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1. Overview of the Cluster

In 2016, the 9th year of the Ludwig Boltzmann Cluster Oncology (LBC ONC), the cluster team has made a number of additional new interesting observations and continued to develop all ongoing projects on leukemic stem cells (LSC) in the hematopoietic malignancies tested. In addition, the team finalized several subprojects in 2016. 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 neoplasms, 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. Finally, the LBC ONC continued to investigate the expression, regulation and control of various immune checkpoint molecules on LSC in the year 2016.

1.1 Introduction and Aims

During the past two decades, numerous studies have shown that most if not all neoplasms are composed of 2 distinct fractions of cells, i) a bulk-population exhibiting a limited capacity of long-term proliferation, and ii) a (much) smaller fraction that has unlimited self-renewal and thus long-term disease-propagating capacity, the so-called neoplastic stem cells (NSC), also known as leukemic stem cells (LSC) in the context of a leukemia. This hypothesis is widely accepted and predicts that anti-cancer therapy (drug therapy or immunotherapy) is curative only when eliminating most or all NSC/LSC. 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. Currently, major attempt are made to identify specific markers and targets in LSC (Figure 1). In the past few years, the LBC ONC has made considerable progress in the phenotyping of LSC in various 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 markers and 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 so far about expression and function of molecular targets in the leukemic stem cells.

General Aims of 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
- Analysis of expression, regulation and pharmacologic control of resistance-mediating immunologic checkpoint molecules in LSC
1.2 Budget of the LBC ONC

As in previous years, the budget plan for 2016 was established in cooperation with the Ludwig Boltzmann (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 total budget of the LBC ONC in 2016 amounted to 648,818.- €.

1.3 Partners and Internal Structure of the Cluster

Partners, Cluster Contract, and Establishment of an Operative Board

As in the previous project-years, two academic partners were involved in the LBC ONC in 2016, the Hanusch Hospital and the Medical University of Vienna (MUW). In 2016 the cluster was based on a new contract in which cluster-related activities and relationships are defined. The contract was signed by all partners and all carrier-institutions involved (MUW, KAV, WGKK). Based on this contract, an official Cluster Board, consisting of major representative of all involved partner-institutions, was established. The cluster organized a first Board Meeting in April 2016.

Project Groups and Scientists

The LBC ONC is running 3 project lines (PL) that were also maintained in 2016: 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) and chronic myelomocytic leukemia (CMML). The following cluster researchers were employed by the LB Society in 2016: Heidrun Karlic (MDS, AML), Barbara Peter (MCL, CML), Karin Bauer (MCL), Katharina Blatt (ALL), Mathias Schneeweiss (AML, MCL), Gregor Eisenwort (AML, CML, MCL), and Emir Hadzijusufovic (MCL, CML). The group organized weekly staff-report meetings, a week-start meeting (Monday 10:00), and a Lecture Series. Members of the LBC ONC were also involved in the organization of several international scientific meetings and actively participated in these workshops.

Administration Team

Administrative work was coordinated by Emir Hadzijusufovic (Administrative Coordinator) and Thomas Grunt (Deputy Coordinator of the LBC ONC). The coordinators of the LBC ONC were supported by our secretary, Sabine Sonnleitner.

Core Facility Groups

All core facilities (CF) of the LBC ONC were maintained and supported our projects in 2016: CF-platforms (PF) included a LSC-sorting PF, a gene/omics PF, a NSG mouse xenotransplantation PF (cooperation with the University of Veterinary Medicine Vienna, Vetmeduni Vienna), and a clinical PF (including a biobank-system and several patient registries; coordination: Wolfgang R. Sperr). According to the master-plan, the LBC ONC also maintained 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: Gregor Hoermann).
1.4 Scientific Advisory Board (SAB)

In cooperation with the LB-Society and the Board, a new international SAB consisting of 3 experts in the field (Michel Arock, Kimmo Porkka, Dominik Wolf) was established in 2016. A first SAB meeting was organized in October 2016. According to the recommendations of the SAB, LBC ONC projects and PL were adjusted in 2016. As per SAB recommendation the PL on lymphoid neoplasms was maintained.

