

## Review Article

# Differentiation Therapy of Myeloid Leukemia: Reversing Maturation Arrest

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### Abbreviations

ABL	:	AblesonOncogene
AML	:	Acute Myeloid Leukemia
APL	:	Acute Promyeloid Leukemia
ATRA	:	All- Trans Retinoic Acid
BPR	:	Break Point Region
CCR	:	Complete Cytogenetic Response
CD	:	Cluster of Differentiation Marker
CLL	:	Chronic Lymphocytic Leukemia
CML	:	Chronic Myeloid Leukemia
CN	:	Cytogenetically Normal
CR	:	Complete Clinical Response
EGFR	:	Epidermal Growth Factor Receptor
FLT3	:	FMS-like Receptor Tyrosine Kinase 3
IL-3R	:	Interleukin-3 Receptor
M0-M7	:	World Health Organization Classifications of Myeloid Leukemia
PML	:	Polymorphonuclear Leukocyte
PMLP	:	Promyelocytic Leukemia Protein
RAR $\alpha$	:	Retinoid Acid Receptor Alpha
RT-PCR	:	Reverse Transcriptase Polymerase Chain Reaction.
SLAMF7( CD 319):	:	Signaling Lymphocyte Activation Molecule Family Present on Plasma Cells
STI-571	:	Serine Tyrosine Kinase Inhibitor-571
UBE1L	:	Ubiquitin Activating Enzyme-E1 Like Protein

VPA : Valproic Acid

### Myeloid Leukemia Stem Cells and Maturation Arrest

Cancers of the myelocytic lineage (granulocytes), as like other cancers, develop from a failure of cell in the differentiation pathway to mature, i.e., maturation arrest [1,2]. This results in continued proliferation and/or failure of these cells to mature into terminally differentiated cells so that the number of the immature cells in the body continues to rise [1].The immature precursors accumulate in the bone marrow and blood, forcing out precursors of normal blood cells (white cells, red cells and platelets) resulting in infections, fatigue (anemia) and bleeding. Treatment of acute myeloid leukemias is directed to the proliferating transit amplifying cell population of the cancer (chemotherapy) which that blocks DNA replication. While this may inhibit proliferation of the transit amplifying cells, the non-proliferating leukemic stem cells are not affected and the leukemia frequently returns when chemotherapy is discontinued (Figure 1). Treatment of cancers with agents that reverse maturation arrest and allow differentiation of the cancer cells is called “Differentiation Therapy” [3].When a cell divides, the two daughter cells can in turn each divide (symmetric division), or one daughter cell can retain the capacity to divide, whereas the other begins the process of differentiation (asymmetric division), or both daughter cells may terminally differentiate [4]. In normal tissue renewal, the number of new cells that is produced by asymmetric division of tissue stem cells is equal to the number of cells that terminally differentiate so that the total number of cells in the tissue remains essentially constant. In cancers, some of the daughter cells that should differentiate into non-dividing cells retain the capacity to divide and do not differentiate and die, so that the number of cells in the cancer increases with time [4].

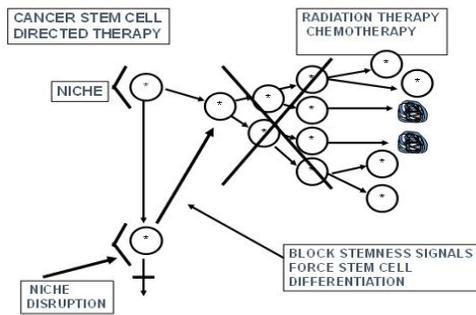


Figure 1.

**Figure 1:** Leukemia stem cells and differentiation therapy. Leukemias consist of three cell populations: stem cells, transit amplifying cells and terminally differentiated (dying) cells. Conventional chemotherapy and radiation therapy is directed to the proliferating transit amplifying cells. This may be very effective in reducing the tumor mass. However, when therapy is discontinued, the leukemia will be reconstituted by the leukemic stem cells which that are resistant to chemotherapy. Two ways are presented to direct differentiation of the cancer stem cells, by directly blocking stemness signals and forcing differentiation or by disruption of the protective niche that tissue stroma provides the stem cells in the bone marrow.

