



15 years Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO): achievements and future perspectives

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Summary Cancer stem cells, also known as leukemic stem cells (LSC) in the context of leukemias, are an emerging topic in translational oncology and hematology. The Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO) was established in 2008 with the aim to translate LSC concepts into clinical practice. Major specific aims of the LBI HO are to identify LSC in various blood cell disorders and to improve anti-leukemic therapies by establishing LSC-targeting and LSC-eradicating approaches with the ultimate aim to translate these concepts into clinical practice. In addition, the LBI HO identified a number of diagnostic and prognostic LSC markers in various

blood cell malignancies. Members of the LBI HO have also developed precision medicine tools and personalized medicine approaches around LSC in applied hematology. As a result, diagnosis, prognostication and therapy have improved in the past 10 years. Major disease models are myeloid leukemias and mast cell neoplasms. Finally, the LBI HO consortium launched several projects in the field of open innovation in science where patient-derived initiatives and their input supported the scientific community. Key aims for the future of the LBI HO are to develop LSC-related concepts and strategies further, with the long-term vision to cure more patients with hematologic malignancies.

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Historical overview and background

In the past 3 decades, cancer-initiating stem cells have been recognized as an emerging new target in applied oncology and hematology [1–6]. Initially, these cancer stem cells (CSC) have been identified and characterized in acute myeloid leukemia (AML) [7–10]. In the context of leukemia, CSC are termed leukemia-initiating (propagating) stem cells or leukemic stem cells (LSC). The concept of CSC/LSC is based on the assumption that each neoplasm (solid or liquid) consists of two distinct fractions of cells, (i) the CSC/LSC and (ii) more mature clonal cells [1–6]. In contrast to more mature neoplastic cells, CSC/LSC have the ability to propagate clonal, neoplastic (cancer/leukemia) cells *in vivo* for unlimited time periods. The long-term disease-propagating ability of CSC/LSC is associated with specific stem cell functions, including self-renewal, the ability of asymmetrical cell division, some (limited) differentiation capacity, and the related ability to propagate one or more sub-clones in a given neoplasm [1–6]. As a result, the CSC/LSC pool exhibits an unlimited capacity to form the bulk of ‘more mature’ cells in a given malignancy. Finally, in common with normal stem cells, CSC/LSC have advanced self-protection capabilities, which prevents their exhaustion and contributes to their resistance against toxins and drug therapies [1–6].

As mentioned, the concept of CSC/LSC was first established in AML [7–10]. However, the LSC concept can also be applied to other forms of leukemia, including acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), chronic lymphatic leukemia (CLL), or mast cell leukemia (MCL) [11–22].

In chronic leukemias, such as CML, the clonal hierarchy and ‘LSC-dependence’ of disease evolution and diversification into subclones, are obvious features. However, the concept of LSC is also relevant in acute leukemias. Over the years, the CSC hypothesis has also been tested in diverse solid tumors, myeloma, and malignant melanomas [23–32]. To a degree, clonal evolution and stem cell hier-

archies can also be demonstrated in these cancer models. However, in advanced (metastatic) cancers and treatment refractory acute leukemias, the stem cell hierarchy is gradually diminishing and the pool of CSC/LSC may increase rapidly over time [23–37]. Another important point is that in advanced neoplasms, CSC/LSC are becoming more and more heterogeneous cell populations, and, depending on stage and type of malignancy, ‘stemness’ may be, or may become, a ‘reversible functional feature’ or a ‘newly acquired functional feature’ of neoplastic (precursor) cells [32–37].

In the past 10 years, a number of markers, driver pathways, and targets have been identified and characterized in CSC/LSC in various disease models. As a result, CSC/LSC have been identified as major target cell population in various cancer models [38–45].

CSC/LSC research has also been promoted by several groups in Vienna, many of them within the Vienna Cancer Stem Cell Club (VCSCC) and its major ‘research-driver’, the Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO) [46, 47]. Indeed, the LBI HO contributed substantially to LSC research between 2008 and 2023 [46].

In 2023, members of the LBI HO celebrated its 15-year anniversary and organized a 15-year jubilee meeting in Vienna. In the current article, we provide an update of the LBI HO and provide information on major aims and project lines as well as research highlights.

