In 2015, the 8th year of our Ludwig Boltzmann Cluster Oncology (LBC ONC), the cluster team has made a number of new fascinating observations and further developed projects on leukemic stem cells (LSC) in various hematopoietic malignancies. In addition, the cluster team was able to finalize several studies on LSC. According to the master-plan of the LBC ONC, projects were performed in 3 project-lines (PL), one dedicated to myeloid neoplasms, one to lymphoid leukemias, and one to mast cell neoplasms. In these studies, new LSC markers and LSC targets have been identified and subsequently have been validated. In addition, LSC-niche interactions and the effects of various targeted drugs on niche cells and LSC were examined.

1. **Overview of the Cluster (LBC ONC)**

1.1. Introduction and Aims

During the past 2 decades, several studies have shown that hematopoietic neoplasms are essentially composed of two distinct fractions of cells, i) a bulk-population exhibiting a limited capacity of long-term proliferation, and ii) a smaller fraction that has unlimited self-renewal and thus long-term disease-propagating capacity, the so-called neoplastic stem cells, also known as cancer stem cells or leukemic stem cells (LSC) in the context of a leukemia. This hypothesis is widely accepted and predicts that anti-cancer therapy is curative only when eliminating most or all stem cells of a given cancer or leukemia. During the past few years, more and more data have shown that LSC fractions represent heterogeneous populations of cells, reflecting plasticity and genetic instability in these cell populations. However, little is known so far about the expression of specific markers and targets in LSC (Figure 1). During the past few years, the LBC ONC and other groups in the field have made considerable progress in the phenotyping of LSC. In particular, we and others have been able to identify a first series of specific markers and targets in LSC in various human leukemias.

The general aims of the LBC ONC are to identify and characterize LSC in various human leukemias, to define target expression profiles in these cells, to validate molecular targets, and to examine the effects of various targeted drugs on growth and survival of LSC. The long-term goal in these projects is to develop curative therapies for human leukemias by applying drugs that recognize and eliminate LSC.
Figure 1
Expression of cell surface and cytoplasmic targets in leukemic cells
Many therapeutic targets have been identified in ‘bulk leukemic cells’. However, little is known about the expression of molecular targets in leukemic stem cells (LSC).

General Aims in LBC ONC Projects:

- Identification, isolation, and characterization of LSC in human leukemias
- Characterization of target expression profiles in LSC in human leukemias
- Delineation of stem cell plasticity and stem cell resistance: relationship to genetic instability and characterization of relevant LSC subclones
- Targeting of LSC using specific targeted drugs and drug combinations
- Evaluation of interactions between LSC and the stem cell niche
- Evaluation of effects of endogenous immune-mediators (cytokines), and death regulators on growth and function of LSC
1.2. Budget of the LBC ONC

The budget plan for 2015 was established in cooperation with the LB-Society. Budget was mainly used to employ personnel and to purchase consumables. Part of the budget was used to invite experts in the field or to send LB society-employed LBC ONC members to conferences and meetings. The LBC ONC budget of 2015 amounted to 548,750.- €.

1.3. Partners and Internal Structure of the Cluster

Partners

As in previous project-years of the LBC ONC, two academic partners were involved in 2015, the Hanusch Hospital and the Medical University of Vienna (MUW).

Project Groups and Scientists

The LBC ONC is running 3 project lines (PL) that were maintained also in 2015: PL1 on myeloid neoplasms, PL2 on lymphoid neoplasms, and PL4 on mast cell neoplasms including mast cell leukemia (MCL). PL1 is also working on normal stem cells and stem cells in myelodysplastic syndromes (MDS). According to the evaluation report and recommendations of the Scientific Advisory Board (SAB), projects on solid tumors and melanomas (PL3) had been finalized in 2011, and most projects on lymphoid neoplasms were completed in 2015. The following researchers were employed in 2015: Heidrun Karlic (MDS, AML), Alexandra Keller (MPN, MCL), Barbara Peter (MCL), Mathias Schneeweiss (AML, MCL), Gregor Eisenwort (AML, CML, MCL), and Emir Hadzijusufovic (MCL, CML). The group organized weekly staff-report meetings (1.5 hours), one week-start meeting (Monday, 10:00-10:20), and a Lecture Series (Friday 16:00). Members of the LBC ONC were also involved in the organization of several scientific meetings and actively participated in these meetings.

Administration Team

Administration was coordinated by Dr. Emir Hadzijusufovic (Administrative Coordinator) and Prof. Dr. Thomas Grunt (Deputy Coordinator of the LBC ONC). They were supported by our secretary, Sabine Sonnleitner.

