ANNUAL REPORT
2010

Ludwig Boltzmann Cluster
Oncology

During the third project-year (2010) of the LB-CO, a number of essential results have been generated, and several major aims have been reached. Substantial new insights into target expression profiles in neoplastic stem cells and stem cell lines in hematopoetic and non-hematopoietic neoplasms have been accumulated as outlined below. In addition, the effects of various targeted drugs have been studied.

1. The Cluster

1.1. Introduction and Aims

During the past decade numerous studies have shown that most if not all neoplasms are composed of two different cell fractions, a bulk population of cells with limited capacity to divide and a second smaller cell population exhibiting the capacity of unlimited growth and self-renewal, the so-called cancer stem cells. This hypothesis predicts that minimal residual disease, relapsing disease, and thus all relevant disease components contain such cancer stem cells, and that any therapy (targeted, conventional, immuno-, etc) is curative only when eliminating (all) cancer stem cells in a given neoplasm. During the past few years, more and more data have shown that in each neoplasm and in each patient, stem cell fractions represent heterogeneous cell populations, reflecting plasticity and genetic instabilty. So far, however, little is known about the expression of relevant targets in cancer stem cells and subclones (Figure 1). From the above mentioned considerations it seems important to learn more about target expression profiles of cancer stem cells and about responses of these cells to targeted drugs and conventional drugs.

The aims of the current project are to define cancer and leukemia stem cells in various human neoplasms, to define target expression profiles for these cells, and to examine the effects of conventional and targeted drugs on growth and survival of neoplastic stem cells. Drugs and markers were selected based on our previous screens and the published literature. The long term goal of the project is to establish new improved (potentially curative) therapies for cancer and leukemias by applying drugs that recognize and eliminate cancer stem cells (all subclones) in a given neoplasm.

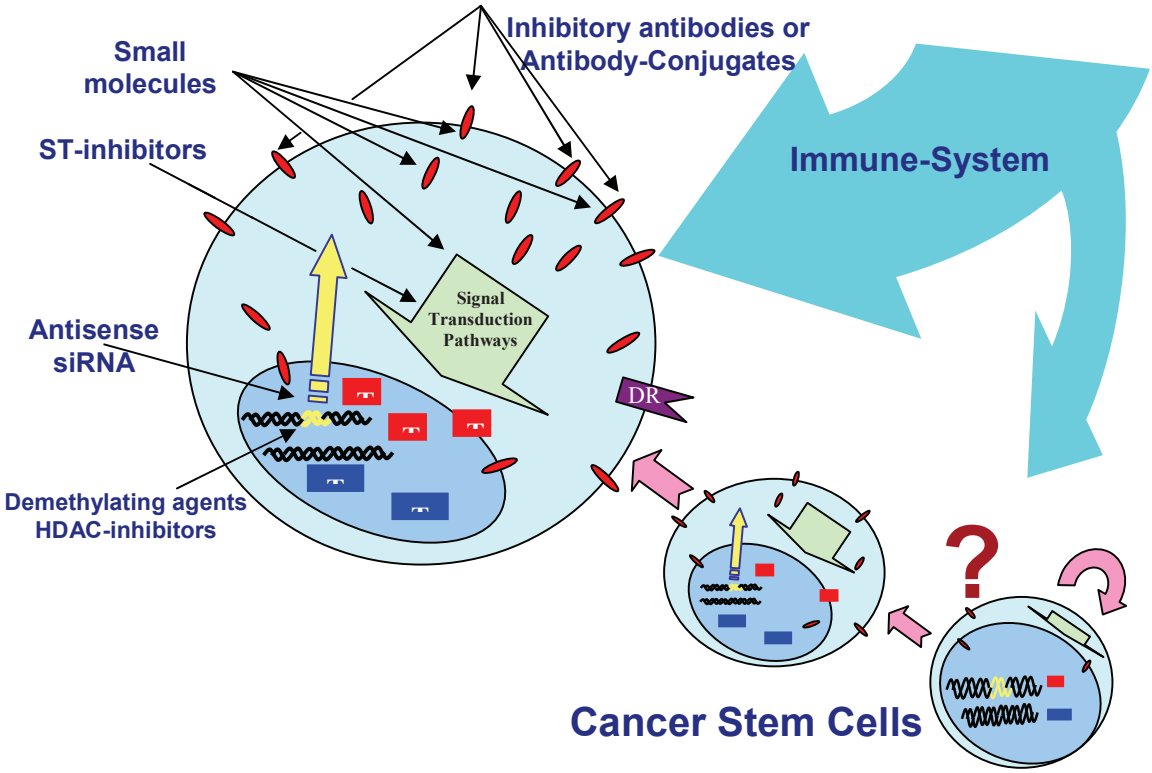

Figure 1
Expression of cell surface and cytoplasmic target antigens in neoplastic cells.
Many therapeutic targets have been identified in bulk cancer cells, but little is known about expression of these target antigens in cancer stem cells.

Major aims of the LB-CO

- Identification and phenotypic characterization of neoplastic stem cells in various hematopoietic neoplasms, solid tumors, melanomas, and mast cell neoplasms

- Evaluation of expression of therapeutic targets in cancer stem cells

- Examination of plasticity of cancer stem cells - phenotypic and functional characterization of relevant subclones

- Effects of conventional drugs and of targeted drugs on neoplastic stem cells

- Identification of most effective drug combinations acting on neoplastic stem cells

- Identification of novel markers of CSC

1.2. Budget Plan

According to the Projects of our LB-CO Cluster, the budget plan was established in cooperation with the LB society. Budget was mainly used to employ personnel (Cluster Postdocs) and key consumables required to run technology platforms, the enrichment and isolation of stem cells, the identification and characterization of novel markers and targets, and to run NSG mouse repopulation experiments (gene chip consumables, NSG mice, and sorting facilities). Part of the budget was used to invite authorities in the field or to send our Cluster Postdocs to conferences, meetings, or cooperating scientists.

1.3. Partners and Internal Structure of the Cluster

Partners

As in the previous 2 years, two scientific partner institutes were involved in the LB-CO in 2010, namely the Hanusch Hospital and the Medical University of Vienna.

Project Groups and Scientists

Within the LB-CO, 4 different project lines have been established: a) myeloid neoplasms, b) lymphoid neoplasms, c) solid tumors, and d) malignant melanoma and other skin neoplasms. Group ´a´ also worked on normal stem cells and myelodysplastic syndromes (MDS). For groups b) through d), each one key scientist is responsible for ongoing projects; for group ´a´, two scientists were employed. The following researchers were involved: Heidrun Karlic (a), Harald Herrmann (a), Rainer Hubmann (b), Sylvia Laffer (c), and Irina Mirkina (d). The group organized weekly scientific meetings (2 hours), one Week Start Meeting per week (Monday, 10:00), and a Lecture Series (Friday 16:00).

Administration

Administration was coordinated by the administrative head, Prof. Dr. Thomas Grunt and our secretary, Sabine Sonnleitner, who handled logistic, administrative, and technical issues in the LB-CO. Technical issues were solved in cooperation with the LB Society.

Core Facility Groups

According to the project plan, core facility (CF) groups were established in cooperation with the Medical University of Vienna. CF-Platforms (PF) included a stem cell sort PF, a gene chip PF, a NOD/SCID (NSG) mouse PF, and a clinical trial PF. CF-PFs were essential for the successful progress of our LB-CO projects in 2010.

1.4. Scientific Advisory Board

Members of the local scientific advisory board (SAB) were constantly informed about the progress in all projects by weekly reporting, and about all strategic and specific decisions made within the LB-CO. For the next period an international SAB will be established.

1.5. Personnel and Career Development

The following Scientists were employed through the LBG in the LB-CO Cluster in 2010:

Heidrun Karlic	Hanusch Hospital
Harald Herrmann	Medical Unviversity of Vienna
Rainer Hubmann	Medical Unviversity of Vienna
Sylvia Laffer	Medical Unviversity of Vienna
Irina Mirkina	Medical Unviversity of Vienna (until September 2010)

In this group, the following career steps were recorded: One promotion to Associate Professor: Heidrun Karlic. Sylvia Laffer is expected to fulfil all criteria for an Associate Professor within a short time. Irina Mirkina was hired by a Biotech Company on a Senior Researcher Position in 2010. Within the group of non-LBG-employed colleagues of the LB-CO, one successful application for Associate Professor can be reported.

