

In Vivo Growth of Mantle Cell Lymphoma Xenografts in Immunodeficient Mice Is Positively Regulated by VEGF and Associated with Significant Up-regulation of *CD31/PECAM1*

(mantle cell lymphoma / angiogenesis / CD31 / VEGF)

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Abstract. Mantle cell lymphoma (MCL) is an aggressive lymphoma subtype with dismal prognosis. New treatments are needed to improve outcome of relapsed/refractory disease. Recently, several drugs targeting at least partially the process of angiogenesis have been successfully tested in the therapy of MCL. Molecular mechanisms that regulate MCL-induced angiogenesis and that might represent potential new druggable targets remain, however, incompletely understood. We established two mouse models of human MCL by subcutaneous xenotransplantation of JEKO-1 and HBL-2 cell lines into immunodeficient mice. Histological analyses of xenografts confirmed their neovascularization. The growth of xenografts was significantly suppressed by single-agent therapy with bevacizumab, monoclonal antibody targeting vascular endothelial growth factor (VEGF). Sub-

sequently, we analysed expression of 94 angiogenesis-related genes in *ex vivo* isolated JEKO-1 and HBL-2 cells compared to *in vitro* growing cells using TaqMan low-density arrays. The most up-regulated genes in both JEKO-1 and HBL-2 xenografts were genes encoding platelet/endothelial cell-adhesion molecule (*CD31/PECAM1*), VEGF receptor 1 (*FLT1*), hepatocyte growth factor (*HGF*), angiogenin (*ANG*) and transcription factor PROX1. The most down-regulated genes in both JEKO-1 and HBL-2 xenografts were midkine (*MDK*) and ephrine B2 (*EPHB2*). In summary, our results demonstrate an important role of angiogenesis in the biology of MCL and provide preclinical evidence of potent anti-MCL activity of bevacizumab. In addition, gene expression profiling of 94 angiogenesis-related targets revealed several *in vivo* up-regulated and down-regulated transcripts. The most differentially expressed target in both MCL tumours was *CD31/PECAM1*. Whether any of these molecules might represent a potential druggable target in MCL patients remains to be elucidated.

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Abbreviations: ANG – angiogenin, B-NHL – B-cell non-Hodgkin lymphoma, bFGF – basic fibroblast growth factor, DAB – diaminobenzidine, EPHB2 – ephrine B2, HGF – hepatocyte growth factor, IMDM – Iscove's modified Dulbecco's medium, MCL – mantle cell lymphoma, MDK – midkine, PBS – phosphate-buffered saline, PGF – placental growth factor, VEGF – vascular endothelial growth factor.

Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (B-NHL) subtype that accounts for approx. 5–10 % of all B-NHL and is characterized by chromosomal translocation t(11;14) leading to over-expression of cyclin D1 with ensuing cell cycle disruption (Pérez-Galán et al., 2011; Smedby and Hjalgrim, 2011). Besides the up-regulation of cyclin D1 and a plethora of additional genetic and epigenetic aberrations, extrinsic signals from the microenvironment have been identified as key pro-survival events that pos-

itively regulate survival, proliferation, migration and/or drug resistance of MCL cells (Kurtova et al., 2009; Medina et al., 2012). Recently, new drugs targeting at least partially the complex process of angiogenesis including bevacizumab, temsirolimus, enzastaurine and lenalidomide were successfully tested in the therapy of relapsed/refractory MCL patients (Morschhauser et al., 2008; Hess et al., 2009; Stopeck et al., 2009; Smith et al., 2010; Witzig et al., 2011; Eve et al., 2012). Blood vessels are important components of the tumour micro-environment and creation of new vessels by tumour-induced angiogenesis positively impacts tumour growth and dissemination. It was shown that lymphoma cells can enhance local neovascularization by paracrine secretion of vascular endothelial growth factor (VEGF). VEGF binds not only to VEGF receptors expressed on endothelial cells, but also to VEGF receptors expressed on the lymphoma cells, thereby stimulating their survival and proliferation in an autocrine fashion (Wang et al., 2004). Several studies reported that cells obtained from patients with MCL express both VEGF and VEGF receptors (Stopeck et al., 2009; Wang et al., 2010). While VEGF remains the most studied pro-angiogenic factor, the roles of other pro- and anti-angiogenic factors (e.g. basic fibroblast growth factor – bFGF, hepatocyte growth factor – HGF, angiopoietins, midkine – MDK, etc.) or cell surface receptors/adhesion molecules involved in angiogenesis (e.g. MET, CD31/PECAM-1, CD105/Endoglin, ephrin B2 – EPHB2) in the biology of lymphomas remain largely unexplored.