Core Facility Groups

All core facility (CF) groups that were established in cooperation with the MUW in the LBC ONC in previous years were maintained, and supported our projects in 2015: these CF-platforms (PF) include a LSC-sort PF (employing a high speed sorter), a gene array PF (including proteomics and sequencing facilities), a NSG mouse Xeno-TX-PF (in cooperation with the University of Veterinary Medicine Vienna, Vetmeduni Vienna), and a clinical PF (including a biobanking-system and disease-registries; coordination: Prof. Dr. Wolfgang R. Sperr). All these CF-PF were essential for the progress in our cluster projects in 2015. According to the master-plan, the LBC ONC has also established a CF-PF dedicated to lentiviral-mediated gene delivery, and the establishment of various stem cell lines, including LSC-like cell lines and iPSC-like cell lines (coordination: Dr. Gregor Hoermann).
1.4. Scientific Advisory Board (SAB)

In collaboration with the LB-Society, the LBC ONC has established an international SAB consisting of three experts in the field (Dominique Bonnet, Joos Jonkers and Charles Theillet). The SAB members also attended the evaluation meeting in December 2013. According to the evaluation report and the recommendations of the SAB, LBC ONC projects were adjusted in 2014 and 2015.

1.5. Personnel and Career Development

The following scientists were employed via the LBG in the LBC ONC in 2015:

- Heidrun Karlic     Hanusch Hospital       01-12/2015
- Barbara Peter     Medical University of Vienna   01-12/2015
- Alexandra Keller   Medical University of Vienna   01-06/2015
- Mathias Schneeweiss  Medical University of Vienna   08-12/2015
- Gregor Eisenwort   Medical University of Vienna   01-12/2015
- Emir Hadzijusufovic  Medical University of Vienna   01-12/2015

Career development steps: Emir Hadzijusufovic received the prestigious TRTH grant of the EHA and ASH dedicated to career development and was promoted to act as Administrative Coordinator and Key Researcher in our LBC ONC. The other LBG-employed researchers, including two new Ph.D. Students, work on their scientific career in the LBC ONC. Among the group of non-LB-Society-employed LBC ONC colleagues, a visit of Karoline Gleixner in the research lab of Steffen Koschmieder in Aachen in 2015 should be mentioned. During her visit, Karoline established several iPSC-like cell lines, and she is currently establishing the iPSC technology in our lab.

1.6. Infrastructure

The infrastructure in 2015 included lab-space in 4 labs (total: 70 m²) dedicated to LSC research at the MUW, several other labs of participating scientists at the MUW, and work space at the Hanusch Hospital. In addition, one office for our secretary (Sabine Sonnleitner) as well as several core facility rooms were made available and were used to run cluster projects in 2015. All in all, the labs and the infrastructure shared by partners as well as the scientific environment of the MUW provided optimal conditions for our LBC ONC projects in 2015.

1.7. Scientific Highlights in the LBC ONC in 2015

Among several different scientific observations and achievements in 2015, the following highlights should be mentioned: 1. Identification of BRD4 and MYC as therapeutic targets in CML, 2. Identification of mechanisms of resistance against the BRD4 blocker JQ1, 3. Characterization of the MCL LSC as a CD34+/CD38- cell, 4. Identification of novel targets on MCL LSC, 5. The protective effect of osteoblast-like niche cells on CML LSC exposed to 2nd generation BCR-ABL1 TKI, and 6. Effects of epigenetic drugs on expression of the checkpoint target PD-L1 in leukemic cells.
1.8. Public Relation

The basic mission of the LBC ONC is to increase awareness and to gain knowledge in the field of leukemic (neoplastic) stem cells (LSC) in human leukemias and other blood cell malignancies, to establish pathogenetic and targeting concepts in these neoplasms, and to establish LSC-eradicating treatment concepts, with the ultimate aim to improve anti-neoplastic therapy in patients with MDS, AML, CML, ALL, and MCL. As in previous years, the LBC ONC team transferred its mission-intention to the public in 2015 through publications and meetings organized by the LB Society. In addition, members of the LBC ONC were involved in the special research program (SFB) F47 of the FWF dedicated to myeloid neoplasms at the MUW and organized an international top-meeting on translational medicine, premalignant conditions and cancer stem cells in Vienna in August 2015.

