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Leukemia stem cells: old concepts and new perspectives

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Abstract

Myeloid leukemias are heterogeneous malignancies in morphology, immunophenotype, genetic and epigenetic alterations, and response to therapy. This heterogeneity is thought to depend on the accumulation of secondary mutations enhancing proliferation/survival and/or blocking differentiation in a small subset of leukemia-initiating cells capable of self-renewal. This model of clonal evolution is based on xenotransplantation studies demonstrating that leukemia can be initiated and maintained in immunodeficient mice by a small subset of purified leukemic cells immunophenotypically similar to normal hematopoietic stem cells and is known as the leukemia stem cell model. Since its original formulation, many studies have validated the main conclusion of this model. However, recent data from xenotransplantation studies in more severely immunodeficient mice suggest that immunophenotype and behaviour of leukemic stem cells is more heterogeneous and “plastic” than originally thought. We will discuss here the evolution of the leukemia stem cell model and its impact for the therapy of patients with myeloid malignancies.

1. Introduction

The concept that leukemia develops from and is maintained by a small population of transformed stem cells organized hierarchically like its normal counterpart has been among the most important in modern oncology and has prompted a lot of studies attempting to validate or disprove the idea that more common non-hematopoietic malignancies follow a similar hierarchical organization. Whether this concept is correct is not an academic dispute but has profound practical consequences since it implies that leukemia (or cancer) can be permanently cured only if therapies can eliminate leukemic (cancer) stem cells.

In its original formulation (Bonnet and Dick, 1997), the leukemia stem cell model was based on the demonstration that a rare population of acute myeloid leukemia (AML) cells immunophenotypically similar to the normal counterpart (the CD34+CD38– subset) was enriched in cells capable of inducing leukemia when injected in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. Leukemia that developed in NOD/SCID mice was immunophenotypically heterogeneous and similar to that seen in the original patients and was serially transplanted in secondary recipient mice, consistent with the concept that transformed stem cells, like their normal counterparts, are capable of self-renewal and differentiation.

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Subsequent studies confirmed that leukemic stem cells (LSCs) are rare and confined to the CD34+CD38[−] subset, although additional antigens capable of enriching for leukemia-initiating cells (LICs) in NOD/SCID mice were identified.

Although widely accepted, early critics argued that the use of xenotransplantation assays to functionally assess the re-populating capability of leukemic cells was subjected to several potential problems; for example, some LSC subsets may not escape residual host immunity, or interact appropriately with the bone marrow microenvironment, or respond efficiently to survival and proliferative signals intrinsic to recipient mice.

In the past few years, data on xenotransplantation assays using purified leukemic cell subsets in more immunodepressed mice such as NOD/SCID mice with a targeted deletion of the γ common chain of the interleukin-2 receptor(IL-2R) (NSG mice) which lack residual natural killer (NK) activity and exhibit an improved environment for the functionality of human hematopoietic stem cells (HSCs), has prompted a reassessment of the leukemia stem cell model. In these mice, cells that promote leukemia formation remain exceedingly low; however, they do not appear to be restricted to the CD34+CD38[−] subset as leukemia-initiating cells (LICs) were identified in the CD34+CD38⁺ subset as well as in the CD34[−] fraction (Sarry et al, 2011; Goardon et al, 2011). Moreover, each subset of leukemic cells that promotes the development of leukemia in NSG mice was capable of reconstituting the phenotypic heterogeneity of the original AML sample. The results of these studies do not challenge the notion that leukemia is maintained by a small cohort of self-renewing cells; however, if confirmed by additional studies, they support the conclusion that: i) self-renewing, LICs reside in primitive as well as more differentiated progenitor cell subsets; and ii) in addition to be hierarchically organized with primitive LICs giving rise to more differentiated blast cells, antigenically differentiated LICs can undergo “de-differentiation”, suggesting that LICs may exhibit some degree of “plasticity”.

In the following sections we will discuss how the leukemia stem cell model has evolved over the years and the implications of this model for disease progression and therapy.