In this study we assessed the dependence of the growth of MCL xenografts on VEGF signalling and analysed changes in the expression of several angiogenesis-related transcripts during *in vivo* growth of MCL xenografts.

Material and Methods

Cell culture

The JEKO-1 cell line was obtained from Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany), the HBL-2 cell line was a kind gift of prof. Dreyling (University of Munich, Germany). Cell lines were cultured in Iscove's modified Dulbecco's medium (IMDM, Lonza, Basel, Switzerland) supplemented with 20% foetal bovine serum (FBS, Lonza) and 1% penicillin/streptomycin.

In vivo experiments

The studies were approved by the institutional Animal Care and Use Committee. Female immunodeficient NOD.Cg-Prkdc^{scid} Il2r^{tm1Wjl}/SzJ mice (Jackson Laboratory, Bar Harbor, ME) were maintained in individually ventilated cages. JEKO-1 and HBL-2 cells were harvested, suspended in PBS, and injected (5×10^6 /mouse) subcutaneously into the left dorsal flank of 8- to 12-week-old mice. Three days posttransplant mice were randomized into control and treatment groups. The treat-

ment group was injected with 300 µg of bevacizumab (Avastin, Roche, Basel, Switzerland) intraperitoneally twice weekly (Mondays and Fridays), the control group was injected with PBS. When tumours in any group reached 3 cm in diameter, animals were sacrificed and tumours weighed.

Proliferation assays

To measure the effect of bevacizumab (Avastin, Roche) on proliferation, we used Quick Cell Proliferation Assay Kit (BioVision, Milpitas, CA) according to the manufacturer's instructions. Briefly, we incubated 20,000 cells with diverse concentrations of bevacizumab (0.1, 1, 10, and 100 µg/ml) for various time periods (0–168 hours) and measured absorbance of the samples after 2-hour incubation with WST-8 reagent.

Immunohistochemistry (IHC)

Mice with grown JEKO-1 and HBL-2 xenografts were post-mortem perfused via left heart ventricle with 4% formalin for *in situ* tissue fixation. Subcutaneous xenografts were excised and fixated in 4% formalin, embedded in paraffin and sectioned into 6–7 µm thick slices. Sections were then routinely stained with haematoxylin/eosin and Masson's blue trichrome. IHC detections using anti-human CD31 (Dako, Glostrup, Denmark) were performed. Antigen retrieval was done in citrate buffer solution (0.291% sodium citrate, 0.05% Tween 20, pH 6.0) using microwave for 3×5 minutes. The standard ABC method was used for detection with diaminobenzidine (DAB). Mayer's haematoxylin was used for counterstaining. Images were collected using an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) equipped with a DP25 digital camera.

Flow cytometry (FCM)

Cell suspensions prepared from subcutaneous xenografts were incubated with fluorochrome-conjugated CD31 antibody (Exbio, Vestec, Czech Republic), washed in PBS and analysed by flow cytometry (FACSCanto II; Becton Dickinson, Franklin Lakes, NJ). Cells stained with the isotype controls served as negative controls.

Human angiogenesis array

JEKO-1 and HBL-2 subcutaneous tumours were passed through a 45-micrometer cell strainer, washed with phosphate-buffered saline (PBS), labelled with human CD45 MicroBeads, and CD45-positive cells (= human MCL cells) were sorted using MACS Columns (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. JEKO-1 and HBL-2 cells cultured *in vitro* were processed in the same way. Total RNA was extracted from *ex vivo* and *in vitro* obtained JEKO-1 and HBL-2 cells by RNeasy Mini Kit (Qiagen, Venlo, Netherlands), and reverse-transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). cDNA samples were mixed with TaqMan Universal