2. Results obtained in the LBC ONC in 2015

2.1. Results obtained in Individual Projects

2.1.1. Identification of BRD4 and MYC as novel molecular targets in CML

In the past 2 years, the LBC ONC has identified BRD4 as a potential drug target in various myeloid neoplasms, including CML. BRD4 and MYC were found to be expressed in primary CML cells as well as in various CML cell lines, including KU812 and K562. Moreover, the LBC ONC was able to show that CD34+/CD38- CML LSC express transcripts for BRD4 and MYC (Figure 2A). The BRD4-targeting drug JQ1 suppressed the proliferation in KU812 cells (IC50: 0.25-0.75 µM) and primary CML cells (0.1-5 µM), whereas K562 cells were found to be JQ1-resistant.

![Figure 2](image)

Expression of BRD4 mRNA in CML LSC and effect of shRNA on growth of KU812 cells
A: Mononuclear cells (MNC), highly purified CD34+/CD38+ progenitor cells, and highly purified CD34+/CD38- stem cells obtained from a patient with chronic phase CML were examined for BRD4 mRNA expression and MYC mRNA expression by qPCR. Results are expressed as percent of ABL mRNA. B: KU812 cells were transduced with 2 shRNAs against BRD4 and one control (renilla-targeting) shRNA by lentiviral gene delivery. The left panel shows the downregulating effect of the shRNAs on BRD4 mRNA expression. Then, cells were mixed 1:1 with empty vector control cells. In the right panel the percent of GFP+ (shRNA-transfected) cells (over time) obtained in these mixing-experiments is shown.
The same effects were obtained with other drugs targeting BRD4, including OTX-015 and I-BET762. In addition, we were able to show that shRNAs directed against BRD4 block proliferation of KU812 cells (Figure 2B). Finally, we were also able to show that these drugs as well as JQ1 induce apoptosis in CML cells. In a next step, we examined the relationship between BRD4 inhibition and MYC expression. In these experiments we found that treatment of KU812 cells or primary CML cells with JQ1 or with BCR-ABL1-targeting drugs (imatinib, nilotinib, dasatinib, ponatinib) is followed by decreased expression of MYC. We also found that shRNAs against MYC suppress the proliferation of KU812 cells and K562 cells. Finally, we found that JQ1 cooperates with BCR-ABL1 tyrosine kinase inhibitors (TKI) in suppressing the proliferation of KU812 cells and K562 cells (Figure 3) suggesting that resistance against JQ1 can be overcome by applying such drug combinations.

![Figure 3](https://example.com/figure3.png)

**Figure 3**
Synergistic growth-inhibitory effects of JQ1 and BCR-ABL1 TKI on CML cells KU812 cells (left panels) and K562 cells (right panels) were incubated in control medium (Co) or in various concentrations of JQ1 and various concentrations of BCR-ABL1 TKI (nilotinib, upper panels; ponatinib, lower panels) alone or in combination at a fixed ratio of drug concentrations at 37°C for 48 hours. Thereafter, cells were harvested and the uptake of \(^3\)H-thymidine was measured. Results are expressed as percent of control and represent the mean±S.D. of triplicates in each experiment. As visible, the drug combinations applied were able to overcome the obvious resistance of K562 cells against JQ1.

Currently, the LBC ONC explores the effects of various BRD4 inhibitors, including JQ1, on MYC expression and growth and survival of primary CML LSC, and the effects of various drug combinations on these cells. The specific question is whether combinations of drugs targeting BRD4, BCR-ABL1, and other major kinase targets, are sufficient to overcome intrinsic resistance of CML LSC. In addition, the LBC ONC will explore drug combination effects on LSC in other myeloid neoplasms.
2.1.2. Identification of mechanisms of resistance against the BRD4 blocker JQ1

During the 8th year of the LBC ONC, the cluster team has also examined mechanisms of resistance against JQ1 and other BRD4 blockers. In these studies, several different principle mechanisms have been identified: in mast cell leukemia (MCL) resistance of certain sub-clones seems to be related to hyperactive KIT. In particular, in the human MCL line ROSA, exposure to the KIT-ligand SCF resulted in partial resistance against JQ1 which was reverted by KIT-inhibition (Wedeh et al, Leukemia 2015;29:2230-2237). By contrast, in CML, resistance to JQ1 may be explained by intrinsic over-expression of BRD4 and MYC in certain sub-clones and cell lines, but also by rapidly restored MYC transcription after drug exposure. These studies are ongoing. A similar mechanism of resistance against JQ1 has been described for AML cells. In particular, in collaboration with the IMP (Johannes Zuber and his team) the LBC ONC was able to show that AML cells may be or may become resistant to JQ1, and that resistance of AML (stem) cells is characterized by their ability to rapidly restore MYC transcription. Subsequent studies revealed that this type of resistance involves activation and recruitment of the WNT signalling pathway (Rathert et al, Nature 2015;525:543-547). Regarding the effects of JQ1 and other BRD4 inhibitors on LSC, and the mechanisms of resistance to these drugs, the LBC ONC has the plan to extend these studies to other disease models, including JAK2-mutated myeloproliferative neoplasms (MPN) and chronic myelomonocytic leukemia (CMML).