2. Xenotransplantation assays with purified AML leukemic subsets: evolution of the leukemia stem cell model.

The impetus for assessing whether leukemic subsets can repopulate hematopoietic organs of immunosuppressed recipient mice stems from pioneering experiments demonstrating long-term reconstitution of multi-lineage hematopoiesis in immunodeficient mice co-implanted with small fragments of human fetal thymus and fetal liver (McCune et al, 1988) or with unfractionated human marrow cells (Kamel-Reid and Dick, 1988). Following these and other studies (including those with sorted populations of human cord blood cells) (Dick et al, 1991; Lapidot et al, 1992), engraftment of leukemic cells in immunodeficient mice was obtained first with unfractionated leukemic cells (Lapidot et al, 1994) and subsequently with purified subsets of AML cells, demonstrating that those capable of initiating leukemia in NOD/SCID mice were restricted to the CD34+CD38[−] subset (Bonnet and Dick, 1997). The latter study also showed that the frequency of LICs was variable but low and that these cells possessed two critical properties typical of the stem cells: they were able to proliferate and

differentiate into blast cells identical to those of the original AML sample and to renew themselves as indicated by induction of leukemia, when re-injected in secondary recipients. Collectively, these studies led to the hypothesis that, in the majority of cases, AML originates from and is maintained by transformed stem cells.

The notion that the LICs were confined to the CD34+CD38⁻ subset remained unchallenged for over a decade, but several recent studies have disputed this assumption.

First, Taussig et al. (2008) showed that the apparent inability of AML CD34+CD38⁺ subsets to induce leukemia in immunodeficient mice was, in part, due to immune-mediated elimination of anti-CD38-labeled cells since pre-treatment of recipient mice with immunosuppressive antibodies restored the leukemia-initiating capability to the CD34+CD38⁺ fraction of several AML samples.

Second, the same group (Taussig et al, 2010) showed that LICs of AML with mutation of the nucleophosmin gene, which is characterized by low CD34 expression, were confined in approximately one-half of cases to the CD34⁻ fraction. Moreover, LICs were found in more than one subset in a significant number of AML samples and were immunophenotypically unstable upon serial transplantation in mice, further supporting the concept that multiple subsets have leukemia-initiating capacity (Taussig et al, 2010).

Evidence that LICs are not restricted to a distinct “stem cell-like” subset was further strengthened by other important studies (Goardon et al, 2011; Sarry et al, 2011; Eppert et al, 2011).

Goardon and colleagues reported that in most AML samples LICs reside in two populations of hierarchically ordered progenitor subsets, CD34+CD38⁻CD90⁻CD45RA⁺ and CD34+CD38+CD90⁻CD45RA⁺ (or “granulocyte-macrophage progenitor (GMP)-like”). The more immature subset (CD34+CD38⁻CD90⁻CD45RA⁺) differentiates in vivo into the more mature “GMP-like” subset while >0.2% of CD34+CD38⁻CD45RA⁺ cells are generated upon injection of “GMP-like” cells, consistent with an in vivo immunophenotypic hierarchy whereby more primitive progenitors give rise to more differentiated progenitors. Of greater interest, the gene expression profile of these two subsets was largely distinct and similar to that of the normal progenitor counterparts rather than HSCs. However, these LIC-enriched subsets expressed a number of genes associated with self-renewal. Collectively, these findings led Goardon and colleagues to propose that in most cases “AML is a progenitor disease where LSC acquire abnormal self-renewal potential”. This model is consistent with that based on findings of MLL-AF9-induced leukemia in mice demonstrating that the LICs originated from the GMP subset but exhibited a gene expression signature enriched in self-renewal-associated genes (Krivtsov et al, 2006).

Recent studies from John Dick’s laboratory (Eppert et al., 2011) using optimized xenotransplantation assays of purified AML subsets in NOD/SCID mice confirmed that the CD34+CD38⁺ subset contained LICs in approximately 50% of the cases. Of interest, the gene expression signature of functionally-defined LICs (eg., those capable of initiating leukemia in NOD/SCID mice) appears to be similar to that of cord blood HSCs. This finding is somewhat different from other observations (Goardon et al., 2011) and it is unclear

whether the different source of HSCs (cord blood versus marrow) used in the two studies may explain this apparent discrepancy.

