukemogenic potential. In general, RARa-PML does not appear to be required for the pathogenesis of APML [Melnick A and Licht JD 1999].

**Variants of RARa gene:** Cases of APML lacking the prototypic t(15;17)(q22;q12) translocations have been rarely described [Melnick A and Licht JD. 1999]. For example, the RARa gene fused to the promyelocytic leukemia zinc finger gene (PLZF) on chromosome 11q23 [Licht JD et al., 1995], the nuclear matrix associated gene (NuMA) on chromosome 11q13 [Wells RA et al., 1996] and nucleophosmin (NPM) on chromosome 5q32 [Redner RL, et al., 1996] have been reported. APML cases involving NPM or NuMA are sensitive to retinoids; APML with the PLZF-RARa rearrangement, on the other hand, is worth noting because it is resistant to ATRA as well as Arsenic Trioxide (Figure 10) and is associated with a relatively poor prognosis [Kitamura K et al., 2000, Sainty D et al., 2000].
Figure 10: The four chromosomal translocations associated with APML. The t(11;17) APML syndrome linking PLZF and RARa is unique among these forms of APML in its resistance to differentiation therapy with ATRA or conventional chemotherapy.

\[ t(5;17)(5q35;17q12) \] translocation. (NPM-RARa) fusion: NPM (nucleophosmin, protein) is a major nonribosomal nucleolar phosphoprotein which is significantly more abundant in tumor and growing cells than in normal resting cells. NPM is not strictly confined to the nucleolus but shuttles like PML between the nucleus and the cytoplasm. NPM has the capacity to oligomerize [Kalantry S 1997]. NPM is expressed in myeloid and lymphoid cell lines. The regulation of its expression in bone marrow hemopoietic precursors or during myeloid and lymphoid terminal differentiation is unknown. Because of its involvement in three different translocations associated with APML, MDS and AML. NPM appears to be a promiscuous partner in translocations specifically associated with lymphohemopoietic tumors [Pandolfi PP 1996].

\[ t(11q23;17q12) \] PLZF-RARa fusion: The RARa gene fused to the promyelocytic leukemia zinc finger gene (PLZF) on chromosome 11q23. Similar to PML-RARa, PLZF-RARa has the capacity to heterodimerize with PLZF. This protein therefore retains the potential capacity to bind DNA, and it could play a significant role in APML leukemogenesis. Patients harboring t(15;17) are uniquely sensitive to treatment with ATRA, which yields complete remission rates of 75% to 95%. They are also highly responsive to conventional chemotherapy. However, APML associated with t(11;17)
shows a distinctly worse prognosis, with poor response to chemotherapy and little or no response to treatment with ATRA [Licht JD et al., 1995].

**T(11;17)(q13;q12)NUMA-RARα fusion:** The *NUMA* gene, located on chromosome 11q13. The gene encoding the *NUMA* protein is the newest reported fusion partner of *RARα* in APML and the first mitotic apparatus protein found to be genetically rearranged in a human malignancy. *NUMA* is also a structural unit of the interphase nucleus. *NUMA* may play a role in apoptosis.

**Secondary Chromosomal changes of t(15;17)(q22;q22)**

**Trisomy 8:** About 30% show trisomy 8 and other additional abnormalities can also occur, particularly in relapse (Figure 11). It remains unclear whether secondary changes occurring at diagnosis are of any prognostic significance [Rooney DE and Czepulkowski BH. 3rd Ed. Page 48].

**Isochromosome 17:** The formation of an isochromosome from the chromosome 17 derivative of t(15;17) is also occasionally seen (Figure 11).

*Figure 11: Representative partial karyotype of trisomy 8 and iso(17)(q10)*

(iii) inv(16)/t(16;16)(p13;q22) : inv (16), and its less common variant t(16;16), are found in 7% of adults with de novo AML in 1982 and are associated with a favorable prognosis and characteristic morphology with relatively good long-term, disease-free survival (DFS) [Paschka P 2008]. Both inv(16)(p13;q22) and t(16;16) involve the *CBFβ* gene, encoding subunit of *CBF*, that is fused with the *MYH11* (myosin heavy chain) gene. Patients with inv(16)/t(16;16) have a unique marrow morphology with the presence of abnormal eosinophils (AML-M4Eo) [Mrozek K and Bloomfield CD 2008]. Arthur and Bloomfield first reported on structural alterations of chromosome 16 referred to as del(16)(q22) in AML patients with marrow eosinophilia which was reclassified as having inversion of chromosome 16.