2.1.3. Validation of novel therapeutic targets in CML and AML

In 2015, members of the LBC ONC continued to validate a number of recently identified molecular targets in LSC in CML and AML (PL1). Two of these targets, CD33 and CD52, were selected and studied in detail. In particular, the LBC ONC was able to show that the CD33-targeting antibody-conjugate gemtuzumab-ozogamicin (GO) and the CD52-targeting antibody alemtuzumab induce growth inhibition and cell death in CD34+/CD38- LSC obtained from patients with AML or CML (Figure 4A). In addition, we were able to show that short-term exposure (2-3 hours) of CML LSC to GO or alemtuzumab results in a reduced ability of these cells (LSC) to initiate and propagate leukemia growth in NSG mice. We also combined these targeted antibodies in order to achieve cooperative anti-leukemic effects on LSC. Indeed when these drugs were combined (GO + alemtuzumab), cooperative (synergistic) anti-leukemic effects were demonstrable (Figure 4B) which is an important observation suggesting that LSC elimination may be achievable by combining targeted drugs directed against (quiescent) LSC. The LBC ONC will continue to study such drug combinations in 2016 and 2017, with the aim to define most effective LSC-eradicating treatment approaches. Several other subprojects related to specific targets detectable on LSC in AML, CML, and ALL, were completed in 2015. These projects include studies on CD26 expression on LSC in CML, effects of CD26-targeting gliptins on CML LSC, studies exploring the expression and function of various drug targets in ALL LSC, and the validation of these targets using various targeted drugs, including the CD20-targeting antibody rituximab, CD52-targeting alemtuzumab, and inhibitors of PI3K and BCL-2. These studies have been summarized in a number of publications that have been submitted for publication or will be submitted within the next few months.
Effects of gemtuzumab-ozogamicin (GO) and alemtuzumab on LSC
A: Primary CML cells obtained from 12 patients were incubated with control medium or medium containing GO (1 µg/ml) for 48 hours. Then, apoptosis of CD34+/CD38- LSC was determined by combined staining for CD34/CD38 and Annexin V by flow cytometry. The image shows the percentage of Annexin V+ LSC in each donor. B: CD34+ CML cells were incubated in control medium, GO (5 µg/ml), alemtuzumab (500 µg/ml), or a combination of both drugs for 1 hour. Then, cells were washed and injected into irradiated NSG mice (5/group). After 26 weeks mice were sacrificed and the percent of engrafted cells examined by flow cytometry. Results are expressed as mean±S.D. of all mice per group. *, p<0.05.

In the next 2 years, the LBC ONC will continue to validate drug targets identified by phenotyping or gene array studies in CML LSC and AML LSC. In addition, the LBC ONC will continue to cross-validate the most promising targets by extending studies to other disease models, including MDS, MPN, and CMML.

2.1.4. Expression of homing receptors on LSC in AML, CML, and MCL

During the project year 2015, the LBC ONC has examined adhesion receptor profiles displayed by LSC in AML, CML, and MCL. In these studies we found that AML LSC, CML LSC, and MCL LSC display several different surface molecules involved in LSC-niche interactions or homing, such as CD44, CD117, CD144, CD184, and ROBO4. Furthermore, the LBC ONC was able to show that various signaling pathways and mechanisms are involved in expression of these surface adhesion receptors. For example, certain demethylating agents, like decitabine, were found to upregulate the expression of CD44 on various cell lines models. These studies are ongoing and will be extended to primary LSC in 2016 and 2017. In addition, the LBC ONC will examine the effects of various receptor-ligands, such as hyaluronic acid (CD44-ligand), SCF (CD117-ligand), SDF-1 (CD184-ligand), or SLIT (ROBO4-ligand) on growth, survival, migration, and homing of LSC. The homing of LSC and of LSC-like cell lines will be examined in various mouse models, including mice deficient in certain adhesion receptors. Preliminary data suggest that CD44 is indeed an important homing molecule for neoplastic (stem) cells in MCL. Other important cell-cell adhesion receptors in advanced mastocytosis and MCL appear to be CD2 and CD58. In fact, CD2/CD58-dependent homotypic adhesion apparently is responsible for the abnormal cluster formation of MC in SM as loss of CD2 promoted leukemic dissemination of HMC-1 cells in a SCID mouse model.
2.1.5. Preclinical validation of molecular targets expressed in MCL LSC