Of interest, comparison of the LICs and HSCs signatures with clinically-annotated gene expression data sets derived from unsorted AML cells revealed that stem cell gene expression profiles persisted in bulk leukemic blasts and were enriched in poor-prognosis AML patients (Eppert et al, 2011).

In a separate study using NSG mice, the authors demonstrated that AML LICs are rare and heterogeneous and, although enriched in the Lin-CD38⁻ subset, the majority was found in the “GMP-like” CD34⁺CD38⁺CD45RA⁺ subset, the “CMP-like” CD34⁺CD38⁺CD45RA⁺CD123⁻ subset, and CD34⁻ subsets (Sarry et al., 2011). Similar to the observations of other studies, each subset injected in NSG mice induced a leukemia with the heterogeneous immunophenotype of the primary sample. The findings of this study differ from those cited above in that they suggest not only in vivo differentiation from primitive into more differentiated progenitors but also a “de-differentiation” capability of more differentiated (immunophenotypically) LICs. If confirmed, this observation could have important implications since it would suggest that LICs have some degree of “plasticity” that could make their targeting considerably more challenging. Leukemic cells (CD45⁺CD33⁺) purified from NSG mice with primary leukemia were capable of inducing leukemia in secondary recipients. A likely explanation for this finding is that each subset with leukemia-initiating capability contains self-renewing cells; however, due to the apparent “plasticity” of LICs, it cannot be excluded that only “de-differentiated” cells are capable of self-renewal and of inducing secondary leukemia. In summary, although based on the use of non-identical xenotransplantation assays, these studies support the general conclusion that AML LICs are rare and do not reside in a specific cell subset (Figure 1).

Much less is known regarding the involvement of stem-progenitor subsets in acute lymphoblastic leukemia (ALL). An elegant study by the Jacobsen's group (Castor et al, 2005) demonstrated that different subtypes of ALL exhibit heterogeneity regarding the cell of origin for transformation based on analysis of fusion genes (eg., the TEL-AML1 translocation is detected in B-cell progenitor subsets while the BCR-ABL translocation that generates the breakpoint cluster region (BCR)/Abelson murine leukemia viral oncogene homolog 1 (ABL1) p210BCR/ABL protein is detected in the stem cell compartment); however, the ability to reconstitute a leukemic process in NOD/SCID mice resided in the committed B-progenitor subset in both cases (Castor et al, 2005). A recent study (Notta et al, 2011) has demonstrated that Philadelphia chromosome (Ph1) ALL samples are heterogeneous in the ability to reconstitute a leukemic process in immunodeficient mice and that this behavior correlates with the presence of specific genetic aberrations (eg., samples with deletion of the CDKN2A/B locus induce a more aggressive disease) and the frequency of LICs; however, multiple, genetically-distinct subclones were identified in the leukemia that developed in xenotransplanted mice, indicating that Ph1 ALL is composed of varying numbers of genetically distinct subclones with different capacity to reconstitute a leukemic process in immunodeficient mice. However, the study did not address the issue of whether different LICs reside in immunophenotypically distinct stem- and/or progenitor subsets and the role of these subsets in leukemia maintenance.

3. LICs and disease progression: lessons from chronic myelogenous leukemia.

Chronic myelogenous leukemia (CML) is universally considered a stem cell disorder based on detection of the Ph chromosome, the genetic lesion responsible for generating the oncogenic BCR/ABL protein that causes the disease (Ren, 2005), in multiple hematopoietic cell types (Haferlach et al, 1997) and classical genetic studies demonstrating clonal glucose-6-phosphate dehydrogenase (G6PD) isoenzyme expression in multiple hematopoietic lineages including B-cells (Fialkow et al, 1977; Martin et al, 1980). Additional evidence in support of the notion that the HSC is the cell of origin in CML derives from studies demonstrating that BCR/ABL can transform stem cell-enriched murine Lin-Sca-1+Kit+ cells but not the GMP subset (Huntly et al, 2006). Moreover, the detection of residual primitive Philadelphia-positive cells in Imatinib-treated CML patients with complete cytogenetic remission (Graham et al, 2002; Bhatia et al, 2003) and the kinetics of disease relapse upon discontinuation of Imatinib in CML patients in clinical remission (Michor et al, 2005) are both consistent with the hematopoietic stem cell being the cell of origin for transformation by BCR/ABL.