The growth rate of a cancer depends on the number of cells in the cell cycle at a given time growth fraction[5]. In Acute Myeloid Leukemia (AML), essentially all the cells are proliferating. After mitoses, most daughter cells immediately start through the cell cycle again and the tumor grows exponentially. In poorly differentiated acute myeloid leukemias, the growth fraction is very high, sometimes approaching 100%, so that survival without therapy is estimated in weeks or months. In more differentiated cancers, some of the cells differentiate into mature cells that no longer divide but instead arrest in a non-proliferating state and do not die. In Chronic Myeloid Leukemia (CML), the growth fraction may be estimated at about 10% and survival without treatment may be months or years. In Chronic Lymphocytic Leukemia (CLL), the growth fraction is less than 1:1000, so that survival without treatment may be decades. Normal blood cell formation and cancer of the blood (leukemia), respectively, provide excellent examples of the principles of maturation arrest and differentiation therapy.

### Normal Blood Renewal

The polymorphonuclear cells of the blood live only a day or so, and are rapidly replaced through proliferation of progenitor cells (myeloblasts) in the bone marrow. The cell lineage of the immature progenitor cells in the bone marrow may be determined morphologically because as the more differentiated precursor cells begin to show characteristic phenotypes of the various mature blood cell types, erythroblasts (red cells), myeloblasts (polymorphonuclear cells), lymphoblasts, and monoblasts [6,7]. The proliferating progenitor cells that give rise to the blood cells are transit amplifying cells. The progenitor cells are progeny of a less differentiated, resting, pluripotent bone marrow stem cell. The existence of an

undifferentiated stem cell for blood cells, “gemeinsame Stammzelle,” was proposed by Pappenheim in 1917 [8]. The cells of the progenitor pool in the bone marrow were identified by their ability to form colonies of various types of blood cells in the spleen after transplantation [9], so-called “Colony Forming Units” [10]. Using this criterion, less than 0.05 percent of the total bone marrow cells can both proliferate and differentiate in vivo into various mature blood cell types [11-13]. The percentage of slowly proliferating “long-term” bone marrow stem cells believed to be the precursors of progenitor cells, as well as possible circulating stem cells for other organs, is even lower than the number of blood cell progenitor cells, approximately 0.005% [14-17]. The long-term bone marrow stem cells normally do not proliferate, but can respond to increased blood cell need, such as after blood loss or residence at high altitude, by proliferating and increasing the pool of transit amplifying cells.

### Leukemias as Models of Maturation Arrest of a Cell Lineage

The root words “leuk” (white) + “emia” (blood) refer to the color of the blood in leukemia when large numbers of white cells have accumulated, changing the color of the blood from red to creamy white. In leukemia, the normal differentiation pathway is blocked at a stage of differentiation at which the cells do not move on to terminal differentiation. This is the basis for the French-American-British and World Health Organization classification of acute myeloid leukemia (Table 1) [18]. For example, if maturation arrest occurs at the level of the myeloid stem cell in Acute Myeloid Leukemia (AML), these are subtypes AML-M0 and AML-M2); at the level of the promyelocyte is subtype AML-M3. In other subtypes, there may be accumulation of Eosinophils (M4eos), Monocytes (M5), Red Blood Cell Precursors (M6) or Megakaryocytes (M7). Chronic Myelogenous Leukemia (CML) is the most differentiated form.

	Name	% of Adult Patients
M0	Undifferentiated Acute Myeloblastic Leukemia	5
M1	Acute Myeloblastic Leukemia with Minimal Maturation	15
M2	Acute Myeloblastic Leukemia with Maturation	25
M3	Acute Promyelocytic Leukemia	10
M4	Acute Myelomonocytic Leukemia	20
M4eos	Acute Myelomonocytic Leukemia with Eosinophilia	5
M5	Acute Monocytic Leukemia	10
M6	Acute Erythroid Leukemia	5
M7	Acute Megakaryocytic Leukemia	5

