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Multi-Parameter Fluorescence-Activated Cell Sorting and Analysis of Stem and Progenitor Cells in Myeloid Malignancies

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Abstract

Owing to the discovery that rare leukemia-initiating cells (or leukemia stem cells, LSC) give origin to and propagate a hierarchical cellular organization of variably differentiated leukemic blasts, the analysis of precisely defined stem and progenitor cells has increasingly gained importance. Emergence of multi-parameter high-speed fluorescence-activated cell sorting (FACS) for the subfractionation of hematopoietic progenitor cells has allowed research on the biology of the cell-of-origin for LSCs and of LSCs as potential (or essential) therapeutic targets that may escape chemotherapy and consequently contribute to disease relapse. This review introduces the current state-of-the-art methods for the fractionation of hematopoietic stem and progenitor cells, with particular focus on myeloid malignancies. As many aspects of human normal and malignant hematopoiesis are frequently modeled in animal studies, we also provide an overview of hematopoietic stem and progenitor cell purification methods that are commonly utilized for research in murine models of disease.

Keywords

Leukemia; Acute Myeloid Leukemia; Myelodysplastic Syndrome; Hematopoietic Stem Cell; Leukemia Stem Cell; Cancer Stem Cell; Leukemia-initiating; Progenitor Cell; Myeloid; FACS

Hematopoietic stem and progenitor cells are defined by key functional properties at the single cell level, including self-renewal, pluripotency, colony-forming capacity, and differentiation. While stem cells are defined functionally, the prospective enrichment of hematopoietic stem cells is based on surface markers and properties to retain or exclude certain intracellular dyes. This ability to separate stem cells has revolutionized stem cell research and its use in clinical practice. During the last two decades, since the seminal discovery that hematopoietic stem cells are contained within the CD34+ compartment [1], our definition and knowledge of hematopoietic stem and progenitor cells has been constantly refined and expanded. In particular, the introduction of the technology of multi-parameter high-speed fluorescence-activated cell sorting (FACS) has rapidly increased our knowledge of the hierarchical organization of hematopoietic differentiation, the cellular and molecular properties of the different stem and progenitor cell populations within this

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hierarchy, and the disruption of normal hematopoiesis in hematologic malignancies. Importantly, the continuous refinement of surface markers for the isolation and analysis of distinct and precisely defined stem and progenitor cell populations in human and murine hematopoiesis has allowed for a careful re-evaluation of the old paradigm that myeloid malignancies are ”stem cell” diseases. While this statement seemed true about 15 years ago, based on the phenotypic definition of stem cells (CD34+) at that time, our significantly improved knowledge of the various stem and progenitor cell types contained within the CD34+ compartment has led to more sophisticated, and leukemia subtype-dependent models of the cellular origin of myeloid leukemias. Proof of the existence of rare subpopulations of leukemia stem cells (LSC), which exhibit properties of self-renewal and give rise to a progeny of more differentiated leukemic blasts, by FACS has revolutionized our understanding of cell growth in AML. Identifying the LSCs and/or the leukemia cell-of-origin for each subtype of leukemia has become the current challenge and prerequisite for accurate diagnosis, reliable prognosis and successful (curative) therapy.

1. Isolation and analysis of human hematopoietic stem and progenitor cells

There are several fundamentally different methods to isolate hematopoietic stem cells (HSC). One relies on the use of combinations of surface markers, another one on the ability of stem cells to efflux intracellular dyes (e.g. Hoechst 33342), and a third one on the presence of elevated levels of the enzyme aldehyde dehydrogenase (ALDH) in stem cells. In the following we will give an overview of these strategies.

1.1 Isolation of normal human hematopoietic stem and progenitor cells based on surface markers

To date, there is no evidence for the existence of a single cell surface marker that is characteristic for HSC and is not expressed on any other hematopoietic cells; it is well possible that such a single marker does not exist. However, isolation of human stem and progenitor cells can be achieved utilizing combinations of surface markers. The discovery, refinement, and functional characterization of such marker combinations has been greatly facilitated by the availability of modern flow cytometry technology and of highly specific antibodies which are conjugated to a constantly expanding array of various fluorophores. Of note, all marker combinations that have been described for the characterization and separation of human stem and progenitor cells yield a relative enrichment of the respective cell type only, but are not specific identifiers at the single cell level. Despite of all progress, it remains challenging to accurately define a human HSC because of the lack of precise single cell-based assays for human cells. The murine transplantation models that are commonly used as a gold standard to assay human stem cells are problematic at the single cell level due to the immunological xeno-reactivity (human-to-mouse) that cannot be totally eliminated even when working with heavily immunocompromised mouse strains.