Based on these data, one may expect that xenotransplantation assays of CML progenitor subsets in immunodeficient mice would be consistent with the leukemia stem cell model showing that LICs reside in the HSC subset. Instead, data on the repopulation ability of CML progenitor subsets in immunodeficient mice are rather limited; although a study has suggested a longer persistence of the CD34+CD38- than of the CD34+CD38+ subset in NOD/SCID- $\beta 2m^{-/-}$ mice (Eisterer et al, 2005), data are inconclusive due to the small number of samples tested. The persistence of normal HSCs in most chronic phase CML patients and the lack of methods for the selective purification of Ph-positive stem cells are thought to be responsible for the poor engraftment of CML cells in immunodeficient mice, and yet it is “paradoxical” that the leukemia stem cell model has not been convincingly validated using primitive cell subsets derived from a stem cell disorder. Perhaps, BCR/ABL lacks the ability to promote self-renewal and this may impair the leukemogenic process in immunodeficient mice xenotransplanted with CML primitive subsets.

The natural course of CML is to evolve from a clinically benign chronic disease (CML-chronic phase) characterized by the expansion of precursor and terminally differentiated myeloid cells in the bone marrow and peripheral blood into a rapidly fatal “blast crisis” (CML-blast crisis) characterized by the accumulation of primitive blast cells of myeloid or, less frequently, B-lineage (Spiers AS, 1995). Treatment with Imatinib and second generation tyrosine kinase inhibitors (TKIs) has had a profound impact on the course of the disease with a marked improvement in long-term survival (Hochhaus et al, 2009; Saglio et al, 2010; Kantarjian et al, 2010); however, some CML patients do not respond to TKIs and progress to blast crisis (Hochhaus et al, 2009) and residual BCR/ABL-expressing stem/progenitor cells persist in most CML patients in clinical remission and undetectable Ph chromosome (Chu et al, 2011), raising the possibility that some of these patients may, ultimately, undergo blast crisis transition.

The mechanisms responsible for chronic phase to blast crisis transition are still not completely understood but it is likely that increased expression of BCR/ABL and secondary genetic abnormalities leading to perturbation of signaling pathways responsible for enhanced self-renewal and differentiation arrest of primitive progenitors are involved (Perrotti and Calabretta, 2004; Perrotti et al, 2010)

Analysis of HSC and progenitor subsets in different stages of the disease has shown that common myeloid progenitors (CMPs) and GMPs but not HSCs are expanded during disease progression (Jamieson et al, 2004). Moreover, these progenitor subsets from CML-BC patients exhibit increased expression and activity of β -catenin and, in contrast to the normal counterpart, are capable of self-renewal, as indicated by re-plating assays (Jamieson et al, 2004). These findings led to the concept that GMPs function as LSCs in blast-crisis CML due to the acquisition of self-renewal potential conferred by enhanced β -catenin activity or, possibly, by other signaling pathways. Consistent with this, genetic or pharmacological inhibition of β -catenin activity impairs the self-renewal of CML stem cells (Zhao et al, 2007; Heidel et al, 2012).

Although there is no formal proof yet that CML-myeloid blast crisis cell subsets can initiate and maintain leukemia in immunodeficient mice, these studies support the existence of a bi-phasic process whereby leukemia is initiated by oncogenic events occurring in stem cells that *per se* do not promote self-renewal and is maintained by secondary mutations in high-proliferative committed progenitors that endow these cells with self-renewal potential. This bi-phasic process may not be limited to CML chronic phase to blast crisis transition; the heterogeneity (by surface antigen expression) of AML LICs validated by xenotransplantation assays suggests that it may be the most common route for myeloid leukemia initiation and maintenance. However, it cannot be excluded that “initiation” and “maintenance” oncogenic mutations both occur in stem cells or that “initiating” mutations occurring in committed progenitors endow these cells, *ab initio*, with the ability to self renew.

