



Ludwig Boltzmann Cluster  
Oncology

# ANNUAL REPORT

## 2014

# **Ludwig Boltzmann Cluster Oncology**

## **Progress Report 2014**

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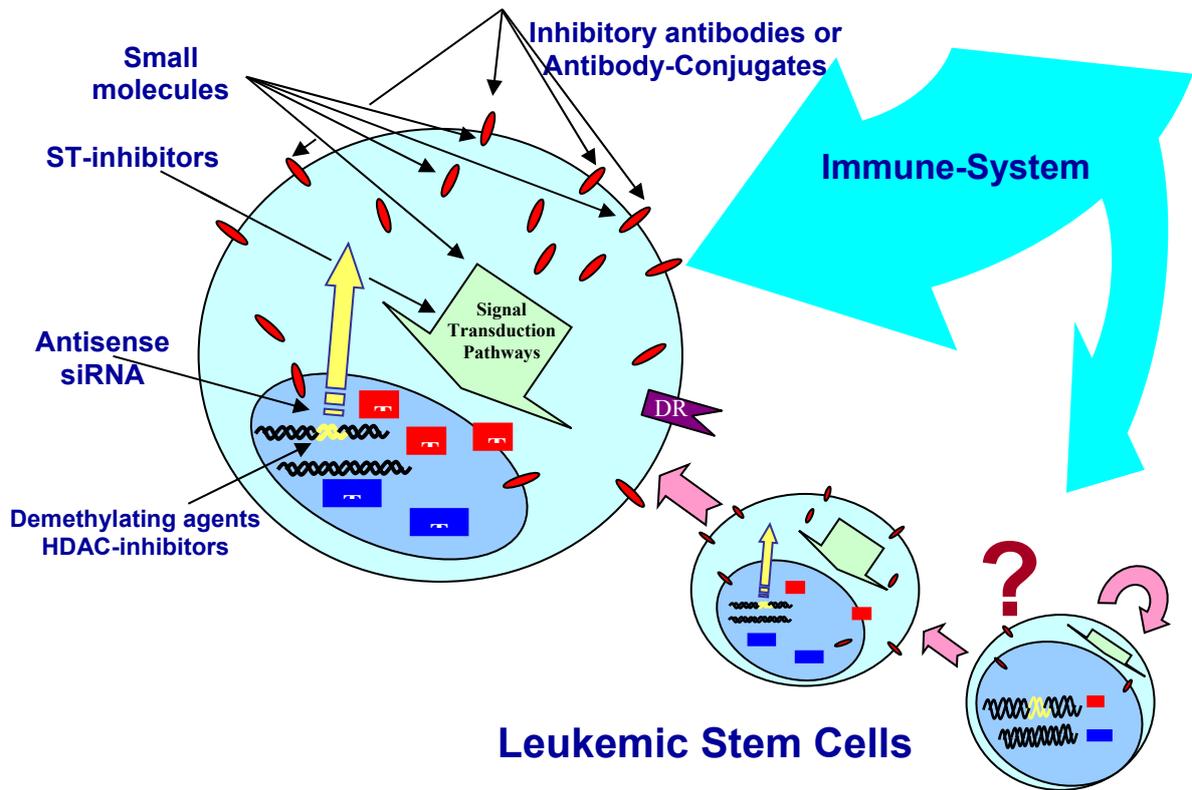
In the 7<sup>th</sup> year of our Ludwig Boltzmann Cluster Oncology (LBC ONC) several projects were conducted and several studies were finalized. The LBC ONC obtained a number of interesting data in 3 project-lines (PL). According to the master-plan of the LBC ONC, myeloid, lymphatic and mast cell leukemias were examined in these projects. Several newly discovered stem cell markers and targets have been validated, and several stem cell-niche interactions were analyzed. The effects of various drugs on stem cells and niche-related cells were also examined, with special focus on the vascular stem cell niche in the leukemic bone marrow in myeloid leukemias.

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

#### **1.1. Introduction and Aims**

In the past 15 years, numerous studies have shown that most if not all neoplasms and leukemias are composed of two distinct fractions of neoplastic cells, a bulk-population with limited capacity to proliferate, and a smaller subset exhibiting the capacity of unlimited self-renewal, the so-called neoplastic stem cells, also referred to as leukemic stem cells (LSC) in the context of a leukemia. This hypothesis is now widely accepted and predicts that anti-cancer therapy is curative only when eliminating most or all LSC in a given 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 populations. So far, however, little is known about the expression of relevant targets in LSC (Figure 1). During the past few years, the LBC ONC and other groups in the field have made considerable attempts to define specific markers and target expression profiles for LSC and to examine responses of these cells to targeted drugs, conventional drugs, and immunotherapy.

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. Drugs and markers were selected based on results obtained in previous years in our LBC ONC projects, and on the available literature. 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 and phenotypic characterization of LSC in various leukemias
- Characterization of target expression profiles in LSC
- 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 cells niche
- Evaluation of effects of endogenous immune-mediators (cytokines) and death regulators on growth and survival of neoplastic stem cells

## **1.2. Budget of the LBC ONC**

The budget plan for 2014 was established in cooperation with the LB-Society. The LB-Society contributed 57%, the Medical University of Vienna (MUW) contributed 41%, and the Hanusch Hospital contributed 2% of the budget. Budget was mainly used to employ personnel (64% of total budget) 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 2014 amounted to 663.156,32 €.

## **1.3. Partners and Internal Structure of the Cluster**

### **Partners**

As in the previous project-years of the LBC ONC, 2 academic partners were involved in 2014, the Hanusch Hospital and the Medical University of Vienna.

### **Project Groups and Scientists**

The LBC ONC is running 3 project lines (PL) that were maintained also in 2014: a) myeloid neoplasms, b) lymphoid neoplasms, and c) mast cell neoplasms including mast cell leukemia (MCL). Group 'a' also worked on normal bone marrow 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 had been finalized in 2011; 2 papers from these studies were submitted in 2014 and will be published. The following researchers were employed in 2014: Heidrun Karlic (MDS/AML), Gregor Eisenwort (AML, CML, MCL), Sabine Cerny-Reiterer (ALL, CML), Barbara Peter (MCL) and Emir Hadzijusufovic (MCL, CML). Unfortunately, Sabine Cerny-Reiterer passed away in November 2014. The group organized weekly staff-report meetings (1.5 hours), one week-start meeting (Monday, 10:00), and a Lecture Series (Friday 16:00). The group was also involved and actively participated in several congresses and other scientific meetings.