1.6. Infrastructure

The infrastructure in 2010 included one lab (30 m^2) dedicated to LSC research at the MedUniWien (head: Peter Valent), several labs of other scientists participating in the LB-CO network, as well as laboratories of the LB-CO partner at the Hanusch Hospital (HK) (head: Heidrun Karlic). In addition, office-space for our secretary (Sabine Sonnleitner) as well as core facility rooms were made available and were used to run LB-CO projects. All in all, the laboratories and infrastructure shared by partners as well as the scientific environment provided optimal conditions for the conduct of our LB-CO projects in 2010.

1.7. Scientific Highlights

Among several scientific highlights and results in the LB-CO the following outstanding observations of 2010 should be mentioned: The characterization of CML stem cells by novel robust marker antigens, the observation that FAS becomes hypermethylated during progression from MDS to AML, and the identification of a CD34+ CLL progenitor cells in our NSG model. Maybe the most important conceptual insight has been that in CML, AML, and ALL, but maybe in most neoplasms, stem cell evolution is a long lasting process that takes place in separate subclones sometimes over decades. This concept has been presented in several reports including one in CCDT and one in Lancet Oncology.

1.8. Public Relation

The Mission of the LB-CO Cluster is to increase awareness and to gain knowledge in the field of neoplastic (cancer/leukemic) stem cells in Hemato/Oncology, to establish solid pathogenetic and target-related concepts around these cells, and to establish stem cell-eradicating concepts, with the ultimate aim to improve curative anti-cancer therapy. The LB-CO Cluster Team was able to transfer this Mission Intention to the Public in 2010 through publications and participation in meetings organized by the LB Society.

2. Results obtained in the LB-CO Cluster in 2010

2.1. Results obtained in Individual Projects

2.1.1. Project Group #I – Myeloid Neoplasms

During the 3rd year of the LB-CO, the numbers of primary samples obtained from patients with AML and MDS increased substantially. A total number of 287 samples (bone marrow or blood) were analyzed until October 2010. Diagnoses were AML (n=125), MDS (n=57), myeloproliferative neoplasms (MPN, n=25), and chronic myeloid leukemia (CML, n=57). In addition, 48 cases of reactive/normal bone marrow and 12 cord blood samples were included. Expression of surface molecules on CD34+ subfractions (CD34+/CD38+, CD34+/CD38-) and CD34- cells were analyzed by multicolor flow cytometry. In a group of patients CD34+/CD38+ cells, CD34+/CD38- cells, and CD34- cells were sorted from mononuclear cells (MNC) to near homogeneity (>98% purity) by high-speed sorting. Isolated cells were subjected to mRNA isolation, qPCR, FISH, ^{3}H-thymidine incorporation experiments (effects of cytokine-ligands and drugs), and gene chip profiling. Wherever possible, FISH was performed to confirm the leukemic nature of sorted cells. Spontaneous and drug-induced apoptosis of AML stem cells was determined by combined staining for surface markers and AnnexinV. A number of AML-related myeloid cell lines were examined, including KG1, HL60, U937, and MO7e. A KG1 subclone containing a CD34+/CD38- and a CD34+/CD38+ subfraction was also examined. In select experiments, these two subfractions were separated by flow cytometry and cultured separately or were subjected to gene chip profiling. Results obtained from gene chip experiments were confirmed by real time PCR and flow cytometry and/or immunohistochemistry. A number of novel potential stem cell markers were identified.

Results

a. Cytokine Receptor Expression
In the 3rd project year, we were able to confirm that in AML patients, CD34+/CD38- progenitor cells display various cytokine receptors and target antigens (Table 1). IL-3R alpha (CD123) and CD44 were found on AML SC in all donors. In most patients, at least a subset of AML stem cells co-expressed the SCF receptor KIT, G-CSFR, endoglin, and FLT3 (Table 1). In the 3rd project year, we were also able to detect the HGF-R c-MET and the OSMß receptor in AML stem cells in a subset of donors. A key finding in year 3 was that neoplastic stem cells in AML (subset of donors) and CML (all donors) express low affinity IL-2 receptor CD25, whereas normal SC do not express substantial amounts of CD25 (Figure 2). In most AML samples, stem cells were found to lack GM-CSFR, M-CSFR, and EPOR (Table 1). Cytokine R expression could largely be confirmed by RT-PCR and gene chip analysis. An interesting finding was that in CML, CD34+/CD38- stem cells express c-MET at the mRNA level, but do not express cell surface c-MET.

Table 1

Expression of key cytokine receptors and target antigens on CD34+/CD38- cells in patients with AML and CML: comparison to normal bone marrow (BM)

		Expression on CD34+/CD38- cells* on		
Antigen	CD	normal BM	AML	CML
LCA	CD45	+	+	+
Hyaluron-R/Pgp-1	CD44	+	+	+
Siglec-3	CD33	+/-	+	+
IL-2-R-alpha	CD25	-	+/-	+
IL-3-R-alpha	CD123	+/-	+	+
G-CSF-R	CD114	+	+	+
GM-CSF-R	CD116	-/+	-/+	-/+
SCF-R/KIT	CD117	+	+	+
IL-3/GM-ßR	CD131	-/+	+/-	+/-
FLT3	CD135	+	+	+
VEGFR/KDR	CD309	-/+	-/+	-/+
IGF-1-R	n.c.	+/-	+/-	+/-
EPO-R	n.c.	-	-	-
OSM-Rß	n.c.	-	-	-
ROBO-4	n.c.	+/-	+/-	+/-
NPDC-1	n.c.	+/-	+/-	+/-
CLL-1	n.c.	-/+	+/-	-/+
IL-1RAP	n.c.	-	-/+	+/-

*Expression of cell surface antigens on immature CD34+/CD38- progenitor cells was examined by flow cytometry using mAb. Abbreviations: R, receptor; IGF, insulin like growth factor; EPO, erythropoietin; OSM, oncostatin M; ROBO-4, Roundabout-4; n.c., not yet clustered.

b. Effects of Cytokines

In the third project year, we extended our analysis on cytokine effects on neoplastic SC in AML and CML. In these experiments we were able to show that G-CSF, GM-CSF, and IL-3 promote ^{3}H-thymidine uptake and thus proliferation in CD34+/CD38- stem cells in AML and CML cells in all donors examined, whereas no effects were seen with EPO. Growth-promoting effects were also observed with the KIT ligand SCF. In addition, we found that the ´effective´ cytokines can inhibit spontaneous apoptosis in AML and CML SC. In particular, spontaneous apoptosis was blocked in all samples when cells were exposed to one of the effective cytokines, i.e. G-CSF, IL-3, or SCF, whereas no effects were seen when rhEPO was added. Since AML and CML SC apparently express IL-2R we also examined whether HGF and IL-2 exert growth/survival-promoting or even inhibitory effects on AML SC and CML SC. In preliminary experiments, no growth-promoting effects of IL-2 were seen. These investigations are ongoing.

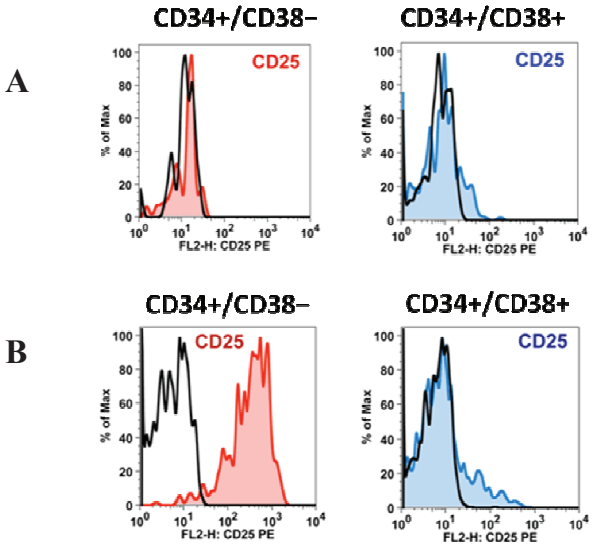

Figure 2
Expression of the IL-2 receptor alpha chain CD25 on CD34+/CD38- CML stem cells.
Expression of CD25 on CD34+/CD38- stem cells and more mature CD34+/CD38+ progenitor cells in normal bone marrow (A) and a patients with CML (B) was analyzed by flow cytometry. The isotype-matched control antibody (black histogram lines) is also shown.