1.5 Personnel and Career Development

The following scientists were employed via the LBG in the LBC ONC in 2016:

- Bauer Karin, Medical University of Vienna, 05-07/2016
- Blatt Katharina, Medical University of Vienna, 01-12/2016
- Eisenwort Gregor, Medical University of Vienna, 01-12/2016
- Hadzijusufovic Emir, Medical University of Vienna, 01-12/2016
- Karlic Heidrun, Hanusch Hospital, 01-12/2016
- Peter Barbara, Medical University of Vienna, 01-12/2016
- Schneeweiss Mathias, Medical University of Vienna, 01-12/2016
- Valent Peter, Medical University of Vienna, 04-12/2016

Career development steps in 2016:

- Emir Hadzijusufovic completed his TRTH grant program course (EHA/ASH) dedicated to career development and advanced to the Administrative Coordinator of our LBC ONC. Karoline Gleixner was able to reach a senior staff position at the MUW. Katharina Blatt received the prestigious Türk Award of the Austrian Society for Hematology and Oncology. Peter Valent, was invited to co-author the WHO Blue Book Chapter on Mastocytosis and to prepare two chapters (Mastocytosis and Eosinophil Disorders) in the world leading hematology textbook ‘Hematology: Basic Principles and Practice, Expert Consult’ (Hofmann & Benz).

1.6 Infrastructure

The infrastructure in 2016 included lab-space in 4 labs (total: 64 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, as in previous years, one office for our secretary (Sabine Sonnleitner: 6 m²) as well as several core facility rooms were made available and were used to run cluster projects in 2016. 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 2016.

1.7 Scientific Highlights in the LBC ONC in 2016

Among several different scientific observations and achievements in 2016, the following highlights should be mentioned: 1. Identification of BRD4 and MYC as regulators of PD-L1 expression in LSC in myeloid leukemias, 2. Identification of a putative LSC in CMML, 3. Translation of KIT as a key target in MCL, and 4. Identification of age-related hematopoiesis and its potential impact in CML.
1.8 Public Relation

One basic mission of the LBC ONC is to increase awareness and to gain knowledge in the field of NSC/LSC in human leukemias and other blood cell malignancies, to establish pathogenetic and targeting concepts in these neoplasms, and to develop 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 2016 through publications and meetings organized by the LB Society. In addition, the LBC ONC maintained all collaborations with groups working in the special research program (SFB) F47 dedicated to myeloproliferative neoplasms and organized an international top meeting on basophil neoplasms and CML in Vienna in March 2016 and an international top meeting on MDS in July 2016. In both meetings, the first day was dedicated to education and was open to the public (without registration fee).

2. Results obtained in 2016

2.1 Results obtained in Individual Projects

2.1.1 Further validation of cell surface markers on LSC in AML and CML

In 2016, the LBC ONC team completed most studies on CD25 expression in LSC in AML and CML. CD25 is expressed on CML LSC in a STAT5-dependent manner and serves as a negative regulator of cell growth (Sadovnik et al, Clin Cancer Res, 2016). A review article on expression and function of CD25 on CML LSC was also completed. Currently, the cluster team explores the prognostic value of CD25 on CML LSC. With regard to CD26, our team also completed most studies on CML LSC. In collaboration with our CML partners in Brno, we found that the numbers of CD26+ LSC in CML correlate with resistance against imatinib (Culen et al, Oncotarget, 2016). In AML, LSC are usually CD26-negative cells. However, we found that in a small subset of patients with FLT3 ITD+ AML, LSC express CD26. In 2017 the LBC ONC will continue to define the diagnostic and/or prognostic value of newly identified LSC markers and their potential therapeutic value in AML and CML. With regard to prognostication, the cluster will ask whether expression (expression levels) of certain surface antigens on LSC correlates with survival and/or progression-free survival. With regard to targets, the cluster will continue to apply shRNA and targeted drugs in vitro as well as in vivo in NSG mice in validation experiments.