Virtually all human stem cell purification methods start with the pre-enrichment for cells expressing the CD34 antigen, which is present on all human stem and progenitor populations. Clinically, CD34+ cells are used for stem cell transplantation. The human CD34+ cell population has the capacity of stable long-term hematopoietic reconstitution in patients who received myeloablative therapy, demonstrating that long-term HSC are contained within the CD34+ compartment. Of note, however, CD34+ cells do not represent a pure population of HSC, despite the sometimes misleading nomenclature in the (clinical) literature which suggests otherwise, as the terms “stem cells” and “CD34+ cells” are often used synonymously. In fact, CD34+ cells represent a very heterogeneous mixture of many different types of hematopoietic stem and progenitor cells, and only about 1% of CD34+ cells are long-term multilineage-repopulating HSC.

The first CD34+ enrichment step can be achieved using immunomagnetic beads, which has the advantage of reducing total cell numbers by one order of magnitude and thus reducing the amount of antibodies (and cost) required for consecutive staining steps. In a second step, virtually all protocols eliminate committed progenitor cells of the various lineages by excluding cells that stain for lineage-specific markers, a strategy commonly referred to as "lineage depletion". For this purpose, all antibodies directed against lineage-affiliated antigens are usually conjugated to the same fluorophore simplifying this negative selection (gating on lineage-negative (Lin-) cells). Antibody cocktails for hematopoietic lineage-depletion usually bind to antigens belonging to clusters CD2, CD3, CD4, CD7, CD8 for the T-cell lineage, CD11b, CD14, CD15 for the myeloid lineage, CD19, CD20 for the B-lymphoid lineage, CD56 (natural killer cell antigen), and Glycophorin A for the erythroid lineage.

In the third step (positive selection), cells are subsequently co-stained with antibodies directed against CD34, CD38, CD90 (Thy-1), CD123 (IL3Rα), and CD45RA. These antigens have been shown to be characteristic for the major types of hematopoietic stem and myeloid progenitor cells, including long-term HSC (LT-HSC), short-term HSC (ST-HSC), common myeloid progenitors (CMP), granulocyte-monocyte progenitors (GMP), and megakaryocyte-erythrocyte progenitors (MEP) [2,3,4,5,6,7,8]. Inclusion of a CD10 antibody also permits identification of common lymphoid progenitors (CLP)[9]. Importantly, when immunomagnetic selection of CD34+ cells was used in the first step, staining of CD34 in the third selection step has to be performed with an antibody directed against a different CD34 epitope (e.g. a class II epitope for immunomagnetic selection, and a class III epitope for FACS staining). Table 1 summarizes established and commonly used surface marker combinations for different types of human stem and progenitor cells.

1.2 Functional isolation of human hematopoietic stem and progenitor cells based on dye efflux

A second approach for the isolation of hematopoietic stem and progenitor cells relies on their ability to exclude certain intracellular dyes such as Hoechst 33342 [10]. This capacity is derived from the presence of ABC/G2 transporters which are selectively expressed at high levels on HSC [11,12]. As a result of dye efflux, HSC demonstrate a typical low staining pattern with Hoechst 33342, which places them "at the side" of the main Hoechst 33342 high-positive population representing all other hematopoietic cell types in a typical FACS dot plot, and has led to their name "side population" (SP) cells. Membrane pump blockers, such as Verapamil (100 μM) or Reserpine (50 μM), are typically used as negative controls, since they cause the disappearance of the side population and can thus assist in appropriate gating. While the majority of research utilizing SP cells has been performed in mice (for review see Challen GA et al. [13]), the SP principle can also be used for the isolation of human stem and progenitor cells [14]. Importantly, human SP cells contain both CD34+ and CD34− cells, but repopulating capacity is restricted to the CD34+ subset [15]. Thus, human SP cells seem to represent a mixture of HSC and other (CD34−) cells without stem cell activity. Therefore, Hoechst 33342 is commonly not recommended as a single marker to identify and sort highly enriched human HSC. However, combinations of Hoechst 33342 with surface markers or ALDH staining have been used [15]. Also, Hoechst 33342 staining is a valuable strategy for enriching cancer stem cells, since SP cells have been shown to contain putative cancer/leukemia stem cells in specimens from patients with hematologic malignancies [16,17].