During the past few years, LBC ONC members have identified LSC in MCL and have subsequently determined the target expression profiles displayed by putative LSC in aggressive systemic mastocytosis (ASM) and MCL. In 2015, members of the LBC ONC were able to further define the phenotype of ASM/MCL LSC. In addition, potentially relevant targets have been subjected to validation in in vitro studies and in vivo experiments in NSG mice. Although the bulk of the MCL clone is composed of CD34-negative mast cells and mast cell-precursors, these cells are unable to initiate or propagate the disease in NSG mice. By contrast, the very small sub-fraction of CD34+ cells (usually <1%) are able to initiate and propagate an MCL-like disease in NSG mice in primary, secondary, and tertiary recipients. A most important finding in 2015 was that among this small fraction of CD34+ cells only the CD34+/CD38- subset engrafts NSG mice with an MCL-like disease. During the past few years, the LBC ONC has partly defined the phenotype and target expression profiles of these cells. Compared to normal stem cells, ASM and MCL LSC display higher levels of CD33, CD52, CD123, and IL-1RAP. In 2015, the LBC ONC has validated the two most promising molecular targets in ASM/MCL LSC, namely CD33 and CD52. We found that antibodies against CD33 and CD52 induce growth arrest and apoptosis in primary ASM/MCL LSC as well as in all human mast cells lines that express the respective target receptor. In addition, we were able to show that the CD33-targeting antibody gemtuzumab-ozogamicin (GO) produces synergistic anti-neoplastic effects on MCL LSC when combined with the KIT-targeting drug PKC412 (Figure 5).

![Figure 5](image)

**Figure 5**
Induction of apoptosis in LSC in ASM and MCL by targeted antibodies
Primary neoplastic cells obtained from 6 patients with advanced SM (ASM, n=3; MCL, n=3) were incubated in control medium (-/-) or in medium containing various concentrations of gemtuzumab/ozogamicin (GO) and/or PKC412 as indicated. After 48 hours, the numbers of apoptotic LSC were determined by gating for CD34+/CD38- cells and staining for Annexin V by flow cytometry. The figure shows the relative numbers of apoptotic LSC (compared to control = 100%) and represent the mean±S.D. of all 6 donors.

In a next step, the effects of GO and alemtuzumab on engraftment of ASM/MCL LSC in NSG mice were examined. In these experiments, we found that both drugs, GO and alemtuzumab, interfere with engraftment and propagation of MCL. Figure 6 shows the effect of alemtuzumab on engraftment of MCL LSC in NSG mice.
Figure 6
Effects of alemtuzumab on engraftment of MCL LSC in NSG mice
Primary MCL cells were preincubated in control medium or in medium containing alemtuzumab (500 µg/ml) at 37°C for 1 hour. Then, cells were demonstrated to be viable (>95%), were washed, and were then injected into the tail vein of irradiated NSG mice expressing the membrane-bound form of human SCF (10^6 cells/mouse; 5 mice per group). After 20 weeks, mice were sacrificed and the percentage of human CD45+/CD117+ MC was determined by flow cytometry. The left panel (A) shows typical dot plot patterns in two mice, suggesting that engraftment was largely suppressed by alemtuzumab-preincubation. The right panel shows the percentage counts of mast cells in all mice in both groups. The difference in the engraftment levels in both groups was found to be statistically significant (* p<0.05).

Finally, the LBC ONC has started to analyze the clonality of the vascular stem cell niche in patients with indolent SM (ISM), ASM, and MCL. Bone marrow endothelial cells (EC) were either collected by cell sorting (CD34+/CD45-) or by microdissection (morphologically identifiable CD34+ EC). Whereas EC derived from patients with ISM did not express KIT D816V, EC in patients with ASM and MCL were found to express the KIT mutant in first experiments. These studies are ongoing.