Origin and structure of the LBI HO

The initial research cluster, the Ludwig Boltzmann Cluster Oncology (LBC ONC), was established in 2008 by merging two Ludwig Boltzmann Institutes working in the field of hematology and oncology: the Ludwig Boltzmann Institute for Leukemia Research and Hematology at the Hanusch Hospital, and the Ludwig Boltzmann Institute for Clinical and Experimental Oncology located at the Medical University of Vienna [46]. In the first 10 years, the LBC ONC conducted a series of major projects in the field of LSC research and expanded substantially. In 2018, the structure of the LBC ONC was refined, and the name changed to LBI HO. During the past 5 years, the LBI HO was able to attract several additional academic partners in Vienna, including the University of Veterinary Medicine

Table 1 Academic Partners in the Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO)^a

Partner	Joined ^a (Year)	Major Contributions to the LBI HO Consortium
Medical University of Vienna	2008	Clinical models, research labs, flow cytometry unit, student education, secretary office, CCC and VCSCC
Hanusch Hospital, Vienna	2008	Clinical models, clinical trials, MDS, MPN and AML center
University of Veterinary Medicine Vienna	2017	Mouse xenotransplantation models (NSG and NSG-related mouse strains), comparative oncology—focus: canine mast cell tumors
Children’s Cancer Research Institute	2018	Molecular diagnostics—focus: Ph+ CML and MPN, mutation specific PCR assays—focus: <i>BCR::ABL1</i> mutant forms

^aThe LBI HO was established on the basis of two academic partners in 2008. In 2017 and 2018 additional partners were invited and joined in the LBI HO

Vienna in 2017 and the Children’s Cancer Research Institute (CCRI) in 2018 (Table 1). In addition, the LBI HO started several fruitful partnerships and collaborations with industrial partners.

The LBI HO consortium is based on a partner board that includes major representatives of all participating partner institutions, including academic partners and industrial partners. In addition, the LBI HO has established a scientific advisory board (SAB) consisting of 3 international experts in the field. The SAB members have been visiting the LBI HO once a year and provide essential input and valuable scientific advice to the LBI HO.

The LBI HO runs seven core facility platforms (CF-PF) in collaboration with academic partner institutions [46]. These platforms focus on management and administration (CF-PF1), flow cytometry and cell sorting (CF-PF2), molecular studies and genetic tests (CF-PF3), mouse xenotransplantation experiments (CF-PF4), education (CF-PF5), clinical investigations (CF-PF6), and viral-mediated gene delivery and cell line models (CF-PF7). The CF-PF are interconnected with each other and with all project lines of the LBI HO and form an essential basis for the successful conduct of research projects within the LBI HO.

The LBI HO is embedded in an active multidisciplinary scientific network in various partner institutions, including the Medical University of Vienna where the LBI HO is interacting with the VCSCC, local core facility units, the division of hematology and hemostaseology, a local stem cell transplantation unit, several collaborating research laboratories, a central routine laboratory, and the Comprehensive Cancer Center (CCC) of the Medical University of Vienna [46]. The LBI HO is also embedded in a robust communication system that interconnects LBI partner institutions and promotes continuous networking and scientific cooperations as well as project discussion. Internal communications in the LBI HO and interactions with external partners are guided and coordinated by the administration team of the LBI. Members of the LBI HO meet regularly in weekly progress report meetings [46]. These meetings are joint meetings with the VCSCC and represent a suitable platform for ongoing scientific discussions and collaborations [46, 47].

Major aims and project lines in the LBI HO

The general scientific goals of the LBI HO are to identify and characterize LSC in various blood cell malignancies, to characterize and validate clinically important (diagnostic and prognostic) markers and therapeutic targets in these cells, and to determine the efficacy of various targeted drugs and drug combinations on proliferation and survival of LSC. During the past 15 years, the LBI HO made significant progress in the phenotypic and functional characterization of LSC in various leukemia models (Table 2). In addition, members of the LBI HO were able to identify and char-

Table 2 Major disease models examined by the Ludwig Boltzmann Institute for Hematology and Oncology (LBI HO)

