Niedersachsenprofessur für Molekulare Hämatopoese

Direktor: Prof. Dr. Karl H. Welte

Tel.: 0511/532-6710 • E-Mail: welte.karl.h@mh-hannover.de • intranet.mh-hannover.de/12105.html

Keywords: Schwere kongenitale Neutropenie, Myelopoese, Granulopoese, Leukämogenese

Forschungsprofil

Das Forschungsprofil der Abteilung Molekulare Hämatopoese wird maßgeblich durch die klinische Forschung und Grundlagenforschung auf dem Gebiet der angeborenen Erkrankungen der Blutbildung und Leukämogenese bestimmt. Mitglieder der Abteilung haben auf diesem Gebiet international beachtete Pionierarbeit geleistet. Beispielsweise wurden neue Genmutationen identifiziert, die in der Entwicklung von Leukämien beteiligt sind (siehe ausführlicher Bericht). Weiter wurden mehrere neue und wichtige Signalwege in der Entwicklung myeloider Zellen und in der Leukämogenese identifiziert. Die Ergebnisse konnten in den hoch renommierten Journalen "Nature Medicine" und "Blood" veröffentlicht werden. Darüber hinaus ist die Europazentrale des Internationalen Registers für die schwere chronische Neutropenie hier angesiedelt. Die Mitglieder der Abteilung sind in mehreren Forschungsnetzwerken federführend beteiligt, dem Bundesministerium für Bildung und Forschung (German Network on Congenital Bone Marrow Failure Syndromes) und der BMBF-geförderten Initiative für seltene Erkrankungen (E-Rare) der EU-Staaten.

Forschungsprojekte

Bortezomib Induces Granulocytic Differentiation of CD34+ Cells from Congenital Neutropenia Patients by Reversing Hyperactivate-STAT5a-Dependent Downregulation of LEF-1: possible clinical application of bortezomib for treatment of neutropenia

Granulocyte colony-stimulating factor (G-CSF) regulates the survival, proliferation, and maturation of granulocytic precursors. The G-CSF receptor (G-CSFR) is a member of the type I cytokine receptor family that triggers the phosphorylation of receptor-associated protein tyrosine kinases, including Janus kinase 1/2 (JAK1/2) and members of the Src kinase family (e.g., Lyn and Syk). The phosphorylation of these tyrosine kinases leads to the activation of a cascade of downstream effector molecules, such as STAT (signal transducer and activator of transcription) proteins, or causes recruitment of various adaptor proteins. These, in turn, mediate activation of downstream pathways, including phosphoinositide 3-kinase (PI3K)/Akt, Ras/Raf/MAPK (mitogen-activated protein kinase, and Nampt (nicotinamide phosphoribosyltransferase)/NAD+/SIRT1 (sirtuin 1).

STAT proteins STAT3 and STAT5 are robustly activated by G-CSFR, but in a different manner and serving different effector functions. STAT3 is activated in a sustained fashion, but activation of STAT5 is transient, with maximal activation levels occurring within 10 to 30 minutes. STAT5 is involved in the maintenance and expansion of human hematopoietic stem/progenitor cells and is crucial for cell survival and proliferation. In myeloid progenitors lacking both STAT5a and STAT5b, the clonal advantage conferred by mutant G-CSFR was found to be abrogated. Constitutive activation of STAT5 results in impaired in vitro myelopoiesis of human hematopoietic stem/progenitor cells in association with downregulation of myeloid-associated factors such as C/EBPα. Moreover, hyperactivation of STAT5 signaling has been implicated in various hematological malignancies, including BCR–ABL-induced chronic myeloid leukemia (CML) and acute myeloid leukemia (AML), and in myeloproliferative disorders, such as chronic myelomonocytic leukemia and polycythemia vera.

Severe congenital neutropenia (CN) is characterized by a maturation arrest of granulopoiesis at the promyelocytic stage. G-CSFR signaling is severely impaired in CN patients. CN patients show a response to G-CSF therapy; however, pharmacologically high doses of G-CSF (1–100 µg/kg body weight/day) are required to increase neutrophil counts. CN patients are at increased risk of developing AML (cumulative incidence, ~15–21%). Whether and how G-CSF affects this predisposition remains unclear. Long-term therapy with pharmacological doses of G-CSF or elevated levels of endogenous G-CSF in plasma of CN patients may cause genomic instability due to increased pressure on cell division and DNA replication. Moreover, G-CSF may lead to a preferential secondary outgrowth of pre-existing cell clones containing mutations, for example in the G-CSFR (CSF3R) gene. Hematopoietic cells from nearly 80% of CN patients who developed leukemia show truncations in the cytoplasmic region of the G-CSF receptor crucial for maturation signaling.