4. Clinical implications of the leukemia stem cell model.

A generally accepted implication of the leukemia stem cell model is that if leukemia is driven by transformed stem cells an effective cure can only be achieved if these cells are completely eradicated. This assumption holds true for CML, the prototypical stem cell disorder, in which the duration of complete hematological and cytogenetic remission is inversely correlated with residual BCR/ABL expression (Breccia and Alimena, 2010). However, BCR/ABL-positive stem/primitive progenitors persist in CML patients with durable cytogenetic and molecular remission (Chomel et al, 2011; Chu et al, 2011); and preliminary evidence indicates that discontinuation of Imatinib therapy leads to late molecular recurrence even in patients with complete molecular response for more than two years (Rousselot et al, 2007; Mahon FX et al, 2010). This suggests that, while a treatment with tyrosine kinase inhibitors (TKIs) may be highly effective in some patients, persistence of BCR/ABL-positive stem cells may foreshadow the development of clinical relapse. For the past ten years, the issue of whether BCR/ABL activity can be suppressed in CML stem cells by treatment with TKIs and if these cells are sensitive to the treatment has been hotly

debated and investigated by the CML research community. A consensus has now emerged that BCR/ABL tyrosine kinase activity is suppressed by treatment of CML stem cells with TKIs and yet these cells remain “insensitive” in that they survive and eventually proliferate, although at the slow pace of stem cell (Corbin et al, 2011; Hamilton et al, 2012). The reasons why CML stem cells are “insensitive” to TKIs are presently unknown; most strategies aimed at eliminating CML stem cells attempt to “re-sensitize” CML stem cells to TKIs through the simultaneous targeting of BCR/ABL and other signaling pathways that may be activated upon BCR/ABL inhibition or self-renewal pathways (eg., the WNT/ β -catenin/Lef or Hedgehog) shown to play a role in BCR/ABL-dependent leukemia (Dierks et al, 2008; Zhao et al, 2009; O’Hare et al, 2012). Activation of p53 (by inhibition of B-cell lymphoma 6 (BCL6) or sirtuin 1 (SIRT1) or inhibition of Bcl-2 family anti-apoptotic proteins has also been shown to enhance the elimination of CML stem cells by TKIs in vitro and in mice (Goff et al, 2013; Hurtz et al, 2011; Li et al, 2012). However, a precise understanding of the mechanisms whereby these approaches may promote the elimination of CML stem cells compared to treatment with TKIs only is lacking, as some of the pathways targeted in CML stem cells are activated by BCR/ABL in a tyrosine kinase-dependent manner and TKIs suppress BCR/ABL activity in CML stem cells. Our laboratory has shown that treatment with TKIs induces autophagy in CD34+CD38– CML cells and that these cells are highly sensitive to treatment with TKIs and autophagy inhibitors such as chloroquine (Bellodi et al, 2009). However, it is still unclear whether : i) CML stem cells are more prone than committed progenitors to undergo autophagy; and ii) if this, in the context of the bone marrow hematopoietic “niche”, provides a survival mechanism against Imatinib-induced cell death.

It is conceivable that CML stem cells may also be targeted by inhibition of pathways activated independently of BCR/ABL tyrosine kinase activity; these may include stroma-dependent signals influencing survival signals in LSCs (Goff et al, 2013) or epigenetic changes induced by active BCR/ABL that persist in spite of effective inhibition of kinase activity or pathways that use kinase-dead BCR/ABL as a “scaffold” (O’Hare et al, 2012). In the latter situation, the elimination of CML stem cells would be improved by treatment with drugs promoting BCR/ABL degradation such as HSP90 inhibitors (Peng et al, 2007).