2.1.6. Regulation of Death Molecules and Checkpoint Targets on LSC

During the past few years that LBC ONC has started to examine interactions between the immune system and LSC in AML and CML. In one project, the effects of NK cells mobilized by IL-2 and histamine have been determined. We found that these NK cells are indeed able to kill AML cells effectively. However, no useful animal model is available. Therefore, we examined patient-derived AML cells and NK cells. The AML patients received IL-2 and histamine as maintenance therapy. Again, the anti-AML effect of NK cells was demonstrable in this study. In 2015, the LBC ONC has started a new subproject dedicated to checkpoint-dependent interactions between LSC and the immune system. Specifically, the LBC ONC will examine expression and regulation of PD-L1 on LSC in AML, ALL, CML, and MCL. In a first project phase, various cytokines and targeted drugs were applied on AML cell lines. We found that PD-L1 expression is largely dependent on IFN-gamma, thereby confirming the available literature. In addition, we found that PD-L1 expression in leukemic cells can be
modulated by various epigenetic drugs. A highlighting example is JQ1-induced suppression of IFN-gamma-dependent expression of PD-L1 in ALL cell lines (Figure 7). The major aim of this subproject is to determine the upregulating and downregulating effects of available drugs on PD-L1 expression in cell lines, primary leukemic cells, and LSC in AML, ALL, CML, and MCL. In addition, the LBC ONC will explore drug effects on PD1 expression on T lymphocytes. This project will be performed together with our collaboration partners at CeMM using a high-capacity screen platform employing a larger drug-panel. Downregulating drugs may per se exert beneficial effects on the immune system. By contrast, PD-L1 upregulating drugs will be combined with checkpoint inhibitors in order to restore T cell effects.

![Figure 7](image)

**Figure 7**
Effects of JQ1 on IFN-gamma-induced PD-L1 expression in ALL cells
The Ph+ ALL cell lines Z119 (left panel - A) and BV173 (right panel - B) were incubated in control medium (co) or in medium containing recombinant INF-gamma (100 U/ml), JQ1 (5 µM) or both agents at 37°C for 48 hours. Thereafter, expression of the checkpoint target PD-L1 was determined by flow cytometry. Results are expressed as staining index (‘1’=100%) and represent the mean±S.D. of three independent experiments. The significance levels are also shown (p<0.05).

### 2.1.7. Niche cells and LSC-niche interactions as potential targets of therapy

In the year 2015, the LBC ONC further examined the potential value of niche cells and LSC-niche interactions as targets of therapy. In this sub-project, the effects of various targeted drugs, including BCR-ABL1 TKI, on growth and viability of vascular cells and osteoblast-like cells are examined. In the past few years, the LBC ONC was able to show that certain BCR-ABL1 TKI, including nilotinib and ponatinib, exert strong growth-inhibitory effects on vascular endothelial cells. In 2015, these studies were extended to osteoblast-like sarcoma cell lines. In these experiments, nilotinib and ponatinib did not induce major growth-inhibitory effects on osteosarcoma cell lines. However, we found that the apoptosis-inducing effects of both TKI on CML cells are clearly reduced when these CML cells are co-cultured with osteosarcoma cells (Figure 8). Preliminary data suggest that this holds also true for CML LSC. In other words, contrasting endothelial cells, osteoblast-like cells are not only resistant against BCR-ABL1 TKI, but also protect CML cells from these TKI.
Figure 8
Effects of BCR-ABL1 TKI on survival of K562 cells in a co-culture system
K562 cells and Cal72 osteosarcoma cells were cultured separately or together in co-culture as indicated. Cells were incubated in control medium (co) or in medium containing ponatinib (10 or 25 nM), nilotinib (100 or 250 nM), or imatinib (1 µM) at 37°C for 48 hours. Thereafter, cultures were harvested and the numbers of apoptotic cells were determined by staining for Annexin V by flow cytometry. Results represent the mean±S.D. from 3 independent experiments. Osteoblast-like Cal72 cells did not respond to these TKI, but rescued K562 cells from TKI-induced apoptosis.

Preliminary data suggest that similar effects are found when primary LSC are examined in such a co-culture system. In 2016, the LBC ONC will continue to explore the effects of various drugs on niche cells and niche-LSC interactions in CML, AML, and MCL. Specifically, the LBC ONC will try to identify drug combinations that can overcome the niche-mediated resistance of CML LSC against TKI. Other studies will explore direct drug effects on niche cells. In 2015, the LBC ONC completed a study on nilotinib effects on endothelial cells which is relevant clinically in the context of vascular side effects observed in patients treated with this TKI. These data have been submitted recently. The effects of ponatinib on endothelial cells (ponatinib is the second BCR-ABL TKI that produces vascular occlusions in patients) are further investigated. The data obtained so far suggest that ponatinib is an even stronger growth-inhibitor of endothelial cells compared to nilotinib. These studies are ongoing.