We have previously shown that the transcription factor LEF-1 (lymphoid enhancer-binding factor 1) plays a definitive role in G-CSF-triggered granulopoiesis. LEF-1 and its target genes, including the myeloid-specific transcription factor C/EBPα, are severely downregulated in promyelocytes of CN patients. However, the mechanism underlying LEF-1 downregulation have remained elusive. LEF-1 belongs to the LEF-1/TCF (T cell factor) family of high mobility group domain-containing transcription factors. Members of the LEF-1/TCF transcription factor family generally act through the canonical Wnt signaling pathway in a functional complex with ß-catenin. Wnt /ß-catenin-independent stimuli, such as tumor growth factor (TGF)-ß and Notch pathways, may also engage LEF-1. Among the proteins known to interact with LEF-1 are ß-catenin, ALY, Pitx2, and Groucho/TLE. Interestingly, Nemo-like kinase (NLK) was shown to antagonize Wnt/ß-catenin signaling by interaction with LEF-1 and LEF-1 phosphorylation. Recent studies have demonstrated that NARF (NLK-associated ring finger protein) interacts with LEF-1, leading to LEF-1 ubiquitination and degradation via the proteasome pathway. Specifically, it was shown that NARF complexes with NLK under steady-state conditions and, in response to an unknown ligand, acts through activation of NLK to exert E3 ubiquitin-ligase activity toward LEF-1. Interestingly, Kojima et al. demonstrated that NLK is activated by G-CSF and interleukin (IL)-6 via STAT3. It has been shown that NLK interacts with STAT3 and that STAT3 activates NLK, serving as a scaffold between activated IL-6 and NLK. Whether hyperactivated STAT5 plays a similar scaffolding role in G-CSF-triggered NLK activation and NLK/NARF-mediated ubiquitination of LEF-1 is unclear.

The normal expression of LEF-1 in monocytes and lymphocytes, whose differentiation is unaffected in CN, suggests the presence of a granulopoiesis-specific mechanism downstream of G-CSF receptor signaling that leads to LEF-1 downregulation. We found that the levels of phosphorylated (activated) STAT5 were significantly elevated in unstimulated CD34+ cells from CN patients compared to those in healthy individuals; the relative increase was even greater in CN/AML patients. Stimulation with G-CSF induces phosphorylation of STAT5 in healthy individuals, an effect that was markedly higher in CN and CN/AML patients (Fig. 1a, b). Previously, we reported elevated levels of JAK2 in myeloid cells of CN patients. Here, we found diminished mRNA expression levels of SOCS3 (suppressor of cytokine signaling 3) as well as elevated G-CSFR mRNA and protein expression in CN myeloid cells compared with healthy individuals, a result in line with elevated phospho-STAT5 (Fig. 1c, d). This enhanced phosphorylation, in turn, would be predicted to lead to STAT5 hyperactivation.