Thus, there is a compelling reason for developing more effective therapies targeting CML stem cells as failure to eliminate these cells by treatment with TKIs is responsible for minimal residual disease which may, ultimately, lead to clinical relapse.

For AML, the translational implications of the leukemia stem cell model are less clear-cut.

A recent study by John Dick and collaborators shows that the gene expression profile of functionally-defined LICs (those inducing leukemia in NOD/SCID mice) is enriched in genes expressed by normal HSCs (Eppert et al, 2011). Moreover, the LIC and HSC signatures are part of the gene expression profile of AML blast from poor-prognosis patients (Eppert et al, 2011). This may have clinical implications, as patients with the LIC/HSC signature may be treated differently from those without the signature. At the moment, it is unclear which treatment may be beneficial to patients with the LIC/HSC signature; it is conceivable that drugs may be selected based on the ability to preferentially suppress the

LIC/HSC signature in AML blasts. In this regard, a recent paper has used a chemical genomic screen to identify compounds that decrease Mcl-1 expression, identified as marker of apoptosis resistance in breast cancer cells (Wei et al, 2012); the apoptosis-inducing effects of these compounds were phenocopied by Mcl-1 RNA interference (Wei et al, 2012).

The question of whether LSCs may be targeted specifically rests on their reliance on a number of signaling pathways associated with the ability to self-renew and the “distinctiveness” of their immunophenotype.

Regarding the first issue, there is growing evidence that certain signaling pathways (eg, WNT/ β -catenin, Sonic Hedgehog, Notch, Bmi-1, NF- κ B) are implicated in self-renewal not only in leukemic cells but also in other tumor types (Becker and Jordan, 2011). Ideally, the most attractive approach would be to target the self-renewal pathway(s) activated in individual patients; however, this may not be necessary if distinct self-renewal pathways are activated in patients' samples with specific genetic abnormalities or if a limited number of self-renewal pathways are activated, regardless of the genomic abnormality characteristic of a specific leukemia subtype.

However, self-renewal pathways activated in LSCs (eg, WNT/ β -catenin and Hedgehog) may be also required by normal hematopoietic cells (Reya et al, 2003; Zhao et al, 2007; Gao et al, 2009; Zhao et al, 2009), raising the possibility that such treatments may be toxic.

The implication of the leukemia stem cell model as originally proposed is that if LSCs reside only in the CD34+CD38[−] subset, antigens preferentially expressed by AML CD34+CD38[−] stem cells may be identified, providing targets for their elimination. Below is a partial list of potential LSC targets (Table 1).

The CD123 antigen (interleukin-3 receptor alpha chain) was the first to be identified in CD34+CD38[−] AML cells (Jordan et al, 2000); targeting of this receptor with a neutralizing monoclonal antibody (7G3) reduced the AML burden and inhibited the re-populating activity of LSCs in NOD/SCID mice (Jin et al, 2009). Moreover, the delivery of toxic conjugates to LSCs through this receptor is now under investigation (Frankel et al, 2008). The C-type lectin-like molecule-1 (CLL-1) is another antigen that can identify residual CD34+CD38[−] leukemic cells in the bone marrow of patients in clinical remission (van Rhenen et al, 2007). A monoclonal antibody against CLL-1 induced antibody-dependent cytotoxicity of primary AML cells, but the effects on LSCs were not reported (Zhao et al, 2010). Studies from Irving Weissman's laboratory showed that LSCs express the CD47 antigen that binds to the macrophage SIRP-alpha protein, blocking the ingestion of leukemia cells by macrophages (Jaiswal et al, 2009); its targeting by a monoclonal antibody suppressed the engraftment of LSCs in NSG mice and induced a marked decrease of bone marrow and circulating AML blasts in NSG mice engrafted with AML cells and treated with the antibody 8–12 weeks later (Majeti et al, 2009). Studies from John Dick's laboratory showed that engraftment of AML (but not normal cord blood or marrow) cells in NOD/SCID mice was impaired by treatment with an anti-CD44 activating antibody (H90), but the effect was more pronounced in mice with low disease burden (Jin et al, 2006). Mechanistically, the effects of this antibody on the re-populating activity of AML cells

appear to be multifactorial in that it inhibits the homing capacity and promotes the monocytic/granulocytic differentiation of LSCs (Jin et al, 2006). Monoclonal antibodies targeting these and other candidate LSC-specific antigens are intensively investigated in pre-clinical models of leukemia and may, ultimately, prove effective in AML patients (Majeti, 2011).