*Figure 12: Representative G banded partial karyotype of inv(16)(p13q22)(upside) and t(16;16)(downside) and ideogramatic explanations of inv(16)*
**CBFB-MYH11 fusion gene:** The resultant fusion product generates a chimeric fusion gene *CBFB-MYH11* which exerts a dominant-negative effect on the core binding factor transcription factor complex, resulting in a block in myeloid differentiation [Kundu M and Liu PP. 2001]. The *CBFa2/CBFB-MYH11* complex also acts as a transcriptional repressor [Hiebert SW and Lutterbach 2001]. Expression of the *CBFB-MYH11* chimeric protein alone is, only, not sufficient for leukemogenesis and additional mutations may be needed to lead to the development of AML [Kundu M and Liu PP. 2001], and AMLs carrying *CBFB* alteration exhibit some specific characteristics. inv(16) is a subtle rearrangement (Figure 12) that can sometimes be overlooked by less experienced cytogeneticists, especially in preparations of suboptimal quality. Occasionally, an inv(16) may be misinterpreted as del(16)(q22) [Mrozek K et al., 2001].

**Secondary Change of inv(16):** Cytogenetically, the *CBFB-MYH11* rearrangement may be associated with trisomy 8, 21, and 22 and less frequently with deletion of the long arm of the chromosome 7 [Byrd JC et al., 2002].

**Trisomy 8:** Trisomy 8 is also found as a secondary change of inv(16) [Litmanovich D 2000].

**Trisomy 22:** Grois and associates [1989] demonstrated that a number of patients with AML-M4 who were initially thought to have trisomy 22 as the only chromosomal abnormality had in fact an undetected inv(16)(p13q22) as the primary aberration. Trisomy 22 has been correlated with FAB M4. It is the most common secondary abnormality in patients with inv(16)(p13q22) and is rare in patients with other
primary aberrations. Thus, it is likely that cases initially found to have a solitary trisomy 22 may also harbor an undetected inv(16)(p13q22), suggesting that correlation of FAB M4 with inv(16)(p13q22) may be higher than previously reported. A more recent study has suggested that the presence of the \textit{CBFβ-MYH11} fusion gene correlates AML with abnormal eosinophil differentiation, irrespective of whether the AML is classified as M4Eo or as other FAB subtypes [Marcucci G et al., 2000]. The favorable impact of trisomy 22 in inv(16) AML also has been reported independently by others [Paschka P 2008].

\textbf{Variants of inv(16)}: Rare variants are t(1;16)(p31-32;q22), t(3;16)(q21;q22), t(5;16)(q33;q22) and are associated with eosinophils anomalies [URL: http://AtlasGeneticsOncology.org/Anomalies/inv16.html].

\textbf{2\textsuperscript{nd} Risk group-Intermediate Risk Group}: The intermediate-risk cytogenetic group includes patients with normal karyotype; t(9;22), 11q23 rearrangements and other numerical and structural abnormalities. Survival rate of patients are usually lower than those of adequately treated patients with t(8;21), inv(16) or t(15;17), but higher than patients with unfavorable chromosome aberrations [Mrozek et al., 2004].

\textbf{(i) Normal Karyotype}: The presence of an exclusively normal karyotype in a diagnostic marrow sample does not mean, however, that cytogenetically normal leukaemic blasts harbour no acquired genetic alterations. AML patients with an apparently normal karyotype may be positive for a leukemia-associated gene fusion resulting from a chromosome rearrangement involving segments smaller than the length of a single band that is thus unrecognizable by standard cytogenetic or SKY analysis [Mrozek K et al., 2001].

\textbf{(ii) T(9;22)}: T(9;22) was observed in 3% of AML patients mostly in AML-M1, AML-M2; 1% in childhood AML. Prognosis is very poor. BCR/ABL has a cytoplasmic localization, in contrast with ABL, mostly nuclear; this may have a carcinogenetic role. The hybrid protein has an increased protein kinase activity compared to ABL. Blast crisis is sometimes at the first onset of CML and those cases may be undistinguishable from true AMLL with t(9;22) and p210 BCR/ABL hybrid. The Ph
chromosome is found in 1-2% of adults with AML. The presence of the Ph chromosome in acute leukaemia carries a poor prognosis [Huret JL 1997].