Disease Model	Abbreviation	Phenotype of Stem Cell-Containing Cell Fraction ^a
Premalignant Neoplasms/Chronic Myeloid Neoplasms:		
Myelodysplastic neoplasms	MDS ^b	CD34 ⁺
Chronic myeloid leukemia	CML	CD34 ⁺ /CD38 ⁻ /CD26 ⁺ /IL-1RAP ⁺
Chronic myelomonocytic leukemia	CMML	CD34 ⁺ /CD38 ⁻
Ph ⁻ myeloproliferative neoplasms	MPN ^c	CD34 ⁺ /CD38 ⁻
Indolent systemic mastocytosis	ISM ^d	CD34 ⁺
Smoldering systemic mastocytosis	SSM ^d	CD34 ⁺
Aggressive Neoplasms/Acute Leukemias^e:		
<i>De novo</i> acute myeloid leukemia	AML	CD34 ⁺
Secondary acute myeloid leukemia	sAML	CD34 ⁺
Ph ⁺ Acute lymphoblastic leukemia	Ph ⁺ ALL	CD34 ⁺
Ph ⁻ Acute lymphoblastic leukemia	Ph ⁻ ALL	CD34 ⁺
Acute mast cell leukemia	MCL	CD34 ⁺ /CD38 ⁻

In all disease models, NSG mice (acute neoplasms) or NSG mice exhibiting human cytokines (chronic myeloid neoplasms) were employed. Engraftment results were assessed after about 2–3 months (acute neoplasms) or 4–6 months (chronic neoplasms)

^aCells that can produce a disease-phenotype in a severely immune-deficient mouse model. In each case, the *in vivo* stem cell behavior of these cells was confirmed or revealed by members of the LBI HO

^bOnly cells obtained from high-risk MDS patients engrafted in immune-deficient mice

^cIn classical, Ph⁻ MPN, cells obtained from polycythemia vera or primary myelofibrosis (*JAK 2*- or *CALR*-mutated) were used in engraftment experiments

^dIn patients with ISM or SSM, engraftment in mice is usually limited to a very small number of cells, corresponding to the extremely long time of mast cell development (months to years)

^eUntil 2021, the LBI HO examined LSC in patients with Ph⁺ ALL and Ph⁻ ALL

acterize pre-leukemic (premalignant) neoplastic stem cells (pre-L-NSC) in various indolent (premalignant) hematologic disease states, including myelodysplastic neoplasms (MDS), myeloproliferative neoplasms (MPN), and systemic mastocytosis (SM) (Table 2). In these projects, the LBI HO consortium was also able to identify and to validate several most promising markers and therapeutic targets in neoplastic stem cells (Table 2). A key aim of the LBI HO is to establish a solid basis for the development of novel LSC-eradicating treatment concepts and to translate these concepts into clinical practice [46]. A long-term vision of the LBI HO is to introduce new curative approaches for patients by applying drugs and drug-combinations or immunotherapies that can eliminate LSC (or even LSC and pre-L-NSC) in these malignancies.

When launching the LBI HO (LBC ONC) in 2008, 4 project lines (PL) were established, one for myeloid neoplasms, one for lymphoid neoplasms, one for solid tumors, and one for skin cancer and mast cell neoplasms [46]. Over the years, the focus of the LBI HO shifted more and more to myeloid neoplasms and mast cell neoplasms, based on project development and scientific performance as well as recommenda-

Table 3 Major translational and clinical concepts studied by the LBI HO: status 2024

Concept	Disease Model(s)
LSC eradication by allo-HSCT	Myeloid neoplasms, advanced mastocytosis ^a
LSC eradication by LSC-directed drugs and drug combinations	Myeloid neoplasms, advanced mastocytosis
Diagnostic LSC phenotyping and monitoring of MRD	CML, Ph+ ALL, AML, advanced mastocytosis
Employing LSC markers and targets for improved prognostication and prediction of treatment responses ^b	Myeloid neoplasms, mastocytosis
Promoting personalized precision medicine approaches and tools from bulk cell-to LSC-assessment	Myeloid neoplasms, advanced mastocytosis
Genetic predisposition and related germ line abnormalities/mutations	Myeloid neoplasms, mastocytosis, mast cell activation syndromes (MCAS)
Mast cell eradication to cure MCAS	Mastocytosis with MCAS (primary MCAS), secondary MCAS, idiopathic MCAS
Open Innovation in Science (OIS)	Myeloid neoplasms, mastocytosis, MCAS
Comparative Oncology/Hematology	Human and canine mast cell neoplasms