2.1.2 Evaluation of target expression profiles in ALL and multiple myeloma

In ALL the cluster team completed most studies on LSC phenotyping. Based on the recommendation of the SAB, however, the cluster continued to validate cell surface targets on ALL LSC. In 2016 the cluster team was able to show that in Ph+ ALL, NSG-repopulating LSC reside not only in a CD34+/CD38- fraction of the clone, but also in the CD34+/CD38+ subset of leukemic cells. Currently, the team explores and compares targeted expression profiles in CD34- and CD38+ ALL LSC fractions by gene array and RNA-seq experiments as well as phenotyping. In patients with Ph+ ALL exhibiting BCR-ABL1p210, LSC display CD25 and CD26. In 2017, the LBC ONC will continue to define the diagnostic and/or prognostic value of newly identified LSC markers in ALL. In multiple myeloma, the cluster
completed all studies on the characterization of putative neoplastic stem cells and drug sensitivities of primary myeloma (stem) cells and myeloma cell lines (Blatt et al, Oncotarget 2016).

2.1.3 Identification of BRD4 as regulator of PD-L1 expression on LSC

In the past two years, the LBC ONC started to examine the expression and regulation of various checkpoint-molecules known to mediate LSC-resistance, such as CD47 or PD-L1, on LSC in myeloid neoplasms. In first experiments, we found that PD-L1 expression on leukemic cell lines is largely dependent on the presence of IFN-gamma (IFN-G). In addition, we found that PD-L1 expression in leukemic cell lines can be modulated by various epigenetic drugs. Whereas demethylating agents like azacytidine or decitabine were found to upregulate PD-L1 expression, the BRD4/MYC blocker JQ1 was found to suppress IFN-G-dependent expression of PD-L1 in several AML and ALL cell lines. In the project year 2016 the LBC ONC also extended these studies to primary LSC in CML, ALL, and AML. We found that JQ1 inhibits the IFN-G-dependent expression of PD-L1 in primary LSC in Ph+ CML and Ph+ ALL (Figure 2).

![Figure 2](image-url)

**Figure 2**

*Effects of JQ1 on expression of PD-L1 in primary LSC in CML and ALL*

A,B: Mononuclear cells (MNC) isolated from 5 CML patients, were incubated in control medium, interferon-gamma (IFN-G, 100 U/ml) or IFN-G plus JQ1 (2.5 µM) at 37°C for 48 hours. Then, expression of PD-L1 on CD34+/CD38− LSC was determined by multi-color flow cytometry. A: One representative experiment (donor) is shown: the blue histogram shows expression of PD-L1 on LSC after incubation with IFN-G, and the red histogram PD-L1 expression on LSC after incubation in IFN-G+JQ1. The grey histogram represents expression of PD-L1 on LSC in control medium. B: Results show PD-L1 expression on CD34+/CD38− LSC in 5 donors. Results were calculated as staining index (mean fluorescence intensity of PD-L1 relative to fluorescence intensity obtained with an isotype-matched antibody) expressed as % of control and represent the mean±S.D. from 5 donors. Grey bar: expression of PD-L1 on LSC in control medium; red bar: PD-L1 expression on LSC after IFN-G incubation; blue bar: PD-L1 expression on LSC after incubation with JQ1; violet bar: PD-L1 expression on LSC after exposure to IFN-G plus JQ1. Asterisk (*): p<0.05. C: MNC were isolated from a patient with Ph+ ALL and incubated in control medium, IFN-G (100 U/ml) or IFN-G plus JQ1 (2 µM) at 37°C for 48 hours. Then, expression of PD-L1 on CD34+/CD38− LSC (expressed as staining index) was determined by multi-color flow cytometry.
These data suggest that BRD4 and its downstream target MYC are involved in the regulation of PD-L1 expression in LSC. Further studies performed in collaboration with the Institute of Molecular Pathology (IMP) Vienna and the University of Melbourne (Australia) confirmed this hypothesis. In addition, we found that this regulation applies also to other hematopoietic neoplasms and their LSC (Hoggs et al, Cell Rep, in press). In the next project period, the LBC ONC will further examine expression of various checkpoint molecules on LSC, and cytokine-dependent and drug-induced regulation of expression of these antigens.