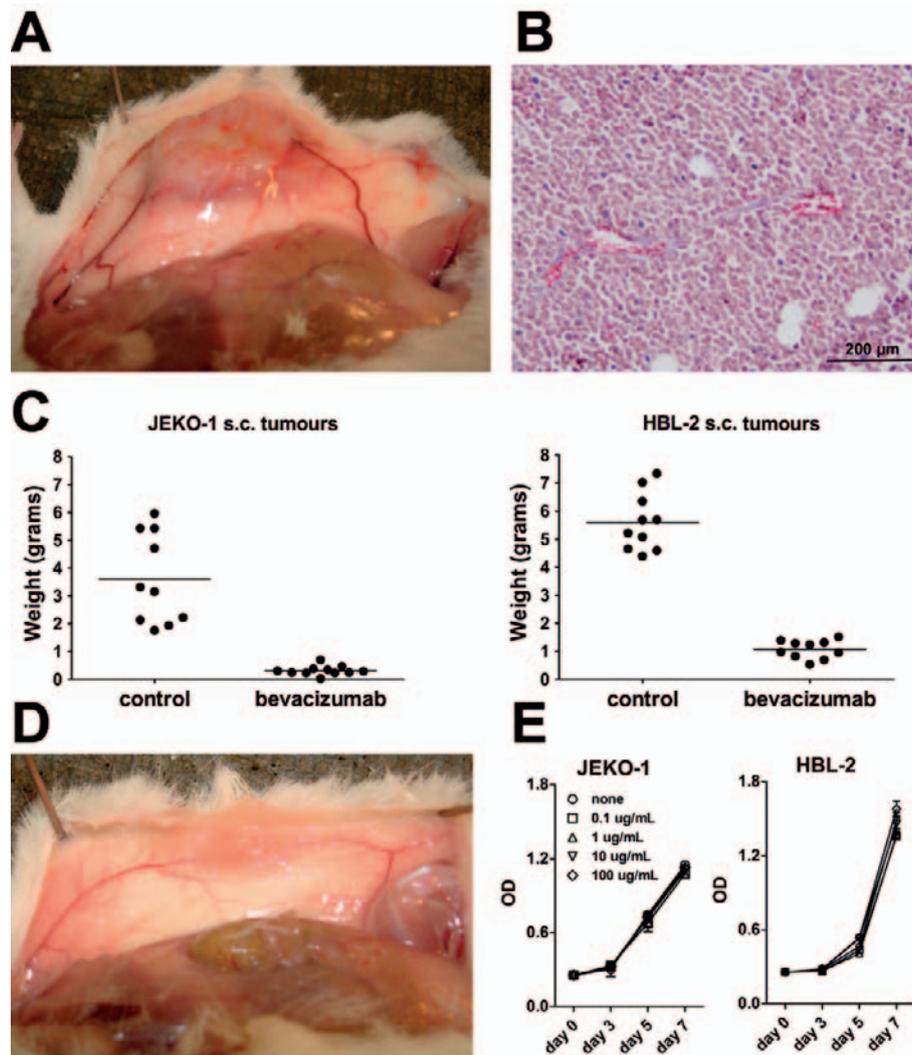


Fig. 1. *In vivo* growth of MCL is associated with neovascularization and is positively regulated by VEGF. **(A)** Vascularized tumour after s.c. xenotransplantation of JEKO-1 cell line into immunodeficient NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mouse. **(B)** A paraffin-embedded tumour section from JEKO-1 xenograft stained with haematoxylin/eosin revealed the presence of blood vessels in the tumour. **(C)** Immunodeficient NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice were subcutaneously injected with JEKO-1 or HBL-2 cell lines. Three days after xenotransplantation mice were randomized and either treated with 300 µg of bevacizumab (Avastin, Roche) intraperitoneally twice weekly, or treated with PBS only (control group). Dots represent weights of individual tumours and lines are plotted at the means. **(D)** A representative macroscopic tumour grown in mice treated with bevacizumab at the time of completion of experimental therapy. **(E)** JEKO-1 and HBL-2 cell lines were *in vitro* treated with bevacizumab (concentrations as indicated) and proliferation was measured as described in Material and Methods.

PCR Master Mix (Applied Biosystems) and loaded into TaqMan Array Human Angiogenesis Panel microfluidic plates (Applied Biosystems). Real-time qPCR was run in a 7900 HT SDS PCR cycler (Applied Biosystems) according to manufacturer's instructions. Data were normalized to the expression of *GAPDH* and *in vitro* cultured MCL cell lines were used as controls.