(iii) 11q23 rearrangements: 11q23 rearrangements include translocations, inversions and insertions with a number of partner sites. Many of these have been shown to disrupt a gene located at 11q23 known as MLL (also known as ALLI, HTRX, HRX or TRX1). This is a recurring rearrangements and/or interstitial deletions mainly observed in both ALL and AML, especially acute monoblastic leukemia (AML-M5) and acute myelomonocytic leukemia (AMML-M4) [Rowley JD.1990, Bitter et al., 1987]. Numerical abnormalities of chromosome 11, such as trisomies or tetrasomies, also result in additional copies of the MLL gene. Despite the large variety of rearrangements involving the MLL gene, the overall prognosis of AML with this abnormality is unfavourable. Therefore, detection of MLL disruption or amplification is much needed for treatment decision. Studying the wide variety of fusion genes involving MLL could also lead to a better understanding of leukemogenesis [De Braekeleer M et al., 2005].

**Distribution of MLL in different FAB subtypes and incidence:**
11q23 rearrangement observed in different percent with AML subtypes. 50% cases in AML-M5, 20% in M4, 10% in M1 or M5 each, 5% in M2. In infant <1 yr was observed more frequently [Rowley JD. 1993]. It is observed equally in both children and adults. Male to female ratio is 0.9. In normal karyotype: partial tandem duplication (in situ) of MLL is present [Huret JL 2003].

**MLL gene:** The MLL gene is expressed at high levels in differentiated myeloid cells, at low levels in stem cells and lymphocytes, but not in erythrocytes. HOX genes are a major group of transcription factors, playing a role in the early stages of development and hematopoietic differentiation, as well as in the later stages of hematopoietic differentiation with a specific pattern of expression in different lineages at various differentiation stages. Translocations involving band 11q23 usually lead to a breakage in the MLL gene. The 5’ part of the MLL gene is retained on the derivative chromosome 11 where it is fused with the 3’ part of the partner gene (Figure 13). Therefore, the active fusion gene (5’ MLL-3’partner) is almost always located in the der(11), except in rare cases of insertion of the 5’ MLL in another chromosome [De Braekeleer M et al., 2005].
Partners of MLL gene: MLL rearrangements are highly variable, because of the great number of different partner genes and the diversity of mechanisms that generate MLL fusion genes with malignant potential. To date, approximately 91 different MLL rearrangements have been described (Figure 14). In more than 55 of them, the MLL partner gene has been identified, of which the most common are AFF1 (alias AF4), MLLT3 (alias AF9), MLLT10 (alias AF10), MLLT4 (alias AF6), MLLT1 (alias ENL, LTG19), and ELL [Sarova I et al., 2009]. In pediatric AML, the main translocations are: t(9;11), t(11;19); t(6;11) and t(10;11) accounting for almost 15-20% of the cases [Balgobind BV. et al., 2009].
It is also reported that 11(q23) might involve genes other than MLL [Giugliano E et al., 2002] and that conventional cytogenetics can barely discriminate between true 11q23 rearrangements clustering within the 11q22~25 region without MLL involvement [Cox MC et al., 2004]. Five translocations account for approximately 80% of MLL rearrangements: t(4;11)(q21;q23), AFF1/MLL; t(6;11)(q27;q23), MLLT4/MLL; t(9;11)(p22;q23), MLLT3/MLL; t(11;19)(q23;p13.1), MLL/ELL; and t(11;19)(q23;p13.3) [Keefe JG et al., 2010].