2.1.4 Disease-modifying impact of the immune system in myeloid neoplasms

In 2016, the LBC ONC completed a sub-project relating to the immune system and its impact on residual leukemic (stem) cells in AML. The cluster team found that maintenance therapy with histamine plus IL-2 induces a substantial expansion of two CD56bright NK cell subpopulations in patients with AML. In addition, we found that therapy with IL-2 and histamine supports the activation of NK cells, and that these NK cells are able to attack and to kill AML blasts (Cuapio et al, Oncotarget 2016). In several patients, therapy with IL-2 and histamine resulted in disappearance of measurable MRD suggesting effects on LSC. Whether IL-2 plus histamine therapy is indeed able to attack and to kill residual AML LSC is currently under investigation. The cluster team also completed their studies on CD52 expression on LSC in MDS and AML. In a pilot study, we examined the effects of the CD52-targeted antibody-type drug alemtuzumab. However, the effects obtained in patients with MDS and AML were unsatisfactory. In addition, when tested in an NSG xeno-transplantation model, alemtuzumab showed no convincing effects on engraftment of AML LSC, whereas in the same experiments, the CD33-targeted antibody-conjugate gemtuzumab ozogamicin (GO=mylotarg) produced major anti-leukemic effects.

2.1.5 Identification and characterization of putative LSC in patients with chronic

In 2016, members of the LBC ONC characterized the cell surface phenotype of putative LSC in CMML. A number of mAb were tested and found to react with CD34+/CD38− and CD34+/CD38+ cells in these patients. The putative LSC (CD34+/CD38−) were found to co-express CD44, CD117, CD123, and CD133 (Table 1). However, CMML LSC did not react with mAb against CD25, CD26, IL-RAP, or CLL-1 (Table 1). An interesting observation was that in patients with CMML who progressed to secondary AML, LSC were found to display CD25 at the time of transformation (Table 1). In these patients, the RAS-signalling pathway is usually activated in leukemic cells. In the project year 2017, the team of the LBC ONC will continue to define marker- and target expression profiles of CMML LSC. In addition, the LBC ONC started to analyze the engraftment of these putative CMML LSC (CD34+ sub-fractions) in NSG mice and MITRG mice (exhibiting human myeloid growth factors). The aims of these studies are to identify a NSG or/and MITRG-engrafting CMML LSC, to define the phenotype of these cells, and to identify clinically relevant target structures in CMML LSC. The long term goal is to improve therapy and prognosis of CMML by introducing LSC-eradicating concepts.
Table 1

Expression of surface antigens on CD34+/CD38− cells in CMML, AML and CML

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CD</th>
<th>CMML</th>
<th>sAML</th>
<th>AML</th>
<th>CML</th>
<th>normal BM</th>
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<td>+/−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DPPIV</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Siglec-3</td>
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<td>+</td>
<td>+</td>
<td>++</td>
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<td>+</td>
</tr>
<tr>
<td>Pgp-1</td>
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<td>+</td>
</tr>
<tr>
<td>IAP</td>
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<td>+</td>
</tr>
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</tr>
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<tr>
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<td>+</td>
<td>−/−</td>
<td>−</td>
</tr>
</tbody>
</table>

Flow cytometry analysis of expression of surface markers and targets on CD34+/CD38− stem cells in patients with CMML (n=5), sAML post CMML (n=3), de novo AML (n=10), CML (n=10), and normal BM. Abbreviations: CMML, chronic myelomonocytic leukemia; sAML, secondary acute myeloid leukemia; CML, chronic myeloid leukemia; BM, bone marrow; DPPIV, dipeptidylpeptidase IV; Pgp1, phagocytic glycoprotein-1; IAP, integrin-associated protein; THY-1, thymocyte antigen-1; G-CSFR, granulocyte-colony stimulating factor receptor; FLT-3, fms like tyrosine kinase 3; PD-L1, programmed death-ligand 1; IL-1RAP, interleukin-1 receptor alpha protein; n.c., not yet clustered: Score of antibody reactivity: ++, strongly expressed on stem cells in most cases; +, clear expression in a majority of cases; +/−, expression in minority of cases or weak expression; −, no expression on stem cells.