LBI HO Ludwig Boltzmann Institute for Hematology and Oncology, *LSC* leukemic stem cells, *allo-HSCT* allogeneic hematopoietic stem cell transplantation, *CML* chronic myeloid leukemia, *Ph+ ALL* Philadelphia chromosome-positive acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *MRD* minimal residual disease

^aAdvanced mastocytosis includes aggressive systemic mastocytosis, systemic mastocytosis with an associated advanced (not mast cell) hematologic neoplasm, mast cell leukemia, and mast cell sarcoma. In contrast to non-advanced forms of mastocytosis, patients with advanced mastocytosis are often treated with cytoreductive drugs, targeted drugs or allo-HSCT

^bSo far only very few predictive LSC markers are used or are examined in translational projects in myeloid or mast cell neoplasms. One example is CD33 expression on LSC which may predict responses of LSC to gemtuzumab ozogamicin. Another example is checkpoint antigen expression on LSC as basis to develop anti-checkpoint therapy concepts

tions provided by the partner board, the SAB, and the evaluation reports. From 2022, scientific projects of the LBI HO are conducted in 2 project lines, one dedicated to myeloid neoplasms in basic research and translational research and one on mast cell neoplasms in basic research and translational research.

The overarching strategic aim of the LBI HO is to establish a multi-disciplinary platform for interactive collaborative research on pre-L-NSC and LSC, and to provide this platform to interested experts and groups in various partner institutions and also to new collaboration partners [46]. In fact, the LBI HO is seeking new collaboration partners, including strong academic partners and suitable industrial collaboration partners. To fertilize the development of cooperative research projects, members of the LBI HO have also organized a series of international meetings and conferences on LSC in the past 15 years.

Another important aim of the LBI HO is to attract top scientists, to educate young scientists interested in LSC research and to shape their career, and to promote junior group leaders and professors working in the field of stem cell research and translational hematology [46]. The LBI HO is also promoting gender medicine aspects in hematopoietic neoplasms, and is also particularly inviting and integrating female researchers in LBI projects.

Finally, an important strategic aim of the LBI HO is to translate research results and concepts into clinical practice [46]. This goal is particularly followed at the Medical University of Vienna and Hanusch Hospital, where clinical markers and targets as well as targeted drugs are tested in observational studies, registry studies and clinical trials, and at the University of Veterinary Medicine, Vienna, where clinical concepts and studies are developed in dogs and other domestic animals. In many of these studies, concepts are

established in animal and the corresponding human neoplasms in parallel, following the principles of comparative oncology within the LBI HO consortium [46]. At the Medical University of Vienna, clinical concepts are developed in collaboration with the CCC, VCSCC, and industrial collaboration partners [46].

During the first 10 years, the LBI HO focused primarily on preclinical models and projects, whereas from 2018, the LBI HO is focusing more and more on translational hematology and the development of clinical concepts around markers and targets displayed by LSC [46]. An overview of projects performed by members of the LBI HO is provided in Table 3.

Examples of contributions of the LBI HO to basic LSC research

Identification of 'stem cell signatures' in patients with premalignant clonal conditions and patients with minimal residual disease during therapy

To test the hypothesis that pre-L-NSC are detectable in patients with early clonal conditions and patients who are successfully treated with anti-leukemic therapy, we examined the phenotype of putative stem cells and related molecular aberration profiles. In a substantial number of patients with early clonal conditions, such as age-related clonal hematopoiesis (ARCH), idiopathic cytopenia with unknown significance (ICUS), or clonal cytopenia with unknown significance (CCUS), bone marrow-derived and circulating CD34⁺/CD38⁻ stem cells display low but detectable levels of CD25 and/or CD123 [48]. Similarly, the residual CD34⁺/CD38⁻ stem cells that can be detected in patients with Ph+ CML who are successfully treated with BCR::ABL1 TKI, often express CD25 and less frequently CD26. Such residual pre-