**Table 1:** French-American-British Classification of Acute Myeloid Leukemia.

## Acute Leukemia

Furth and Kahn in 1937 proved that acute leukemia of mice could be transplanted by a single cell [19]. In 1955, Makino and Kano [20] obtained clones of tumor cells from single leukemic cells. These findings combined with the observations that most of the cells of AML look like immature blasts and that the rapid clinical course indicated exponential growth, led many to conclude at that time that each cell in AML is a leukemic stem cell capable of synchronous division. However, this does not appear to be true of human AMLs. There is considerable variability in the ability of human AML cells to initiate new colonies in vitro [21] or to produce tumors in SCID mice [22,23]. For example, Lapidot et al. [23] found only 1 in 250,000 cells and Bonnet et al [22] found only between 0.2 and 200 per 106 AML cells to be able to engraft the leukemia in SCID mice. Sutherland et al [21] found between 0.2 and 2,600 colony forming units per 106 AML cells, analogous to short term hematopoietic progenitor cells, and 0.02 to 2 long term Culture Initiating Cells (LTC-IC), which likely are analogous to hematopoietic stem cells. Thus, the average incidence of true cancer stem cells in human AML appears to be about 1/100,000. These leukemic stem cells express the CD34+CD38- phenotype as do normal hematopoietic stem cells [21,22], although CD34- stem cells have been found both in AML and in normal hematopoietic populations [23].

The low incidence of leukemic stem cells and morphologic homogeneity of the numerous large blast cells reflect the clinical course and the response to chemotherapy of AML. First, most of the leukemic cells are immature blast cells, suggesting they are in the cell cycle (transit amplifying cells). Second, the tumor expands rapidly with the kinetics of a high growth fraction of the cells. Third, over 99.9% of the cells are killed by chemicals that interfere with DNA synthesis. These observations indicate that most of the cells of AML are in the cell cycle at any given time, yet only 1/100,000 cells are capable of transplanting the leukemia. Most of the leukemic cells are progenitor or transit amplifying cells rather than true stem cells [3]. In their transit-amplifying capacity, they contribute to the growth rate in vivo, but are unable to function as stem cells to recreate the tumor after passage in vitro or in vivo.

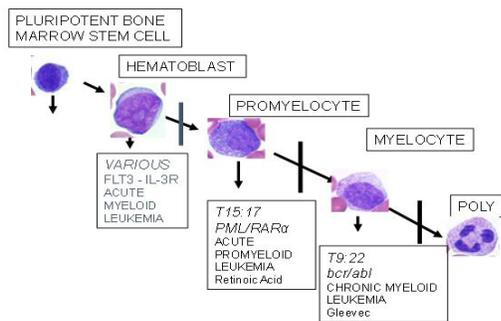
## Chronic Leukemia

In CML on the other hand, maturation arrest is at a more differentiated level than in AML. Because many of the daughter cells cannot divide, the growth fraction of the tumor is about 1 in 100. However, the non-proliferating daughter cells do not die, but rather they accumulate in the bone marrow and blood at various stages of maturation [24]. This process results in a much slower accumulation of cells and a longer course of the disease than in AML (see below). The growth fraction of chronic lymphocytic leukemia is even lower, similar to that of normal lymphocyte renewal (about 0.005%). The lesion in CLL is failure of the leukemic cells to die. The clinical course of leukemia is determined by the rate of re-

placement of the normal bone marrow progenitor cells with leukemic cells. This replacement results in a loss of the normal cells and their function. Loss of red blood cells leads to anemia; loss of platelets leads to bleeding; loss of polymorphonuclear cells leads to decreased resistance to infection. Leukemia with a high growth fraction (e.g., AML) will replace the normal marrow cells rapidly (in months), whereas leukemia with a low growth fraction (e.g., CLL) may take years to produce symptoms [25,26].

