

Cancer Treatment and Research

Steven T. Rosen, M.D., Series Editor

Robert H. Lurie Comprehensive Cancer Center
Northwestern University Medical School

Acute Myelogenous Leukemia

Genetics, Biology and Therapy

Edited by
Lalitha Nagarajan

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Lalitha Nagarajan
Editor

Acute Myelogenous Leukemia

Genetics, Biology and Therapy

 Springer

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The Leukemia Stem Cell

Zeev Estrov

Introduction and Historical Perspective

In a meeting held in the Charité Hospital in Berlin in 1909, Alexander Maximow postulated that all circulating blood cells arise from a single lymphocyte-like cell [68]. An almost identical hypothesis was proposed by Artur Pappenheim in 1917 [78]. This hypothesis was tested years later by numerous investigators who demonstrated that all hematopoietic cells arise from a single hematopoietic stem cell (HSC) [54, 75, 90, 116]. Our knowledge of the leukemogenic process has benefited from hematopoiesis research. Identification and characterization of the HSC led to the theory that leukemia is a stem cell disease, i.e., that leukemia arises from a neoplastic HSC.

As far as we know, leukemia has always existed. The first patient reported to exhibit symptoms most likely attributable to chronic lymphocytic leukemia was a 63-year-old Parisian lemonade salesman named Monsieur Vernis [108]. At first there was not much interest in this disease, and Armand Velpeau's report drew little attention. However, within a few years, other reports followed. The first cases of chronic myelogenous leukemia (CML) were reported by Donne and Craigie [30, 25], and the clinical characteristics of several patients with different forms of leukemia were published by John Hughes Bennett [5–9], who first used the term “leucocythemia.” The word ‘leukämie’ was coined by Rudolph Virchow who by then had described several patients with this disease [110–113]. Several decades passed before the morphological features of leukemia cells were defined and the pathophysiology of leukemia deciphered.

The stem cell concept has been applied to non-hematopoietic tissues. Embryonic stem cells and non-hematopoietic, tissue-specific stem cells have been isolated, characterized, and extensively studied [60]. Cells isolated from embryonic tissue, gametes, and fertilized eggs have been found to have

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totipotent and pluripotent capacities [47]. Although these two capacities are dissimilar, the cells received identical names from different investigators, and to avoid confusion a common terminology was agreed upon. According to the recently established nomenclature (Table 1), adult tissue-specific stem cells (such as HSCs) that are capable of generating and replacing terminally differentiated cells within their own tissue boundaries are termed multipotent (adult) stem cells.

Table 1 Stem cell nomenclature

<i>Totipotent stem cells:</i> The zygote (fertilized egg) (<i>totus</i> : entire). These cells have the capacity to give rise to every tissue associated with the embryo and, eventually, the adult
<i>Pluripotent stem cells:</i> The blastocyst (starting 4 days after fertilization) (<i>plures</i> : several). These cells can give rise to all tissue cells except the embryo's outer layer (the trophoblast) and the embryo-supporting placenta
<i>Multipotent stem cells:</i> Somatic (adult, tissue-specific) stem cells

The Hematopoietic Stem Cell

Concept and Definition

The hematopoietic system is thought to originate from multipotent HSCs capable of reproducing themselves (through a process termed “self-renewal”) and producing a hierarchy of downstream multilineage and unilineage progenitor cells that differentiate fully into mature cells [28, 58, 115]. The concept arose from studies exploring the formation of hematopoietic colonies in spleens of irradiated mice. It has been known since the late 1940s that exposing mice to ionizing radiation results in the generation of macroscopic hematopoietic spleen colonies [51]. In the early 1960s, Till and McCulloch [104] and their colleagues [3, 105] demonstrated that a spleen colony is generated from a single cell called a spleen colony-forming unit (CFU-S). It was demonstrated later that cells arising from a single CFU-S can rescue a lethally irradiated mouse, and subsequent limiting-dilution studies established that a single cell is capable of repopulating the entire hematopoietic system [54, 90, 72]. These studies defined the HSC as a cell that possesses self-renewal and clonogenic abilities and the capacity to differentiate into multiple lineages. HSCs with the capacity for both long-term and short-term repopulation of the mouse hematopoietic system have been identified. Similar studies with human bone marrow cells, performed in the severe combined immunodeficiency (SCID) mouse model, yielded similar results. HSCs are thought to be rare quiescent cells that, upon demand, give rise to progenitor cells (characterized in vitro and termed CFUs), which are destined to generate fully differentiated cells. The division of HSCs is thought to be either symmetric, producing two identical daughter cells that are either both stem cells or both progenitor cells, or

asymmetric, producing an HSC and a progenitor with diminished self-renewal capacity but with the ability to enact clonal expansion and maintain the circulating blood cell population [28, 74, 86, 110].