### **Administration**

As in previous years, administration was coordinated by the administrative head of the LBC ONC, Prof. Dr. Thomas Grunt. He was supported by our secretary, Sabine Sonnleitner, who handled all technical and minor administrative issues in the Cluster.

### **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 2014: these CF-Platforms (PF) include a LSC-sort PF (employing a high speed sorter), a gene array PF (including proteomics and sequencing facilities), a NOD/SCID (NSG) mouse Xeno-TX-PF (in cooperation with the University of Veterinary Medicine Vienna, Vetmeduni), and a clinical PF (including a biobanking-system and disease-registries; coordination: Prof. Dr. Wolfgang R. Sperr). The CF-PF were essential for the progress in our cluster projects in 2014. According to the master-plan of the LBC ONC, we also establish a CF-PF dedicated to lentiviral-mediated gene delivery and the establishment of various stem 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 have also attended the evaluation meeting in Vienna in December 2013. According to the evaluation report and the recommendations of the SAB, LBC ONC projects have been adjusted in 2014.

## 1.5. Personnel and Career Development

The following scientists were employed via the LBG in the LBC ONC in 2014:

Heidrun Karlic	Hanusch Hospital	01-12/2014
Gregor Eisenwort	Medical University of Vienna	01-12/2014
Sabine Cerny-Reiterer	Medical University of Vienna	01-11/2014
Barbara Peter	Medical University of Vienna	01-12/2014
Emir Hadzijusufovic	Medical University of Vienna	01-12/2014
Alexandra Keller	Medical University of Vienna	01-12/2014

Career development steps: Harald Herrmann, previously employed in the LBC ONC via the LB-Society, gained a full position at the Radiation Department of our University. The other LBG-employed Postdocs work on their scientific career in the LBC ONC environment. Within the group of non-LB-Society-employed LBC ONC colleagues, the promotion of Gregor Hoermann to group leader at the Molecular Biology section of the Laboratory Medicine Department (MUW) should be mentioned.

## 1.6. Infrastructure

The infrastructure in 2014 included lab-space in 4 labs (total: 70 m<sup>2</sup>) 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 2014. 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 2014.

## 1.7. Scientific Highlights in the LBC ONC

Among several different scientific achievements in 2014, the following highlights should be mentioned: 1. The mast cell-eradicating effect of imatinib in vitro and in patients with Ph<sup>+</sup> chronic myeloid leukemia, 2. The identification of the serum tryptase level as a prognostic marker in freshly diagnosed CML, 3. The identification of IL-1RAP as a stem cell marker in MCL, 4. The full characterization of novel human MCL-like mast cell lines: MCPV and ROSA, and 5. The identification of the vascular niche as a novel potential target of TKI therapy in CML.

## 1.8. Public Relation

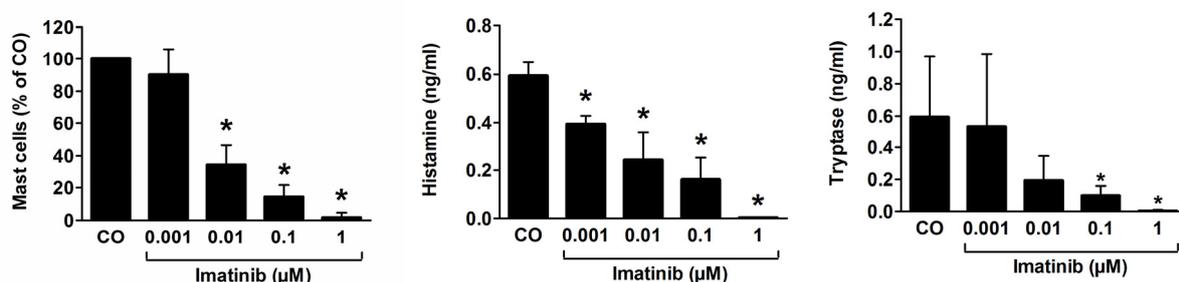
The basic mission of the LBC ONC is to increase awareness and to gain knowledge in the field of neoplastic/leukemic stem cells (NSC/LSC) in leukemias and other blood cell malignancies, to establish pathogenetic and targeting concepts in these neoplasms, and to establish LSC-eradicating treatment concepts, 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 2014 through publications and meetings organized by the LB Society. In addition, members of the LBC ONC were involved in a new special research program (SFB of the FWF) on myeloid neoplasms at the MUW and organized an international top-meeting on CML eradication in Vienna in July 2014.

## 2. Results obtained in the LBC ONC in 2014

### 2.1. Results obtained in Individual Projects

#### 2.1.1. Imatinib produces Mast Cell Deficiency in Patients with CML

In a substantial number of patients with CML, the BCR/ABL tyrosine kinase inhibitor (TKI) imatinib is able to suppress the disease for prolonged time periods. Based on discontinuation studies, it seems as if imatinib is also able to suppress LSC in many of these patients. However, the mechanism of suppression is not known as it seems unlikely that imatinib is capable of eradicating all neoplastic stem cells (LSC) in CML. In order to learn more about potential effects of imatinib on bone marrow (BM) cells, including niche cells and niche-related cells, we screened for additional effects of imatinib on various cell types relevant to LSC-niche interactions. In initial experiments we were unable to demonstrate an effect of imatinib on vascular endothelial cells. In a second step, we were able to show that imatinib profoundly suppresses stem cell factor (SCF)-dependent growth and development of human mast cells (MC) from their (normal) progenitor cells in an *in vitro* bioassay (Figure 2).