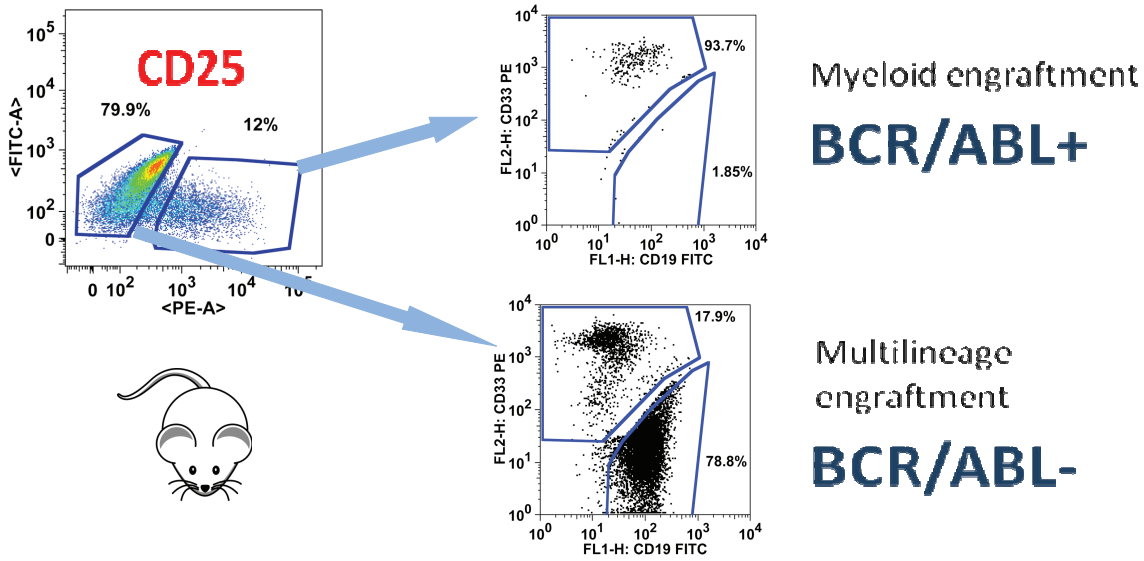

Figure 3
Selective engraftment of BCR/ABL+ cells from CD34+/Lin-/CD25+ cells
Freshly isolated bone marrow cells from a patient with CML (at diagnosis) were separated into a CD34+/Lin-/CD25- (normal progenitors) and a CD34+/Lin-/CD25+ fraction by cell sorting. Then, cells were injected i.v. into irradiated NSG mice. After 4 weeks, engrafted cells were examined by flow cytometry and PCR. CD25+ stem cells produced a BCR/ABL+ granulocytic engraftment, whereas CD25-negative stem cells produced multilineage engraftment with normal cells, that were CD19+ and CD33+, and found to lack BCR/ABL.

To reconfirm the specificity of IL-2R/CD25 for CML progenitor cells, NSG engraftment experiments were performed. As visible in Figure 3, CD34+/Lin-/CD25- cells obtained from patients with newly diagnosed CML produced BCR/ABL-negative multilineage engraftment, suggesting that indeed normal stem cells reside within the CD25-negative fraction of SC in these patients. By contrast, the CD34+/Lin-/CD25+ cells produced a granulocytic BCR/ABL+ engraftment suggesting that CML stem cell indeed reside within the CD25+ fraction of progenitor cells.

c. Effects of Cytokine Receptor-Targeting mAb

We also continued our work on neutralizing anti-cytokine receptor antibodies in the third year of our LB-CO project. However, so far, no growth-inhibitory effects of these mAb on spontaneous growth of AML cells, CML cells, or leukemic cell lines could be demonstrated. We also examined the effects of anti-CD44 antibodies on growth and viability of AML cells and AML cell lines. However, again, no effects of anti-CD44 antibodies were found. In a next step, we examined the effects of various antibody-toxin conjugates. Here we continued to focus on the CD33-targeted antibody construct gemtuzumab/ozogamicin (GO, mylotarg) (see below). Currently, we are extending these studies to antibody constructs directed against CD25 and other target antigens.

d. Evaluation of Effects of Gemtuzumab/Ozogamicin (GO) on CML Stem Cells

In the first 2 years of our project, we examined the expression of CD33 on AML and CML stem cells, and started to examine the effects of gemtuzumab/ozogamicin (GO) on these cells. In the third project year we have extended these investigations and found that in almost all patients with AML and CML, leukemia-repopulating stem cells reside within the CD33+ fraction of the clone. Moreover, we were able to show that GO (mylotarg) induces apoptosis and growth-arrest in CD34+/CD38- stem cells in AML and CML in most donors. As expected the response to GO was found to correlate with expression of CD33. In fact, in those patients in whom CD33 was clearly expressed on leukemic cells, GO produced a strong effect, whereas in patients, in whom CD33 expression levels were low, the effects of GO were clearly less pronounced (Figure 4).

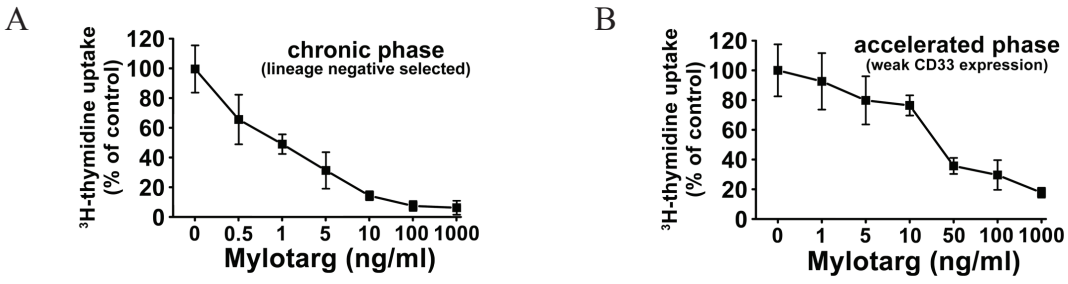

Figure 4

Dose-dependent inhibitory effect of mylotarg on growth of CD34+/CD38- CML stem cells (SC). Results represent the mean±S.D. of triplicates in one donor in whom SC expressed huge amounts of CD33 (A) and a second donor in whom SC expressed only low amonts of CD33 (B).

e. Evaluation of Novel Stem Cell Markers: ROBO-4 and NPDC-1

In the 3rd project year, we continued to examine the expression of the 2 novel stem cell markers that we had identified by gene array analysis in this project, ROBO-4 and NPDC-1, on normal and neoplastic SC. We found that both markers are expressed not only on neoplastic SC in AML and CML, but also on normal SC (Table 1). This means in contrast to IL-2R/CD25, neither ROBO-4 nor NPDC-1 are disease-specific SC marker-antigens. We then examined the specificity of CLL-1 and IL-1RAP for neoplastic SC. Similar to CD33 and CD123, CLL-1 and IL-1RAP seem to be overexpressed on leukemic SC in AML and CML when compared to normal SC (Table 1). The HGF receptor c-MET was also detectable in CML- and AML SC. However, unexpectedly, this target receptor was only expressed at the mRNA level but not on the surface of SC. Similarly, in most donors tested, MDR-1 was found to be expressed only at the mRNA level but not on the surface of leukemic SC, which explains why these SC respond well to GO.

f. Effects of Conventional Antineoplastic Drugs

We also continued our investigations on effects of conventional cytotoxic drugs on AML and CML SC. In these experiments, ARA-C and fludarabine were applied alone and in combination with each other or with other anti-leukemic drugs. Among other results, we found that ARA-C, fludarabine, and clofarabine promote apoptosis in CD34+/CD38- cells in most donors. In addition, we found that in all patients tested, strong cooperative effects were obtained when these drugs were combined. ARA-C and fludarabine were also found to inhibit the cytokine-induced ^{3}H-thymidine uptake in sorted AML stem cells in all donors. We also extended our studies to several new anti-AML agents, including the PI3-kinase inhibitor NVP-BEZ235, the multikinase-inhibitor PKC412, and the pan-bcl-2-inhibitor obatoclax. Most of these agents showed moderate but no substantial effects on AML cells and CML cells. Currently the effects of these agents alone or in combination on growth and survival of AML- and CML stem cells are examined.