## Gene Rearrangements and Maturation Arrest in Leukemia

Many gene rearrangements and/or mutations have been identified in human leukemia; gene rearrangements result in gene fusion and formation of new cellular products that block further differentiation [27-31]. Of the many rearrangements or mutations reported up to 264 different mutations [32], three specific examples can be used to illustrate maturation arrest, at various levels of activation (Figure 2). These rearrangements produce translocation of a promoter and a structural gene, resulting in activation of expression of the rearranged gene or formation of a gene fusion product. There are now three major classes: 1. those that activate kinases and cause proliferation (FLT3, TP53, c-KIT, k-RAS); 2. those that block apoptosis (NPM1, CEBPA, BCL-2); and 3. those that regulate DNA methylation (DNMT3A, TET2, IDH, 26-32). In most instances, mutation in one gene will produce a chronic leukemia or a myelodysplasia which may develop into an acute leukemia after a long time [31,32]. However, mutation in one class predisposes to mutation in a gene in the other class. The combination of a mutation in a gene in class I and a gene in class II results in a rapidly growing acute leukemia. This is due to combination of a proliferation signal with cell survival signal (blocks apoptosis). The presence of more than one lesion in acute leukemia (most patients have up to 15 mutations) complicates targeted differentiation therapy (see below). The nature of the genetic lesion can be directly related to the clinical course of the type of leukemia. For example, various genetic changes in AML are expressed at the myeloid blast cell (hemocytoblast) or precursor (mono/myeloid leukemia) level. This leads to rapid accumulation of immature blast cells of various blood cell types. The malignant cell is the multi/pluripotent tumor progenitor cell with the capacity to differentiate into multiple types of blood cells [33,34]. Although there are exceptions, the genetic lesion is present in many other cells of the body, but the malignant phenotype is only expressed when the genetic lesion is activated. An illustrative example of this principle is the development of B-cell lymphomas in transgenic mice that express oncogenes under the control of the Immunoglobulin (Ig) promoter [35]. The Ig-oncogene transgene is present in every cell of the mouse, but is only expressed with when the Ig promoter is activated. Since the Ig promoter is not activated until the level of B-cell differentiation, the malignant effect is manifested as B-cell lymphoma [35], but not in cells earlier in the differentiation pathway.



**Figure 2:** Gene Translocation and Stages of Maturation Arrest of Three Human Myeloid Leukemias. Depicted is the maturation lineage of myelogenous cells with stages of maturation at which translocated genes become activated. Constitutive activation of transactivation kinases at the myeloid stem cell stage (hematocytoblast) causes acute myeloid leukemia; activation at the promyelocyte stage causes acute promyeloid leukemia; and activation at the myelocyte stage causes chronic myeloid leukemia. Acute leukemias usually have more than one mutation (see Table 1). Maturation arrest in CML is at the level of the myelocyte. About 10% of the leukemic daughter cells in CML retain the capacity to divide (asymmetric division) and most daughter cells differentiate to more mature granulocytes. However, these non-proliferating daughter cells do not fully mature, do not function normally, and do not die. The molecular lesion is the *bcr/abl* translocation, which results in constitutive activation of a tyrosine kinase present in myelocytes. Treatment may be effected using a modified monoclonal antibody that blocks this kinase (imatinib, GLEEVECGleevec®). In APL there is maturation arrest at the promyelocyte level because of the action of a fusion protein. This arrest may be alleviated by treatment with all trans-retinoic acid (ATRA), which results in degradation of the fusion protein. Maturation arrest in AML is at the common myeloid stem cell. At this level of differentiation, almost all of the leukemic daughter cells retain the capacity to divide, but most do not retain stem cell properties, i.e., ability to form colonies in vitro or grow after transplantation into SCID mice. Maturation arrest is due to constitutive activation of the IL-3R due to mutations in components of the IL-3R signaling pathway, such as FLT3. Treatment may be attempted by small molecules which that block the activation mutant FLT3.

## Chemotherapy

Treatment of leukemia is directed toward the inhibition of proliferation using anti-metabolic drugs. The rapid response to chemotherapy of acute leukemia indicates that these cancers are composed of mostly of cells that are actively proliferating [28-32]. The present treatment for AML is the so-called 7 + 3 [32] or 3 + 7 [36] infusions of high doses of cytarabine (7 days) plus 3 doses of anthracyclines (3 days). Cytarabine inhibits DNA and RNA polymerases and interferes with DNA synthesis. Anthracyclines intercalates between base pairs of the DNA/RNA strand, inhibits topoisomerase II, generates free oxygen radicals and induces de-histonization of chromatin. Even with 2 cycles of this induction therapy up to 50% of AML patients retain residual disease [37], and even those with complete remission require “consolidation therapy”, i.e., further treatment [36]. Thus, chemotherapy alone

is now effective for about a 50% 5-year survival rate for AML patients under 60 years of age and 12% for patients over 60 years of age, even after additional treatment with other drugs such as tyrosine kinase inhibitors (sorafenib), monoclonal antibodies (Anti-CD33) and/or stem cell transplantation [36].