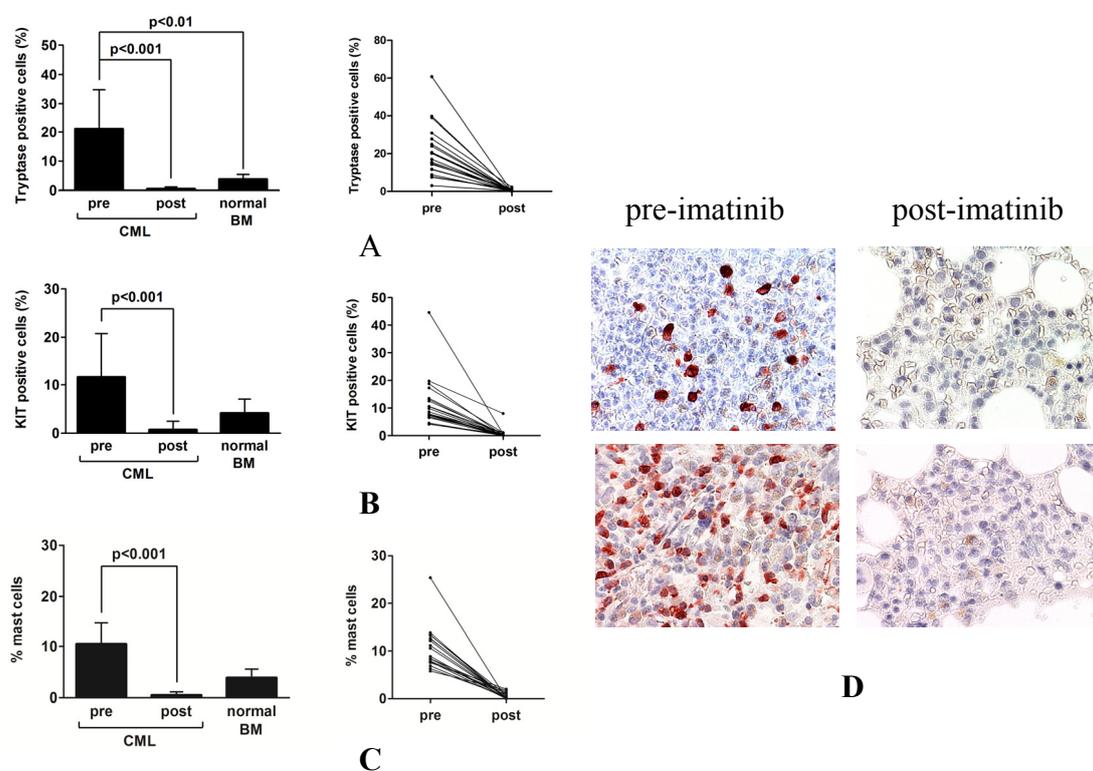


**Figure 2**

Imatinib inhibits SCF-induced *in vitro* differentiation of human mast cells

Isolated cord blood MNC ( $1 \times 10^6/\text{ml}$ ) were cultured in medium containing SCF (100 ng/ml) in the presence or absence (CO) of various concentrations of imatinib (0.001-1 μM) at 37°C for 28 days. Thereafter, the total numbers of mast cells (left panel), total (cellular) histamine levels (RIA) and total tryptase levels (FEIA) were measured. Results represent the mean±S.D. from 3 independent experiments. Asterisk:  $p < 0.05$ .

We were also able to show that long-term treatment of CML patients with imatinib is associated with a profound deficiency of BM mast cells (MC). The numbers of MC (tryptase+ or KIT+ cells) in the BM of newly diagnosed patients with CML exceed the numbers of MC in the normal BM by far (Figure 3). After successful long-term treatment with imatinib, defined by major (MMR) or complete (CMR) molecular response for at least 24 months, the numbers of tryptase+ and KIT+ cells decreased significantly ( $p < 0.001$ ) compared to pre-treatment values (Figure 3). MC numbers in the BM of long-term-treated CML patients were even lower compared to MC numbers in the normal BM ( $p < 0.01$ ) (Figure 3). Since MC development and survival is a long-lasting process and MC may persist in normal tissues for several years, we also examined BM sections of patients treated with imatinib for less than 1 year. In this cohort of patients ( $n=6$ ) the numbers of tryptase+ and KIT+ MC also decreased slightly compared to pre-treatment values, but the decrease was not significant.



**Figure 3**

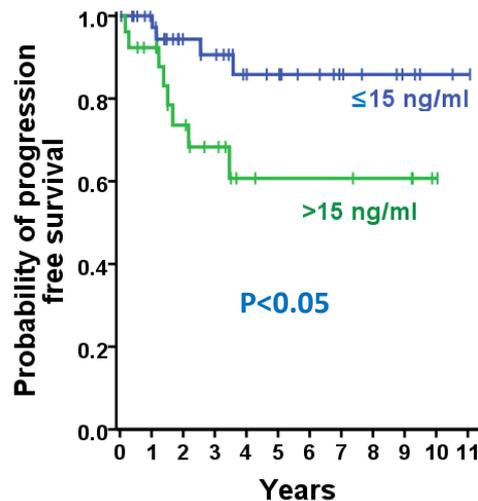
Imatinib induces mast cell (MC) deficiency in the bone marrow (BM) of patients with CML. BM was obtained from patients with CML ( $n=23$ ) at diagnosis and at the time of MMR/CMR and at least 2 years on therapy with imatinib. In addition, control BM section ( $n=5$ ) were analyzed. Serial BM sections were stained with antibodies against tryptase (A) or KIT (B) or by Giemsa-staining (C). Percentages of tryptase+ and KIT+ cells (MC) relative to all nucleated BM cells were determined by microscopy. Results in the left panels represent the mean  $\pm$  S.D. from all donors before and after therapy, and a comparison to normal BM samples ( $n=5$ ), and the right panels show the percentages of tryptase+ MC and KIT+ MC in each individual patient before and after therapy. In C, results in the left panel represent the mean  $\pm$  S.D. from all donors and the right panel shows the percentages of MC in each individual patient. D: Examples of BM sections stained for tryptase (upper panels) and KIT (lower panels) at diagnosis (upper and lower left panels) and at the time of re-investigation (upper and lower right panels) by indirect immunohistochemistry.