L-NSC that escape TKI therapy may or may not display *BCR::ABL1* mRNA. In some of these patients *BCR::ABL1* is not detectable, but ARCH-type mutations can be identified. The resulting hypothesis is that residual early pre-L-NSC (pre-*BCR::ABL1* stage of CML) express these mutations. As a result of successful therapy with *BCR::ABL1*-targeting drugs, most dominant subclones are eliminated, whereas pre-L-NSC, bearing ARCH mutations, often persist. Since these mutations also predispose to the occurrence of vascular occlusive events, members of the LBI HO defined the actual risk in these cases. Indeed, there is a correlation between the presence of ARCH mutations and vascular adverse events in CML patients treated successfully with nilotinib, regardless of *BCR::ABL1* mRNA levels [49].

When following patients with early (indolent) myeloid neoplasms, the expression levels of CD25 often increase when the disease is progressing to an aggressive neoplasm/leukemia. For example, in CMML, LSC display low or negligible levels of CD25, whereas in patients with secondary AML (sAML) following CMML, LSC express large amounts of CD25 [22]. In patients with primary (*de novo*) AML, LSC exhibit CD25 in roughly 50% of all cases, whereas in CML, CD25 is almost invariably expressed on LSC in all patients [48]. In the disease models analyzed, including Ph+ CML, CD25 expression on leukemic cells apparently depends on STAT5-activation [50].

Identification of specific aberrant (diagnostic) phenotypes of LSC in myeloid leukemias

During the past 15 years, members of the LBI HO screened over 5000 samples of patients with myeloid neoplasms (as well as control samples) for expression of cell surface markers and targets on stem- and progenitor cells by flow cytometry [14, 19, 21, 22, 48, 50, 51]. In these studies, the LBI HO team was able to identify a number of aberrant surface antigens that are specifically expressed on pre-L-NSC or LSC in various types of myeloid neoplasms, including Ph+ CML, Ph-MPN, CMML, MDS, AML, SM, and MCL. In Ph+ CML, putative LSC display CD25, CD26, CD33, CD93, CD123, and IL-1RAP [48, 50, 51]. In AML, LSC often express CD25, CD96, and CD371 (CLL-1) [48]. In addition, in FLT3-mutated AML, LSC may also express CD26 [48]. In most myeloid neoplasms, including MPN and CML, pre-L-NSC and LSC also display low levels of CD274 (PD-L1) [45, 52–54]. When exposed to interferon-gamma (IFN- γ) and/or tumor necrosis factor alpha (TNF- α), pre-L-NSC and LSC express significant amounts of this resistance-mediating checkpoint antigen [45, 52–54]. It is also important to note that pre-L-NSC and LSC also display several other major (resistance-related) checkpoint antigens, such as CD47 and also several known drug targets, such as CD9, CD44, or CD52 [48].

Overall, LSC phenotyping revealed disease-specific cell surface profiles, which forms the basis for diagnostic stem cell phenotyping (LSC typing) and for the development of LSC-targeting (improved curative) therapies.

Identification of the osteoblast as a major site of LSC resistance in ph+ CML

In myeloid neoplasms, the stem cell niche in the bone marrow is composed of various structural cells, including fibroblasts, endothelial cells, macrophages, other stromal cells, and endosteal-lining cells (osteoblasts) [55–57]. These niche-forming cells reportedly are involved in the differentiation, distribution, and function of normal and neoplastic stem cells and contribute to LSC resistance [55–57]. Whereas stromal cells have repeatedly been reported to contribute to LSC resistance in CML and AML, little is known about the role of other niche cells. More recently, members of the LBI HO found out that osteoblastic cells (endosteal cells) play a major role in resistance of LSC against *BCR::ABL1*-targeting TKI in patients with Ph+ CML [58]. In fact, when co-cultured with these cells, CML LSC can no longer be driven into apoptosis by nilotinib or ponatinib [58]. Osteoblast-mediated resistance of LSC in Ph+ CML is dependent on several oncogenic pathways, including the PI3-Kinase-mTOR pathway and the BRD4-MYC axis [58, 59].