The malignant genetic change is also present in the “Resting” or G0 stage stem cells that are not proliferating when the drug is given. Because the resting leukemic stem cells are not affected by one cycle of treatment, successful treatment may require four or more cycles of anti-proliferative therapy to catch all the leukemic stem cells. Even so, up to 40% of patients will require total bone marrow ablation and stem cell transplantation from a normal donor, to eliminate all the primitive resting cells that have the genetic lesion. This implies that the leukemia arises in the reserve hematopoietic stem cells, which can only be eliminated by total bone marrow ablation [32,36]. Differentiation therapy is an alternative approach to leukemia treatment which that does not directly kill the proliferating cells but induces cells with arrested maturation to mature and die.

## Differentiation Therapy of Leukemia

With the recognition that leukemia is the result of maturation arrest of cells at an immature differentiation stage, a therapeutic approach directed toward removing the maturation block and allowing differentiation could be a more specific and effective therapy than the traditional treatment approach of treatment with cytotoxic drugs. Differentiation therapy has led to great success in CML and Acute Promyeloid Leukemia (APL), but has not yet been effectively applied to most AMLs.

## Chronic Myeloid Leukemia

In 1960, Nowell and Hungerford [38] made a major breakthrough in the understanding of molecular mechanisms in cancer, by identifying a distinct chromosomal abnormality, the Philadelphia Chromosome, in cases of CML. The Philadelphia Chromosome is formed by a gene rearrangement, resulting in the appearance of a “new” chromosome [39,40]. The gene rearrangement in CML places the *bcr* (break point region) next to the *abl* (Ableson) oncogene, resulting in production of a fusion protein (BCR-ABL). The fusion product of *bcr-abl* is an oncogenic protein that localizes to the cytoskeleton and provides tyrosine kinase activity [39]. This results in activation of downstream signaling molecules such as phosphoinositide 3-kinase [40], and constitutive proliferation of cells at the myelocyte stage of differentiation [40-42] (Figure 2). The cells in CML accumulate relatively slowly and are identified as immature and differentiated cells in the granulocytic series myelocytes, band cells [41,42]. The leukemic cell may differentiate into recognizable mature cell types, but they live much longer than normal polymorphonuclear cells and do not functionally normally. Characterization of the specific fusion tyrosine kinase responsi-

ble for CML led to the development of a specific small molecule inhibitor, imatinib mesylate (Gleevec®, STI-571), which binds to the fusion protein and neutralizes the tyrosine kinase activity [43,44]. This breakthrough in treatment provides proof-of-principle of “Targeted Cancer Therapy” [44,45]. Blocking of the activation kinase allows the cells to complete differentiation and die in a few days as do normal mature granulocytes. Administration of imatinib to patients with CML results in an 80% Complete Cytogenetic Response (CCR) in newly diagnosed patients, and a 40% CCR in patients who have relapsed after failure of interferon-alpha therapy [45]. Even after CCR, residual disease can be detected by RT-PCR, so that complete cure is not yet achieved and imatinib treatment must be maintained or the leukemic cells will re-accumulate [43]. This indicates that the leukemic stem cell has not been eliminated by imatinib. However, those leukemic progenitor cells that depend on bcr-abl kinase activation are forced to differentiate. Most patients with a CCR and maintained on imatinib remain stable, but some develop mutations in the kinase domain of the bcr-abl gene, resulting in a fusion product that is no longer inhibited by imatinib. The bcr-abl translocation has multiple subtypes that depend on varying position of the breakpoints, but each of these results in a fusion gene product that is blocked by tyrosine kinase inhibitors [46]. The patients at risk for failure of imatinib treatment become candidates for reduced-intensity conditioning stem cell transplantation, using lower than high-dose chemotherapy for preparation for transplantation [47,48].