+11: It is found in 1% of AML and MDS as well; M1, M2, and M4; therapy related AML; MDS evolving towards AML; partial tandem duplication (in situ) of MLL; visible dup(11q) also occur. This trisomy reflects poor prognosis.

t(6;11)(q27;q23): This translocation is found in 5% of cases; mostly; children and young adults; it is male predominance and the gene involved in 6q27 is AF6 play role in signal transduction. This translocation has poor prognosis.

t(9;11)((p23;q23): It represent in 1/4 of cases; found in AML mainly in M5 (70%), or M4 (10%); the gene involved in 9p22 is AF9, a transcription activator [Huret JL et al., 1997]. The MRC and the Southwest Oncology Group classify the risk for patients
with AML with t(9;11) as intermediate and poor, respectively. Some clinical trials reported that patients with t(9;11) fared better than patients with v11q23 [Byrd JC et al., 2002, Mrozek K et al., 1997], whereas other studies failed to identify differences [Tanaka K et al., 2001, Grimwade D et al., 1998]. These discrepancies probably reflect the marked biologic heterogeneity of 11q23 aberrations. Many v11q23 translocations are rare translocations, the clinical impact of specific single variants is difficult to extrapolate, even from large studies on 11q23 [Cox MC et al., 2004].

**t(10;11)(p12;q23):** It is found in 5% of cases frequently in AML-M4 or AML-M5; from infants and children to (rare) adult cases; the gene involved in 10p12 is AF10, a transcription activator. This translocation has worse prognosis [Huret JL 2003].

**t(11;19)(q23;p13.1):** It is found in 5% of cases mainly in M4 or M5 de novo and therapy related AL; mainly adults are affected; the gene involved in 19p13.1 is ELL, a transcription activator. This translocation associated with worse prognosis [Huret JL 2003].

**t(11;19)(q23;p13.3):** It is found in 5% of cases; ALL, biphenotypic AL and AML (M4/M5 mainly); therapy related AL; T-cell ALL at times, these T-cell cases are the only cases of t(11;19) with an excellent prognosis, mostly found in infants (half cases), and other children (altogether 70%), or young adults. The gene involved in 19p13.3 is ENL, a transcription activator [Huret JL 2003].

**(iv)**Numerical abnormalities observed in intermediate risk group

*Trisomies:*
Trisomy 8 is the most common abnormality. Trisomy or partial trisomy 1q is a ubiquitous finding both in hematological disease and solid tumors; In myeloid disorders it is most often seen in Myeloproliferative disorder (MPD), but when seen in AML in association with der(1;7). Other recurrent trisomies are 4, 6, 9, 11, 13, 14, 15, 19, 21 and 22. Trisomy 4 is associated with AML-M4 but the others are more widespread and are also encountered in chronic disease, indeed +9 and +14 are...
typical of MPD and MDS. Trisomy 11 usually seen in acute leukemia and is likely to be associated with $MLL$ rearrangement. Most instances of +13 are in undifferentiated or minimally differentiated leukemia. Trisomy 15 is an occasional finding in MDS but has also been seen in patients without confirmed hematological disease. Trisomy 21 is not characteristic of any disease type and like trisomies of chromosome 19 and chromosome 9 is often a feature of chromosome evolution (Figure 15).

*Figure 15: Representative Partial karyotype of different trisomies.*

**Trisomy 8:** A number of chromosomal abnormalities have been identified in AML which are closely associated with distinct morphological and clinical subsets of this disease. Among these abnormalities, trisomy 8 (+8) is the most frequent numerical aberration in AML, occurring at a frequency of 10-15%. It is frequent in myelodysplasia and myeloproliferative syndromes. When present, it exists as the sole anomaly in 40% of AML patients, in combination with simple chromosomal changes in 35%, and as a component of a complex karyotypic picture in the remaining 25% of AML patients. It occurs either as the sole anomaly or together with other clonal chromosome aberrations. Only few studies considered the cytogenetic heterogeneity of the cases showing that prognosis in presence of trisomy 8 appeared to be dependent on the other associated clonal cytogenetic changes [Jaff N et al., 2007].

**Trisomy 4:** Sole trisomy 4 is a rare chromosomal abnormality associated with a specific subtype of AML and secondary (treatment related) AML with myelomonocytic morphology. It has been found with the same frequency in the M1-M2 and M4 FAB subtypes. It is generally found in association with $t(8;21)$ and it is a recurrent abnormalities and has no significant prognosis, with exception of the cases bearing c-kit mutations who are associated with a rapid disease progression [Beghini A, 2000].
Patients with trisomy 4 appear to be poor compared with the intermediate risk cytogenetics [Gupta V et al., 2003]. Patients with trisomy 4 have unique clinical and experimental features and a poor prognosis [Pan JL et al., 2007].