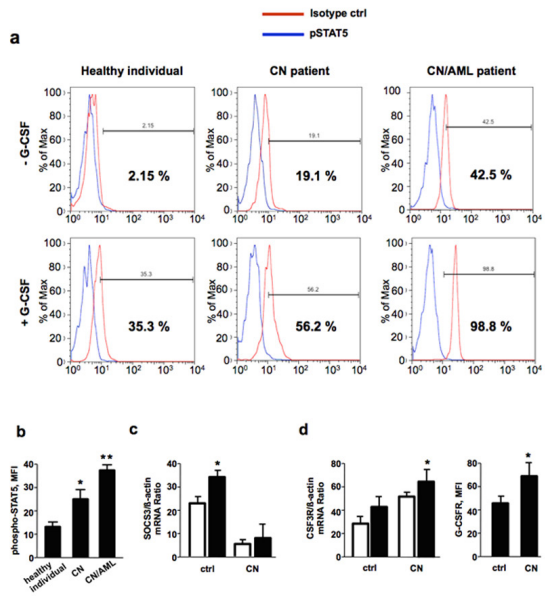

Fig 1: G-CSF-triggered phosphorylation of STAT5 is elevated in myeloid cells of CN patients; caSTAT5a inhibits mRNA expression of LEF-1 and its target gene C/EBP.(a, b) The levels of phospho-STAT5 in myeloid cells were assessed by FACS analysis of CD34+ cells from healthy individuals (n = 4), CN patients harboring ELA2 (n = 3) or HAX1 (n = 3) mutations, and CN patients who developed AML (n = 3) after incubating with or without recombinant human G-CSF (10 ng/ml) for 10 min. Cells were immunostained with an anti-phospho-ST A T5 (Y694) antibody and isotype control antibody. (a) Representative histograms showing phSTAT5 (blue) and isotype (red) staining are depicted. (b) Bars show mean fluorescence intensity (MFI) of phospho- STAT5 (Y694) staining in CD34+ cells treated with G-CSF as described above. Data represent means ± s.d. and are derived from two independent experiments, each in triplicate (*P < 0.05, **P < 0.01). (c) SOCS3 mRNA expression in CD33+ cells from G- CSF-treated healthy individuals (n = 3) and G-CSF-treated CN patients (n = 6) was measured by qRT-PCR. SOCS3 mRNA levels were normalized to those of ß-actin and are presented as arbitrary units (AUs). Data represent means ± s.d. and are derived from two independent experiments, each in triplicate (*P < 0.05). (d) G-CSFR mRNA and G- CSFR protein surface expression in CD33+ cells of G-CSF-treated healthy individuals (n = 3) and G-CSF-treated CN patients (n = 6). mRNA expression was measured by qRT- PCR. G-CSFR mRNA levels were normalized to those of ß-actin and are expressed as AUs. Data represent means ± s.d. and are derived from two independent experiments, each in triplicate (*P < 0.05).

We further investigated the effects of activated STAT5 on LEF-1 expression and functions in hematopoietic progenitor cells. We measured LEF-1 and C/EBPα mRNA levels in CD34+ hematopoietic progenitor cells transduced with retroviral constructs expressing (1) WT STAT5a; (2) caSTAT5a, containing H299→R and S711/716→F mutations (STAT5a1*6) and possessing constitutive tyrosine kinase activity; (3) STAT5a Y-F MUT, in which tyrosine 694 of WT STAT5a was mutated; or (4) control retrovirus (ctrl-rv). We found a dramatic downregulation of LEF-1 and C/EBPα in caSTAT5a-transduced cells compared to samples transduced with WT STAT5a, Y-F STAT5a MUT, or ctrl-rv constructs (Fig. 2a).

To analyze the effects of hyperactivated STAT5 on LEF-1 promoter activity, we generated a reporter gene construct comprising 4000 bp of the human LEF-1 promoter, which contains three STAT5 binding sites and five LEF-1 binding sites (Fig. 2b). Co-transfection of HEK293 cells with a LEF-1 expression plasmid and the LEF-1 promoter-luciferase reporter construct resulted in upregulation of LEF-1 promoter activity, confirming LEF-1 autoregulation (Fig. 2b). Co-transfection with caSTAT5a and LEF-1 expression plasmids inhibited LEF-1-dependent autoregulation of the LEF-1 promoter, whereas transfection with caSTAT5 alone had no effect (Fig. 2b). Moreover, co-transfection with WT STAT5a or STAT5a Y-F MUT had no inhibitory effect on LEF1-mediated LEF-1 promoter autoregulation (Fig. 2b).