1.3 Identification of human hematopoietic stem and progenitor cells based on aldehyde dehydrogenase (ALDH) levels

Elevated levels of aldehyde dehydrogenase (ALDH) have been found in murine and human hematopoietic stem and progenitor cells [18], as well as in putative cancer stem cell populations [19]. These findings form the basis for utilizing intracellular detection of ALDH activity as a method to identify stem cells. Commonly used protocols apply a cell-permeable fluorescent substrate to identify cells with high ALDH activity. The product of the enzymatic reaction is a highly charged molecule that cannot easily leave the cell and thus accumulates in cells with high ALDH activity [20,21]. NOD-SCID mouse repopulating capacity is particularly enriched in $ALDH^{high}$ Lin- negative cells [22]. Therefore, the ALDH method is usually combined with lineage depletion. Others have successfully combined ALDH and SP staining to obtain a higher enrichment of stem cells [15]. In summary, selection of cells with high ALDH activity leads to a reasonable relative enrichment of stem and progenitor cells only when combined with other markers, and is not the method of choice when used as a single marker.

2. Analysis of stem and progenitor cells in myeloid malignancies, including acute myeloid leukemia

The analysis and sorting of hematopoietic stem and progenitor cells in patients with myeloid malignancies is challenging for several reasons. Although cytogenetic, molecular genetic, and immunophenotypic findings suggest that some stem and progenitor cell compartments are part of the malignant clone in acute myeloid leukemias (AML), myelodysplastic syndromes, and also myeloproliferative diseases [23,24,25,26,27,27,28,29], the exact involvement of the distinct subsets of stem and progenitors is still unclear, and might vary in different diseases and disease subtypes. Another challenge lies within the still open question whether the markers that are used in normal hematopoiesis are equally applicable to define the differentiation hierarchy in leukemic hematopoiesis. Nonetheless, analysis and sorting of hematopoietic stem and progenitor cell compartments has been successfully utilized in myeloid malignancies to investigate gene expression changes that contribute to leukemogenesis and may underlie leukemic transformation at the stem or progenitor cell level [30,31,32,33,34]. In view of the discovery of leukemia-initiating cells (or leukemia stem cells, LSC) in myeloid malignancies, the physical separation of stem and progenitors from the leukemic bulk population has become a necessity, with implications both for research on LSC and the monitoring of minimal residual disease (MRD) at the stem cell level.

Several normal stem cell markers have been proposed for the identification and enrichment of populations that contain LSC, which are operationally defined as the cells that repopulate NOD-SCID mice in xenotransplantation assays (SCID leukemia-initiating cells, SL-IC). Of note, the use of lineage-marker depletion cocktails is controversial because of the potential aberrant expression of certain lineage markers on LSC in some disease subtypes. However, linage depletion might still be useful to define the leukemia-cell-of-origin populations (defined as the earliest cells carrying clonotypic aberrations, which have not yet been fully transformed to LSC). Several studies have demonstrated that LSC in AML are predominantly contained within immature hematopoietic cell compartments that are CD34+CD38− [35,36]. LSC were furthermore shown to be negative for Thy-1 (CD90) as well as HLA-DR expression [37,38], thus closely resembling the immunophenotype of HSC in normal hematopoiesis. Acute promyelocytic leukemia (APL) represents an exception as the CD34+CD38− cell compartment cannot be transplanted into NOD-SCID mice suggesting that APL is a disease that originates from committed myeloid progenitors rather than early stem cells [39]. Recent data in AML with nucleophosmin (NPM) mutation

suggest that LSC, even within one molecular disease subtype, can be heterogenous [40]. Both CD34+ and CD34− LSC fractions from some NPM-mutated AML cases initiated leukemia when transplanted into immunodeficient mice.

