NEOPLASIA
www.neoplasia.com

Volume 20 Number 6 June 2018 pp. 632–642 632

Phenotyping and Target Expression Profiling of CD34 $^{+}$/CD38 $^{-}$ and CD34 $^{+}$/CD38 $^{+}$ Stem- and Progenitor cells in Acute Lymphoblastic Leukemia

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Abstract

Leukemic stem cells (LSCs) are an emerging target of curative anti-leukemia therapy. In acute lymphoblastic leukemia (ALL), LSCs frequently express CD34 and often lack CD38. However, little is known about markers and targets expressed in ALL LSCs. We have examined marker- and target expression profiles in CD34 $^{+}$/CD38 $^{-}$ LSCs in patients with Ph $^{+}$ ALL (n = 22) and Ph $^{-}$ ALL (n = 27) by multi-color flow cytometry and qPCR. ALL LSCs expressed CD19 (B4), CD44 (Pgp-1), CD123 (IL-3RA), and CD184 (CXCR4) in all patients tested. Moreover, in various subgroups of patients, LSCs also displayed CD20 (MS4A1) (10/41 = 24%), CD22 (12/20 = 60%), CD33

Abbreviations: ALL, acute lymphoblastic leukemia; BM, bone marrow; CML, chronic myeloid leukemia; GO, gemtuzumab-ozogamicin; LSC, leukemic stem cell; MNC, mononuclear cell; NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ; OS, overall survival; PB, peripheral blood; Ph, Philadelphia chromosome; SCT, stem cell transplantation; TKI, tyrosine kinase inhibitor.

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Received 8 February 2018; Revised 12 April 2018; Accepted 13 April 2018

1476-5586/18

https://doi.org/10.1016/j.neo.2018.04.004

(Siglec-3) (20/48 = 42%), CD52 (CAMPATH-1) (17/40 = 43%), IL-1RAP (13/29 = 45%), and/or CD135 (FLT3) (4/20 = 20%). CD25 (IL-2RA) and CD26 (DPPIV) were expressed on LSCs in Ph^+ ALL exhibiting $BCR/ABL1_{p210}$, whereas in Ph^+ ALL with $BCR/ABL1_{p190}$, LSCs variably expressed CD25 but did not express CD26. In Ph^- ALL, $CD34^+/CD38^-$ LSCs expressed IL-1RAP in 6/18 patients (33%), but did not express CD25 or CD26. Normal stem cells stained negative for CD25, CD26 and IL-1RAP, and expressed only low amounts of CD52. In xenotransplantation experiments, $CD34^+/CD38^-$ and $CD34^+/CD38^+$ cells engrafted NSG mice after 12–20 weeks, and targeting with antibodies against CD33 and CD52 resulted in reduced engraftment. Together, LSCs in Ph^+ and Ph^- ALL display unique marker- and target expression profiles. In Ph^+ ALL with $BCR/ABL1_{p210}$, the LSC-phenotype closely resembles the marker-profile of $CD34^+/CD38^-$ LSCs in chronic myeloid leukemia, confirming the close biologic relationship of these neoplasms. Targeting of LSCs with specific antibodies or related immunotherapies may facilitate LSC eradication in ALL.

Neoplasia (2018) 20, 632–12

Introduction

Acute lymphoblastic leukemia (ALL) is a life-threatening malignancy defined by leukemic expansion and accumulation of lymphoid blast cells in hematopoietic tissues, including the bone marrow (BM), spleen, and lymph nodes [1,2]. The clinical course and prognosis vary among patients, depending on age, comorbidities, the variant of ALL, and cytogenetic and molecular features. In approximately one third of all adult patients, ALL cells display the Philadelphia chromosome (Ph) and the associated *BCR/ABL1* oncogene [1–5]. In most cases, leukemic cells display the p190-form of BCR/ABL1, whereas in a smaller group of patients, $BCR/ABL1_{p210}$ is found.