g. Identification of Hsp32/HO-1 as a Novel Target in AML Cells and AML SC

During the third project-year, we extended our investigations on Hsp32 expression and function in AML cells and AML SC. We found that HO-1 is constitutively expressed in primary leukemic cells in most AML patients, and that Hsp32 is also expressed in immature CD34+/CD38- AML SC in all patients examined. Hsp32 expression in sorted AML cells and AML SC was demonstrable by qPCR analysis as well as by immunostaining experiments. The levels of Hps32 mRNA were higher in AML SC when compared to normal sorted bone marrow SC or more mature AML cells. Finally, we were able to confirm that the Hsp32-targeting drugs pegylated zinc-protoporphyrin (PEG-ZnPP) and styrene-maleic-acid-encapsulated ZnPP (SMA-ZnPP) inhibit growth of AML cells in vitro as well as in vivo in our xenotransplant model. Growth inhibition produced by SMA-ZnPP and PEG-ZnPP was found to be associated with apoptosis. In the 3rd year of our project we have also shown that SMA-ZnPP cooperates with ARA-C and other anti-leukmic drugs in producing growth inhibition and apoptosis in AML cells in vitro as well as in vivo in our NSG xenotransplant mouse model.

2.1.2. Project Group #II – Lymphoid Neoplasms

In project line II, three neoplasms were analyzed: chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia, and multiple myeloma. A major focus until 2010 was CLL, as in this disease the group was able to identify a putative neoplastic stem cell. These studies have been largely completed in the third project year. Other studies focussed on expression of Hsp32 in ALL cells and multiple myeloma cells.

a. Identification of a potential CLL Stem Cell (CLL SC)
During the 3rd year of our LB-CO project we continued to characterize the putative stem cells in patients with CLL by multicolor flow cytometry. In these analyses, we included a larger series of patients, a broad panel of antibodies, and various assays including FISH and molecular studies. In all CLL donors examined, we were able to identify a small population of CD34+/CD19+/CD5+ cells in the bone marrow and/or peripheral blood. These cells were also identified in the blood of healthy controls. However, the numbers of these progenitors were significantly higher in CLL patients (mean: 85 cells/µL) compared to controls (mean: 0.6 cells/µL). As assessed by molecular studies and FISH, these CD34+/CD19+/CD5+ cells are clonal cells and thus indeed belong to the CLL clone.

b. In vitro Expansion of Putative CLL Stem Cells
It is well known that long term growth of CLL cells depends on the presence of a stroma cell layer that can support the survival and growth of these cells through cytokine production and direct cell-cell interactions. In the current project, a co-culture system was used to define the long-term growth potential of CD34+/CD5+/CD19+ cells in CLL. In these experiments, the putative CLL stem cells initiated long term growth in vitro. In addition, when CD34+ cells were depleted from CLL bulk samples, the remaining CD34-negative cells were unable to induce long-term growth of CLL cells in this co-culture assay. An interesting observaiton was that the purified CD34+/CD5+/CD19+ cells were found to proliferate in vitro, but did not differentiate into mature CLL cells under the culture conditions and over the time period tested (several months). All in all, these data suggest that CLL long term growth-initiating cells reside within the CD34+ fraction of the leukemic (CD5+/CD19+) CLL clone. However, it remains unknown which factors support differentiation of clonal CD34+ progenitors into mature CLL cells.

c. Expression of Molecular Targets and Cytokine Receptors on Putative CLL SC
Next, we examined the expression of cytokine receptors and potential surface targets on CD34+/CD5+/CD19+ progenitor cells by multi-color flow cytometry. In these analyses we were able to show that these putative CLL stem cells co-express substantial amounts of CD10, CD20, CD23, CD44, CD52, and CD133 (Table 2). This is of particular interest as for several of these targets, targeted antibodies or other targeted drugs are available. Expression of CD10 confirmed that CD34+/CD19+/CD5+ cells are immature B-lineage cells, a result that that was supported by the observation that these cells co-expressed the IL-7 receptor CD127. Most of the other cytokine receptors tested (G-CSF-R, GM-CSF-R, KIT, IL-3R) were not expressed or only weakly expressed on CD34+ CLL stem cells.

Table 2

Expression of target antigens on CD34+/CD19+/CD5+ cells in patients with CLL: Summary

Antigen	CD	Surface Expression on CD34+/CD19+/CD5+	Surface Expression on bulk CLL cells	targeted drug available
CALLA	CD10	+ (32-100%)	-	-
B1	CD20	+/- (12-94%)	+	+
FcεRII	CD23	+ (72-100%)	+	+
Hy-R/Pgp-1	CD44	+ (94-100%)	+	-
CAMPATH	CD52	+/- (16-100%)	+/-	+
AC133	CD133	+ (33-96%)	-	-

CLL, chronic lymphocytic leukemia; Hy-R, hyaluronan receptor.

d. Effects of Targeted Antibodies and other Targeted Drugs on CLL cells

In the 3rd project year, we continued to examine the effects of various conventional and various targeted drugs on growth of CLL progenitor cells. These experiments were performed using the co-culture assay described above. So far, we were able to show that PI3 kinase inhibitors, certain conventional drugs like fludarabine and lenalidomide, and the targeted antibody mab-campath (anti-CD52 antibody) can partly inhibit the growth of CD34+ CLL progenitor cells in the co-culture system. In addition, we found, that combinations of these drugs are more effective than single agent treatment, which confirms clinical observations. Notably, it is well known that in advanced CLL, major cytoreductive effects can only be achieved when applying drug combinations.

e. Studies on the Repopulating Capacity of CD34+ CLL Cells

In order to demonstrate that CD34+/CD19+/CD5+ cells are CLL-initiating progenitor cells, NSG mouse repopulation experiments were conducted. These experiments were continued in the 3rd project year. In these experiments, the putative CLL stem cells (CD34+/CD19+/CD5+) were injected into NSG mice (i.v. or subcutaneously) together with autologous bone marrow-derived stromal cells. In 3 experiments, we were indeed able to detect engraftment of putative CLL stem cells in NSG mice as assessed by flow cytometry. However, engraftment was marginal and composed of immature cells and was not accompanied by the development of a full-blown leukemia (CLL) in these mice. Where detectable, most of the engrafted cells were CD34+/CD19+/CD5+ cells, and only a few cells were found to be CD19+/CD34- cells (more mature clonal CLL cells). These data suggest that the development phase of CLL from their progenitors (CLL stem cells) may take a longer time period possibly exceeding the life time of the host. Our co-culture experiments would also be in favor of such a hypothesis. Alternatively, the micro-environment in the mouse (and in the co-culture) did not provide all factors (cytokines) required to induce differentiation of CD34+ CLL stem cells into more mature CLL cells.

f. Identification of HO-1 as a Novel Target in ALL cells

In the 3rd project year, we continued our work on HO-1/Hsp32 expression in ALL cells. Leukemic cells from patients with Ph+ ALL (n=10) and Ph- ALL (n=10) were examined. In addition, in the 3rd project year, we were able to purify CD34+/CD38- stem cells in patients with ALL. Finally, we also continued our studies on Ph+ ALL cell lines (Z-119, BV-173, TOM-1, NALM-1) and Ph- ALL cell lines. In all cell lines and all patients tested, we reconfirmed that ALL cells express the Hsp32 protein as well as Hsp32 mRNA. The Hsp32-inductor hemin was found to promote the expression of Hsp32 in leukemic cells in all samples analyzed. We were also able to show that leukemic progenitors obtained from patients with Ph+ ALL express HO-1/Hsp32 (Figure 5).

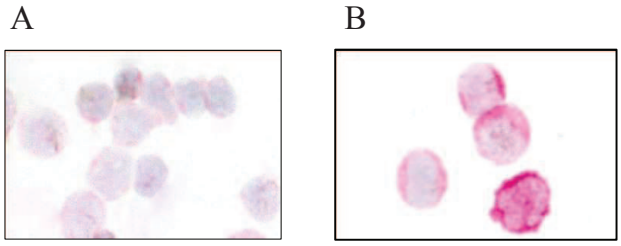

Figure 5

Expression of HO-1/Hsp32 in leukemic progenitor cells in a patient with Ph+ ALL

CD34+/CD38- ALL progenitor cells were purified by flow cytometry and then incubated in control medium (A) or hemin, 10 µM (B) for 8 hours. Thereafter, cells were spun on cytospin slides and stained with an antibody against HO-1. Indirect immunocytochemistry.