Figure 3

Analysis of CD34+/CD38− LSC for expression of CD25 and CD26 in CMML and sAML

CD34+/CD38− LSC were examined for expression of CD25 (upper panels) and CD26 (lower panels) (blue histograms) in a patient with CMML (left) and a patient with sAML (post CMML, right panels) by multicolor flow cytometry. The black open histograms represent the isotype-matched control antibody. CMML, chronic myelomonocytic leukemia; sAML, secondary acute myeloid leukemia; MFI, mean fluorescence intensity; PE, phycoerythrin.
2.1.6 Validation of KIT as a molecular target in mast cell leukemia (MCL)

In 2016, the team of LBC ONC continued to validate KIT as a potential target of therapy in advanced SM and MCL. A major problem in MCL is that neoplastic stem cells exhibit not only mutant forms of KIT (in most cases KIT D816V) but also additional oncogenic mutations in other target genes, such as SRSF2, ASXL1 or RUNX1, and that these additional mutants and the related pathways mediate resistance against various TKI. In addition, MCL LSC exhibit intrinsic stem cell resistance against most TKI. During the past 5 years, members of the LBC ONC were able to show that the multi-kinase blocker midostaurin (PKC412), that is active against KIT D816V, induces growth arrest and apoptosis in MCL cells and various MCL cell lines. However, we also found that midostaurin exerts only weak effects on MCL LSC. Therefore, drug combinations were applied. We found that combinations consisting of midostaurin and alemtuzumab or midostaurin and GO induce synergistic anti-neoplastic effects on MCL LSC and various MCL cell lines. The cluster team was also involved in the preparation and conduct of a global Phase II trial exploring the effects of midostaurin in patients with advanced SM and MCL. In this study, midostaurin was found to suppress mast cell expansion and mast cell-induced organ damage associated with advanced SM and MCL. In addition, mediator-related symptoms improved during treatment with midostaurin (Gotlib et al, N Eng J Med 2016). The cluster team was able to show that treatment with midostaurin blocks IgE-dependent histamine release in mast cells and basophils in vitro and in vivo, and that these effects are in part attributable to the strong inhibitory effects of the drug on SYK activation and other IgE-receptor downstream targets (Peter et al, Leukemia, 2016). However, after an initial hematologic response that was seen in most patients, several patients were found to develop a hematologic relapse. Whereas several different mechanisms of resistance against midostaurin (intrinsic LSC-resistance, metabolite-effects, additional mutations, activation of other signaling pathways) may be responsible for these relapses, the cluster team was able to show that each of these mechanisms can be overcome by applying certain drug-combinations. Currently, the cluster-team explores the in vivo effects of these combinations on MCL LSC in vivo in NSG-SCF mice.