Statistical analysis

The data were analysed in GraphPad Prism (GraphPad Software, La Jolla, CA). The values represent means ± SD of at least three independent experiments. The differences between experimental groups were determined by Student's *t*-test.

Results

In vivo growth of MCL is positively regulated by VEGF

To study angiogenesis during engraftment and growth of MCL we established and characterized two mouse models of human MCL after subcutaneous (s.c.) xenotransplantation of MCL cell lines JEKO-1 and HBL-2 into immunodeficient NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice. Grown MCL tumours (approx. 2–3 cm in diameter) were subjected to histological analyses that confirmed neovascularization of the xenografts (Fig. 1A, B). VEGF is the single most important pro-angiogenic fac-

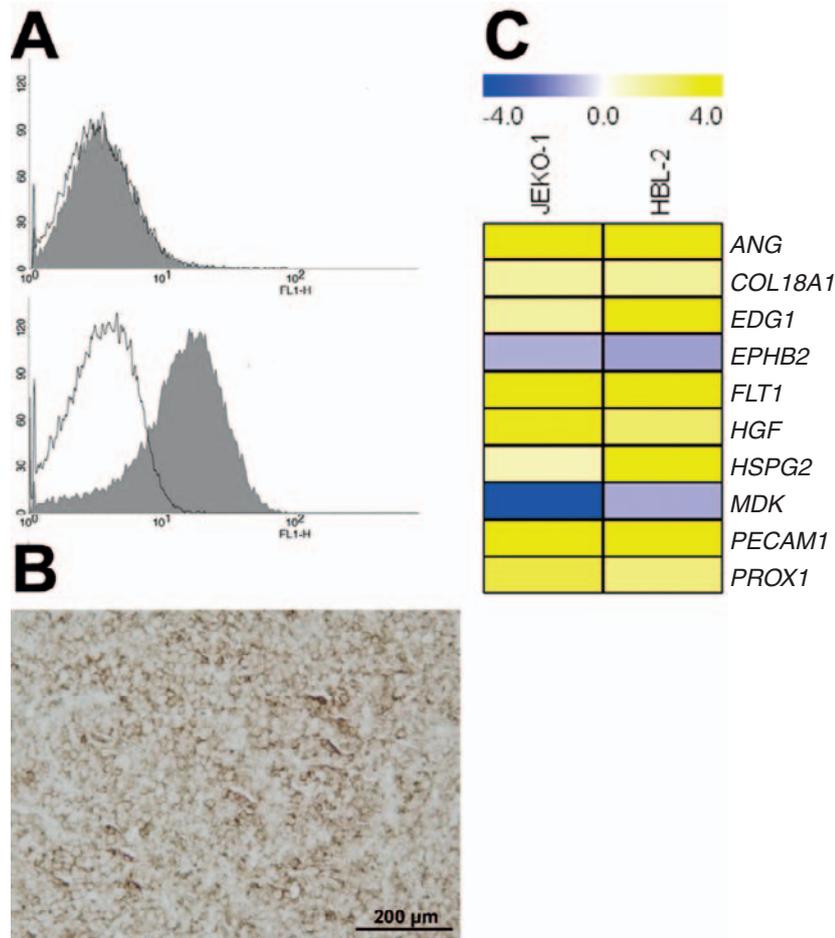


Fig. 2. *CD31/PECAM1* is the most up-regulated gene during *in vivo* growth of mantle cell lymphoma xenografts in immunodeficient mice. **(A)** Flow cytometry analysis of *CD31/PECAM1* expression (full histograms) on the surface of JEKO-1 cells cultured *in vitro* (upper histogram) and obtained *ex vivo* from JEKO-1 xenografts (lower histogram). Black line empty histograms represent cells stained with the control isotype antibody. Histograms shown are representative examples from three independent experiments. **(B)** A paraffin-embedded tumour section from JEKO-1 xenograft stained with human anti-*CD31* antibody revealed diffuse positivity of *CD31* antigen. **(C)** The expression of angiogenesis-related genes was assessed in JEKO-1 and HBL-2 cells obtained *ex vivo* from s.c. xenografts using TaqMan Low-Density Human Angiogenesis Array. Columns represent cell lines and rows correspond to genes. Colour changes within a row indicate expression levels relative to the *in vitro* cultured control cell lines (data analysed using MeV4 software, colour scale limits: logFC = ± 4, blue down-regulation, yellow up-regulation).