We also applied siRNA against Hsp32 in ALL cell lines and extended these studies in the 3rd project-year. The resulting knock-down of Hsp32 was found to be associated with reduced growth and an increase in apoptotic cells compared to control siRNA (Figure 6).

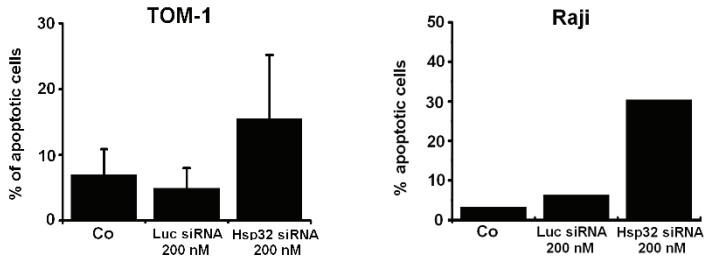

Figure 6

Effect of siRNA-induced Hsp32/HO-1 knockdown in ALL cell lines

The lymphoblastic cell lines TOM-1 (Ph+, left panel) and Raji (Ph-, right panel) were left untreated (Co) or were transfected with control siRNA against luciferase (Luc siRNA) or siRNA against Hsp32 (200 nM). After transfection, the percentage of apoptotic cells was determined by light microscopy. Results represent the mean±S.D. from three independent experiments. The siRNA-induced HO-1 knock-down was confirmed by Western blotting (not shown).

Two pharmacologic inhibitors of Hsp32, pegylated zinc protoporphyrine (PEG-ZnPP) and styrene maleic acid-micelle-encapsulated ZnPP (SMA-ZnPP) were applied. During the first 2 years of our project we were able to show that both drugs inhibit the proliferation of BCR/ABL+ and BCR/ABL- ALL cell lines (IC_{50} 1-10 µM). These effects were confirmed for primary ALL cells in a larger series of patients with Ph+ ALL and Ph- ALL. No major differences were found when comparing results in imatinib-sensitive and imatinib-resistant patients. Our drug combination experiments were also continued in the 3rd project year. In these experiments, the Hsp32-targeting drugs were found to cooperate with imatinib and nilotinib as well as with bendamustine in producing growth inhibition and apoptosis in all ALL cell lines. All in all, these results suggest that Hsp32 is a novel molecular target in ALL. Finally, we examined the apoptosis-inducing effects of PEG-ZnPP and SMA-ZnPP in more detail. In these experiments, we found that both drugs induce apoptosis in all ALL cell lines tested. Apoptosis was confirmed by microscopy as well as Tunel assay (Figure 7).

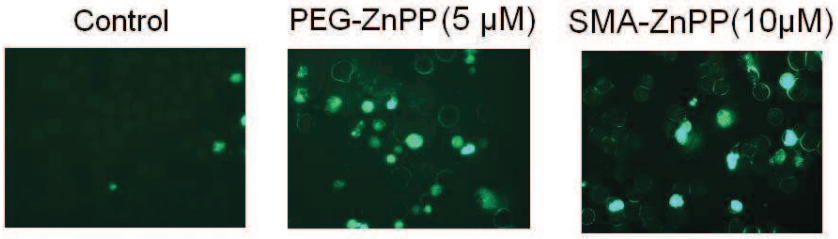

Figure 7
Effects of PEG-ZnPP and SMA-ZnPP on viability of Z-119 cells
The Ph+ ALL cell line Z-119 was incubation in control medium (Control), PEG-ZnPP (5 µM), or SMA-ZnPP (10 µM) at 37°C for 48 hours. Then, cells were subjected to Tunel assay. As visible, both drugs were found to induce apoptosis in a considerable number of leukemic cells.

g. Evaluation of Myeloma Progenitor Cells and Effects of Targeted Drugs
During the 3rd year of our project, we started to explore the phenotype of potential myeloma stem cells. Based on the results obtained in CLL and ALL, we were first interested to learn whether CD34+ myeloma-initiating progenitor cells can be identified. However, after a thorough examination of myeloma-derived bone marrow samples, we were unable to detect a subset of CD34+ cells that would co-express myeloma-associated antigens. Next, we examined CD20+/CD138- cells and CD27+/CD138- cells. However, again, no consistent population of myeloma-progenitor cells could be detected. In another series of experiments, we examined the phenotype of 4 myeloma cell lines, and their response to various targeted drugs. In these experiments, the following drugs showed consistent and strong growth-inhibitory effects in all cell lines as well as in primary myeloma cells: the PI3K/mTOR blocker NVP-BEZ235 (Figure 8), the Hsp70-inhibitor 17AAG, and the HDAC inhibitor vorinostat. Currently ongoing work is performed to define what drug combinations are producing synergistic growth-inhibitory and pro-apoptotic effects.

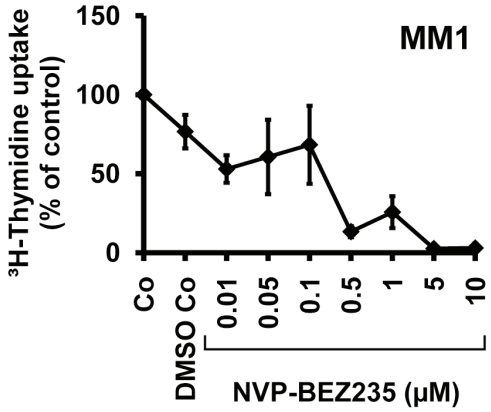

Figure 8
Effects of NVP-BEZ235 on growth of MM1 myeloma cells
MM1 cells were cultured in control medium (Co), with or without solvent control (DMSO) or various concentrations of NVP-BEZ235 for 48 hours. Then, ^{3}H-thymidine uptake was measured. Results are expressed as percent of control and show mean±S.D. values from 3 independent experiments.

2.1.3. Project Group #III – Solid Tumors

In the third year of our LB-CO project we continued to examine the expression of various stem cell markers and stem cell related phenotypes in diverse tumor cell lines, including colon cancer cell lines and breast cancer cell lines. Expression of cell surface targets was examined by multi-color flow cytometry, and expression of intracellular targets visualized by Western blotting and immunocytochemistry. The following results were obtained:

a. Expression of CD133 in HCT116 cells is Accompanied by Expression of CD26 and is Associated with a Growth Advantage
In the first 2 project years, we found that unfractionated HCT116 cells are composed of 2 distinct subpopulations, a CD133+ and a CD133-negative fraction. Both populations express the *RAS* mutation 38G>A and displayed an almost identical surface-phenotype, including CD44, CD166/ALCAM, CD326/EpCAM, and IGFR-1. In addition, both HCT116 subfractions display several surface target antigens, including EGFR/ErbB1, ErbB2, ErbB4, and c-MET. In long term culture, CD133+ HCT116 cells were found to outnumber CD133-negative cells in all experiments, even when sorted "CD133-negative" cells were examined. In gene chip-analysis we had identified CD26 as one of the 'most upregulated' genes within the CD133+ subfraction. Therefore, we focused on this target antigen in our third project year. In a first step we were able to confirm that CD26 is expressed on the cell surface of HCT116 cells, and that expression of CD26 correlates with expression of CD133, although no complete match was seen. The CD26 antigen, also known as Dipeptidylpeptidase IV, has recently been implicated as a prognostic marker in colon cancer and other solid tumors.

b. Expression of CD133 or CD26 is not Associated with Resistance to ErbB Blockers

Both CD133+ and CD133- HCT116 cells were found to be completely resistant against various kinase blockers including reversible ErbB-inhibitors erlotinib, lapatinib, and gefitinib, and partly resistant against irreversible ErbB inhibitors, such as pelitinib, canertinib, or BIBW2992. Multiple fractionation-experiments revealed that HCT116 cells are composed of a responsive and a resistant subpopulation. However, expression of CD133 was not invariably associated with resistance against irreversible ErbB blockers, and a CD133 siRNA failed to overcome drug resistance in HCT116 cells. Moreover, gene chip analysis revealed that although several growth-related pathways are expressed differentially, resistance-related genes showed no preferential expression in the CD133+ fraction. Whereas the irreversible ErbB blockers inhibited ErbB-phosphorylation and several downstream signaling molecules in all fractions, no major effect on ERK phosphorylation was seen in drug-resistant cells. Together, these data show that expression of CD133 in HCT116 cells is associated with a growth advantage but not with resistance against ErbB blockers. Similar observations were made with CD26. We also asked whether CD26 or CD133 is a growth-regulating gene. To address this question, siRNA and shRNA were applied. However, knock-down of CD26 or CD133 did not result in growth inhibition. Figure 9 shows the effect of CD133 siRNA on growth and viability of HCT116 cells, and on responses to ErbB-targeting drugs.