### Acute Promyelocytic Leukemia (M3)

Another example of differentiation therapy of myeloid leukemia is treatment of APL with All-Trans-Retinoic Acid (ATRA), which removes the maturation block caused by the fusion protein. APL is a cancer involving the transit amplifying precursor cells of the myelocytic lineage expressing markers of the M3 type (promyelocyte). The molecular lesion in APL is often the t(15:17) gene rearrangement involving the nuclear retinoic acid receptor alpha (RAR $\alpha$ ). This results in production of a fusion protein between the Promyelocytic Leukemia Protein (PML) and the RAR $\alpha$  (PML/RAR $\alpha$ ). The PML-protein is normally found in a discrete, circumscribed nuclear structure called a nuclear body in cells of the myeloid series [49,50]. The PML/RAR $\alpha$  fusion product inhibits activation of the RAR $\alpha$  [51], and disrupts the nuclear bodies [52]; also, without its ligand (retinoic acid), the fusion product also functions as a constitutive transcriptional repressor, blocking promyelocytic differentiation [53]. The APL fusion gene product induces genes that maintain the stem cell phenotype, and it represses DNA repair genes, resulting in a mutator phenotype that enhances tumor progression by increasing the incidence of additional mutations [54]. All Trans-Retinoic Acid (ATRA) reacts with the RAR $\alpha$  in

the fusion protein, upregulates the Ubiquitin Activating Enzyme-E1 Like Protein (UBE1L), and triggers degradation of the PML/RAR $\alpha$  fusion protein [55]. This process activates RAR $\alpha$ -mediated transcription, allows re-formation of the PML-nuclear body, and stimulates differentiation and eventual apoptosis of APL cells [56,57]. Currently, about 90% of newly diagnosed patients with APL achieve complete remission, and over 70% are cured by ATRA therapy [58] either with or without concomitant chemotherapy with methotrexate and cytarabine [58-65]. The addition of ATRA to arsenic trioxide is the standard treatment for APL with complete remission rates of 95-100% [36]. However, patients with molecular weight variant-APL (translocation between chromosome 11 and 17 or 5 and 17) have cure rates of 80%, and they may benefit from addition of gemtuzumab, ozaogamicin or anthracyclines [36].

### Acute Myeloid Leukemia

AML includes various leukemias caused by the accumulation of hematopoietic transit amplifying cells arrested at the early myeloid stem cell (hematocytoblast) differentiation of hematopoiesis (Figure 2). Included in the classification of AMLs are many leukemias with different genetic lesions (Table 2). Most of these involve gene translocations resulting in constitutive activation of signal transduction molecules and, along with cytogenetic abnormalities, may be used to establish prognosis and select therapy (Table 3). AML is generally divided into those that present under the age of 18 (childhood and adolescent AML) and those that present after the age of 18 (adult AML) [66]. AML occurs more frequently in older adults than in children, but may appear at all ages. There are at least 150 different genetic changes in each pediatric AML [67], including gene duplications in the FMS-like receptor tyrosine kinase for acute myeloid leukemia described in more detail below (FTL3). Because most of the cells of AML are proliferating at any given time, the standard treatment of AML is cytotoxic chemotherapy with drugs such as cytarabine and mitoxantrone, which kill proliferating cells by interfering with DNA synthesis. Cytotoxic chemotherapy is still the most effective for treatment of AML, but only about half of pediatric patients and one-third of adult patients can be cured [66-68]. Since the gene rearrangements in AML are present not only in the leukemic progenitor cells but also in the more primitive hematopoietic stem cell and because this type of cytotoxic chemotherapy is directed toward proliferating cells, the resting pluripotent leukemic stem cell is not affected, [24,28,29]. In the patients that relapse, the AML cells regenerate from the leukemic stem cell after the course of chemotherapy is completed [69-73]. As with other AMLs, selected patients may be cured by allogeneic bone marrow transplantation [74].

AML with t(8:21)(q22;q22); RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBEB-MYN11
Acute promyelocytic leukemia (APL) with t(15;17)(q22;q12); PML-RARA
AML with t(9;11)(q22;q23); MLLT3-MLL
AML with t(6;9)(p23;q34); DEK-NUP214
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RNP1-EV11
AML (megakaryocytic) with t(1:22)(p13;q13); RBM15-MLK1
Provisional entity: AML with mutated NPM1
Provisional entity: AML with mutated CEBPA
PLUS HUNDREDS OF UNCOMMON CHROMOSOMAL ABERRATIONS
From: Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood 127: 2391-2405, 2016

**Table 2:** World Health Organization Classification of Acute Myeloid Leukemia (AML) with Recurrent Genetic Abnormalities.