The BRD4-MYC axis as a driver of LSC resistance in myeloid neoplasms

Drug resistance of LSC against targeted drugs and other therapies remains a problem in applied hematology. During the past 10 years, members of the LBI HO have examined the mechanisms of LSC resistance and developed strategies to overcome LSC resistance by applying drug combinations and by disrupting key pathways and targets contributing to resistance. In these studies, the BRD4-MYC pathway turned out to be a key driver of LSC resistance in several malignancies, including AML and CML [58, 60–62]. For example, in Ph+ CML, BRD4 and MYC contribute to niche-induced resistance of LSC as well as to intrinsic LSC resistance, acquired (mutation-induced) resistance, and immunologic resistance [58]. Similar data have been collected in other myeloid neoplasms, including AML. Members of the LBI HO were also able to show that BRD4 degraders can completely disrupt the BRD4-MYC pathway in LSC, which is important as these cells frequently have or develop resistance against small molecule type BRD4 inhibitors [58].

Examples of contributions of the LBI HO to clinical translation

From KIT to KIT-targeting treatment concepts

During the past 15 years, members of the LBI HO have established the anti-neoplastic activity profiles of various KIT-targeting drugs in the context of advanced SM and mast cell activation. The first compound tested, imatinib, turned out to be less effective, since the *KIT* mutation D816V confers resistance against this TKI [63, 64]. However, midostaurin (PKC412) was found to exert profound effects on KIT D816V as well as growth and survival of neoplastic mast cells obtained from patients with advanced SM [63, 65]. In addition, members of the LBI HO were able to show that neoplastic stem cells in SM display KIT and KIT D816V and that midostaurin is also able to suppress the growth and viability of these stem cells [21]. Finally, members of the LBI HO were able to demonstrate that midostaurin blocks IgE-mediated histamine secretion in human mast cells and basophils [66, 67]. Based on these results and other data obtained in other major research laboratories, midostaurin was further developed in preclinical and clinical studies [68] and was finally approved for the treatment of patients with advanced SM. In subsequent studies, the LBI HO also examined the activity profiles of other KIT D816V-targeting drugs, including avapritinib [69]. Similar to midostaurin, avapritinib is able to suppress the growth and survival of neoplastic mast cells and IgE-mediated histamine release in neoplastic mast cells and basophils [69]. However, compared to midostaurin, avapritinib is a stronger inhibitor of KIT D816V and a more potent agent regarding its inhibitory effects on mast cell expansion in advanced SM. The LBI HO is also continuing to test the efficacy profiles of novel KIT-targeting drugs.

Identification of CDK4/CDK6 as key vulnerability of BCR::ABL1 T315I-transformed cells

In Ph+ CML, a remaining challenge is the occurrence (selection) of the multi-resistant mutant form T315I of BCR::ABL1, especially when expressed together with other *BCR::ABL1* mutations in compound configuration. In the past 10 years, several attempts have been made to overcome BCR::ABL1 T315I-mediated resistance in CML. A first clue to the critical mechanisms was the observation that BCR::ABL1 T315I is a weak (even growth-inhibitory) oncogene that needs additional pro-oncogenic machineries to expand clonal stem and progenitor cells [70]. Subsequently, members of the LBI HO were able to show that BCR::ABL1 T315I-mediated resistance of CML cells is triggered by activation of cyclin-dependent kinases (CDK), especially CDK4 and CDK6 [71]. In addition, the team of the LBI HO found that the CDK4/6-targeting drug palbociclib as well as hydroxyurea (HU), a potent

inhibitor of CDK4/6 expression, counteract growth and survival of CML cells and Ba/F3 cells exhibiting BCR::ABL1 T315I alone or BCR::ABL1 T315I in compound configuration with other mutant forms of BCR::ABL1 [71, 72]. In addition, HU was found to induce selective, complete and long-lasting suppression of BCR::ABL1 T315I-bearing subclones in patients with TKI-resistant CML [71]. Finally, members of the LBI HO were also able to show that CDK-targeting drugs cooperate (or even synergize) with BCR::ABL1 T315I-targeting drugs (ponatinib and asciminib) in inhibiting the growth and viability of CML cells expressing the T315I mutant [71, 72].