In spite of these encouraging results, the nature of the LSCs raises legitimate questions on the feasibility of their elimination. First, LSCs reside in more than one population of blast cells and the more primitive LSCs can generate a more differentiated (and immunophenotypically distinct) progeny still capable of self-renewal ; second, each LSC subset, regardless of its immunophenotype, can reconstitute a leukemic process in immunodeficient mice that “mimics” the heterogeneity of the original AML. Whether different cell subsets in the secondary leukemia contain LSCs is presently unknown; however, the current understanding of the biology of LSCs suggests that these cells are more “plastic” than originally thought, based on the assumption that they all resided in the CD34+CD38– subset and were “hierarchically” related to more differentiated subsets lacking self-renewal capacity. It is possible that the “heterogeneity” of the LSCs is exaggerated by the reliance on expression of the CD34 and CD38 antigens for their purification and that, once new and more reliable tools become available, LSCs will be found to reside in a “discrete” cell subset amenable to selective targeting. However, within the limits of today’s tools, normal and leukemic stem cells do not appear to share the same “hierarchical” organization, supporting the concept that LSCs are functionally heterogeneous. If the “plasticity” of LSCs is confirmed by additional studies, it will be much more challenging to target these cells based on their immunophenotype, and novel approaches will be needed.

5. Summary.

Based on xenotransplantation studies in immunodeficient mice, the leukemia stem cell model postulates that, like normal hematopoiesis, leukemia is maintained by a small population of immunophenotypically distinct and hierarchically organized LSCs. Over the years, experiments carried out in more severely immunodepressed mice did not change the initial conclusion that LSCs are rare. However, the notion that functional LSCs (i.e., cells capable of initiating leukemia in immunodeficient mice) reside only in the CD34+CD38– subset has been disputed; a large body of evidence now supports the concept that LSCs are heterogeneous in that they can also reside in less primitive progenitor subsets, based on immunophenotype and gene expression profiles. Although more differentiated, these cells are also characterized by expression of self-renewal genes typical of HSCs, a finding consistent with their leukemia maintenance capacity in immunodeficient mice. Moreover, recent observations suggest that leukemia initiated in immunodeficient mice by more differentiated LSCs recapitulates the heterogeneity of the AML samples from which LSCs are derived. If confirmed, this unexpected “plasticity” would make much more challenging to develop anti-leukemia therapies based on targeting the LSCs.

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NORMAL MYELOID HEMATOPOIESIS

AML

Figure 1.

Heterogeneity of LICs. LSCs functionally defined as capable of generating leukemia in immunodeficient mice (LICs) are immunophenotypically heterogeneous. Although some LICs may be similar to HSCs, the majority reside in hierarchically-organized more differentiated subsets. In a study (reference 49), CD34⁻ LSCs reproduced in vivo the heterogeneity of the original AML sample, consistent with “LSC plasticity”. Within the hematopoietic hierarchy, immunophenotypically-distinct LICs may arise as consequence of oncogenic mutations conferring different properties depending on the cell in which the mutation has occurred (eg., proliferative advantage to HSCs; self-renewal to “GMP-like” cells).

Table 1

LSC antigens targeted by monoclonal antibodies.

Antigen	Monoclonal antibody	Effect	Refs.
CD123 (IL-3R alpha chain)	7G3	Reduced AML burden in NOD/SCID mice	Jin et al. (2009)
CLL-1 (C-type lectin-like molecule-1)	1075.7	AML cytotoxicity	Zhao et al. (2010)
CD47	B6H12	Reduced LSC engraftment in NSG mice	Majeti et al. (2009)
CD44	H90	Reduced AML engraftment in NOD/SCID mice	Jin et al. (2006)