Isolation of HSCs

Because it was thought that a single cell is capable of repopulating the hematopoietic system, several investigators have attempted to isolate HSCs by using phenotypic cell-surface markers associated with defined lineages and developmental stages of hematopoietic cells. Using high-speed multi-parameter flow cytometry, distinct cell populations were purified and collected for functional analysis. Several cell-surface proteins thought to be specific to primitive cells, such as CD34, CD133, and CD150, and combinations of cell-surface markers such as Lin⁻c-kit^{high}Thy^{low}Sca⁺ [76, 93, 103], Lin⁻Thy⁺CD34⁺CD38^{-/low}, or the SLAM family receptors [57] have been used to identify HSCs. Other techniques to isolate HSCs utilize cellular physical characteristics or enzymatic activity, such as the extrusion of Hoechst dye 33342 (like the activity of P-glycoprotein that is encoded by the *MDR* gene) identifying “side population cells” and the use of BODIPY aminoacetaldehyde to assess aldehyde dehydrogenase activity [39, 46, 56, 57, 96].

HSC Characterization

To test the stem cell characteristics of a cellular population, it is necessary to purify the cells to functional homogeneity and to demonstrate that every single purified cell is capable of reconstituting the entire hematopoietic system. In addition, it is necessary to show that every single cell of a phenotypically identical cellular population isolated from one animal is capable of reconstituting the hematopoietic system in another animal, i.e., that the stem cells that repopulated the hematopoietic system self-renew (Fig. 1). These goals cannot be reproducibly reached using any of the currently available stem cell fractionation techniques because not every cell of the phenotypically identical cell population is a functional stem cell. Rather, the end products of these fractionation assays are cells that are enriched with HSCs. Remarkably, some cells of the “stem cell-depleted” cellular fraction are also functional stem cells, as demonstrated in studies with CD34 fractionation. CD34 was the first HSC “marker,” and CD34 fractionation has been used clinically for HSC enrichment. Remarkably, hematopoietic stem cell transplantation has been successfully performed with a population of CD34-negative cells [120, 121], suggesting that functional HSCs are present within the CD34-negative cell fraction. Thus, a stem cell should be defined by its functional capacity, not by phenotype, surface marker expression, or other cellular characteristics.

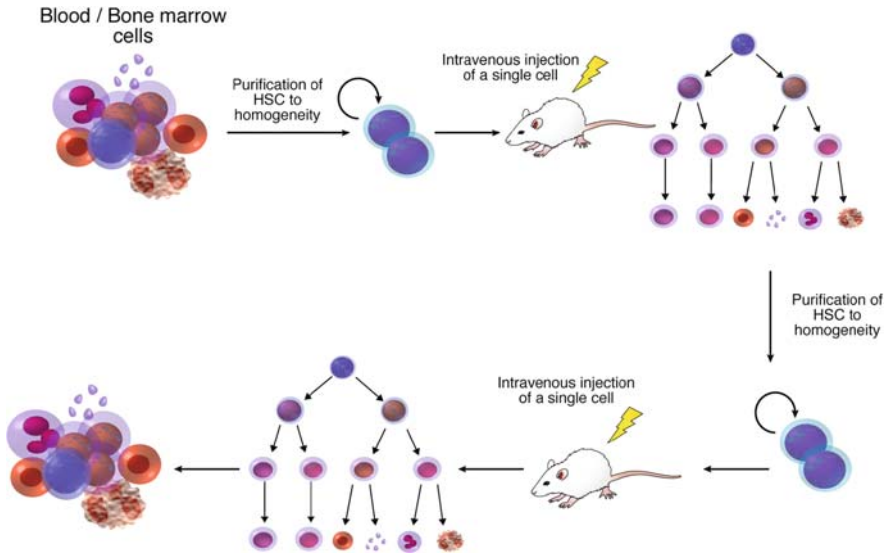


Fig. 1 Functional assessment of a purified population of HSCs. To confirm that fractionated cells are HSCs, every single cell of that homogenous population should have the capacity to repopulate the hematopoietic system in a lethally irradiated animal and, in addition, every single blood or bone marrow cell isolated from the irradiated animal by the same method should be capable of reconstituting all blood and bone marrow elements of an identical lethally irradiated animal (second-generation reconstitution)