We were also able to show that tryptase mRNA levels and KIT mRNA levels in BM samples decreased significantly during successful long-term treatment with imatinib in all patients with CML tested. Tryptase and KIT mRNA levels were found to be even lower when compared to that found in normal BM samples ( $p < 0.01$ ). In a next step, we asked whether the effect of imatinib on MC is a systemic effect. In order to address this question, we measured serum tryptase levels before and after treatment with imatinib in a subset of patients, and compared post-treatment levels to pre-treatment levels and tryptase levels in healthy controls. Tryptase levels at diagnosis were found to be higher compared to tryptase levels in healthy controls ( $p < 0.01$ ). After treatment with imatinib, serum tryptase levels decrease significantly in all patients when compared to pre-treatment levels ( $p < 0.001$ ) or to tryptase levels in controls ( $p < 0.05$ ). In several cases, tryptase decreased to very low or even undetectable levels. Together, these data provide evidence that long-term treatment with imatinib results in systemic MC deficiency, and that this effect may be mediated by inhibition of the KIT kinase. We next examined the influence of long-term imatinib therapy on growth and differentiation of other blood cells. However, no significant changes in white blood cell numbers were found when comparing long-term treated CML patients with normal blood counts. In a next phase of the project, we asked whether imatinib would also decrease MC numbers in mice. Two different mouse strains were employed, C57BL/6J and BALB/c. In both strains, injection of imatinib resulted in a time-dependent decrease in MC in various organ-sites, including the skin and intestinal mucosa. Imatinib effects in MC growth and survival were published in Cerny-Reiterer et al. (Oncotarget in press). Currently, the effects of other BCR/ABL TKI (nilotinib, ponatinib) on MC growth and survival are examined.

### **2.1.2. Identification of Tryptase as Prognostic Marker in CML**

During the past few years, the LBC ONC tried to establish novel biomarkers in clinical hematology. Most of these markers are expressed by LSC. However, in the past few years, we were also interested in markers reflecting the BM microenvironment and the stem cell niche. Based on the intriguing effects of imatinib on MC and tryptase levels, we examined the clinical impact of tryptase in CML in more detail. To address this question, serum samples from 79 CML patients (chronic phase=CP,  $n=69$ ; accelerated/blast phase=AP/BP,  $n=10$ ) were analyzed. The median tryptase level was 12.5 ng/mL (range: 1.4-67.7 ng/mL). In 33 patients (41.8%), elevated tryptase levels ( $>15$  ng/mL) were detected. Significantly higher median tryptase levels were recorded in patients with advanced CML (AP/BP: 29.9 ng/mL; range 8.1-67.7) compared to CP (11.7 ng/mL, range 1.4-65.5;  $p < 0.05$ ). Serum-tryptase levels  $>15$  ng/mL were found in 38% of the CP patients and in 70% of our AP/BP patients. Significant differences in tryptase levels were also found when comparing prognostic risk-groups, i.e. Sokal (low, 6.3 ng/mL; intermediate, 10.7 ng/mL; high, 24.7 ng/mL), Hasford (low, 8.5 ng/mL; intermediate, 14.7 ng/mL; high 20.2 ng/mL), and EUTOS subgroups (low, 10.3 ng/mL; high, 33.2 ng/mL;  $p < 0.05$ ). In a next step, the CP group was examined separately. Twenty-six CP patients (38%) had an elevated serum-tryptases, and 43 (62%) a normal enzyme level. In the cohort with elevated tryptase, 30.8% (8/26) were found to progress, compared to 9.3% (4/43) in the normal tryptase group ( $p < 0.05$ ). The

5-year progression-free survival (PFS) was 86% and 61% in the low and high tryptase group, respectively (Figure 4). Marked differences were also seen when calculating event-free survival (EFS), whereas no significant difference in overall survival (OS) was found ( $p>0.05$ ) which is best explained by the fact, that most of the relapsing patients can now-a-days be rescued by second- and third-generation BCR/ABL TKI +/- stem cell transplantation. Of the 11 patients who died during the observation period, six were in the high-tryptase group and five in the low-tryptase group. The 5-year OS rates were 87% for both groups (Sperr et al, Am J Cancer Res, 2014).



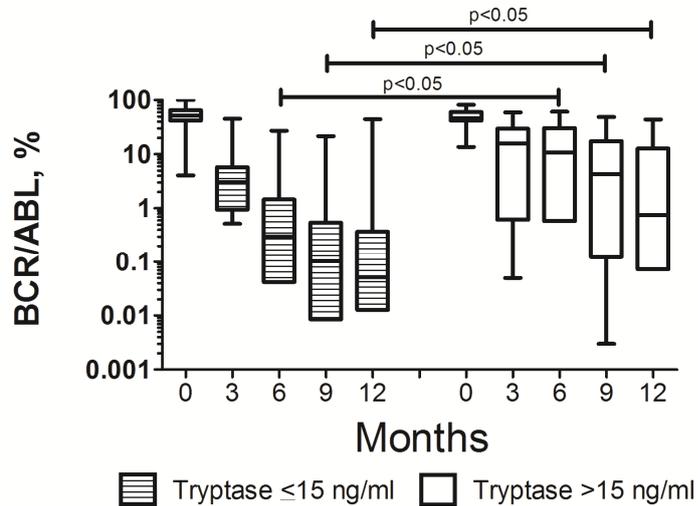
**Figure 4**

Influence of serum tryptase on progression-free survival (PFS) in CP CML

Patients with a normal serum-tryptase level ( $\leq 15$  ng/mL; blue line) and patients with elevated serum-tryptase levels ( $>15$  ng/mL, green line) were compared. A significant difference in PFS was found in this comparison ( $p<0.05$ ).

In a next step, serum tryptase levels were correlated with other prognostic variables. We found a positive (expected) correlation between serum-tryptase levels and basophils, and between tryptase levels and peripheral blood (PB) and BM blast cell counts in CML CP. Canonical analysis of the relationship of these parameters and PFS showed that tryptase had the highest weight, and subsequent multivariate analyses confirmed that tryptase was an independent prognostic variable with regard to PFS. We also measured tryptase mRNA levels in circulating leukocytes of 43 patients (CP,  $n=36$ ; AP,  $n=7$ ). In all CML patients tested, PB leukocytes expressed tryptase mRNA. As expected, tryptase mRNA levels varied from patient to patient and were found to correlate with serum-tryptase levels ( $p<0.001$ ).