tor. We clearly demonstrated that monotherapy of both JEKO-1- and HBL-2-xenografted mice with anti-VEGF monoclonal antibody bevacizumab significantly suppressed growth of the xenografts compared to untreated controls (Fig. 1C, also compare 1A and 1D). In contrast, *in vitro* exposure of JEKO-1 and HBL-2 cells to bevacizumab did not impact the proliferation rate (Fig. 1E).

CD31/PECAM-1 is the most up-regulated gene during in vivo growth of mantle cell lymphoma xenografts in immunodeficient mice

Based on these results we decided to use the mouse models of MCL to analyse *in vivo* induced changes in gene expression of selected angiogenesis-related targets. Ninety-four angiogenesis-related genes were assessed in MCL cells obtained *ex vivo* from s.c. MCL

xenografts compared to *in vitro* cultured MCL cell lines using TaqMan Low-Density Human Angiogenesis Array. The most up-regulated gene in both JEKO-1 and HBL-2 xenografts was the gene encoding platelet/endothelial cell-adhesion molecule (*CD31/PECAM1*, fold change: 148.9 ± 19.4 , and 127.6 ± 10.5 , respectively). The up-regulation of *CD31/PECAM-1* protein was confirmed by flow cytometry and immunohistochemistry (Fig. 2A, B). Other genes up-regulated in both JEKO-1 and HBL-2 were genes encoding VEGF receptor 1 (*FLT1/VEGFR1*, fold change: 23.3 ± 6.5 , and 117.5 ± 0.7 , respectively), hepatocyte growth factor (*HGF*, fold change: 8.9 ± 2.7 , and 5.0 ± 0.4), angiogenin (*ANG*, fold change: 11.1 ± 1.2 , and 11.5 ± 13.2), *CD105/Endoglin* (*EDG1*, fold change: 2.6 ± 0.2 , and 21.1 ± 4.9) or transcription factor *PROX1* (fold change: 6.6 ± 1.9 , and 4.0 ± 0.4). The most down-regulated genes in JEKO-1 and

HBL-2 included genes encoding midkine (*MDK*, fold change: 0.06 ± 0.05 , and 0.45 ± 0.01) and ephrin B2 (*EPHB2*, fold change: 0.47 ± 0.05 , and 0.41 ± 0.05). All of the tested angiogenesis-related genes up-regulated or down-regulated during *in vivo* growth of both JEKO-1 and HBL-2 by at least 2-fold are shown in Fig. 2C.

Discussion

In this study we established and characterized two mouse models of human MCL by s.c. xenotransplantation of MCL cell lines JEKO-1 and HBL-2 into immunodeficient mice. We showed that MCL xenografts were neovascularized and that treatment with anti-VEGF antibody bevacizumab resulted in significant tumour growth suppression. These data confirmed VEGF as an important druggable target in MCL. Importantly, bevacizumab did not impact the *in vitro* proliferation rate of MCL cell lines, suggesting an angiogenic switch during *in vivo* growth of MCL xenografts. Profiling of 94 angiogenesis-related genes revealed several targets differentially expressed in both MCL tumours compared to the corresponding MCL cell lines, including *CD31/PECAMI*, *FLT1*, *HGF*, *ANG*, *PROX1* (up-regulated), and *EPHB2*, *MDK* (down-regulated). *CD31/PECAMI* was the most differentially expressed (up-regulated) gene in both JEKO-1 and HBL-2 xenografts. Protein over-expression of CD31/PECAM-1 was confirmed by both flow cytometry and immunohistochemistry. CD31/PECAM-1 is an immunoglobulin superfamily member expressed on the surface of endothelial cells, platelets and leucocytes (Jackson, 2003). CD31/PECAM-1 and CD34 represent established molecular markers used for evaluation of tumour microvessel density. Expression of CD31/PECAM-1 was, however, also reported on the surface of malignant lymphocytes (Gallay et al., 2007; Boyd et al., 2009; Akers et al., 2010; Poggi et al., 2010). Only recently, CD31/PECAM-1 was reported over-expressed on MCL primary cells (Boyd et al., 2009). This finding, together with significant up-regulation of CD31/PECAM-1 on MCL cells during *in vivo* growth in this current study suggest that CD31/PECAM-1 might play an important role in the biology of MCL. FLT-1 is a receptor tyrosine kinase binding VEGF and placental growth factor (PGF). Despite the fact that FLT-1 was described in the literature as a largely negative regulator of angiogenesis, its expression on leukemic cells was recently reported to promote cell proliferation and tumour growth (Fragoso et al., 2006).