Several studies have extended the analysis to markers usually not found in normal hematopoiesis, in an approach to identify unique markers for the prospective isolation of LSC populations that are not contaminated with normal hematopoietic stem and progenitor cells. The successful purification of LSC populations would open new opportunities both for the clinical monitoring of LSC (e,g., post chemotherapy) and the targeted research of LSC, with the ultimate goal to develop strategies for their therapeutic eradication and thus for a lasting cure of leukemia. For instance, the interleukin-3 receptor alpha chain (IL3Rα, CD123) was found to be highly expressed on CD34+CD38− cells of patients with AML, and only CD123+ cells were capable of establishing and maintaining leukemia in NOD-SCID recipient mice [41]. When exploited therapeutically, targeting of CD123 with a monoclonal antibody led to a profound reduction of LSC engraftment in NOD-SCID mice and prolonged survival [42]. An intracellular marker with potential therapeutic implications is NF-kappaB which was shown to be constitutively activated in LSC of patients with AML. Targeting of NF-kappaB led to selective killing of CD34+CD38− LSC but not normal CD34+CD38− cells [43]. Another marker proposed to specifically target LSC is the CD44 surface molecule [44]. Utilizing CD44 antibodies, the interaction of LSC with their bone marrow microenvironment (niche) could be inhibited leading to a marked reduction of leukemic repopulation in vivo in a NOD-SCID transplantation model. Another study showed that the CD96 antigen is expressed on CD34+CD38− cells from AML patients in the majority of cases, while it is only detected at very low levels on rare normal LT-HSC (Lin-CD34+CD38−CD90+)[45]. In this study, LSC activity in a Rag−/−gamma(c)−/− xenotransplantation model was restricted to CD96+ cells in 4 out of 5 cases, suggesting that CD96 might be a promising LSC-specific therapeutic target. The C-type lectin-like molecule-1 (CLL-1) has also been proposed to aid in the discrimination of normal and leukemia stem cells. CLL-1 was shown to be expressed on leukemic blasts as well as CD34+CD38− cells from patients with AML, but not on normal CD34+CD38− cells [46]. CD34+CLL-1+ cells engrafted NOD-SCID mice and gave rise to CLL-1+ leukemic blasts. Important for monitoring of MRD, a high CLL-1+ fraction at the time of remission were associated with early relapse. Furthermore, the same group showed in a separate study that CLL-1 was capable of distinguishing AML side population (SP) cells from normal SP cells [16]. Recently, it was reported that the CD47 antigen is overexpressed on Lin-CD34+CD38−CD90− LSC from patients with AML compared with normal HSC and multipotent progenitors (MPP), and that CD47 expression within the Lin-CD34+CD38− population distinguishes normal and leukemia stem cells [33]. Targeting of CD47 with monoclonal antibodies disrupted stem cell properties of AML LSC, most likely by inducing phagocytosis of LSC, since CD47 is upregulated in circulating normal and leukemia stem cells to avoid phagocytosis [47].

Taken together, several marker combinations have been shown to be capable of enriching cell populations for LSC. Such strategies are instrumental for our improved understanding of the molecular biology of LSC and have led to the rational development of therapies that target and eliminate these cells [48,49]. Those LSC-directed strategies include antibody- or ligand-based approaches for targeted drug delivery, therapeutic interference with the LSC-niche interaction, targeted induction of phagocytosis, and selective modulation of aberrantly activated or silenced transcriptional and epigenetic networks. Despite significant progress and highly encouraging initial results, more research on normal and leukemia stem and progenitor cells is warranted in order to tackle LSC efficiently in the various genetic and epigenetic subtypes of AML and myelodysplastic syndromes.

3. Isolation of murine stem and progenitor cells for research and analysis of genetic models of leukemia

Murine hematopoietic stem and progenitor cells are the best studied adult, tissue-specific stem cells and much of our current knowledge of stem cells is derived from observations that were originally made in murine hematopoiesis. The study of murine hematopoietic stem cells and LSC in genetically defined models of leukemia remains a very powerful tool for basic and preclinical research because of the possibility to assess cellular functions at the single cell level in a variety of assays, including congenic murine transplantation assays.

Improved *ex vivo* culture systems have enabled the thorough characterization of hematopoietic cell growth, lineage-commitment, differentiation and function of isolated primary human and murine hematopoietic stem and progenitor cells. To date, the knowledge of the murine hematopoietic system is more advanced than our understanding of human hematopoiesis. This is due to the fact that the most informative studies analyzing hematopoiesis utilize suitable *in vivo* models, such as genetically modified mice or congenic bone marrow transplantation assays. The development of immunocompromised “humanized mice”, which allow for the engraftment of human hematopoietic cells, has been an important step in facilitating more informative studies characterizing primary human hematopoietic cells. These novel mouse strains are under constant improvement regarding their ability to support long-term, multilineage engraftment of primary human hematopoietic cells [52]. More efficient experimental *in vivo* models combined with the continuous refinement of cell surface markers for the isolation of distinct stem and progenitor cell populations in human and murine hematopoiesis will ultimately allow for a comprehensive identification and precise characterization of important mechanisms that regulate normal and aberrant stem and progenitor cell function.