**Trisomy 10:** Trisomy 10 is a rare nonrandom cytogenetic abnormality found in association with AML. The hematological and clinical features associated with this finding have not yet been clearly defined. A literature review revealed 13 cases of trisomy 10 in AML, some reported as a minority component of a more comprehensive AML study and therefore lacking a full description of both clinical and hematological features [Llewellyn IE et al., 2000].

**Trisomy 11:** Trisomy 11 is found in M1, M2, M4, therapy related AML and MDS evolving towards AML.

**Trisomy 13:** Trisomy 13 is a recurring but rare chromosomal abnormality in AML. It frequently occurs as the sole karyotype anomaly. Several studies have shown an association between trisomy 13 and morphologic and immunophenotypic undifferentiated leukemia, in particular within the rare FAB subgroup AML-M0. The majority of cases with trisomy 13 show low remission rates [Fernando PGS et al., 2007].

**Trisomy 21:** It is a second most frequent acquired trisomy after trisomy 8. No specific phenotype but possibly a slight higher incidence in monocytic phenotypes (AML-M4 and M5, chronic myelomonocytic leukemia (CMML). AML-M7 with acquired +21 is exceptional, whereas AML-M7 is frequent in Down syndrome. Trisomy 21 as sole abnormality has an unfavorable prognosis; not a single patient achieve long term disease free survival. When associated with other recurrent chromosome changes, it does not modify the prognosis of these abnormalities. Gene(s) involved in trisomy 21 associated leukemia is (are) unknown [Viguie F 2001]. The incidence of trisomy 21 is similar (4.1%-6.7%) in AML, CML, myeloproliferative disorders, MDS, chronic lymphoproliferative disorders, and malignant lymphomas; it is substantially higher (14.8%) in ALL. Acquired trisomy 21 is the only karyotypic abnormality in only
0.4%. Trisomy 21 has never been reported as the sole anomaly in a solid tumor [Mitelman F et al., 1990].

**Trisomy 22:** Many of the cases of trisomy 22 in the literature may actually have had this as a secondary aberration in addition to an undetected inv(16). Indeed, one third of the AMLs with +22 have visible rearrangements of chromosome 16 [Mitelman 1994]. Further evidence pointing in the same direction comes from the observation that most +22 cases are AML-M4 [Mitelman F 2nd Ed.].

**Monosomies:**

*Loss of sex chromosome:* Loss of the X as the sole abnormality is a very rare finding, loss of the Y is frequently observed in all myeloid malignancies. It appears equally common in elderly male without hematological malignancy and should not be taken as evidence of a malignant change when seen alone. Loss of the sex chromosomes is commonly observed associated with t(8;21) and less frequently loss of Y is associated with the t(9;22) translocation. In both cases, these changes are seen at diagnosis but have no apparent impact on prognosis.

**Deletions:** A number of recurrent deletions, sometimes in the form of unbalanced translocations, are seen in myeloid disease (*Figure 16*). Mostly in conjunction with more specific changes or as part of a complex karyotype. Chromosomes involved are 5q, 7q, 9q, 11q, 12p, 13q, 16q, 17p and 20q. It has been recently demonstrated that some apparent deletions are in fact the result of subtle unbalanced translocations and it may be important to distinguish between these in the future. Many deletions are associated with evolving disease and involve the loss of tumor suppressor genes or putative tumor suppressor genes. The occurrence of some deletions in disease of different lineage is consistent with loss of tumor suppressors with general influence.

*Figure 16: Recurrently deleted chromosomes.*
**Del(9)(q):** It is seen infrequently as the sole abnormality in myeloid leukemia, particularly AML, del(9)(q) is commonly observed as a secondary change, particularly in association with the t(8;21). It is desirable to rule out concomitant translocations by molecular means if t(8;21) is not apparent. The long arm deletion is variable in size and no common deleted segment has, as yet been found.