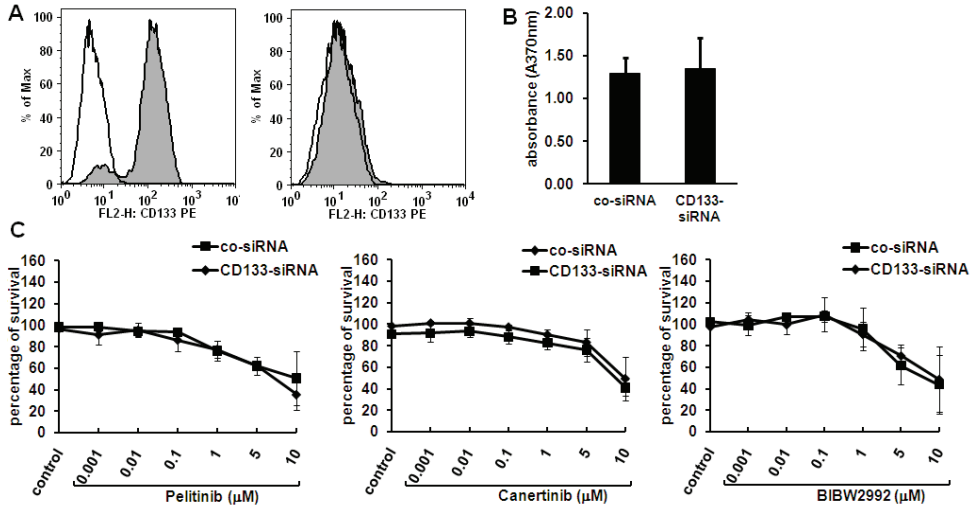

Figure 9

Effect of CD133 siRNAs on growth and responses to ErbB-targeting drugs

A: CD133+ HCT116 cells were transfected with siRNAs against CD133. Flow cytometry confirmed the complete knock-down of surface CD133. B: As determined by BrdU incorporation assay, the siRNA-induced knock-down of CD133 did not lead to a reduced growth/viability. C: After transfection with control siRNA or CD133 siRNA, HCT116 cells were incubated with various concentrations of irreversible ErbB blockers pelitinib, canertinib, and BIBW2992, or control medium at 37°C for 72 hours. Results show the percentage of survival measured by MTT assay and represent the mean±S.D. of triplicates of three independent experiments.

c. Role of the RAS Pathway in Drug Resistance in HCT116 cells

Since the RAS/MEK/ERK pathway is activated in HCT116 cells, we asked whether this pathway is differentially regulated in CD133+ and CD133- cell fractions and whether the RAS pathway mediates resistance against ErbB-targeting drugs. In a first step, we confirmed by Western blotting that HCT116 cells display pERK. We also found that the ErbB blockers applied inhibited ErbB-phosphorylation and several downstream signaling molecules in HCT116 cells, but did not inhibit ERK phosphorylation. We then extended our investigations to HCT116-subfractions that responded differentially to irreversible ErbB blockers in some of our experiments. In these experiments, we found that in the CD133-negative more responsive fractions, the irreversible ErbB blockers indeed caused deactivation of ERK1 (Figure 10). These data suggest that the RAS/MEK/ERK pathway may be critical to drug resistance in HCT116 cells.

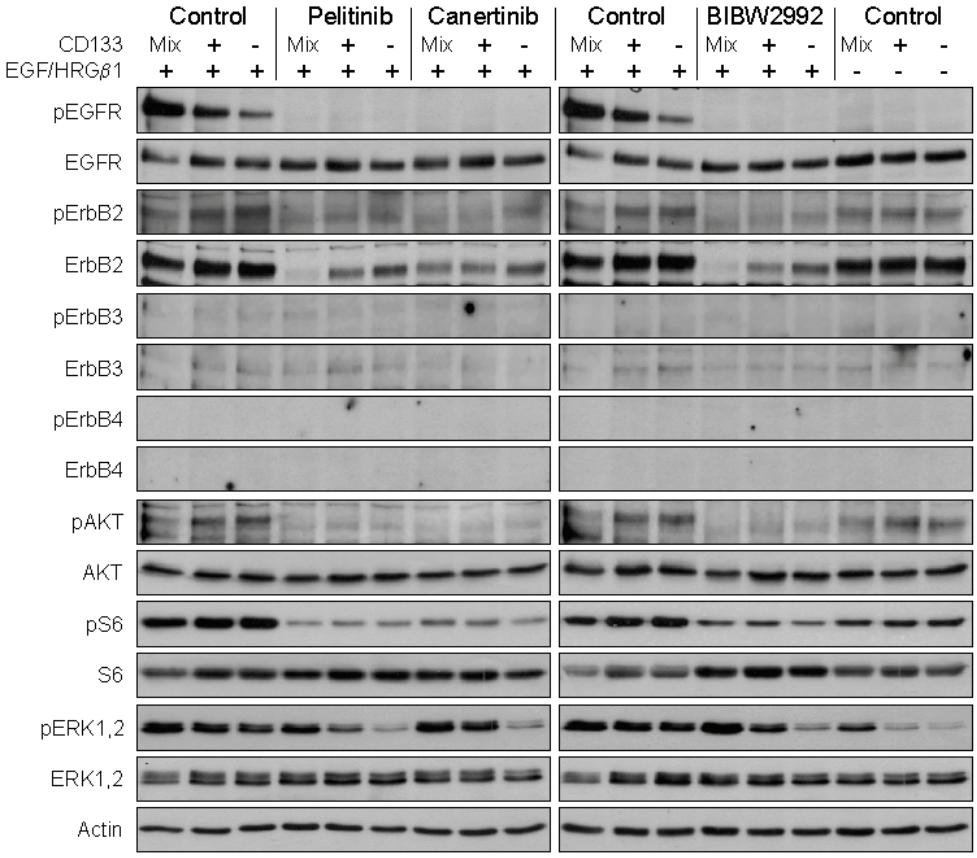

Figure 10

Effects of ErbB blockers on downstream signalling networks in HCT116 cells

Unfractionated (Mix) and sorted (CD133+, CD133-) HCT116 cells were incubated with 5 µM pelitinib, 10 µM canertinib, 10 µM BIBW-2992, or with 0.1% DMSO (Control) for 6 hours. Then, expression and phosphorylation of ErbB receptors and downstream effectors AKT, S6, and ERK1,2 were examined by Western blotting. Actin served as loading control.