In a next step, we compared BCR/ABL mRNA levels and tryptase levels in the follow-up. In both groups of patients (elevated tryptase versus normal tryptase) a marked decrease in BCR/ABL was observed over time. However, in the group of patients with normal tryptase levels ( $<15$  ng/ml), the decrease in BCR/ABL was much faster during the first year of therapy, compared to patients with serum-tryptase  $>15$ ng/ml. These differences in BCR/ABL levels in the two groups of patients remained statistically significant at 6, 9 and 12 months (Figure 5).



**Figure 5**

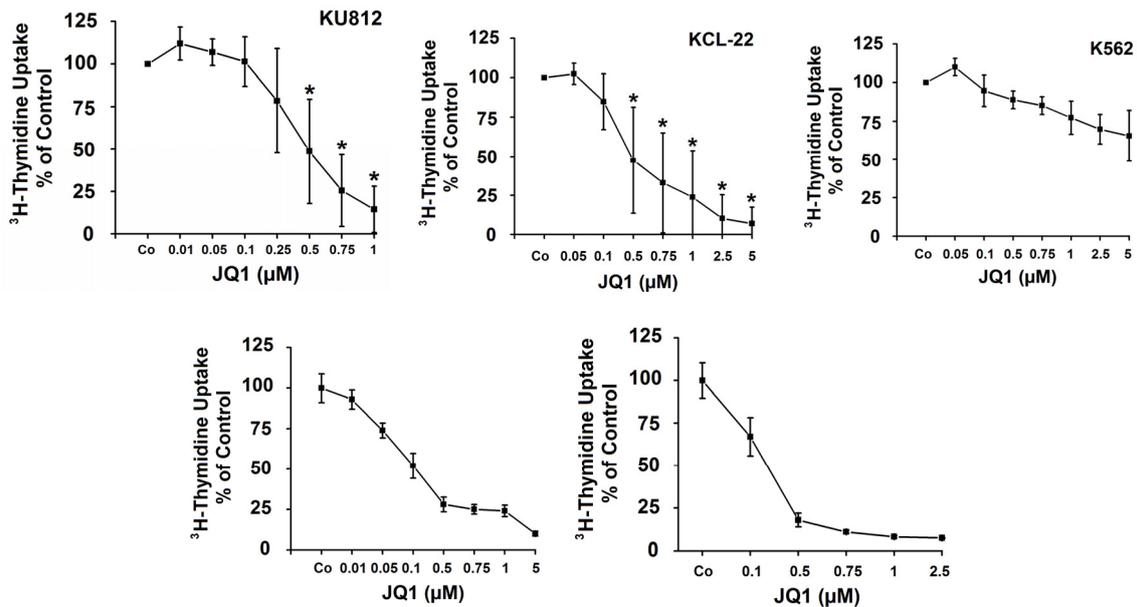
BCR/ABL levels in CML patients with normal or elevated serum tryptase

BCR/ABL mRNA levels, determined by qPCR (% of ABL and expressed according to the international scale, IS) decreased markedly in both groups of patients, namely those with normal tryptase levels (left boxes) and elevated serum tryptase levels (right open boxes). The differences BCR/ABL levels in the two groups, observed at 6, 9 and 12 months, were found to be statistically significant ( $p < 0.05$ ).

To evaluate whether the prognostic value of the EUTOS score in CML-CP would increase by including tryptase as prognostic variable, we replaced the percentage of basophils (known to produce tryptase) by serum-tryptase levels. Using this modified EUTOS-T score, 61 patients with CML-CP (88.4%) were classified as low risk and 8 patients (11.6%) as high risk. Significant differences were found between the two groups regarding PFS and EFS (each  $p < 0.0001$ ). The 5-year PFS rates were 84% and 0% for the low and high risk group, respectively. Of all patients, 11.5% (8/61) of the low risk group and 62.5% (5/8) of the high risk group progressed. EFS rates at 5 years were 75% and 0% in low and high risk patients, respectively. All in all these data suggest that the serum tryptase level at diagnosis is a novel potent prognostic variable in CML. So far the basophil count was considered the top prognostic marker in these patients. However, immature basophils (typically expanding in advanced CML) may easily escape microscopic detection, whereas these cells express and release tryptase. Together our data suggest that the serum tryptase test is a new promising biomarker in Ph+ CML and a robust prognostic variable.

### 2.1.3. Validation of Novel Therapeutic Targets in CML and AML

In the project year 2014, the LBC ONC continued to validate the recognized target structures in LSC in CML and AML. In CML, the LBC ONC examined the potential value of BRD4 as a novel target of therapy. Initial data suggest that BRD4 triggers MYC expression in CML cells and that the BRD4 blocker JQ1 inhibits growth and survival of primary CML cells and CML LSC. However, the concentrations required to achieve anti-leukemic effects are relatively high and some of the CML cell lines did not respond to JQ1 (Figure 6). Therefore, drug combination experiments have been initiated. Preliminary data suggest that JQ1 and BCR/ABL TKI synergize with each other in producing anti-leukemic effects on CML cells, including LSC. These studies are ongoing.



**Figure 6**

Effects of JQ1 on proliferation of 3 CML cell lines and primary CML cells

The Ph<sup>+</sup> CML cell lines KU812, KCL-22 and K562 (upper panels) as well as primary mononuclear BM cells obtained from two patients with Ph<sup>+</sup> CML in chronic phase (lower panels) were incubated in control medium (Co) or in medium supplemented with increasing concentrations of JQ1 at 37°C for 48 hours as indicated. Then, uptake of <sup>3</sup>H-thymidine was measured. Results are expressed as percent (%) of control (Co) and represent the mean±S.D. of at least 3 independent experiments (upper panels) are mean±S.D. of triplicates (lower panels). Asterisk (\*) indicates: p<0.05 compared to control.