**Del(12)(p):** These are usually secondary changes, also associated with abnormalities of chromosomes 3, 5, 7 and 17. Such deletions or unbalanced translocations may be subtle and difficult to detect [Berger et al., 1986]. 12p changes also often seen in MDS and ALL. Mostly present in addition to other aberrations, t/del(12)(p) is the sole anomaly in roughly one fifth of the cases. There is no marked FAB subgroups preference [Mitelman F 2nd ed].

**3rd Risk group-Adverse group:**
Adverse or high-risk group includes abnormalities of complex karyotype (more than 3) abnormalities, del(7q)/monosomy7 , del(5q)/monosomy5, abnormalities of chr.3q, del(9q) without t(8;21), 17p, 20q, or 21q abnormalities and t(6;9).

**(i) Complex karyotype:** Cytogenetic findings at the time of diagnosis are among the most important independent prognostic factors in patients with AML, both adults and children. While t(15;17)(q22;q21), t(8;21)(q22;q22) and inv(16)(p13;q22)/t(16;16)(p13;q22) are associated with a relatively favorable clinical outcome, several recurrent abnormalities, such as inv(3)(q21q26)/t(3;3)(q21;q26) or t(6;9)(p23;q34), bestow a very poor prognosis. In addition to specific chromosome rearrangements that confer an adverse prognosis, a subset of patients whose treatment outcomes have been consistently very poor presents with a complex karyotype defined as the presence of three or more (in some studies, five or more) chromosome abnormalities. This group of patients are not well characterized cytogenetically or molecularly because, in addition to recognizable chromosome abnormalities, such as deletion of the long arm of chromosome 5, monosomy 7, or trisomy 8, these complex karyotypes contain sometimes numerous, aberrations that could only be recognized partially or not at all using standard cytogenetic analysis with G- or R-banding. Such aberrations include marker chromosomes, i.e., abnormal chromosomes in which no part can be identified, ring chromosomes of unknown
origin, homogeneously staining regions, double minutes, and unbalanced translocations, in which the origin of translocated chromosome material cannot be established. The application of molecular-cytogenetic techniques, such as multicolour spectral karyotyping (SKY), multiplex fluorescence in situ hybridization (M-FISH), and cross-species color banding, that allow simultaneous display of all human chromosomes in different colors, as well as comparative genomic hybridization (CGH) and array CGH (a-CGH), enabled more precise characterization of complex karyotypes. This also has led to the identification of several genes whose expression is altered as a result of genomic imbalances in patients in this cytogenetic group. Approximately 20% of patients with t(8;21) and about 10% of patients with inv(16)/t(16;16) and of those with t(15;17) would be considered to have a complex karyotype because they harbour two or more secondary aberrations in addition to their respective primary translocation or inversion. However, since several studies have shown that patients with the aforementioned abnormalities constitute separate biological and clinical entities, and that increased karyotype complexity in these cytogenetic groups does not affect adversely clinical outcome in a manner comparable to other patients with three or more (or five or more) abnormalities, the category "complex karyotype" excludes patients with t(8;21), inv(16)/t(16;16), and t(15;17). This definition was adopted by studies analyzing the impact of cytogenetics on clinical outcome in AML and those focused on improved characterization of genetic rearrangements using M-FISH. The incidence of complex karyotypes increases with age. Schoch et al [2005] estimated that the incidence of karyotypically complex AML with three or more aberrations in the German population was 0.05 per 100,000 people aged 21 to 30 years, but it was almost 25 times higher—1.15 per 100,000 people—in the 61 to 70 years age group. Compared with de novo AML, complex karyotype are approximately twice as common in secondary AML, both therapy-related and evolving from antecedent hematologic malignancy. Even though three is the lowest number of chromosome aberrations necessary to consider a karyotype as complex, the vast majority of patients harbour a higher number of aberrations, which in rare cases can reach as many as 30 [Mrozek K 2008].
(ii) Monosomy 7: AML cases with monosomy 7 (-7) and del(7q) comprise a heterogeneous subgroup (Figure 17 A). The association of losses in 7q with myeloid leukemia suggests that this region contains a tumor suppressor gene or genes whose loss of function contributes to leukemic transformation or tumor progression. The -7/del(7q) aberrations frequently coexist with complex karyotypes such as -5/del(5q) and trisomy 8 [Woo KS 2009]. It is relatively common in AML (seen in 3% of cases as the sole abnormality and in 12% of cases overall). It does not appear to be associated with FAB subtype. Del(7)(q) is a common finding in AML and MDS, although more frequently observed in conjunction with abnormalities of chromosome 5 in therapy related disease. Breakpoints in the long arm are variable, with the most commonly reported deletions at 7q22-q36 and at 7q32-q36. Substantial numbers of these deletions have subsequently been identified as unbalanced translocations. All these abnormalities are usually associated with poor prognosis. (Figure 17 C).