In a next step we asked whether drug resistance in HCT116 cells can be overrided by application of MEK inhibitors. To address this question, several different MEK inhibitors were applied. These studies are ongoing and will be completed within the next few months. First data suggest that MEK inhibitors block HCT116 cell growth and synergize with pelitinib and other ErbB blockers in producing growth inhibition and apoptosis. In these experiments, so far, no differences in responses were seen when comparing CD133+ and CD133- HCT116 cells.

d. Identification of mTOR and VEGF as Key Targets in Solid Tumor Cells

In the first 2 project years, we examined the production and secretion of VEGF in various cancer cell lines, and the role of VEGF as a mediator of transendothelial tumor migration. Ssupernatants from various neoplastic cells including breast-, pancreatic-, lung-, colon-, ovarian-, and cholangiocellular carcinoma were examined. In each case, VEGF was found to accumulate in supernatants of cultured tumor cells over time, and tumor-derived and recombinant VEGF were found to induce endothelial leakiness and tumor cell-endothelial transmigration *in vitro*. These data have been published (Prager et al, Mol Oncol, 2010). In the third project year we have examined the mechanisms of transendothelial migration in detail. In first experiments, transmigration was blocked successfully by an anti-VEGF antibody. Then, we asked whether integrins are required for transmigration. Indeed, several different integrin-blockers were found to inhibit transendothelial migration of tumor cells in our experiments. These studies are ongoing and will be completed within the next few months. Finally, we asked whether primary tumor cells and cancer stem cells also undergo transmigration. Indeed also primary tumor cells transmigrate through endothelial layers against VEGF. Currently, we examine whether these transmigrated cells have tumor-initiating capacity when injected into NSG mice, in order to learn whether tumor stem cells undergo transmigration against VEGF. These studies will also be completed within the next few months.

e. Identification of Heat Shock Protein 32 (HO-1) as a Novel Key Target in Solid Tumor Cells and Cancer Stem Cells

In the first 2 project years, we were able to show that HO-1 is expressed in various cancer cells and cancer cell lines, including glioblastoma, lung cancer, breast cancer, pancreatic, hepatocellular, colon, and ovarian carcinoma, as well as in various leukemias and leukemic cell lines. We also found that HO-1 is expressed in leukemic stem cells and immature colon adenoma stem cells. In Ba/F3 cells, diverse oncogenes were found to lead to an increased expression of HO-1, and a HO-1-specific siRNA was found to lead to reduced viability and induction of apoptosis in all cell lines tested. Finally, treatment of cancer cells and cell lines with SMA-ZnPP, a pharmacologic inhibitor of HO-1, resulted in reduced proliferation and apoptosis. Here we were also able to show that SMA-ZnPP is able to synergize with other anti-neoplastic drugs in producing apoptosis in various cancer cells and cell lines. In the 3rd project year we have completed all these investgations in this subproject of our LB-CO.

2.1.4. Project Group #IV – Malignant Melanoma and other Skin Neoplasms

In the 3rd project year we continued to analyze the phenotype of human malignant melanomas, and completed most of our studies on the identification and validation of major targets on melanoma (initiating) cells. In addition, we continued to screen for effects of novel targeted drugs and drug combinations.

a. Surface Phenotyping of Human Melanoma Cells
In the 3rd project year we continued to phenotype human melanoma cells (freshly isolated from patients, xeno-passaged, or cultured) as well as various melanoma cell lines including A375, 607B, Mel-Juso, and SK-Mel28. Normal epidermal foreskin melanocytes served as control. Surface antigen expression was analyzed by monoclonal antibodies (n=30) and multicolor flow cytometry. Melanoma cells were defined as CD45-/CD31- cells coexpressing one or more melanoma markers (CD146, CD166, or CD63). Confiming our first data we found that in all melanomas, neoplastic cells express the hyaluronan receptor CD44. In addition, we were able to show that melanoma cells consitently express the adhesion receptors ICAM-1 (CD54) and CEACAM-1 (CD66a) as well as Endoglin (CD105). Whereas all melanoma cell lines were found to stain positive for L1 (CD171), patient-derived melanoma cells were L1-negative cells in all samples.

b. Expression of Cytokine Receptors on Melanoma Cells
In all patients examined freshly isolated melanoma cells expressed ErbB3/Her3, and in most patients (80%), melanoma cells stained positive for IGF-1R. ErbB3 was also expressed on melanoma cells in most samples tested (Table 3). All in all, ErbB3/Her3 was expressed on melanoma cells in 13 out of 16 patient-derived melanoma samples analyzed. By contrast, the IGF-1R was only expressed in 7 of 15 melanomas tested. An interesting observation was that the EPO-R was expressed on freshly isolated melanoma cells in all patients. However, the levels of EPO-R expressed on melanoma cells varied from patient to patient (range: 4% to 40%). A most intriguing finding was that the EPOR is expressed on a distinct subpopulation of melanoma cells. This distinct melanoma cell subset also co-expressed CD24 and ErbB4 as evidenced by flow cytometry. Moreover, we were able to detect the NGF-R (CD271) on a subset of melanoma cells, and found that this NGF-R+ subset overlaps with the EPO-R/CD24+ subset of melanomas. Another interesting observation was that TGFß1-R related antigen endoglin (CD105) is expressed on cultured melanoma cells in all patients examined, whereas in freshly isolated melanomas, CD105 was only expressed at low level or was undetectable. None of the other cytokine receptors tested were consistently expressed on patient-derived melanoma cells. Melanoma cell lines were found to display a similar profile of cytokine receptors when comparing to patient-derived melanomas. Normal melanocytes differed substantially from melanoma cells in cytokine receptor expression. In particular, normal melanocytes were found to express high levels of SCF receptors KIT (CD117), but did not express the EPO-R or ErbB4. All in all, melanoma cells display a unique composition of cytokine receptors and differ from normal skin melanocytes in expression of EPO-R and KIT.

Table 3

Expression of Cytokine Receptors on Human Skin Melanoma Cells

Cytokine Receptor	CD	Skin Melanoma	Normal Skin Melanocytes
KIT	117	-	+
EPO-R	n.c.	+/-*	-
ErbB1	n.c.	-	n.t.
ErbB2	n.c.	-	n.t.
ErbB3	n.c.	+	+
ErbB4	n.c.	+/-*	-
IGF1-R	n.c.	+/-	+/-
Endoglin	105	+/-	+
c-MET	n.c.	-	n.t.
G-CSF-R	114	-	-
IL-3Ralpha	123	-	n.t.
FLT3	135	-	n.t.

*expressed on a distinct subpopulation. n.c., not clustered; n.t., not tested.

c. Evaluation of the Melanoma-Initiating Potential of Melanoma Cell-Subpopulations
Recent data suggest that up to 40% of all melanoma cells may exhibit melanoma-initiating capacity i.e. melanoma stem cell characteristics. We therefore screened for a potential stem cell marker that would identify up to 40% of all melanoma cells in primary tumor samples. Of all markers tested, only a few fulfilled this criterion: EPOR, CD24, NGF-R, and ErbB4. Most of these antigens identified the same subpopulation (or at least overlapping subpopulations). When injecting to NSG mice, we found that highly enriched (resorted >99% pure) EPOR+ melanoma cells form melanomas (again EPOR+) in NSG mice. However, the EPOR-negative cells also formed melanoma lesions when injected into NSG mice in these experiments. These data confirm recently published results. All these data suggest that melanoma stem cells may reside within distinct subpopulations, but most surface markers are not sufficient to predict melanoma-initiating capacity.

d. Effects of Targeted Drugs on Growth and Survival (Apoptosis) of Melanoma Cells
In the third project year we also extended our investigations on targeted drugs. We were able to show that obatoclax, VX-680, and 17-AAG, produce substantial growth-inhibitory effects in melanoma cell lines as well as in primary melanoma cells. The most potent compound was obatoclax (IC_{50} <1 μM), followed by 17-AAG and VX-680 (IC_{50} ranging between 0.5 and 10 μM). These effective agents were also found to induce apoptosis in melanoma cell lines as well as in primary melanoma cells, as evidenced by flow cytometry and Tunel assay. Ongoing experiments should clarify whether drug combinations (combining effective agents) can exert synergistic growth-inhibitory effects.

e. Other Skin Neoplasms: Mastocytosis

In the 3rd project year we continued to study mastocytosis asa model of a stem cell disease driven by a mutant form of KIT. Notably, in most patients with systemic mastocytosdis (SM) the transforming *KIT* mutation D816V is detectable. Aggressive variants and mast cell leukemia are rare entities. During the course of the LB-CO project, we asked whether CD34+/CD38-/KIT+ progenitor cells in mastocytosis patients would express surface target antigens. In these experiments we were able to show that SM progenitors (exhibiting the KIT mutant D816V) not only co-express KIT but also CD33 and CD44 similar to leukemic stem cells in AML and CML. In a next step, we screened for effects of various targeted drugs on growth and survival of neoplastic mast cells in SM. Confirming previous data, the KIT tyrosine kinase inhibitors dasatinib and PKC412 were found to block the growth of neoplastic mast cells in SM, including cell lines expressing KIT D816V. Other targeted drugs with growth-inhibitory effects (on mast cells) identified were the Mcl-1/Bcl-2/Bcl-xL blocker obatoclax, the proteasome inhibitor bortezomib, and the multikinase-blocker piceatannol that was found to inhibit STAT5-activation in neoplastic mast cells. We were also able to show that most of these drugs like bortezomib synergize with the KIT inhibitor PKC412 in producing growth inhibition and apoptosis in neoplastic mast cells (Figure 11). Currently, the effects of these drugs are tested on CD34+/CD38- cells obtained from patients with SM.