In the following, we give an overview of the most frequently used methods to analyze and fractionate murine hematopoietic stem and progenitor cell populations by FACS.

3.1 Isolation of murine hematopoietic stem and progenitor cells by cell surface marker detection

Although gene expression profiling studies have been performed on various stem and lineage-committed progenitor cell populations, no single cell surface receptor could be identified that is exclusively expressed on only murine hematopoietic stem or certain subsets of committed progenitor cells. The use of complex combinations of cell surface markers is required for purification and enrichment of either hematopoietic stem or progenitor cells. Hematopoietic stem as well as myeloid and lymphoid lineage committed progenitor cells can be isolated from the murine bone marrow by first excluding mature cells expressing the following antigens: CD3, CD4, CD8a, CD19, Ter119, Gr-1, CD11b, and B220. The resulting cells are referred to as lineage negative (Lin-) cells. This Lin- cell population contains all immature hematopoietic cells; besides hematopoietic stem cells (HSC) also multipotent progenitors (MPP), lymphoid-primed multipotent progenitors (LMPP), common lymphoid progenitors (CLP), common myeloid progenitors (CMP), granulocyte-monocyte progenitors (GMP), and megakaryocyte-erythrocyte progenitors (MEP). While the overall separation strategy is very similar to that of human hematopoietic cells in principle, the surface markers for murine stem and progenitor cells are substantially different.

Protocols for the isolation of lineage committed hematopoietic progenitor cells utilize the differentiation stage-specific expression of the receptor tyrosine kinase c-Kit (CD117) and the stem cell antigen-1 (Sca-1 or Ly6A/E) on Lin- hematopoietic stem and progenitor cells [53]. Cells that are Lin- and negative for Sca-1, but highly express c-Kit contain all myeloid

progenitor populations [54]. Differential expression of the glycoprotein CD34 and of the Fc-gamma receptor II/III (CD16/CD32) allows for the isolation of CMP (Lin-/Sca-1-/cKit+/CD34+/FcγII/IIIdim), GMP (Lin-/Sca-1-/cKit+/CD34+/FcγII/III+) and MEP (Lin-/Sca-1-/cKit+/CD34−/FcγII/III-) cells (Table 3) [55]. Lymphoid lineage committed progenitor cells are purified by including the Interleukin-7 receptor alpha chain (IL7Rα or CD127) as a marker. CLP are highly enriched in the Lin-/cKit+/Sca-1lo/LI7Rα+ cell population (Table 3) [56].

Protocols for the isolation of murine HSC utilize different marker combinations including Thy-1 (CD90), Flk2 (Flt3), CD34, and SLAM markers (for review see [57]). The stringency of enrichment of hematopoietic stem cells varies depending on the marker combination used (see Table 4). Lin-/cKit+/Sca-1+ cell populations (LKS) contain less than 1 in 10 hematopoietic stem cells capable of reconstituting the lymphoid and myeloid compartments of lethally irradiated recipient animals for more than 10 weeks (long-term repopulating HSC, LT-HSC). Subdivision of the LKS population using differential expression of CD34 leads to further enrichment to approximately 1 in 5 LT-HSC in LKS CD34− cells [58]. Roughly the same degree of additional stem cell enrichment can be achieved by using the thymus-cell-antigen 1 (Thy-1, CD90) in addition to the LKS markers [59,60]. The combination of Thy-1 or CD34, with the FMS-like tyrosine kinase 3 (Flk2, Flt3, or CD135) marker, plus LKS markers, allows for the separation of LT-HSC (Lin-Kit+Sca1+Flk2-CD34− or Lin-Kit+Sca1+Flk2-Thy1.1dim) from ST-HSC (Lin-Kit+Sca1+Flk2-CD34+, or Lin-Kit+Sca1+Flk2-Thy1.1dim population) [60]. Moreover, it has been shown that multipotent (lymphoid and myeloid) progenitors (MPP) are highly enriched in the Lin-Kit+Sca1+Flk2+CD34+, or Lin-Kit+Sca1+Flk2+Thy1.1- populations [60]. Finally, Lin-cKit+Sca-1+Flk2^{high} cells contain highly enriched lymphoid-primed multipotent progenitors (LMPP) [61].