2.1.7 Identification and potential impact of pre-leukemic stem cells in CML

Recent data suggest that hematopoietic ageing is associated with an accumulation of somatic mutations in myeloid stem cells and that acquisition of such mutations is associated with an increased risk i) to develop a myeloid malignancy and ii) to develop a severe cardiovascular disease. Correspondingly, the incidence of cardiovascular events increases with age and is higher in patients suffering from certain myeloid neoplasms. We have recently shown that patients with chronic myeloid leukemia (CML) treated with nilotinib have an increased risk to develop severe cardiovascular events. We also found that nilotinib exerts pro-atherogenic and growth-inhibitory effects on endothelial cells. More recently we detected age-related loss-of-function (LOF) mutations (TET2, DNMT3A, ASXL1) in CML patients and found that these mutations cluster in patients who develop severe cardiovascular events during nilotinib-therapy. These LOF mutations are detectable in CML patients not only at the time of diagnosis but also after drug-induced debulking of the dominant Ph+ clone, suggesting that the earliest pre-leukemic (neoplastic) stem cells are involved. In consecutive studies, the cluster made several attempts to identify the phenotype of these premalignant neoplastic stem cells. We found that during successful treatment with a BCR-ABL1-targeting drug, such as imatinib, most CD25+/CD26+/IL-RAP’ LSC disappear rapidly and are no longer detectable after 3 months. However, despite disappearance of BCR-ABL1 and CD26’ LSC, smaller or larger subsets of CD34+/CD38- cells co-expressing CD25 in an aberrant manner, are often detectable in these patients (Figure 4). Whether
these cells are pre-leukemic neoplastic stem cells remains at present unknown. In 2017, the cluster team will examine whether these CD25+ stem cells contain age-related somatic mutations and whether these cells or their precursors represent early, pre-leukemic (Ph-) CML stem cells.

Figure 4
Detection of CD25 on residual stem cells in patients with CML
Expression of CD25 and CD26 on CD34+/CD38− stem cells after treatment with imatinib (400 mg daily) for 3-12 months in 10 patients with newly diagnosed CML. A: BCR-ABL1 mRNA levels (as % of ABL1 expressed as IS) are indicated by the green vertical bars, CD25-expression by red-colored boxes and CD26-expression by blue-colored boxes. As visible, CD25+ stem cells were detectable even in patients in whom BCR-ABL1 had disappeared and stem cells were all CD26-negative cells. B: Expression of CD25 (upper histograms) and CD26 (lower histograms) on CD34+/CD38− stem cells at diagnosis (left images) and during therapy (right images) in one representative patient with CML.

2.1.8 Studies on pre-leukemic neoplastic stem cells in the MDS-context.
During the past few years, the cluster has worked on MDS as a model of premalignant (stem) cells. In our in vitro experiments, we were able to show that the putative CD34+ stem cells of MDS co-express several markers and targets, including CD25, CD33, and CD44. In clinical studies, the cluster tried to correlate stem cell phenotypes and expression of certain surface targets (such as CD33 or CD52) with survival and with clinical responses to targeted and conventional therapies, including demethylating agents or alemtuzmab. These studies are ongoing. In addition, the cluster has a long-lasting interest to define new prognostic factors predicting AML evolution and survival in untreated MDS patients. In one subproject, the cluster examined the time-dependent changes of the risk to transform to AML and to survive during follow-up. Unexpectedly, the differences in the risk of ‘IPSS-R low risk patients’ and ‘IPPS-R high-risk patients’, determined at diagnosis, was found to decline gradually over time and was no longer detectable after an observation period of 5 years. Although this result can be explained in part by a selection of the surviving ‘high-risk’ patients, the clinical impact of this observation is obvious (Pfeilstöcker et al, Blood 2016). The cluster team currently tries to identify clinical and laboratory variables (including mutation profiles) and stem cell markers through which this apparently ‘IPPS-R high risk’ population that exhibits a low transformation rate can be identified at diagnosis.
2.1.9 Niche cells and LSC-niche interactions as potential targets of therapy

In the year 2016, the LBC ONC continued to examine niche-related resistance of LSC against various targeted drugs and conventional anti-neoplastic drugs. We found that the apoptosis-inducing effects of nilotinib and ponatinib on CML LSC are almost completely diminished when these cells are co-cultured with osteosarcoma-like cell lines. In other words, osteoblastic niche cells are not only resistant against BCR-ABL1 TKI, but also protect CML LSC from apoptosis-inducing TKI effects. The same result was obtained in various CML cell lines, including KU812 and K562. In consecutive experiments, the cluster team was able to show that the protective niche-effect on CML cells can in part be reverted by addition of the BRD4/MYC blocker JQ1, suggesting that BRD4 and/or MYC are involved in niche cell-induced resistance. When testing CML cell survival in the presence of microvascular endothelial cells (HMEC-1), similar results were obtained. Again, the apoptosis-inducing effects of nilotinib and ponatinib on CML cell lines were found to diminish when these cells were co-cultured together with the microvascular endothelial cell line HMEC-1 (Figure 5). These data suggest that both the osteoblastic (endosteal) niche and the vascular stem cell niche exert clinically relevant, protective effects on CML cells. In the project year 2017, the cluster will analyze the molecular mechanisms underlying niche-induced resistance of CML LSC, and will extend these investigations to other disease models, including AML and ALL. Furthermore, the cluster will examine the effects of various BRD4/MYC blockers, including next generation D-BET inhibitors.