Dissection of niche-targeting effects of BCR::ABL1-directed TKI

The other unsolved issue in Ph+ CML is the avoidance of TKI-induced adverse side effects, especially TKI-related cardiovascular events [49, 73]. During the past few years, members of the LBI HO screened for potential adverse effects of novel and established BCR::ABL1 TKI in various *in vitro* models. Whereas nilotinib and ponatinib turned out to exert pro-atherogenic and growth-inhibitory effect on vascular endothelial cells [49], bosutinib and dasatinib were less effective, and asciminib did not show any inhibitory or pro-atherogenic effects on human endothelial cells [59]. This observation has recently been confirmed in smaller and larger clinical trials, suggesting that the LBI HO-based pre-testing of targeted drugs, including BCR::ABL1-directed TKI is of clinical value and of predictive significance.

Immunotherapies directed against LSC to improve curative treatment approaches

During the past 10 years, a number of immunotherapy approaches have been developed, including targeted antibody-based therapies, bi-specific engager antibodies, and CAR-T cell therapies. In addition, several efforts have been made to improve stem cell transplantation (SCT) approaches. Indeed, in most myeloid and mast cell neoplasms, SCT still remains the only established curative therapeutic approach. By contrast, only very few studies have shown encouraging effects of antibody-based or CAR-T cell therapies in myeloid neoplasms.

One example is gemtuzumab ozogamicin (GO), an antibody-toxin conjugate (CD33+ γ -calicheamicin) that exerts major anti-neoplastic effects in myeloid stem and progenitor cells. Members of the LBI HO were able to show that AML LSC and CML LSC as well as LSC in advanced mast cell neoplasms display CD33, and that GO is able to induce apoptosis in these cells [21, 45, 48, 54, 74–76]. Unfortunately, however, normal hematopoietic stem cells also display CD33, albeit at a lower level compared to LSC. This small therapeutic window allows for treatment with GO at

Table 4 Major Conferences and other CSC Meetings organized by the LBI HO

Title and Topic of Meeting ^a	Place & Year	Publication ^b
CSC Symposium 2008	Vienna 2008	–
Year 2011 Working Conference on CSC	Vienna 2011	[5, 76]
10 Year Jubilee Meeting of the VCSCC	Vienna 2012	[not listed ^c]
Ph+ CML: from LSC Eradication to Cure	Vienna 2014	–
From Cellular Basis and Targets to Targeted Therapies	Vienna 2015	[77]
Classification and Nomenclature of Clonal Conditions	Vienna 2015	[77]
Workshop on MDS and pre-MDS Conditions	Vienna 2016	[not listed]
10 Year Jubilee Meeting of the LBC ONC	Vienna 2018	[46]
Working Conference on CMML	Vienna 2018	[not listed]
Precision Medicine in Hematology Meeting	Vienna 2019	[not listed]
Cell Therapies and Stem Cells in AML and CML	Vienna 2020	[45]
20 Year Jubilee Meeting of the VCSCC	Vienna 2022	[47]
15 Year Jubilee Meeting of the LBI HO	Vienna 2023	This document

CSC cancer stem cells, *LBI HO* Ludwig Boltzmann Institute for Hematology and Oncology, *VCSCC* Vienna Cancer Stem Cell Club, *CML* chronic myeloid leukemia, *MDS* myelodysplastic neoplasms (syndromes), *LBC ONC* Ludwig Boltzmann Cluster Oncology (precursor of LBI HO), *CMML* chronic myelomonocytic leukemia, *AML* acute myeloid leukemia

^aIn each conference, the first day included an education session that was open to the public and free of registration fees

^bPosition papers were prepared to summarize the event and the most important conference outcomes in form of consensus statements

^cBecause of space limitation, several position papers have not been included in the list of references

moderate doses, but may not allow for CAR-T cell-based therapy unless a SCT rescue is applied. Indeed, GO at higher doses reportedly induces severe and long-lasting cytopenia, especially when combined with poly-chemotherapy.

Currently, the LBI HO is seeking more specific LSC targets that can be employed in CAR-T cell-based treatment approaches or antibody-based therapy using high doses of the drug.