The Hematopoietic Stem Cell Niche

Bone and bone marrow are intrinsically linked. Morphological and functional studies suggest that HSCs are located proximal to the endosteal surface of trabecular bone (reviewed in [71]). Several studies have demonstrated that osteoblast cells are required for this localization. Furthermore, HSCs express a calcium-sensing receptor. Stem cells lacking this receptor failed to localize to the endosteal niche and did not function normally after transplantation, highlighting the importance of the ionic mineral content of the bone itself and of the bone-derived matrix in the lodgment and retention of HSCs within the endosteal niche. However, HSCs function in the absence of osteoblasts. Extramedullary hematopoiesis is frequently found in patients with myeloproliferative disorders and in transgenic mice where osteoblast cells have been ablated, the marrow is aplastic, and extensive extramedullary hematopoiesis occurs. Thus, HSCs can survive and function in tissues that have no osteoblasts. The contributions of other cellular elements, such as stromal cells, osteoclasts, or perivascular cells, have been characterized. For example, it has been shown that HSCs can be recruited to a “vascular niche” that serves as an “extramedullary niche.” Experiments with parabiotic mice have demonstrated that HSCs circulate between the

blood and the bone marrow. Therefore, the existence of multiple types of HSC niches is not surprising. Whether different niches affect HSC function differently has yet to be determined.

The Leukemia Stem Cell

Cancer Stem Cell: Concept and Definition

The cancer stem cell hypothesis was proposed about 150 years ago by Rudolf Virchow and Julius Cohnheim, who argued that cancer results from the activation of dormant embryonic tissue remnants [23, 109].

The notion that leukemia stem cells (LSCs) might exist emerged from experiments performed in the early 1970s showing that only a subset of leukemia cells was capable of in vitro proliferation [70, 73, 98]. These in vitro studies, together with the in vivo studies described below, suggested that among the entire population of leukemia cells there are rare cells with the potential for self-renewal that drive the expansion of the leukemic clone. These LSCs are thought to exhibit characteristics similar to those of normal HSCs, with the exception that LSCs do not necessarily differentiate into different lineages. The current dogma is that all leukemia cells are derived from LSCs and that the descendents of these cells are clones.

It is thought that LSCs, like normal HSCs, give rise to differentiated daughter progenitor cells (termed CFU-Ls) that differentiate into leukemic blasts that lose their self-renewal capacity (Fig. 2). However, defects in the cellular machinery of CFU-Ls usually eliminate their ability to differentiate fully into morphologically and phenotypically mature cells. As a result, the leukemic cell population consists of undifferentiated and variably differentiated leukemia cells. The

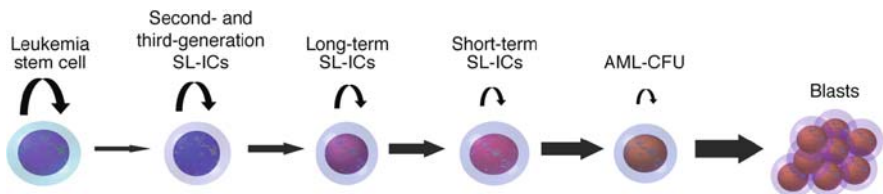


Fig. 2 Proposed hierarchal differentiation of LSCs: Rare LSCs with self-renewal capacity (*curved arrows*) give rise to SCID leukemia-initiating cells (SL-ICs) capable of initiating leukemia in second- and third-generation mice. These SL-ICs give rise to long-term SL-ICs capable of repopulating the marrow of identical second-generation mice. The short-term SL-ICs are capable of repopulating the marrow of the injected animal and give rise to AML colony-forming units (AML-CFU) that have a low rate of self-renewal. During this hierarchal differentiation process, the proliferation capacity (*straight arrows*) of the leukemic cells increases (adopted from [49] and [83]). This model is based on the assumption that human leukemia cells that engraft in SCID and NOD/SCID mice carrying LSC characteristics

degree of differentiation of leukemia cells has been used to identify their lineage (for example, myeloid versus lymphoid). Indeed, the current clinical classification of leukemias is based on the presence of “differentiation markers” in the leukemia blasts [10, 107].