2.2. Publications in 2015 – Overview

A number of original publications and review articles have been published in the project year 2015. Among these are several first- and/or senior authorships in Blood, Clinical Cancer Res, Leukemia, and other top journals. In addition, LBC ONC members co-authored in Nature and in the N Engl J Med. We like to point out that several of these studies were conducted in collaboration with groups of the LBI for Cancer Research, St. Anna Children’s Hospital, and CeMM, in several instances within the special research program (SFB) F47 coordinated by us (P. Valent). Finally, members of the LBC ONC were involved in the conduct of several clinical studies in 2015. A list of publications is provided in a separate file.
2.3. Patents

No patents were filed during the period 2015 in our LBC ONC consortium. However, the cluster is actively scouting for patentable results.

2.4. Attending Conferences and Meetings

Members of the LBC ONC attended several important national and international meetings and conferences in the field of Hematology and LSC Research. In addition, members of our cluster organized and participated in an international meeting on translational medicine, premalignant conditions and LSC (Paul Ehrlich Memorial Meeting – celebrating the 100th Death Day of Paul Ehrlich) in Vienna in August 2015.

2.5. Lectures and Presentations

Researchers of the LBC ONC presented their data in a series of invited lectures and other presentations in national and international top conferences and workshops in 2015. Highlighting examples are invitations to main Education Lectures at the Annual Meeting of the European Hematology Association (EHA) in Vienna in June 2015 (topic CML stem cells) and at the Annual Meeting of the American Society of Hematology (ASH) in Orlando, USA (topic of education: mast cell disorders) in December 2015. In addition, members of the LBC ONC presented their concepts and data in the Paul Ehrlich Memorial meeting in August 2015 in Vienna. This meeting can be regarded as the highlight-event in Vienna in the LBC ONC in 2015, as several major new concepts around stem cells and premalignant conditions were presented to the community. A list of all presentations is provided in a separate file (publications).

3. Additional Information

3.1. Scientific Collaborations in the LBC ONC

Scientific Collaborations within the Cluster
In the past 8 years, several important internal collaborations have been established within the scientific environment of the LBC ONC. These collaborations have been used by members of the LBC ONC in 2015, with the aim to strengthen our cluster projects. Collaborations between the Hanusch Hospital and the MUW were maintained in 2015. These interactive projects include, among others, collaborations on MDS, on the osteoblastic stem cell niche, and on drug effects in MCL. In addition, ongoing cooperations with the Departments of Laboratory Medicine (MCL models), Pathology (stem cell-niche interactions), Internal Medicine I (leukemias, MPN, and myeloma) and Radiation Therapy (leukemia models) were maintained in 2015.

Scientific Collaborations with other Groups in Vienna
During the past 8 years, the LBC ONC established several essential collaborations with a number of groups working in the field of stem cell research and translational hematology in Vienna. These collaborations were also maintained in 2015. Examples
are projects conducted together with our colleagues at CeMM (Giulio Superti-Furga and Stefan Kubicek), the LBI for Cancer Research (Richard Moriggl and Lukas Kenner), the Childrens’ Cancer Research Institute (Thomas Lion and his team), the Vetmeduni Vienna (Veronika Sexl, Michael Willmann & Thomas Rülicke), and the LB Cluster for Cardiovascular Research (Johann Wojta and his team). Several of these collaborations also relate to the SFB program F47 dedicated to MPN (Speaker: P. Valent). The LBC ONC will intensify these collaborations in the period 2016-2017. One important strategic goal is to prolong SFB F47 into a second phase (2017-2020) and to include the Vetmeduni Vienna as a new partner of our LBC ONC.

3.2. Organization of Conferences and Meetings

Members of the LBC ONC were actively involved in the organization of national and international conferences and meetings, including the Annual Meeting of the European Competence Network (ECNM) in Munich (October 2015), the Annual meeting of the Austrian Competence Network on Mastocytosis (AUCNM) in Graz (October 2015), and the Paul Ehrlich Memorial Meeting in Vienna (August 2015). The Paul Ehrlich Meeting included a symposium on the development of targeted drug therapies and a workshop on premalignant neoplastic conditions and neoplastic stem cells. As mentioned, researchers of the LBC ONC actively participated in all these meetings and presented their concepts and data. Finally, the LBC ONC contributed actively to the Meet Science Meeting of the LB Society in April 2015.