Hepatocyte growth factor (HGF) and its receptor tyrosine kinase MET are oncogenes involved in the biology of many cancers, including lymphomas (Mahtouk et al., 2010). Angiogenin (ANG) is a potent inducer of angiogenesis and its high concentration correlated with poor outcome in patients with NHL (Fang et al., 2011). Transcription factor PROX1 plays pivotal roles during lymphatic development, but its role in lymphoma-induced (lymph)angiogenesis remains largely unexplored (Yoshimatsu et al., 2011). Midkine (MDK), the most *in*

vivo suppressed gene in this study, is a heparin-binding growth factor. *MDK* was, however, reported to be up-regulated in many types of cancer cells (Tsutsui et al., 1993). Ephrin B2 (*EPHB2*) is a receptor tyrosine kinase involved in the maintenance of tissue architecture via regulating cell adhesion, motility, survival and angiogenesis (Zou et al., 1999). The precise role of *EPHB2* in cancer growth and metastasizing remains controversial. *EPHB2* appears to act as a tumour suppressor in some types of cancer, while in other types it behaves as a classical oncogene. Both loss of *EPHB2* expression and *EPHB2* over-expression or mutation were associated with increased tumour aggressiveness or cancer progression (Jubb et al., 2005; Lugli et al., 2005; Guo et al., 2006; Sikkema et al., 2012). Strong expression of *EPHB2* was recently reported on CD34⁺ haematopoietic stem cells (Lazarova et al., 2006). There are, however, no published data concerning *EPHB2* expression in hematologic malignancies.

It must be taken into account that the gene expression analysis was performed on CD45-sorted human MCL cells only. Pro- and anti-angiogenic factors expressed by murine microenvironment might contribute to stimulation or blockage of MCL-induced angiogenesis and/or MCL survival/growth.

In summary, our results demonstrate a significant role of angiogenesis in the biology of MCL, and provide pre-clinical evidence of potent anti-tumour activity of bevacizumab, thus confirming VEGF as a key druggable angiogenesis target in MCL. In addition, gene expression profiling of 94 angiogenesis-related targets revealed several *in vivo* up-regulated and down-regulated transcripts, including *CD31/PECAMI*, *FLT1*, *HGF*, *ANG*, *PROX1*, and *MDK*, *EPHB2*, respectively. Whether any of these molecules might represent a potential druggable target remains to be elucidated.

References

- Akers, S. M., O'Leary, H. A., Minnear, F. L., Craig, M. D., Vos, J. A., Coad, J. E., Gibson, L. F. (2010) VE-cadherin and PECAM-1 enhance ALL migration across brain microvascular endothelial cell monolayers. *Exp. Hematol.* **38**, 733-743.
- Boyd, R. S., Jukes-Jones, R., Walewska, R., Brown D., Dyer, M. J. S., Cain, K. (2009) Protein profiling of plasma membranes defines aberrant signaling pathways in mantle cell lymphoma. *Mol. Cell Proteomics* **8**, 1501-1515.
- Eve, H. E., Carey, S., Richardson, S. J., Heise, C. C., Mami-dipudi, V., Shi, T., Radford, J. A., Auer, R. L., Bullard, S. H., Rule, S. A. J. (2012) Single-agent lenalidomide in relapsed/refractory mantle cell lymphoma: results from a UK phase II study suggest activity and possible gender differences. *Br. J. Haematol.* **159**, 154-163.
- Fang, S., Repo, H., Joensuu, H., Orpana, A., Salven, P. (2011) High serum angiogenin at diagnosis predicts for failure on long-term treatment response and for poor overall survival in non-Hodgkin lymphoma. *Eur. J. Cancer.* **47**, 1708-1716.
- Fragoso, R., Pereira, T., Wu, Y., Zhu, Z., Cabeçadas, J., Dias, S. (2006) VEGFR-1 (FLT-1) activation modulates acute