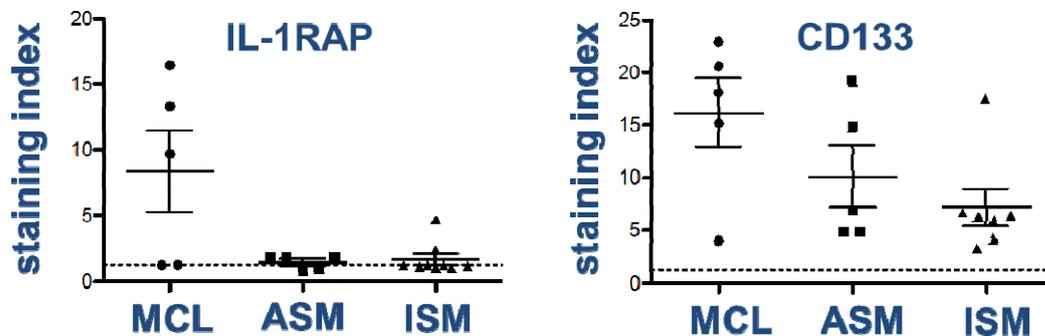
In 2015, the LBC ONC will examine BRD4 expression in JAK2-mutated MPN cells. In addition, the LBC ONC will finalize validation experiments performed with various targeted antibodies, including GO and alemtuzumab (AML, CML), and other targeted drugs, such as vildagliptin (CML). Finally, in 2015, AML LSC and CML LSC will systematically be examined for expression of various cell surface adhesion receptors and homing molecules (integrins, selectins and their ligands, CD44), with the long term aim to detect functionally relevant antigens that can be employed as potential therapeutic targets in future studies. Several of these projects will be conducted in collaboration with SFB F47 dedicated to CML and MPN.

#### 2.1.4. Acute Lymphoblastic Leukemia (ALL)

In the 7<sup>th</sup> project year of the LBC ONC, a solid xenotransplantation model for primary ALL LSC was established and various targeted drugs were tested for their ability to counteract the engraftment of ALL LSC in NSG mice. In these experiments, we were able to show that alemtuzumab blocks the engraftment of ALL LSC in those patients in whom LSC express CD52. Our *in vitro* experiments confirmed the LSC-depleting effect of this drug. Moreover, we were able to show that in a subgroup of patients with Ph<sup>+</sup> ALL, LSC express CD33, and that the CD33-targeted drug mylotarg (gemtuzumab/ozogamicine=GO) inhibits the growth and survival of ALL LSC. By contrast, no substantial effects were seen with the CD20-targeted antibody rituximab. In another subproject, we have continued to explore the effects of targeted drugs directed against the PI3-Kinase and drug directed against members of the BCL2 family. Our studies on ALL cells and ALL LSC will be completed in 2015.

### 2.1.5. Identification of IL-1RAP as a Novel LSC Marker in MCL

During the past few years, members of the LBC ONC were able to establish the cell surface marker profile of LSC in patients with MCL. In addition, we were able to define the phenotype of putative LSC in MCL. As assessed by *in vitro* testing and NSG xenotransplant experiments, LSC reside within a small CD34<sup>+</sup> fraction of the MCL clone. This fraction was detected in patients with acute or subacute MCL, but was hardly or not detected (is probably too small to be detected) in patients with chronic MCL. In the 6<sup>th</sup> and 7<sup>th</sup> year of our LBC ONC, we examined the phenotype of putative MCL LSC in more detail. In 2014, the LBC ONC was able to show that in a subset of patients with MCL, CD34<sup>+</sup>/CD38<sup>-</sup> LSC aberrantly display IL-1RAP (Figure 6A). In addition, we were able to show that MCL LSC express higher levels of CD133 when compared to normal stem cells (Figure 6B). A similar upregulation was observed with Siglec-3 (CD33) and CD52. The other cell surface markers and targets, including CD13 and CD44, were expressed on MCL LSC and normal BM stem cells without major differences in expression levels. Interestingly, in contrast to CML LSC, MCL LSC do not express the IL-2RA (CD25) or DPPIV (CD26). Currently, the LBC ONC team examines the effects of various targeted drugs (anti-CD33, anti-CD52) on engraftment of LSC in NSG<sub>SCF</sub> mice. Preliminary data suggest that the CD52 targeting antibody alemtuzumab interferes with engraftment. In addition, the LBC ONC will screen for additional novel targets and markers in MCL LSC in 2015. Finally, the LBC ONC will examine clonality and target expression profiles of niche cells in MCL.



**Figure 6**

Expression of IL-1RAP and aC133 (CD133) on LSC in mast cell leukemia (MCL) Bone marrow cells were obtained from patients with MCL, aggressive SM and indolent SM. Expression of IL-1RAP (left panel) and CD133 (right panel) on CD34<sup>+</sup>/CD38<sup>-</sup> stem cells was determined by multicolor flow cytometry. The staining index refers to an isotype-matched control antibody. As visible, LSC in MCL aberrantly display IL-1RAP. In addition, these cells express CD133 in excess over LSC in ASM or ISM and SC in normal BM (not shown).

### 2.1.6. Further Characterization of Novel Human Mast Cell Lines

In the past 5 years the LBC ONC has established a number of human MCL-like cell lines, including ROSA, ROSA<sub>KIT-D816V</sub>, and the MCPV series (MCPV1-MCPV4). Whereas MCPV represent an immature stage of MC development, augmented by hyperactive RAS, ROSA is a novel mature mast cell line expressing the IgE receptor.

Both cell lines serve as valuable tools in the LBC ONC and support various projects in our consortium, and both cell lines will help to keep the numbers of mouse experiments to a minimum. In 2014, both cell lines were published in collaboration with our partners in SFB F47 (Hoermann et al, FASEB J 2014;28:3540-3551) and our collaboration partners in Paris with Michel Arock (Saleh et al, Blood 2014;124:111-120). In 2014, the LBC ONC has also continued to screen these cell lines for expression of relevant cytoplasmic, epigenetic and cell surface targets. Among others, the following interesting targets have been identified: MCL1, BRD4, MYC, JAK2, and STAT5. These targets are currently being validated in these cell lines, and also in HMC-1 cells and in canine mastocytoma cell lines (C2 and NI-1) in the LBC ONC.