Before BCR/ABL1 blockers had been introduced in clinical practice, patients with Ph^+ ALL had a quite unfavorable prognosis [3–5]. However, since the advent of imatinib and other more effective BCR/ABL1-targeting tyrosine kinase inhibitors (TKIs), the prognosis of Ph^+ ALL has improved substantially [3,6–14]. Nevertheless, not all patients respond to chemotherapy or/and to targeted drugs [8–12,14]. Depending on age, co-morbidities and donor-availability, stem cell transplantation (SCT) is recommended for high-risk patients [15–20]. The overall treatment plan may include chemotherapy with subsequent SCT as well as BCR/ABL1-targeting drugs [16,18,19]. However, despite SCT and other treatment options, not all patients with ALL can be cured. Therefore, current research is attempting to identify new drug-targets and novel treatment approaches, including immunotherapies and other targeted therapies, with the hope to improve treatment outcome and prognosis.

An emerging new target of therapy in clinical hematology is the leukemic stem cell (LSC). The concept of LSCs has been established with the intention to explain cellular hierarchies in leukemic clones, and to improve drug therapy through the elimination of disease-initiating cells [21–27]. The LSC-hypothesis is based on the assumption that leukemias are organized hierarchically, with more mature cells programmed to undergo apoptosis after a limited number of cell divisions, and LSCs which have self-renewal and thus unlimited disease-propagating ability [21,23–25]. In Ph^+ chronic myeloid leukemia (CML), LSCs are considered to reside within a $CD34^+/CD38^-$ fraction of the clone [22,23,28,29]. In ALL, the phenotype of LSCs is less well defined. In adult patients with Ph^+ ALL, NOD/SCID-repopulating LSCs supposedly reside within a $CD34^+/CD38^-$ compartment [30–32]. However, in other (childhood) variants of ALL, NOD/SCID-repopulating LSCs may also be detectable in other $CD34^+$ sub-fractions or even in $CD34^-$ populations [31–33]. Overall, little is known about markers and target expression profiles in ALL LSCs.

The aim of the current study was to establish the phenotype and target expression profile of LSCs in Ph^+ and Ph^- ALL in adults. Our data show that depending on the type of ALL, LSCs exhibit unique phenotypes and variable combinations of aberrantly expressed surface targets which may assist in LSC purification and the development of LSC-eradicating treatment strategies.

Material and Methods

Patients and Cell Lines

Peripheral blood (PB) and/or BM samples were collected in 49 patients with ALL and 10 with Ph^+ CML. The patients´ characteristics are shown in Supplementary Table S1. All patients gave written informed consent before blood or BM was obtained. The study was approved by the ethics committee of the Medical University of Vienna. The following cell lines were used: the Ph^+ cell lines Z-119 (RRID: CVCL_IU88), BV-173 (RRID: CVCL_0181), TOM-1 (RRID: CVCL_1895) and NALM-1 (RRID: CVCL_0091), the Ph^- cell lines RAJI (RRID: CVCL_0511), RAMOS (RRID: CVCL_0597), REH (RRID: CVCL_1650) and BL-41 (RRID: CVCL_1087), the CML cell line CML T1 (RRID: CVCL_1126), and the myeloid cell line M-07e (RRID:CVCL_2106) expressing or lacking BCR/ABL1. A detailed description is provided in the Supplement.

Monoclonal Antibodies (mAb) and Other Reagents

A detailed description of reagents used in this study is provided in the Supplement. A list of mAb employed is shown in Supplementary Table S2.

Flow Cytometry and Cell Sorting

Flow cytometry was performed on heparinized BM or PB cells or MNCs to characterize the phenotype of $CD34^+/CD38^-$ and $CD34^+/CD38^+$ cells as described [29,34,35]. The gating-strategy is shown in Supplementary Figure S1 and the antibody-combinations applied in Supplementary Table S3. In selected patients with Ph^+ ALL (n = 6), Ph^- ALL (n = 6), and CML (n = 3), $CD34^+/CD38^-$ and