- lymphoblastic leukemia localization and survival within the bone marrow, determining the onset of extramedullary disease. *Blood* **107**, 1608-1616.
- Gallay, N., Anani, L., Lopez, A., Colombat, P., Binet, C., Domenech, J., Weksler, B. B., Malavasi, F., Heralut, O. (2007) The role of platelet/endothelial cell adhesion molecule-1 (CD31) and CD38 antigens in marrow microenvironmental retention of acute myelogenous leukemia cells. *Cancer Res.* **67**, 8624-8632.
- Guo, D. L., Zhang, J., Yuen, S. T., Tsui, W. Y., Chan, A. S. Y., Ho, C., Ji, J., Leung, S. Y., Chen, X. (2006) Reduced expression of EphB2 that parallels invasion and metastasis in colorectal tumours. *Carcinogenesis* **27**, 454-464.
- Hess, G., Herbrecht, R., Romaguera, J., Verhoef, G., Crump, M., Gisselbrecht, C., Laurell, A., Offner, F., Strahs, A., Berkenblit, A., Hanushevsky, O., Clancy, J., Hewes, B., Moore, L., Coiffier, B. (2009) Phase III study to evaluate temsirolimus compared with investigator's choice therapy for the treatment of relapsed or refractory mantle cell lymphoma. *J. Clin. Oncol.* **27**, 3822-3829.
- Jackson, D. E. (2003) The unfolding tale of PECAM-1. *FEBS Lett.* **540**, 7-14.
- Jubb, A. M., Zhong, F., Bheddah, S., Grabsch, H. I., Frantz, G. D., Mueller, W., Kavi, V., Quirke, P., Polakis, P., Koeppen, H. (2005) EphB2 is a prognostic factor in colorectal cancer. *Clin. Cancer Res.* **11**, 5181-5187.
- Kurtova, A. V., Tamayo, A. T., Ford, R. J., Burger, J. A. (2009) Mantle cell lymphoma cells express high levels of CXCR4, CXCR5, and VLA-4 (CD49d): importance for interactions with the stromal microenvironment and specific targeting. *Blood* **113**, 4604-4613.
- Lazarova, P., Wu, Q., Kvalheim, G., Suo, Z., Haakenstad, K. W., Metodiev, K., Nesland, J. M. (2006) Growth factor receptors in hematopoietic stem cells: EPH family expression in CD34+ and CD133+ cell populations from mobilized peripheral blood. *Int. J. Immunopathol. Pharmacol.* **19**, 49-56.
- Lugli, A., Spichtin, H., Maurer, R., Mirlacher, M., Kiefer, J., Huusko, P., Azorsa, D., Terracciano, L., Sauter, G., Kallioniemi, O. P., Mousses, S., Tornillo, L. (2005) EphB2 expression across 138 human tumor types in a tissue microarray: High levels of expression in gastrointestinal cancers. *Clin. Cancer Res.* **11**, 6450-6458.
- Mahtouk, K., Tjin, E. P. M., Spaargaren, M., Pals, S. T. (2010) The HGF/MET pathway as target for the treatment of multiple myeloma and B-cell lymphomas. *Biochim. Biophys. Acta* **1806**, 208-219.
- Medina, D. J., Goodell, L., Glod, J., Gélinas, C., Rabson, A. B., Strair, R. K. (2012) Mesenchymal stromal cells protect mantle cell lymphoma cells from spontaneous and drug-induced apoptosis through secretion of B-cell activating factor and activation of the canonical and non-canonical nuclear factor κ B pathways. *Haematologica* **97**, 1255-1263.
- Morschhauser, F., Seymour, J. F., Kluijn-Nelemans, H. C., Grigg, A., Wolf, M., Pfreundschuh, M., Tilly, H., Raemaekers, J., van 't Veer, M. B., Milpied, N., Cartron, G., Pezzutto A., Spencer, A., Reyes, F., Dreyling, M. (2008) A phase II study of enzastaurin, a protein kinase C β inhibitor, in patients with relapsed or refractory mantle cell lymphoma. *Ann. Oncol.* **19**, 247-253.