### **2.1.7. Identification of the Vascular Niche as Potential Target of TKI Therapy**

During the 7<sup>th</sup> project-year, the LBC ONC continued to investigate the effects of various targeted drugs on niche-related cells. Recent data suggest that certain BCR/ABL TKI can promote atherosclerosis and provoke vascular occlusive events in patients with Ph+ CML. Therefore, we examined the effects of these TKI (nilotinib, ponatinib) on vascular endothelial cells. Indeed, in patients with CML, the microvascular density (MVD) and thus angiogenesis decrease during treatment with nilotinib. Moreover, nilotinib and ponatinib were found to suppress the growth and viability of vascular endothelial cells *in vitro*, whereas imatinib showed no substantial effects on endothelial cells. Based on these observations, one emerging hypothesis is that nilotinib and ponatinib exert superior anti-leukemic effects in CML patients (over imatinib) not only because of their stronger effects on BCR/ABL but also because of their additional effects on the vascular stem cell niche (BM angiogenesis). The notion that CML LSC expand and survive independent of BCR/ABL would favor this hypothesis. The LBC ONC will test this exciting new hypothesis during the next few years. In 2014, the LBC ONC validated several of the recognized TKI targets in the context of vascular safety and anti-niche effects. In addition, the LBC ONC started to screen for BCR/ABL-targeting TKI that act anti-leukemic but would spare niche cells, and, more importantly, TKI that exert anti-niche effects but would not exert any pro-atherogenic effects on vascular cells. In this screen, we found that bosutinib, a TKI known to spare several potentially relevant targets (mediating side effects when blocked) exerts a weaker effect on endothelial cells compared to nilotinib or ponatinib. Another TKI, designed to spare most vascular targets, was also found to act anti-leukemic without showing any major effects on endothelial cells. Of the various targets detected, KDR, Tie-2/TEK and ABL2 were identified as the most relevant ones. However, although prolonged exposure to nilotinib was followed by a decreased expression of KDR in endothelial cells, no direct (physical) binding of KDR to nilotinib could be demonstrated. In consecutive experiments, KDR was identified as a secondary target of nilotinib, and ABL2 was identified as the relevant intermediate-substrate. In particular, nilotinib was found to block ABL2 activity, and ABL2 iRNA was found to block KDR expression in vascular endothelial cells. In 2015, the LBC ONC will continue to study the effects of various TKI on niche cells (endothelial cells, osteoblasts). In addition, the LBC ONC will examine clonality of niche cells in patients with CML and MCL. Finally, we will try to establish immature totipotent cell lines and iPSC-like cell lines from the BM of patients with CML and MCL.

## **2.2. Publications in 2014 – Overview**

A number of original publications and review articles have been published in the project year 2014. Among these are several first- and/or senior authorships in Blood, Clinical Cancer Res, Haematologica, Oncotarget, and other top journals. It should be pointed out that several of these studies were conducted in collaboration with groups of the LBI for Cancer Research (LBI-CR), the St. Anna Children's Hospital and the CeMM, working in the special research program SFB F47 on MPN coordinated by P. Valent. Finally, members of the LBC ONC were involved in several clinical research programs and studies in 2014. A list of publications generated in 2014 is provided in a separate file.

## **2.3. Patents**

No patents were filed during the period 2014 in our LBC ONC consortium. However, the cluster is actively scouting for potentially '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 a special CML meeting, and discussed novel strategies aimed at eradicating CML LSC, in Vienna in 2014.

## **2.5. Lectures and Presentations**

Members of the LBC ONC consortium presented their data in a number of invited lectures and other presentations in national and international top conferences and workshops in 2014. The effects of nilotinib and ponatinib on vascular endothelial cells and vascular events in patients with CML were presented in an oral lecture at the Annual Meeting of the European Hematology Association (EHA) in Milano (June 12-15, 2014). The LBC ONC also presented preclinical data in a special meeting dedicated to CML LSC eradication in Vienna. Based on this meeting, several companies have agreed to consider clinical eradication trials in collaboration with our CML trial group. 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

During the past few years, several internal collaborations have been established in the scientific environment of the LBC ONC. These collaborations have also been exploited by members of our consortium in 2014, with the aim to strengthen LBC ONC projects. Collaborations between the Hanusch Hospital and the MUW were

maintained in 2014. These cooperative projects include, among others, collaborations 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 and myeloma) and Radiation Therapy (leukemia models) were maintained in 2014.

#### Scientific Collaborations with other Groups in Vienna

During the past few 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 cooperations were maintained and extended in 2014. Highlighting examples are projects conducted together with our colleagues at CeMM (Giulio Superti-Furga), the LBI for Cancer Research (LBI-CR) with Richard Moriggl and Lukas Kenner, the St. Anna Childrens' Hospital with Thomas Lion the Vetmeduni (Veronika Sexl, Michael Willmann & Thomas Rüllicke), and the LB Cluster for Cardiovascular Research (Johann Wojta). Several of these collaborations are embedded in the SFB program F47 dedicated to MPN (Speaker: P. Valent). The LBC ONC has the plan to intensify these collaborations in the period 2015-2017. The strategic goals are to prolong the SFB into its second phase (2017-2020) and to invite the Vetmeduni to join as a new partner of our LBC ONC cluster.

### **3.2. Organization of Conferences and Meetings**

As mentioned above, members of the LBC ONC were actively involved in the organization of various national and international conferences and meetings, including a basic science seminar (topic: novel targets in hematopoietic malignancies) in the Annual Spring-Meeting of the Austrian Society for Hematology and Oncology (Innsbruck, April, 2014), EHA meeting in Milano (Cancer Stem Cell Working group) and a CML meeting dedicated to LSC eradication strategies in Vienna in July 2014. LBC ONC members actively participated in all these meetings. The CML meeting was a highlighting event, since several different new treatment strategies, developed in the LBC ONC consortium in recent years, were presented, and based on these presentations, several clinical trials will probably be initiated within the next few months. Finally, the LBC ONC contributed actively to the Health Science Meeting of the LB Society in December 2014.