Major conferences and other meetings organized by the LBI HO

During the past 15 years, members of the LBI HO organized a series of international scientific meetings, including several working conferences and workshops on CSC/LSC and/or on personalized medicine and precision medicine in hematology (Table 4). In these meetings, international top experts participated and exchanged concepts, data and expert opinion, with the aim to establish or refine (update) definitions and nomenclatures around CSC/LSC, pre-L-NSC, and to discuss the emerging new fields of personalized medicine and precision medicine in hematology. Some of these meetings focused on distinct types of myeloid neoplasms, such as MDS, mastocytosis or CMML.

Additional important topics discussed in these meetings were premalignant stages of cancer/leukemia, the development of curative cell therapies and immune therapies, comparative oncology, and open innovation in science (OIS). In each meeting, the first day was open for students and the interested public and consisted of several education sessions, whereas the following days were closed and essentially restricted to the faculty of the meeting. With regard to CSC/LSC definitions and terminologies, the most important and most influential working conference was organized in 2011, the 'Year 2011 Working Conference on CSC' (Table 4). In this conference, major authorities in the field discussed the definitions and terminologies as well as the heterogeneity of neoplastic stem cells, including CSC and LSC [5]. In addition, stem cell assays and limitations in currently used xenotransplantation models were discussed in this conference [5]. Finally, the faculty presented a proposal for the classification of NSC into premalignant NSC and malignant NSC. In the context of a leukemia, these cells are classified as pre-L-NSC and LSC [5, 77]. This concept is also consistent with the assumption that CSC/LSC evolution is a step-wise process that takes several years or even decades and is triggered by molecular drivers and co-driving passenger mutations in one, more, or many different sub-clones [5]. This concept has several clinical implications and explains nicely the biology of a multi-mutated neoplasm, including genomic plasticity and the heterogeneity of primary and secondary cancer lesions, lineage switches of cancer cells, and the unique molecular features of relapsing disease [5, 43, 77].

In 2015, members of the LBI HO and VCSCC organized a meeting to discuss and establish a global classification of clonal conditions, from early clonal lesions to fully developed, overt malignancies (Table 4). In this project, the faculty members extended the basic concept of dividing neoplastic stem cells into premalignant and malignant (cancerous) stem cells, to diseases in general, resulting in the delineation of neoplasms into premalignant (indolent) neoplasms and malignant (aggressive) neoplasms [78] which is in line with the classification of hematologic and non-hematologic neoplasms provided by the WHO.

In 2018, the members of the LBI HO organized a 10-year jubilee meeting of the LBI HO, at that time named still LBC ONC [46]. In June 2023, members of the LBI HO celebrated the 15-year jubilee of the LBI HO in Vienna (Table 4).

Summary and future perspectives

During the past 15 years, the LBI HO has established the phenotype and target expression profiles of pre-L-NSC and LSC in various blood cell malignancies, including myeloid leukemias, MDS and MPN as well as mast cell neoplasms, including MCL. Based on these achievements, members of the LBI HO were also

able to isolate NSC to define major functional and immunological vulnerabilities through which these cells can be detected and can be eliminated using specific targeted drugs. As a result, the diagnostic and prognostic impact of LSC has been assessed in various cancer models, and improved LSC-eradicating (more curative) drug therapies have been developed. Several of these therapies are based on drug combinations directed against cooperative signaling pathways representing key vulnerabilities when blocked together, or antibody-based therapeutic approaches to overcome multiple forms of LSC resistance and even LSC dormancy. In addition, the LBI HO validated diagnostic LSC-based tools and new prognostic stem cell markers and patterns, including genetic abnormalities, serologic parameters and flow cytometry-patterns detecting key markers or minimal residual (LSC-containing) disease. The LBI HO has also defined premalignant phases and cells in various myeloid malignancies. Finally, the LBI HO identified mechanisms underlying resistance of pre-L-NSC and LSC in various disease models and developed strategies to overcome stem cell resistance. In the next few years, the LBI HO will continue to define the clinical value of druggable vulnerabilities of NSC/LSC in various hematopoietic neoplasms and will try to translate diagnostic, prognostic and LSC-eradicating treatment concepts into clinical application.

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