In an attempt to simplify murine HSC purification, Kiel and colleagues in 2005 found cell surface receptors of the signaling lymphocyte attractant molecule (SLAM) family, including CD150, CD48, and CD244, to be differentially expressed in functionally distinct stem and progenitor cells. According to this schema, HSC (LT- and ST-HSC) were highly purified as CD150+/CD244−/CD48− cells, yielding an enrichment comparable to that when LKS markers were used in combination with CD34, Thy-1, or Flk2 (30% single cell reconstitution frequency), while MPP were CD244+/CD150−/CD48− [62]. Combining these newly identified SLAM markers with the classic LKS markers highly enriched LT-HSC in the Lin-Kit+Sca1+CD150+CD48− population (approximately 1 in 2 cells with LT-HSC capacity) [62], representing the most effective marker combination for LT-HSC to date.

Other novel HSC cell surface markers have recently been proposed, such as the endothelial cell adhesion molecule (Esam) that is highly expressed on hematopoietic stem and multipotent progenitor cells in mice and humans [63]. Further evaluation of these, and combinations with existing markers, might ultimately allow for a precise definition of murine LT-HSC at the single cell level.

3.2 Isolation of murine hematopoietic stem and progenitor cells based on dye efflux combined with cell surface marker expression

Several alternative methods for HSC isolation have been developed to potentially increase HSC purity, but also to simplify HSC isolation protocols for technical and cost-effectiveness reasons. In particular, strategies have been explored that try to avoid the need of simultaneous determination of expression of >10 cell surface markers. Analogous to the isolation of human HSC, murine HSC can be identified based on the ability to actively export Hoechst 33342 dye [10], resulting in “side population” (SP) stem cells. In recent

years, protocols have been developed that combine Hoechst efflux with the detection of HSC cell surface markers [65]. Combination of LKS markers with SP (so called SP^{LKS}) highly enriches for LT-HSC. The combination of SP with SLAM markers (SP^{SLAM}) is suitable for defining subpopulations that are functionally distinct with respect to lineage commitment as well as their proliferative status. CD150+ SP cells proliferate less and demonstrate a slightly higher reconstitution potential compared to CD150− SP cells [65].

Conclusion

During the last two decades our definition and knowledge of hematopoietic stem and progenitor cells has been constantly refined and expanded. Nowadays, modern flow cytometry applications combined with single cell assays are the key techniques allowing for molecular and functional examination of hematopoietic stem, multipotent progenitor as well as lineage-committed cells. Current protocols for the isolation of both human and murine hematopoietic stem and progenitor cells as well as leukemia stem cells rely on either the detection of distinct combinations of surface markers, or on particular cellular characteristics, such as the ability to efflux intracellular dyes or the presence of significantly elevated levels of enzyme activity. The simultaneous use of cell surface markers and functional features permits the analysis of a broad spectrum of distinct hematopoietic stem and progenitor cells in normal hematopoiesis as well as in leukemia. Importantly, several cell surface markers have been discovered that allow for the prospective isolation of populations enriched for LSC activity in myeloid malignancies. Catalyzed by these novel experimental tools, our functional and molecular knowledge of purified LSC populations is growing rapidly and provides the basis for the development of drugs that specifically target these leukemia-initiating cells. Furthermore, modern FACS technology now permits the monitoring of minimal residual disease and, thus, the response to therapy at the stem cell level in the framework of clinical trials.

PRACTICE POINTS

- Hematopoietic stem and progenitor cells are defined by key functional properties at the single cell level, including the capacity of self-renewal, pluripotent differentiation, and colony-formation.
- The simultaneous use of cell surface markers and functional features allows for the analysis of a broad spectrum of distinct hematopoietic stem and progenitor cells in normal hematopoiesis as well as in leukemia.
- Particularly in myeloid malignancies, the use of high-speed multiparameter flow cytometry has provided proof for the existence of a rare subpopulation of leukemia-initiating stem cells with the ability to propagate the leukemic bulk population.
- Identifying the LSC for each type of leukemia is a necessary challenge for the understanding of the biology of LSCs and the development of effective LSC-directed treatments.

RESEARCH AGENDA

- The separation of leukemia stem cells (LSC) still awaits the characterization of unique markers that will allow for the isolation of LSC without contamination by normal hematopoietic stem and progenitor cells.