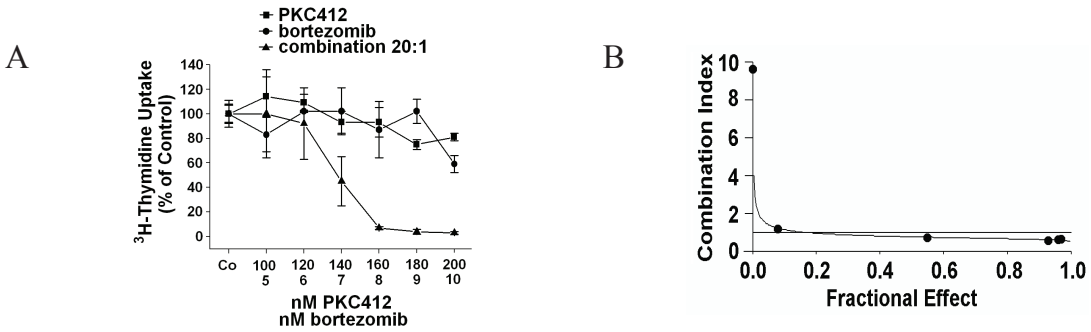

Figure 11

A: HMC-1.2 cells (KIT D816V+) were incubated in control medium (Co) or in medium containing drugs at 37°C for 48 hours. After incubation with PKC412 (■), bortezomib (●) or drug combinations (▲), cells were analyzed for ^{3}H-thymidine uptake. Results show ^{3}H-thymidine uptake as percentage of control (100%) and represent the mean±SD of triplicates. B: Using CalcuSyn software, analyses of dose-effect relationships of PKC412 and bortezomib in HMC-1.2 cells were calculated. A combination index (CI) less than 1 indicates synergism.

In other studies, we identified STAT5 as a major regulator of KIT-dependent signalling in neoplastic mast cells, and CD30 as novel marker of advanced mastocytosis. In addition, we identified Btk and Lyn as KIT-independent targets in neoplastic mast cells. Finally, we were able to show that NPM/ALK and IL-9 cooperate in producing a mastocytosis-like disease in mice, which is of interest as neoplastic mast cells express CD30 and ALK.

Strategic Aims reached in the first 3 years of the LB-CO Cluster:

Several strategic aims formulated for the Cluster were reached within the first 3 years. Probably the most important aim was to establish a robust xeno-transplantation model for various human neoplasms. In fact, the models used in previous studies, i.e. the NOD/SCID model, was found to have several limitations, and in many neoplasms (e.g. AML, CML, and in melanomas), a reproducible repopulation could not be demonstrated. We and others have shown that NSG mice provide a better engraftment model to study tumor- and leukemia-intiating cells. In particular, when using these mice, reproducible engraftment of myeloid leukemias and solid tumors can be achieved.
Another strategic aim was to establish phenotypes indicating stem cell-enriched cell populations in various neoplasms and to purify these cells to homogeneity. In myeloid leukemias, a flow-chip-flow approach revealed several novel and more specific stem cell markers. In CLL, we were able to identify a hitherto unrecognized (immature) progenitor cell that co-expresses CD34 and may be a true CLL stem cell. In solid tumors, most stem cell markers could not be verified. Here, further work has to be done to identify novel robust CSC markers. In melanomas, tumor-initiating cells may reside within the EPOR+ fraction, but no robust stem cell marker was identified in our studies. However, more recently, Boiko et al. have shown that CD271+ cells are enriched in melanoma-initiating cells (Nature 2010;466:133) which matches with our results as CD271+ cells usually represent a subpopulation of EPOR+ cells. A third aim was to define target expression profiles and to examine the effects of various targeted drugs on tumor and leukemic cells and their progenitors. This goal was also reached using various targeted drugs. Most of these studies are ongoing and are currently extended to drug combinations.
For the next period the most important strategic aim will be to establish cancer stem cell lines in order to extend to more omics-approaches and to start subclone analyses.

2.2. Publications and Reports – Overview

A number of key publications have been published in the project year 2010, including 15 original manuscripts, 10 review articles, and numerous other publications. These articles reflect both the scientific activities in the cluster as well as the numerous cooperations that the cluster consortium has established. Notably, in several instances, the cluster was able to contribute substantially to publications by sharing vital data on neoplastic stem cells where other cooperating groups did not have the opportunity to study certain targets or markers at the stem cell level: one highlighting example is the demonstration that neoplastic stem cells in MDS express CD44. Other examples are the expression of Stat5 mRNA in CD34+ cells in CML or expression of CD30 and the IL-9 receptor on neoplastic mast cells in patients with systemic mastocytosis. A list of publications generated in 2010 is provided in a separate file.

2.3. Patents

No patents were filed in 2010 in our LB-CO Cluster.

2.4. Conferences

Members of our consortium attended several national and international conferences in the fields of Hematology, Oncology, and Cancer Stem Cell Research. In addition, members of our consortium, in most instances the scientific coordinator, were involved in the organization of national and international conferences and meetings.

2.5. Lectures and Presentations

Members of the LB-CO consortium delivered a number of lectures and presentations at national and international conferences and meetings, inlcuding numerous invited lectures. A list of all presentations is provided in a separate file (publications).

3. Additional Information

3.1. Scientific Cooperations

Scientific Cooperation within the Cluster LB-CO
During the first 3 years of the project, several new scientific cooperations have been established in the LB scientific environment. These cooperations have been extended in the 3rd project year, and have been used to strengthen our projects. We have especially extended our strong ongoing cooperation with our colleagues from the departments of dermatology (mastocytosis and mast cell leukemia), pathology (leukemias, cytogenetics), hematology (leukemias and myeloma), and cancer research (colon cancer).

Scientific Cooperation with other Groups in Vienna
During the first 3 project years, the core cluster group has established new important cooperation or intensified pre-existing scientific cooperation with a number of groups working in the field of oncology and translational research in Vienna. These cooperations were extended in 2010. Cooperations that should be highlighted are the ongoing cooperation with our colleagues at the CeMM (Prof. Giulio Superti-Furga), the LBI for Cancer Research (Prof. Richard Moriggl), and the Vet.Med.Wien (Dr. Michael Willmann & Prof. Thomas Rülicke). We have recently also started a new collaboration-project (MDS/AML) with the Cluster Translational Oncology (Prof. Klaus Geissler).

Other Cooperations
No other new cooperations have been established in 2010.

3.2. Organization of Conferences and Meetings

As mentioned above, members of our consortium were involved in the organization of national and international conferences and meetings. The Cluster also organized a Progress Report Meeting of the LB-CO in Februar 2010 in Vienna.

3.3. Education and Ph.D. Program

The Ph.D. program of the MedUniWien is coordinated sucessfully by Brigitte Marian who is a key member of our LB-CO consortium. Members of our LB-CO consortium were also involved in teaching and training of students and postdocs in 2010.

4. Aims for the Next Time-Period

Based on results obtained in the first three years of our LB-CO project (2008-1010) and the evaluation report (reviewers´ suggestions) the LB-CO cluster will focus on neoplastic stem cells in leukemias and pre-leukemic conditions in the next project period (2011-2013). Major disorders that will be analyzed are MDS, MPN, AML, CML, ALL, and Mast Cell Leukemia (MCL). Drug-targets, markers, and targeted drugs will be selected based on our previous data and the published literature. In addition, we will initiate a genome-wide screen for pro-oncogenic lesions in various disease models and follow our subclone-and-latency hypothesis of cancer stem cell development. The long-term goal of the project is to develop new improved (potentially curative) therapies for leukemias and related disorders by applying drugs that recognize and eliminate leukemic stem cells.

5. Publications

The Cluster LB-CO published a series of publications in top and standard peer-review journals.

Original Manuscripts

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