2.2 Publications in 2016 – Overview

Several original publications and review articles were published by the cluster team in 2016. Among these are several first- and/or senior authorships in Blood, Leukemia, Clinical Cancer Research and other top journals. In addition, members of the LBC ONC co-authored in the N Engl J Med, Blood and other top journals. Several of these studies were conducted in collaboration with the LBI for Cancer Research, the Research Institute of Molecular Pathology (IMP), and the Center for Molecular Medicine (CeMM) of the Austrian Academy of Science. In addition, members of the LBC ONC maintained their collaborations with groups involved in the special research program on myeloproliferative neoplasms (SFB F47) of the Medical University of Vienna. Finally, members of the LBC ONC were involved in several clinical studies in 2016. A list of publications is provided in a separate file.
2.3 Patents

No patents were filed during the period 2016 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, cancer research, and LSC research. In addition, members of our cluster organized and participated in two international top meetings in Vienna in 2016, one dedicated to basophil neoplasms and CML (March 2016) and one to pre-leukemic conditions and MDS in July 2016.

2.5 Lectures and Presentations

Researchers of the LBC ONC presented their data in invited lectures and other presentations in various national and international top conferences and workshops in 2016. Highlighting examples are invitations to give Education Lectures at the Annual Meeting of the EPNM in Verona (October 2016) and Annual Meeting of the American Society of Hematology (ASH) in San Diego, USA (December 2016). In addition, members of the LBC ONC presented their concepts and data in a Working Conference on basophil neoplasms and in a Working Conference on MDS and pre-MDS conditions. 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 LBC ONC (Internal Collaborations)

In the past 9 years, a number of essential internal collaborations have been established within the scientific environment of the LBC ONC. These collaborations were exploited and maintained in 2016, with the aim to strengthen our cluster projects. The close collaborations between the Hanusch Hospital and the MUW were also maintained in 2016. 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 (AML, ALL, MPN) and Radiation Therapy (long term culture and niche cells) were all maintained in 2016.

Scientific Collaborations with other Groups in Vienna

During the past 9 years, the LBC ONC established several important collaborations with a number of groups working in the field of stem cell research and translational cancer research in Vienna. These collaborations were also maintained in 2016. Examples are projects conducted together with our colleagues at CeMM (Giulio Superti-Furga, Georg Winter, Christoph Bock, Stefan Kubicek), the LBI for
Cancer Research (Richard Moriggl, Lukas Kenner), the Children’s Cancer Research Institute (Thomas Lion), the Vetmeduni Vienna (Veronika Sexl, Michael Willmann, Thomas Rülicke), and the LB Cluster for Cardiovascular Research (Johann Wojta). Several of these collaborations also relate to the SFB program F47 dedicated to MPN. The LBC ONC will exploit and intensify these collaborations in 2017. One important strategic goal is to prolong SFB F47 into the next funding period (2018-2021) 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 and conduct of national and international top meetings, including a Working Conference dedicated to basophil neoplasms in Vienna (March 2016), a Working Conference on MDS and pre-MDS conditions in Vienna (July 2016), the Annual Meeting of the European Competence Network (ECNM) in Verona (October 2016), and the Annual Meeting of the Austrian Competence Network on Mastocytosis (AUCNM) in Vienna (September 2016). Finally, the LBC ONC contributed actively to the LBG Meeting for Health Sciences in Vienna (November 2016).