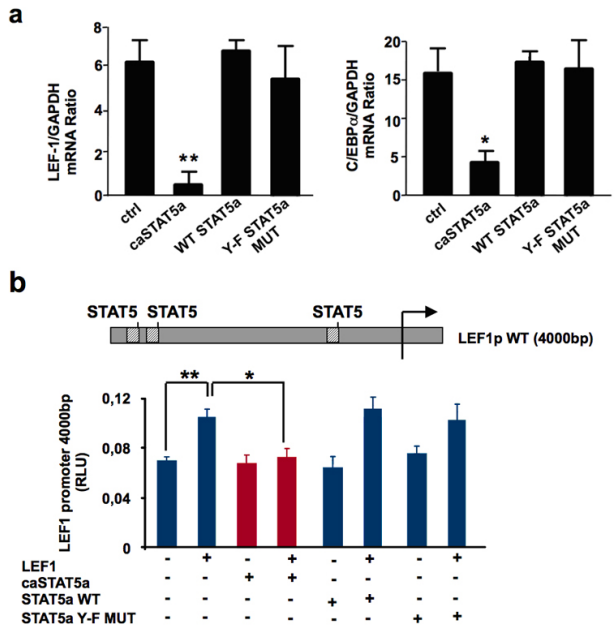

Fig. 2: caSTAT5a inhibits the transcriptional activity of the LEF-1 promoter. (a) CD34+ hematopoietic progenitor cells were transduced with a retroviral expression construct for caSTAT5a, WT STAT5a, STAT5a Y-F MUT, or ctrl-rv. After 60 hours of culture in X-VIVO 10 medium (Cambrex) supplemented with 20 ng/ml of interleukin-3 (IL-3), 20 ng/ml of IL-6, 20 ng/ml of thrombopoietin, 50 ng/ml of stem cell factor, 50 ng/ml of Flt3 ligand and 10 ng/ml of G-CSF, GFP-positive cells were sorted, and LEF-1 and C/EBP mRNA levels were measured by qRT-PCR. LEF-1 and C/EBP mRNA levels were normalized to those of GAPDH and are expressed as AUs. G-CSFR surface expression was measured by FACS. Data represent means ± s.d. and are derived from two independent experiments, each in triplicate (*P < 0.05, **P < 0.01). (b) A LEF-1 reporter construct containing a 4000-bp upstream region of the LEF-1 gene harboring three binding sites identified in ChIP assays (top inset) was generated. The effects of exogenously expressed caSTAT5a, WT STAT5a, or STAT5a Y-F MUT on LEF-1 autoregulation were then tested in HEK293 cells co-transfected with the LEF-1 reporter construct. Data represent means ± s.d. and are derived from three independent experiments, each in triplicate (*P < 0.05, **P < 0.01).

We further demonstrated that constitutively active STAT5a (caSTAT5a) inhibited LEF-1-dependent autoregulation of the LEF-1 gene promoter by binding to the LEF-1 protein, recruiting NLK and the E3 ubiquitin-ligase NARF to LEF-1, leading to LEF-1 ubiquitination and a reduction in LEF-1 protein levels (Fig. 3).

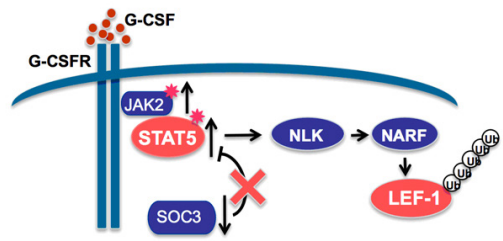

Fig. 3: Schematic representation of the caSTAT5a-dependent degradation of LEF-1 protein in myeloid cells of CN patients. In CN patients, daily treatment with high pharmacological doses with G-CSF results in hyperactivation of STAT5a by phosphorylation owing to elevated JAK2 and diminished SOCS3 expression levels. Hyperactivated STAT5 activates NLK, promoting NLK–NARF interactions and leading to recruitment of the NARF-NLK complex to LEF-1 protein and subsequent LEF-1 ubiquitination and degradation.

Intriguingly, the proteasome inhibitor bortezomib reversed the defective G-CSF-triggered granulocytic differentiation of CD34+ cells from CN patients in vitro, an effect that was accompanied by restoration of LEF-1 protein levels and LEF-1 mRNA autoregulation (Fig. 4 a, b). Taken together, our data define a novel mechanism of LEF-1 downregulation in CN patients via enhanced ubiquitination and degradation of LEF-1 protein by hyperactivated STAT5. Based on these findings, bortezomib could be applyed by congenital neutropenia patients.