Models of Leukemogenesis

The currently accepted paradigm of leukemogenesis rests on the theory that leukemia arises from a single cell and is maintained by a small population of LSCs [37, 49, 69]. It is thought that normal HSCs themselves can undergo leukemic transformation. First, in normal HSCs, the machinery for self-renewal is already activated, and second, stem cells persist for a long time; therefore, the opportunity to accumulate mutations in these cells is greater than that in mature, short-lived cell types. Nonetheless, restricted progenitors could potentially be transformed, either by acquiring mutations that cause them to self-renew or by inheriting existing mutations from the preleukemic stem cells and then acquiring additional mutations themselves. Indeed, Jamieson et al. [53] reported that in chronic myelogenous leukemia, the LSC is the differentiated progenitor CFU-granulocyte-macrophage (CFU-GM).

Two models of leukemogenesis have long been proposed. According to the stochastic model, leukemia consists of a homogenous population of immature cells and a few cells that can either self-renew or proliferate in a stochastic manner [61, 86, 104]. In contrast, in the hierarchy model, leukemia consists of a heterogeneous population, within which only a small percentage of LSCs generate leukemia clones and sustain the disease. Recently, a third model was proposed [83]. According to this model, mature leukemia cells can de-differentiate and regain functional LSC capacity [53]. Whereas the first two models hold that only a few immature cells sustain leukemia, the third model allows that mature cells can regain self-renewal capacity. One might envision leukemia as a disease in which the phenotypically mature and immature neoplastic cells, characterized by genomic instability, multiply and form new leukemic clones selected to expand based on their proliferation and survival capacity. Such cells might at times either be quiescent or be proliferate; and during this process, they may differentiate or self-renew without adhering to the “rules” of the normal hematopoietic system.

LSC Phenotype and Function

The phenotypic and functional properties of normal HSCs have been used to study LSCs [15, 29, 42] (Wissman 2000). Classic studies of human acute myeloid leukemia (AML) have shown that AML consists of a heterogeneous population of cells with a small percentage of quiescent (non-cycling) cells [22, 114], of

which only 0.1–1% harbor the capacity to initiate leukemia when injected into SCID or non-obese diabetic (NOD)/SCID mice [15, 62]. In most AML subtypes, except for promyelocytic leukemia (AML-M3), the cells capable of transplanting leukemia in NOD/SCID mice have been shown to have a $CD34^+CD38^-$ or $CD34^+CD38^{+low}$ phenotype similar to that of normal HSC, whereas more mature $CD34^+CD38^+$ leukemic blasts failed to engraft [12, 15]. Using clonal tracking of retroviral-transduced normal and leukemic cells in NOD/SCID mice, it was demonstrated that both normal and LSC compartments were composed of individual stem cell classes that differed in their repopulating and self-renewal capacities [44, 49]. These elegant studies demonstrated that a fractionated population of phenotypically identical human leukemia cells engrafting in NOD/SCID mice consists of cells with different capacities. Some cells, termed NOD/SCID leukemia-initiating cells (SL-ICs), could be harvested and, when injected into a second and third generation of identical mice, engrafted and induced leukemia. A “marker” to determine whether a certain cell is a short-term or long-term SL-IC has not been identified.

Other combinations of cell-surface markers [75] and other techniques, including the extrusion of Hoechst dye 33342, have been used to identify LSCs [16, 39, 56]. Several studies suggest that HSCs and LSCs share some cell-surface markers but not others. For example, HSCs and LSCs both express CD34, CD71, and HLA-DR. However, Thy-1 (CD90) and c-Kit (CD117) are expressed on HSCs but not on LSCs, and CD123 the interleukin-3 receptor- α are expressed on LSCs but not on HSCs [12, 13, 55]. Remarkably, cytogenetically abnormal LSCs have been found in the $CD34^+CD90^+$ populations in several patients with AML, and in rare cases, $CD34^-$ cells as well as $CD34^+$ cells have successfully engrafted and initiated human leukemia in mice [17, 85, 102]. In acute promyelocytic leukemia, unlike in other myeloid leukemias, the characteristic translocation has been observed in $CD34^+CD38^+$ but not in $CD34^+CD38^-$ cells [15, 41].