3.3 Education and Ph.D. Program

Like in previous project years, members of the LBC ONC were actively involved in teaching master students, M.D. students, and Ph.D. students in 2016. Our students exploited these teaching facilities provided by the LBC ONC and its partners at the Medical University of Vienna. All our Ph.D. students are 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 coordinator of the CF-PF dedicated to student’s education in our LBC ONC.

4. Outlook and Aims for 2017

Following the master-plan of the LBC ONC, results obtained in the past 2 years, and the recommendation of the SAB, the LBC ONC will continue to study expression and function of novel markers and targets in LSC in MDS, AML, CML, MCL, and other myeloid neoplasms. One new disease model that will be studied extensively in the LBC ONC is CMML. The cluster team will make every effort to identify NSG and/or MITRG-engrafting LSC in CMML and to define markers and target expression profiles in these cells. In addition, based on the recommendation of the SAB, the LBC ONC will re-activate and continue projects on ALL. The LBC ONC team also considered to re-activate some of the projects on solid tumors (checkpoint molecules). However, based on the report and recommendations of the SAB, the LBC ONC decided to keep the solid tumor project line closed in 2017. The specific topics of our LBC ONC projects will all be maintained in 2017. These include niche-related cells and LSC-niche interactions, niche-mediated resistance of LSC, drug effects on niche cells, drug effects on LSC-niche interactions, and expression and regulation of resistance-mediating checkpoint molecules on LSC. The LBC ONC will continue to screen for natural factors and drugs that either up-regulate or down-regulate expression of CD47, PD-L1 and other checkpoint molecules on
LSC in 2017. In addition, our team will continue to apply drug combinations with the aim to identify strong inhibitors of cytokine- and drug-induced PD-L1 expression in LSC in AML, CML, MDS, and ALL. In 2015 and 2016, the LBC ONC has established facilities to generate iPSC-like cell lines from patients with CML and MCL. These cell line models will be used to define mechanisms of differentiation of very immature (pluripotent) neoplastic stem cells into hematopoietic cells and niche-related cells and to identify targets and drug-actions through which this differentiation process can be blocked. During the past few years, the LBC ONC has established several projects and international collaborations in the new important field of Comparative Oncology. The plan for 2017 is to intensify these projects and to establish an international consortium working in the field of canine mast cell tumors. A first preparative meeting has recently been organized by the LBC ONC. 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) in 2017. With regard to targets, the LBC ONC will continue to focus on clinically relevant diagnostic markers and target antigens in 2017. Another aim is to identify specific immunological targets that can be exploited using targeted antibodies, bi-specific antibodies, or CAR-T cell therapy. 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 2017 is to include the Vetmeduni Vienna as academic partner and to invite additional academic and industrial partners to join the LBC ONC in the next period. Strategic aims of the LBC ONC are to prepare for the next period, to fulfil the master plan describe above, and to maintain all collaborations and interactions required for the successful conduct of our LBC ONC projects.
5. Publications

Researchers of the LBC ONC have published a series of publications in peer-review journals in 2016, including manuscripts in Blood, Leukemia, Clinical Cancer Research, Am Journal of Hematology, Oncotarget, and N Engl J Med. A complete list of publications is provided below.

Original Manuscripts


Review Articles


Oral Presentations / Lectures


Sperr WR. Differential Diagnosis of Tryptase-Positive Leukemias. Workshop on Basophil Disorders, CeMM, Vienna, Austria. March 3-4, 2016.


Valent P. Basophilia as Prognostic Variable and Active Trigger of Disease Progression in CML and other MPN. Workshop on Basophil Disorders, CeMM, Vienna, Austria. March 3-4, 2016.


Peter B. Molecular Targets of PKC412 and clinical relevance. Annual Meeting of the ECNM. Verona, Italy. October 27-29, 2016.


Abstracts

The LBC ONC has published a total of 69 abstracts in the year of 2016