### **3.3. Education and Ph.D. Program**

As in previous years, members of the LBC ONC were actively involved in teaching master students, M.D. students and Ph.D. students in 2014. Our students exploited all the 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). Our Ph.D. students are all integrated in the oncology-related Ph.D. program of the Medical University of Vienna, coordinated by Brigitte Marian, who is a valuable member and CF-PF coordinator (dedicated to student's education) in our cluster.

#### **4. Outlook and Aims for the Next Project-Year**

Based on the master-plan of the LBC ONC and results obtained in 2014 (and in previous project years) the LBC ONC will continue to focus on novel markers and targets in LSC in myeloid and mast cell leukemias. In addition, the LBC ONC will focus on niche-related cells, including vascular (endothelial) cells, osteoblasts, endosteal cells, and other microenvironmental cells of the BM. Following the evaluation report 2013 and recommendations of the SAB, the LBC ONC will complete its projects on lymphoid neoplasms in 2015. Strategic aims for 2015 are to establish additional stem cell line models, to intensify research on stem cell-niche interactions and to translate several LSC-related models from a preclinical phase of development into clinical application. 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), Cluster for Cardiovascular Research (vascular stem cell niche) and the Cluster for Translational Oncology. With regard to targets, the LBC ONC will continue to validate clinically important cell surface target antigens, including CD25, CD26, CD33, CD44, CD52, CD117, CD123, and CD133. In several instances, preclinical concepts will be translated into clinical practice using the clinical trial platform of the LBC ONC. We will also continue to validate important cytoplasmic target antigens, such as BRD4, the PI3 kinase, RAS or STAT5 in our LBC ONC projects in 2015. Another major aim will be to identify LSC antigens that play a role in LSC-niche interaction and specifically in LSC homing and redistribution. The cluster is also examining the expression of various markers and targets in niche-related cells in the BM of patients with MDS, AML, CML and MCL. Markers and targets will be compared in various phases of the disease and will be correlated with clinical parameters and outcomes. Finally, we will continue to study genomic diversification in LSC in our leukemia models in order to confirm our subclone-and-latency hypothesis of LSC evolution. The long-term goal in all LBC ONC projects is to develop new improved (curative) therapies for leukemias by applying drug combinations that have the capacity to target LSC and potentially also niche-related cells in these leukemias. The strategic aim of the LBC ONC regarding its structure and size for 2015-2017 is to extend the cluster to additional academic and industrial partners. The LBC ONC is currently seeking potential partners that are capable and interested to develop LSC research with us.

#### **5. Publications**

As mentioned above, the LBC ONC consortium published a series of publications in peer-review journals in 2014, including manuscripts in *Blood*, *Clinical Cancer Research*, or *Oncotarget*. A complete list of publications is provided below.

# Ludwig Boltzmann Cluster Oncology

## Publications 2014

### Original Manuscripts

Machherndl-Spandl S, Sega W, Bösmüller H, Germing U, Gruber Ch, Nachtkamp K, Reinecke P, Sperr WR, Wimazal F, Müllauer L, Sotlar K, Horny HP, Tüchler H, Valent P, Krieger O. Prognostic impact of blast cell counts in dysplastic bone marrow disorders (MDS and CMML I) with concomitant fibrosis. **Ann Hematol.** 2014;93:57-64. **IF: 2.396**

Peter B, Cerny-Reiterer S, Hadzijusufovic E, Schuch K, Stefanzl G, Eisenwort G, Gleixner KV, Hörmann G, Mayerhofer M, Kundi M, Baumgartner S, Sperr WR, Pickl WF, Willmann M, Valent P. The pan-Bcl-2 blocker obatoclox promotes the expression of Puma, Noxa, and Bim mRNA and induces apoptosis in neoplastic mast cells. **J Leukoc Biol.** 2014;95:95-104. **IF: 4.568**

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Gadermaier E, Flicker S, Blatt K, Valent P, Valenta R. Possible therapeutic potential of a recombinant group 2 grass pollen allergen-specific antibody fragment. **Allergy.** 2014;69:261-264. **IF: 5.883**

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Valent P. Mastozytose: Aktuelle Studien und ECNM Registry. 13. Onkologische Wintergespräche January 17-18, 2014. Salzburg, Austria.

Grunt TW. Liaison between signaling and metabolism in ovarian cancer: ErbB/PI3K and fatty acid synthase. Cancer Genetics and Pharmacogenomics, Brunel University, January 21, 2014. London, UK.

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Eisenwort G. Identification and Characterization of a Neoplastic Stem Cell in Human Mast Cell Leukemia. OeGHO Frühjahrstagung, Linz, Austria. Young Investigator Meeting. April 11.-12., 2014.

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Wedeh G. The Epigenetic Reader BRD4 Serves as a Novel Marker and Target in aggressive Systemic Mastocytosis and Mast Cell Leukemia. 19th Congress of the European Hematology Association, Milan, Italy, June 12.-15., 2014.

Valent P. Biology of CML LSC and status of research. PH<sup>+</sup>CML: From Stem Cell Eradication to Cure Workshop, Vienna Austria, July 3.-4., 2014.

Hadzijusufovic E. Targeting the Vascular SC-Niche in CML. PH<sup>+</sup>CML: From Stem Cell Eradication to Cure Workshop, Vienna Austria, July 3.-4., 2014.

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Valent P. Elevated tryptase in non-mastocytosis disorders. Annual meeting of the European Competence Network for Mastocytosis, Odense, Denmark. September 3.-6, 2014.

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Sperr WR. Update and practical procedures. Annual meeting of the European Competence Network for Mastocytosis, Odense, Denmark. September 3.-6, 2014.

Valent P. Relevant toxicities of novel BCR/ABL1 TKI: Clinical data, mechanisms and management. Annual Meeting of the German, Austrian and Swiss Society for Hematology and Oncology, Hamburg, Germany, October 10.-14, 2014.

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