The NOD/SCID mouse studies described above suggest that AML, like normal hematopoiesis, is indeed organized as a hierarchy, which is initiated and maintained by an LSC that gives rise to SL-ICs with short-term and long-term repopulating capacities that, in turn, give rise to cells with abnormal differentiation programs, leading ultimately to the production of blasts and abnormally differentiated leukemia cells [28] (Fig. 2). However, Taussig et al. [101] showed recently that cells expressing well-established mature myeloid markers (CD33 or CD13) could function as SL-ICs, thereby questioning the validity of surface marker expression as a predictor of stem cell function. Taussig’s data agree with previous studies that have demonstrated that cells devoid of self-renewal capacity, such as committed progenitors and mature cells, could be targets for leukemic transformation. For example, activation of promoter elements of several mature myeloid-specific human genes (like MRP8, CD11b, or cathepsin G) induces human leukemias in transgenic mouse models [18, 52, 119]. Recently, Cozzio et al. [23] showed that the leukemic fusion gene MLL-ENL, which results from the t(11;19) translocation,

induced the same type of leukemia whether transduced into HSCs, common myeloid progenitors (CMPs), or granulocyte-macrophage progenitors (GMPs). Furthermore, the fusion gene MOZ-TIF2 has also recently been shown to contribute to the transformation of both HSCs and committed myeloid progenitors. Thus, the activation of an appropriate oncogene in a mature hematopoietic cell could transform the cell into an LSC with self-renewal capacity. In contrast, So et al. [92], using a different fusion partner of MLL, GAS7, showed that only the transduction of murine HSC, and not CMP or GMP, resulted in the production of mixed-lineage leukemias in mice. Therefore, a universal phenotype for LSCs may not exist, and patient-to-patient variation in cell-surface protein expression may be the rule.

Of note, a similar heterogeneity exists in HSC gene expression: genes thought to form a stem cell gene signature have been identified; however, dissimilar data suggest that the existence of such a signature may be premature [50, 80, 82]. Because stem cell-specific properties, such as self-renewal, quiescence, and proliferation, are not governed by genes that are specific to stem cells, it has been proposed that there is a “stem cell state” rather than a “stem cell portrait” in the hematopoietic system [125]. The “stem cell state” may represent a transient and potentially reversible state that cells can assume in response to the correct trigger. Using a similar concept, one can argue that there is an “LSC state” rather than an “LSC phenotype,” a concept that is supported by the well-described lineage “infidelity” in human leukemias [91]. Whereas defining the sets of conditions that pertain to the LSC state may allow the development of strategies to eliminate LSCs or render them inconsequential, the identification of specific genes and surface markers expressed solely by LSCs may be difficult or impossible.

Taken together, these data suggest that LSCs should be characterized by function rather than by phenotype and be referred to as “leukemia-initiating cells.” It is unclear whether studies performed using SCID or NOD/SCID mouse models reflect normal human physiology and/or pathophysiology. Thus, the overall conclusion that can be drawn from those studies is that the capability of a leukemia cell to initiate and sustain leukemia in humans, regardless of its phenotype, physical characteristics, or function in immune-deficient mice, is the most important biological feature of the cells that we refer to as LSCs.

Molecular Pathways Regulating HSCs and LSCs

The current dogma is that self-renewal is the hallmark property of stem cells in both normal and neoplastic tissues. Therefore, the molecular pathways that regulate this self-renewal capacity have been studied extensively. Several genes, transcription factors, and cell-cycle regulators modulate the self-renewal, proliferation, and differentiation of HSCs [95, 123]. The genes SCL, GATA-2,

LMO-2, and AML-1 (also known as CBFA2 or RUNX1) govern the transcriptional regulation of early hematopoiesis, and the deregulation of these genes through chromosomal aberrations plays a key role in leukemogenesis. For example, the gene encoding the transcription factor SCL is the most frequent target of chromosomal rearrangements in children with T-cell acute lymphoblastic leukemia [4]. SCL is normally expressed in HSCs and immature progenitors and is downregulated during differentiation, and its abnormal activation might initiate malignant transformation [64]. Similarly, abnormal activation of AML-1, a gene that is required for normal hematopoiesis, as a result of translocation t(8;21) and the generation of the fusion protein AML-ETO is thought to be leukemogenic [67, 77]. The fusion protein AML-ETO has been shown to induce stem cell self-renewal [26]. However, increased self-renewal is of no apparent pathogenic consequence, presumably because secondary mutations are necessary for the expression of the leukemic phenotype [26].