(iii) Del(5)(q): Deletion 5q (Figure 17 B) is a well recognized abnormality in myeloid leukemia and when seen as the sole abnormality, it marks a subset of MDS patients, usually with benign dysplasia. Typically they are elderly patients, predominantly female. Del5q occurring alone is a rare finding in AML (<1%) and MPD but is commonly seen in complex karyotypes (3-4% AML) usually involving abnormalities of 3, 7, 12 and 17. In secondary leukemias such combinations occur in approximately 80% of cases. Although the breakpoint is variable, a consistently deleted region at 5q31.1 is found in cases of AML and MDS associated with secondary leukemia and poor prognosis. Del5q syndrome in MDS appears to have a common breakpoint at 5q32, apparently linked to a different gene. Del(5)(q) appears to be a new nonrandom chromosomal translocation, specifically associated with childhood AML with a differentiated blast cell phenotype [Jaju RJ 1999].

Figure 17: Representative G banded partial karyotype of different structural abnormalities.
(iv) **Abnormalities of 3q21 and 3q26:** A variety of rearrangement at 3q21 is seen in different myeloid diseases. The majority juxtaposes 3q21 and 3q26 but less common translocations occur. Survival is generally poor. Consideration of all abnormalities at 3q21 or q26 as a single category may be misleading as molecular studies have shown a variety of genetic events.

It is observed in AML, MDS, CML as well as other myeloproliferative disorders; involvement of 3q26 in balanced rearrangements is highly suggestive of EVII and/or MDS1/EVII rearrangement. As a consequence, balanced aberrations involving 3q26 are mainly detected in myeloid malignancies which have been described in up to 5% of unselected patients with myeloid malignancies. It is often associated with young age at diagnosis, trilineage dysplasia; dysmegakaryopoiesis and prior treatment with alkylating agents. Generally 3q26 rearrangements are associated with adverse prognosis [Poppe B et al., 2003].

(v) **t(6;9):** This translocation was identified by Rowley and Potter in 1976. It is mainly observed as sole abnormality. The t(6;9)(p23;q34) is a rare recurrent abnormality. AML with t(6;9)(p23;q34) has unique clinical and laboratory features and its prognosis is poor in most cases [Wang Y et al., 2010].

(vi) **20q:** It is a common observation found in myeloid disorders. This is more frequently found in MPD. This abnormality is also present in 5% of MDS patients and rarely in AML, particularly in AML-M6. It is often difficult to define the breakpoints accurately and heterogeneity of breakpoints has been demonstrated. The deleted segment lies between 20q11 and 20q12.

Cytogenetic analysis performed at diagnosis is generally recognized as the single most valuable prognostic factor in AML. Characterization of adult patients with AML according to presentation karyotype provides an important basis for selection of therapy. For example, the outcome for patients with t(15;17) APML has substantially improved with the use of all-trans-retinoic acid in combination with chemotherapy, but the drug is without benefit for patients lacking this translocation. Molecular cloning of the breakpoints involved in these abnormalities has led to the discovery of
many genes that are now known to be converted into leukemia genes by the mechanisms of either gene activation or gene fusion. New methods and characterized specific genetic markers have opened a new era also in detection of minimal residual cells.

To date, acquired chromosome abnormalities have been reported in more than 7800 patients with AML worldwide [Mitelman F 2000]. The distribution of chromosome changes is highly non-random. Certain chromosomes, chromosome regions and bands are involved in AML-associated aberrations much more frequently than others. Among the various aberrations reported in AML patients, those that occur recurrently, have been seen as the sole abnormality in at least some cases and are rarely or never present in other types of haematological and non-haematological neoplasms are considered to be the most important. They have been termed primary aberrations and are assumed to play an essential role in the early stages of leukemogenesis [Mrozek K et al., 2001].