PROGNOSTIC RISK GROUP	Cytogenetic Profile and Molecular Abnormalities
FAVORABLE	t(8:21)(q22;q22) with no c-KIT mutation
	Inv(16)(p13;q22)
	t(15;17)(q22;q12)
	Mutated NPM1 without FLT3-ITD (CN-ALL)
	Mutated biallelic CEBPA (CN-AML)
INTERMEDIATE	t(8:21)(q22;q22) with c-KIT mutation
	CN-AML other than those listed above or below
POOR	tp53 mutation
	CN with FLT3-ITD
	CN with DNMT3A
	CN with KMT2A-PTD
	inv(3)(q21q26.2)
	t(6;9)(q23;q34)
	11q abnormalities other than t(9;11)
	-5 or del (5q)
	-7
	Complex karyotype
CN – CYTOGENETICALLY NORMAL	
Modified from Kouchkovsky D, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. 2016, 6k e441:doi:10.1038/bcj.2016.50 .	

**Table 3:** Prognosis Based on Cytogenetic and Molecular Profile.

With an understanding of the genetic causes and molecular mechanisms responsible for AML, it should be possible to develop more specific and effective therapy based on reversing the maturation arrest responsible for the accumulation of immature cells in AML [31]. Although an oversimplification, many of the activating mutations in AML result in constitutive activation of the IL-3 receptor (IL-3R) related signaling pathway for cell activation. During normal hematopoiesis, this signaling pathway is essential for proliferation and differentiation at the myeloid progenitor (hemocytoblast) level, the progenitor cell for red cells, polymorphonuclear cells and monocytes [24]. Constitutive activation of this early transit amplifying cell type leads to an increase in immature (blast) cells in the myeloid lineage, including erythroblasts, myeloblasts and promonocytes. The gene rearrangement is present in even less mature cells in the bone marrow, but the effect is only seen at the stage of maturation at which IL-3R activation is critical. New and novel therapies for AML include agents that promote differentiation (Azacitidine), small molecules that inhibit signal transduction (thymidine kinase inhibition) and antibody directed cytotoxicity (anti-CD-33 immunotoxin).

### Differentiation Therapy of other AMLs

The change from a stem cell to a mature cell is associated with removal of methyl groups for DNA bases and addition of acetyl groups to the histone DNA protein coat. This change the conformation of the DNA and allows transcription of genes that promote differentiation. One of the effects of constitutive signal activation in AML is that DNA remains methylated. Azacitidine is a DNA hypomethylating agent that appears to induce differentiation of AML cells. It has been approved for treatment of myelodysplastic syndrome [75], an early form of AML, and clinical trials with histone deacetylase inhibitors are now underway [32,36]. Since AML results from an over-proliferation of progenitor cells as well as a failure to differentiate, use of differentiation agents, such as azacitidine, will most likely be more successful if done in combination with inhibitors of signal transduction. Differentiation therapy using histone deacetylase inhibitors Valproic Acid (VPA) and All-Trans Retinoic Acid (ATRA) has some clinical benefit in older, poor risk patients with myeloid leukemia [76].

### Signal Induction Inhibition

As with CML, specific targeted inhibition of the signal transduction pathway of AML cells leads to inhibition of the activation of the cell, loss of proliferation and apoptosis; there are properties of normally differentiating PMLs. This may also be considered differentiation therapy, and blocking signal transduction results in loss of maturation arrest and differentiation of the leukemic cells. One of the common mutations in adult AML is in one of the RAS family of proteins, 21-KD guanine-nucleotide binding proteins.

RAS proteins require several post-translational steps including addition of a farnesyl lipid moiety required for translocation of the RAS protein to the plasma membrane and activation. Agents that inhibit farnesyl transferase block this step, prevent activation and allow the cells to differentiate [76]. Inhibitors of farnesyl transferase (tipifarnib®) have been reported to induce remissions in 20% of older patients with poor prognoses [77] but have had limited effects in phase 2 clinical trials [78].