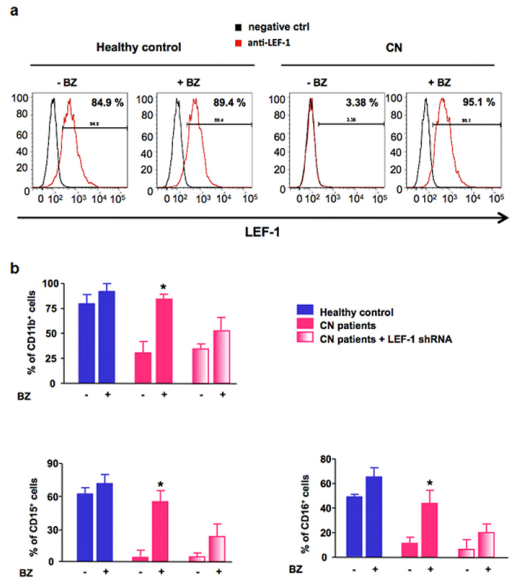

Fig. 4: Restoration of defective LEF-1 expression and activity by treatment of CN CD34+ progenitors with bortezomib. (a, b) In vitro G-CSF-triggered granulocytic differentiation of CD34+ cells from CN patients (CN) and healthy individuals was performed in the presence or absence of bortezomib (10 nM). (a) Intracellular expression levels of LEF-1 protein detected using FACS analysis, as described in Material and Methods. Representative histograms as well as percentages of positive cells are depicted. (b) Some cells from CN patients were transduced with lentivirus-based LEF-1 shRNA constructs. Surface expression of the granulocyte-specific markers, CD11b, CD15 and CD16, were assessed using FACS. Data represent means ± s.d. and are derived from two independent experiments, each in duplicate (*$P < 0.05$).

■ Projektleitung: Prof. Julia Skokowa, MD, PhD; Kooperationspartner: Cornelia Zeidler SCNIR, Johann Meyer Department of Experimental Hematology, Malcolm A. S. Moore Cell Biology Program, Sloan-Kettering Institute, New York, NY, USA.; Förderung: Deutsche Krebshilfe, REBIRTH Cluster of Excellence MHH

Weitere Forschungsprojekte

E-Rare-Verbund: Angeborene Neutropenien mit Mutationen im ELA2-GEN: Klinische Genotyp-Phänotyp Analyse

■ Projektleitung: Zeidler, Cornelia (Dr. med.); Förderung: BMBF, Projektträger im DLR

E-Rare-Verbund: Angeborene Neutropenien mit Mutationen im ELA2-GEN: Epigenetische Veränderungen zusätzlich zu ELANE-Mutationen

■ Projektleitung: Welte, Karl (Prof. Dr. med.); Förderung: BMBF, Projektträger im DLR

Die Analyse der Ursachen der Leukämieentstehung bei Patienten mit schwerer angeborener Neutropenie und de novo AML`s

■ Projektleitung: Skokowa, Julia (Prof. Dr. med. PhD); Förderung: Madeleine Schickedanz Kinderkrebsstiftung

Severe Chronic Neutropenia International Registry

■ Projektleitung: Zeidler, Cornelia (Dr. med.), Welte, Karl (Prof. Dr. med.); Kooperationspartner: Dale, David, University of Washington, Seattle, USA; Förderung: National Institute of Health (NIH), USA

Analysis of the role of NAD+ -dependent protein deacetylase SIRT2 in the pathogenesis of acute myeloid leukemia (AML)

■ Projektleitung: Welte, Karl (Prof. Dr. med.); Förderung: Deutsche José Carreras Leukämie-Stiftung e.V.

Mechanismen der STAT5-Hyperphosphorylisierung, des darauffolgenden Abbaus des LEF-1 Proteins und deren Rolle für die leukämische Transformation bei Patienten mit schwerer angeborener Neutropenie und de novo AML

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD), Welte, Karl (Prof. Dr. med.); Förderung: Deutsche Krebshilfe

Comparison of the hematopoietic differentiation of iPS cells generated from CN and CyN patients carrying same ELANE mutations

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD), Welte, Karl (Prof. Dr. med.); Förderung: Exzellenzcluster REBIRTH

The role of LEF-1 transcription factor and its interaction partner HCLS1 in the inhibition of cellular senescence of leukemic cells

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD), Welte, Karl (Prof. Dr. med.); Förderung: Deutsche José Carreras Leukämie-Stiftung e.V.

Zuteilung von Rechenkontingent am HLRN: Sequence Search in Human Pedigrees with Familial Cancer Syndromes on Whole- and Exome Genome Scale

■ Projektleitung: Welte, Karl (Prof. Dr. med.), Ünalan, Murat (Dr. med., PhD); Förderung: HLRN - Norddeutscher Verbund zur Förderung des Hoch- und Höchstleistungsrechnens

Mechanisms of myeloid differentation and leukemogenesis

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD); Förderung: Exzellenzcluster REBIRTH

Anschaffung von Chips für die Sequenzierung des menschlichen Genoms

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD); Förderung: Benekids e.V.