- The cell-of-origin for LSC, which can be part of the hematopoietic stem cell compartment or a committed progenitor, as proven in APL, must be established for each type of hematologic malignancy.
- The ultimate goal of stem cell research in leukemia is the development of therapeutic measures which target LSC and thus prevent the recurrence of disease post therapy.

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Table 1

Commonly used surface marker combinations for the detection of the different types of human hematopoietic stem and progenitor cells

Cell population	Marker combination
Total HSC	Lin-CD34+CD38−
Long-term HSC (LT-HSC)	Lin-CD34+CD38−CD90+
Short-term HSC (ST-HSC)	Lin-CD34+CD38−CD90−
Common lymphoid progenitors (CLP)	Lin-CD34+CD10+CD45RA+
Total myeloid progenitors	Lin-CD34+CD38+
Common myeloid progenitors (CMP)	Lin-CD34+CD38+CD123+CD45RA−
Granulocyte-monocyte progenitors (GMP)	Lin-CD34+CD38+CD123+CD45RA+
Megakaryocyte-erythrocyte progenitors (MEP)	Lin-CD34+CD38+CD123−CD45RA−

Table 2

Proposed leukemia stem cell markers in AML

Proposed leukemia stem cell marker	Reference
CD34+CD38−	Lapidot et al. 1994[35] Bonnet et al. 1997[36]
CD34+CD38−CD90−	Blair et al. 1997[37]
CD34+CD38−CD71-HLA-DR-	Blair et al. 1998[38]
CD34+CD38−CD123+	Jordan et al. 2000[40]
CD34+CD38−CD44+	Jin et al. 2006[44]
CD34+CD38−CD96+	Hosen et al. 2007[45]
CD34+CD38−CLL-1+, or SP CLL-1+	Van Rhenen et al. 2007[46] Moshaver et al. 2008[16]
Lin-CD34+CD38−CD90−	Wang et al. 2005[50] Majeti et al. PNAS 2007[33]
Lin-CD34+CD38−CD90−CD47+	Majeti et al. 2009[51]

Table 3

Commonly used surface marker combinations for detection of committed hematopoietic progenitor cell populations in mice

Cell population	Marker combination
Hematopoietic progenitor cells (LKS)	Lin-Kit+Sca1−
CLP	Lin-Kit+Sca1+IL7Rα+
Total myeloid progenitors	Lin-Kit+Sca1−
CMP	Lin-Kit+Sca1−CD34+FcγRII/IIIdim
GMP	Lin-Kit+Sca1−CD34+FcγRII/III+
MEP	Lin-Kit+Sca1−CD34−FcγRII/III-

Table 4

Commonly used surface marker combinations for detection of stem and multipotent progenitor cell populations in mice

Cell population	Marker combination	LT-HSC frequency [%]	Reference
Hematopoietic stem cells (LKS)	Lin-Kit+Sca1+	<10	
Hematopoietic stem cells (SLAM)	CD150+CD244−CD48− CD150+CD41−CD48−	30 45	Kiel et al. 2005[62] Kiel et al. 2005[62]
LT-HSC	Lin-Kit+Sca1+Flk2-CD34−, or Lin-Kit+Sca1+Flk2-Thy1.1dim	22 20	Osawa et al. 1996[58] Spangrude et al. 1988[54], Kiel et al. 2005[62]
LT-HSC (“SLAM LKS”)	Lin-Kit+Sca1+CD150+CD48−	47	Kiel et al. 2005[62]
LT-HSC (“SLAM Flk2 LKS”)	Lin-Kit+Sca1+Flk2-CD48−	N/A	Yilmaz et al. 2006[64]
ST-HSC	Lin-Kit+Sca1+Flk2-CD34+, or Lin-Kit+Sca1+Flk2-Thy1.1dim	-	Osawa et al. 1996[58] Christensen et al. 2001[60]
HSPC (Hematopoietic stem and progenitor cells)	Lin-Kit+Sca1+Flk2-	-	Rasko et al. 1995[59]
Multipotent progenitors (MPP)	Lin-Kit+Sca1+Flk2+CD34+, or Lin-Kit+Sca1+Flk2+Thy1.1-	-	Christensen et al. 2001[60]
MPP (SLAM)	CD150-CD244+CD48−	-	Kiel et al, 2005[62]
Lymphoid-primed multipotent progenitors (LMPP)	Lin-Kit+Sca1+Flk2++	-	Adolfsson et al. 2001[61]