Inhibitors of FMS-Like Receptor Tyrosine Kinase 3 (FLT3), a transmembrane tyrosine kinase, are being tested in pediatric AML in combination with chemotherapy. FLT3 acts through Src family tyrosine kinases [32,33,79]. FLT3 also interacts with a nuclear fusion protein formed from other gene translocations, known as the mixed-lineage leukemia gene (MLL), such as 11q23 [80]. The formation of dimers between these proteins transforms hematopoietic precursors in vitro and may be a critical signal for maturation arrest in AML types M4 and M5. Approximately 30% of AML patients have an activating mutation of FLT3 [81]. Small molecule inhibitors of FLT3 such as sorafenib selectively kill transformed cells that have activating mutations of FLT3 [32]. These inhibitors are not as effective as Gleevec in CML. Since CML may depend solely on bcr-abl activation, and AML usually involves more than one activation or an activation step and an apoptosis inhibitor [32,36], combination of FLT3 inhibitors with other drugs should be more effective. In clinical trials, several FLT3 inhibitors have induced biological responses manifested by a large reduction in numbers of peripheral blood leukemic cells, but complete remissions are very rare and the biological effect is of limited duration [68]. Thus, FLT3 inhibition therapy may be of limited value and may need to be combined with other approaches (azacitidine, chemotherapy, bone marrow transplantation), or FLT3 inhibitors may need to be combined with small molecule of other leukemia genes. This is an active field of investigation and it is hoped that some approach to “differentiation therapy” may eventually be used in combination with one of the more conventional chemotherapy regimens [82].

### Antibody-Directed Cytotoxicity

A new specific therapy for pediatric AML now being tested is humanized anti-CD33 antibody conjugated with the drug calicheamicin, a potent cytotoxic agent that cleaves double-stranded DNA (examples of this combination are gemtuzumab, and ozogamicin, Mylotarg) [83,84]. CD33 is a differentiation marker present on myeloid cells but not on normal hematopoietic stem cells. The anti-CD33 in the immunoconjugate is designed to deliver the cytotoxic agent to the myeloid leukemia cells. This appears to be effective in pediatric patients with advanced CD33-positive myeloid leukemia [83], but more studies are needed to determine if this approach will lead to improved therapy. A critical advance was the hypothesis that the stem cells of myeloma are protected from che-

motherapy by interaction with bone marrow stromal cells (niche).

### Niche Disruption (Multiple Myeloma)

Multiple myeloma is caused by maturation arrest at the last stage of B-cell development, plasma cells. It produces multiple lesions in the bone marrow (root, myelo-). It is notable for production of monoclonal immunoglobulins, immunoglobulin light chains (Bence-Jones proteins), lytic bone lesions, hypercalcemia and renal failure. A critical advance in understanding the biology of this disease was made when it was found that the malignant stem cells of myeloma are protected from the effects of chemotherapy by interaction with bone marrow stromal cells [85], the hematopoietic stem cell niche [86]. The myeloma stem cells form junctions with stromal cell through ligand-receptor interactions. This allows the stromal cells to supply survival factors, such as IL-6, VEGF, IGF-1, etc. to the myeloma cells. Disruption of the binding between stromal cells and myeloma stem cells can be accomplished by adding drugs such as bortezomib or thalidomide. Thus, induction therapy for MM is bortezomib or thalidomide based combinations with vincristine, doxorubicin and dexamethasone [87]. In selected refractory cases bone marrow transplantation may be added and can prevent recurrence in patients who are minimal disease free [84]. The use of bortezomib, and lenalidomide (thalidomide) has increased the overall survival from about 3 years to over 10 years [88]. Recently immunotherapy in the form of two monoclonal antibodies, elotuzumab and daratumumab, in combination with thalidomide and dexamethazone has been added to the mix [84]. Elotuzumab reacts with SLAMF7 on plasma cells and daratumumab reacts with CD38, a late B-cell marker. These may also interfere with the stromal-MM precursor cell interaction.

### Conclusions

If the malignant cells of cancers are progenitor cells arrested at an immortal stage of differentiation, then it should be possible to treat cancers by inducing differentiation [3]. If tumor cells can be forced to differentiate and to cease proliferation, then their malignant potential will be controlled. In this review, the application of differentiation therapy to three forms of myeloid leukemia as well as multiple myeloma shows both the promise of and the current problems with this approach. The treatment of chronic myeloid leukemia with imatinib mesylate (Gleevec), of acute promyelocytic leukemia with ATRA, and of some acute myeloid leukemia with hypomethylating agents or with small molecules that block signal transduction (FLT3-inhibitors), are examples of specific molecular targeted therapies that block cell activation and reverse the maturation arrest of the leukemic cells. In addition, disruption of the protective stem cell niche provides a way to remove protection for stem cell survival in the bone marrow and increase effectiveness of treatment for multiple myeloma. This approach may also have some benefit in other leukemia.

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