CHIP Sequenzierungsanalyse Genom-weiter genregulatorischer Effekte durch leukemogene Genmutationen bei Patienten mit schwerer angeborener Neutropenie

■ Projektleitung: Ünalan, Murat (Dr. med.); Förderung: Dieter Schlag Stiftung

Zytokin-Stipendium

■ Projektleitung: Skokowa, Julia (Prof. Dr. med., PhD); Förderung: Tumorzentrum der MHH

Originalpublikationen

Gupta K, Kuznetsova I, Klimenkova O, Klimiankou M, Meyer J, Moore MA, Zeidler C, Welte K, Skokowa J. Bortezomib induces granulocytic differentiation of CD34+ cells from congenital neutropenia patients by reversing hyperactivate-STAT5a-dependent downregulation of LEF-1. Blood 2014;DOI: 10.1182/blood-2012-09-456889

Hennig C, Ilginus C, Boztug K, Skokowa J, Marodi L, Szaflarska A, Sass M, Pignata C, Kilic SS, Caragol I, Baumann U, Klein C, Welte K, Hansen G. High-content cytometry and transcriptomic biomarker profiling of human B-cell activation. J Allergy Clin Immunol 2014;133(1):172-180

Klimenkova O, Ellerbeck W, Klimiankou M, Unalan M, Kandabarau S, Gigina A, Hussein K, Zeidler C, Welte K, Skokowa J. A lack of secretory leukocyte protease inhibitor (SLPI) causes defects in granulocytic differentiation. Blood 2014;123(8):1239-1249

Möricke A, Lauten M, Beier R, Odenwald E, Stanulla M, Zimmermann M, Attarbaschi A, Niggli F, Schrappe M. Prediction of outcome by early response in childhood acute lymphoblastic leukemia. Klin Padiatr 2013;225 Suppl 1:S50-6

Riehm H, Gadner H, Welte K. Die West-Berliner Studie zur Behandlung der akuten lymphoblastischen Leukämie des Kindes-Erfahrungsbericht nach 6 Jahren. Klin Padiatr 2013;225(Suppl. 1):S16-29

Schrappe M, Möricke A, Reiter A, Henze G, Welte K, Gadner H, Ludwig WD, Ritter J, Harbott J, Mann G, Klingebiel T, Gruhn B, Niemeyer C, Kremens B, Niggli F, Debatin KM, Ratei R, Stanulla M, Beier R, Cario G, Schrauder A, Zimmermann M. Key treatment questions in childhood acute lymphoblastic leukemia: results in 5 consecutive trials performed by the ALL-BFM study group from 1981 to 2000. Klin Padiatr 2013;225 Suppl 1:S62-72

Skokowa J, Welte K. Defective G-CSFR signaling pathways in congenital neutropenia. Hematol Oncol Clin North Am 2013;27(1):75-88, viii

Stankov MV, El Khatib M, Kumar Thakur B, Heitmann K, Panayotova-Dimitrova D, Schoening J, Bourquin JP, Schweitzer N, Leverkus M, Welte K, Reinhardt D, Li Z, Orkin SH, Behrens GM, Klusmann JH. Histone deacetylase inhibitors induce apoptosis in myeloid leukemia by suppressing autophagy. Leukemia 2014;28(3):577-588

Welte K. Forschung in der Pädiatrischen Hämatologie [Deutschland, 1970-2000]. Klin Padiatr 2013;225(Suppl. 1):S45-9

Zeidler C. Primäre und sekundäre Neutropenie. Z Rheumatol 2013;72(7):663-668

Abstracts

2013 wurden 14 Abstracts publiziert.

Promotionen

Dittrich, Tino (Dr. rer. nat.): Role of NAMPT/Sirt dependent deacetylation of tumor suppressors p53 and FOXO3a in AML cells.

Wissenschaftspreise

Skokowa, Julia (Prof. Dr. med.): Zytokin-Preis.

Auszeichnungen

Welte, Karl (Prof. Dr. med.): Ehrenmitglied der Deutschen Gesellschaft für Hämatologie und Onkologie.

Skokowa, Julia (Prof. Dr. med.): Auswahl für Best-of-ASH (American Society of Hematology).

Klimiankou, Maksim: ASH, Abstract Achievement Award.

Klimenkova, Olga: ASH, Abstract Achievement Award.

Samareh Abolhasani, Bardia: ASH, Abstract Achievement Award.

Weitere Tätigkeiten in der Forschung

Welte, Karl (Prof. Dr. med.): Mitglied des Hochschulrates der MHH; Vorsitzender des wissenschaftlichen Beirates der Deutschen José Carreras Leukämie Stiftung; Mitglied der Deutschen Akademie der Naturforscher Leopolodina.

Skokowa, Julia (Prof. Dr. med., PhD): Editorial Board Mitglied von Blood Journal; Reviewer Nature Medicine und Blood.