Other transcription factors such as the Homeobox (Hox) genes, which include HoxB4, and the Wnt-signaling pathway have well-described roles in regulating the self-renewal and differentiation of HSCs [85, 124]. HoxB4 is abundantly expressed in HSCs, plays a role in HSC expansion, and declines as terminal differentiation proceeds [88]. Of note, deregulated expression of Hox family members such as HoxA9 is commonly observed in AML [38, 63]. The Wnt-signaling pathway, whose activity is critical to the development of several organs, plays an important role in the regulation of hematopoietic stem and progenitor cell function [85, 94]. Overexpression of β -catenin, a downstream activator of the Wnt signaling pathway, expands the HSC pool [86], and activation of the Wnt pathway increases the expression of transcription factors and cell-cycle regulators important in HSC renewal, such as HoxB4 and Notch-1 [31, 85].

The Notch/Jagged pathway modulates extracellular regulatory signals controlling HSC fate [2]. Members of the Notch family have critical roles in keeping HSCs in an undifferentiated state and may act as gatekeepers for factors governing self-renewal and lineage commitment [81]. Of interest, the gene encoding the Notch receptor is rearranged by recurrent chromosomal translocations in some patients with T-cell acute lymphoblastic leukemia [32]. Transcription factors and cell-cycle regulators associated with oncogenesis, such as Bmi-1 and Sonic hedgehog (Shh), regulate the proliferation HSCs and LSCs [99, 106]. Bmi-1, a member of the Polycomb family [65], is expressed in normal and leukemic HSCs and regulates self-renewal by modulating the activity of genes governing proliferation, survival, and lineage commitment [66, 79]. Although direct evidence for the role of Shh in the regulation of self-renewal is lacking, *in vitro* studies demonstrated HSC self-renewal activity of Shh in combination with various growth factors [11]. Recent data suggest that PTEN (phosphatase and tensin homologue deleted on chromosome ten), a negative regulator of the phosphatidylinositol 3 kinase (PI3K) pathway, has essential roles in restricting the activation of HSCs, in lineage fate determination, and in the prevention of leukemogenesis [122]. Furthermore, it has been shown recently that PTEN dependence could distinguish HSCs from LSCs [118].

Taken together, these data suggest that differential expression of several transcription factors controls the fate of HSCs and plays a critical role in the determination of self-renewal, differentiation, and lineage commitment. These pathways are under the control of various intracellular stimuli as well as cytokines and stromal factors from adjacent cells in the bone marrow. Further studies of these transcription factors in HSCs and the mechanisms causing their deregulation are likely to provide us with better targets for therapy.

Cellular HSC and LSC Regulators

Within the hematopoietic microenvironment, early progenitors are thought to be maintained and compartmentalized in a specific location in the endosteal lining of the bone cavities. However, extramedullary niches have also been described, as discussed above. Extramedullary disease is occasionally found in several leukemias. Although it is well established that the leukemogenic process is bone marrow derived, it is not clear whether, as in normal HSCs, an LSC niche exists.

Normal hematopoietic and leukemia cells proliferate in response to several cytokines and hematopoietic growth factors [34, 84]. Thus, the regulation of normal and leukemic hematopoiesis is the result of multiple processes involving cell–cell and cell–extracellular matrix interactions and the actions of specific growth factors and other cytokines as well as intrinsic modulators of hematopoietic development. Despite some important progress, the genetic and cellular factors that influence stem cells to differentiate into developmentally restricted progenitor cells or to self-renew to replace cells that have become committed to differentiation are still poorly understood.

Clinical Significance

LSCs, Drug Resistance, and Relapse

Despite the development of several new agents that effectively reduce the tumor burden in patients with leukemia, relapse continues to be the most common cause of death, particularly in patients with AML. Therefore, most, if not all, patients who attain complete remission still harbor disease-sustaining cells. Because these residual leukemia cells cannot be detected by conventional techniques (such as light microscopy), the term minimal residual disease (MRD) is used to describe them [33]. Current efforts directed at detecting and quantifying MRD are based on the assumption that eradication of disease-sustaining cells will improve treatment outcomes [35, 100]. The question of why neoplastic cells with disease-sustaining capacity survive chemotherapy has prompted extensive research in recent decades.