- Pérez-Galán, P., Dreyling, M., Wiestner, A. (2011) Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era. *Blood* **117**, 26-38.
- Poggi, A., Prevosto, C., Catellani, S., Rocco, I., Garuti, A., Zocchi, M. R. (2010) Engagement of CD31 delivers an activating signal that contributes to the survival of chronic lymphocytic leukaemia cells. *Br. J. Haematol.* **151**, 252-264.
- Sikkema, A. H., den Dunnen, W. F., Hulleman, E., van Vuurden, D. G., Garcia-Manero, G., Yang, H., Scherpen, F. J., Kampen, K. R., Hoving, E. W., Kamps, W. A., Diks, S. H., Peppelenbosch, M. P., de Bont, E. S. (2012) EphB2 activity plays a pivotal role in pediatric medulloblastoma cell adhesion and invasion. *Neuro Oncol.* **14**, 1125-1135.
- Smedby, K. E., Hjalgrim, H. (2011) Epidemiology and etiology of mantle cell lymphoma and other non-Hodgkin lymphoma subtypes. *Semin. Cancer Biol.* **21**, 293-298.
- Smith, S. M., van Besien, K., Karrison, T., Dancey, J., McLaughlin, P., Younes, A., Smith, S., Stiff, P., Lester, E., Modi, S., Doyle, L. A., Vokes, E. E., Pro, B. (2010) Temsirolimus has activity in non-mantle cell non-Hodgkin's lymphoma subtypes: The University of Chicago phase II consortium. *J. Clin. Oncol.* **28**, 4740-4746.
- Stopeck, A. T., Unger, J. M., Rimsza, L. M., Bellamy, W. T., Iannone, M., Persky, D. O., Leblanc, M., Fisher, R. I., Miller, T. P. (2009) A phase II trial of single agent bevacizumab in patients with relapsed, aggressive non-Hodgkin lymphoma: Southwest oncology group study S0108. *Leuk. Lymphoma* **50**, 728-735.
- Tsutsui, J., Kadomatsu, K., Matsubara, S., Nakagawara, A., Hamanoue, M., Takao, S., Shimazu, H., Ohi, Y., Muramatsu, T. (1993) A new family of heparin-binding growth/differentiation factors: increased midkine expression in Wilms' tumor and other human carcinomas. *Cancer Res.* **53**, 1281-1285.
- Wang, E. S., Teruya-Feldstein, J., Wu, Y., Zhu, Z., Hicklin, D. J., Moore, M. A. S. (2004) Targeting autocrine and paracrine VEGF receptor pathways inhibits human lymphoma xenografts in vivo. *Blood* **104**, 2893-2902.
- Wang, L., Shi, W. Y., Wu, Z. Y., Varna, M., Wang, A. H., Zhou, L., Chen, L., Shen, Z. X., Lu, H., Zhao, W. L., Janin, A. (2010) Cytostatic and anti-angiogenic effects of temsirolimus in refractory mantle cell lymphoma. *J. Hematol. Oncol.* **3**, 30-33.
- Witzig, T. E., Vose, J. M., Zinzani, P. L., Reeder, C. B., Buckstein, R., Polikoff, J. A., Bouabdallah, R., Haioun, C., Tilly, H., Guo, P., Pietronigro, D., Ervin-Haynes, A. L., Czuczman, M. S. (2011) An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. *Ann. Oncol.* **22**, 1622-1627.
- Yoshimatsu, Y., Yamazaki, T., Mihira, H., Itoh, T., Suehiro, J., Yuki, K., Harada, K., Morikawa, M., Iwata, C., Minami, T., Morishita, Y., Kodama, T., Miyazono, K., Watabe, T. (2011) Ets family members induce lymphangiogenesis through physical and functional interaction with Prox1. *J. Cell. Sci.* **124**, 2753-2762.
- Zou, J. X., Wang, B., Kalo, M. S., Zisch, A. H., Pasquale, E. B., Ruoslahti, E. (1999) An Eph receptor regulates integrin activity through R-Ras. *Proc. Natl. Acad. Sci. USA* **96**, 13813-13818.