3.3. Education and Ph.D. Program

Like in previous years, members of the LBC ONC were actively involved in teaching master students, M.D. students, and Ph.D. students in 2015. Our students exploited these teaching facilities provided by the LBC ONC and its partners on Campus, and attended the main lecture series of the newly established SFB program F47 dedicated to myeloid neoplasms (MPN) in 2015. Our Ph.D. students are also actively participating in the Ph.D. program ‘Malignant Diseases’ of the Medical University of Vienna, coordinated by Brigitte Marian, who is a valuable member of our cluster and the CF-PF coordinator (dedicated to student’s education) of the LBC ONC.

4. Outlook and Aims for the Next Project-Year (2016)

Following the master-plan of the LBC ONC and results obtained in 2014-2015 the LBC ONC will continue to focus on novel markers and targets in LSC in AML, CML, MCL, and other myeloid neoplasms. In addition, the LBC ONC will continue to focus on niche-related cells and LSC-niche interactions in 2016. One special topic will be niche-mediated mechanisms of resistance of LSC that seems to be provided especially by osteoblast-like cells. Following the recommendation of the SAB, the LBC ONC has completed all projects on lymphoid neoplasms in 2015 and will publish these data in 2016 and 2017. Moreover, the LBC ONC has completed a sub-project on AML – NK cell interactions. In 2015, the LBC ONC has identified PD-L1 as a new inducible drug target on LSC in AML and CML. Since PD-L1 expression in neoplastic cells has attracted considerable attention in the cancer community in recent years, and since our
preliminary data suggest that PD-L1 expression in leukemic cells is regulated not only by the oncogenome and certain cytokines, like IFN-gamma, but also by various targeted drugs, we decided to implement PD-L1 expression and regulation on LSC as a major new subproject in the LBC ONC. Specifically, the LBC ONC will screen for drugs that either up-regulate or down-regulate PD-L1 expression on neoplastic cells and their LSC. These screens will in part be performed in a new collaboration with our partners at CeMM. In consecutive experiments, various drug combinations will be applied with the aim to identify strong inhibitors of cytokine- and drug-induced PD-L1 expression in LSC. Further strategic aims for 2016 are to establish new stem cell line models, including an iPSC-like cell line model for human leukemias, to extend several of our concepts, including MCL LSC to the canine system thereby implementing the new field of Comparative Oncology in the LBC ONC, and to translate LSC-related models from a preclinical phase of development into clinical application. A good example is expression of CD25, CD26, and IL-1RAP on CML LSC. Based on the specificity of these surface-antigens for CML LSC, LSC-phenotyping has recently been translated into routine diagnostic algorithms in our department. Moreover, the LBC ONC will maintain all important collaborations with other clusters and institutes of the LB Society, including the LBI-CR (leukemia models), LBI for Osteology (stem cell-niche interactions), and Cluster for Cardiovascular Research (vascular stem cell niche). With regard to targets, the LBC ONC will continue to validate clinically important cell surface target antigens, including CD25 (IL-2RA), CD26 (DPPIV), CD33 (Siglec-3), CD44 (Pgp-1), CD52 (Campath-1), CD117 (KIT), CD123 (IL-3RA), CD133 (AC133), CD184 (CXCR4), and CD274 (PD-L1). In addition, the cluster will continue to validate promising cytoplasmic target structures identified in our projects, including BRD4, PI3 kinase, JAK2, or STAT5 in 2016. Another aim is to identify specific antigens that play a role in LSC-homing and LSC-redistribution in AML and CML. Finally, the LBC ONC has the plan to extend his concepts to certain other myeloid neoplasms, including MPN and CMML. The specific plan is to establish the phenotype and target expression profile of LSC in these malignancies, and to compare results with LSC phenotypes obtained in AML and CML. Expression of markers and targets will be correlated with clinical outcomes (survival and progression-free survival) and with prognostic variables. The long-term goal is to develop new improved (curative) treatment strategies by applying drugs and drug combinations that have the capacity to target LSC-niche interactions, to target niche-related cells, and finally to eradicate LSC. Regarding its structure, the aim of the LBC ONC for 2016-2017 is to include additional academic and industrial partners. So far, the LBC ONC has invited one academic partner and two companies to join as new cluster partners.

5. Publications


Review Articles


**Other Publications: Letters, Editorials, Book-Chapters, Meeting Reports**


**Oral Presentations / Lectures**


Grunt TW. Cancer cell lipid metabolism and oncogenic signaling in ovarian cancer. Research Retreat of the Comprehensive Cancer Center Vienna, Vienna, Austria, February 27, 2015.


Abstracts


Grunt TW. Cancer cell lipid metabolism and oncogenic signaling in ovarian cancer. Research Retreat of the Comprehensive Cancer Center Vienna, Vienna, Austria, February 27, 2015.


