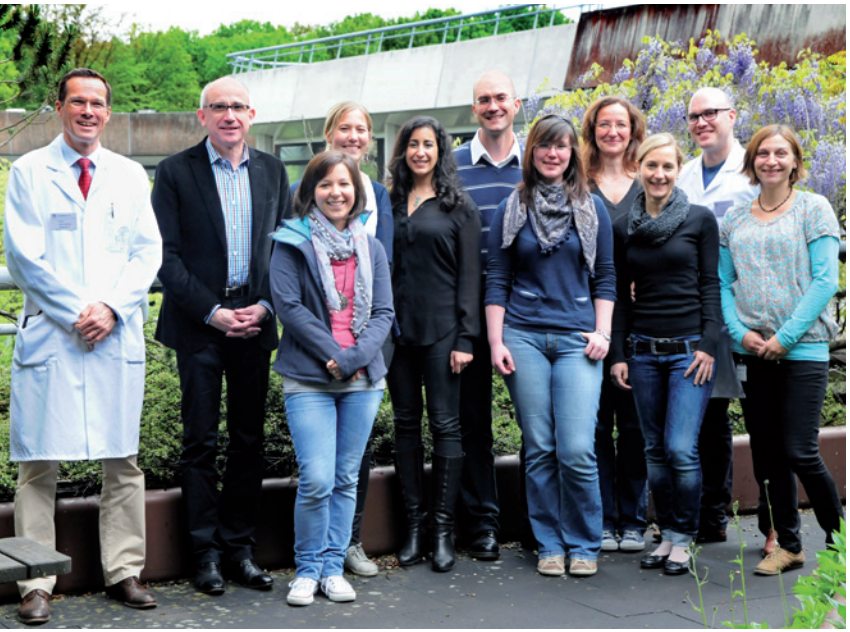

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Department of Internal Medicine III

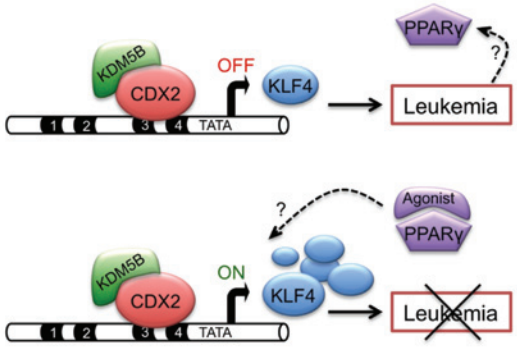
Characterization of Pathogenic Lesions and Development of Novel Therapies in Patients with Hematopoietic Malignancies and Solid Tumors.

Head: Hartmut Döhner

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, but the underlying pathomechanism remains unclear. Billy Jebaraj focuses on: (i) mutations in signaling pathways in CLL; and (ii) the pathogenic link between telomere attrition in a genetically related disease known as mantle cell lymphoma (MCL). Telomere length was found highly variable in MCL, and telomere dysfunction in MCL was evident from a comparison with normal B-cells but had no significant association with any biological or clinical feature. This was in contrast to CLL where a significant correlation of short telomeres with poor prognostic subgroups was confirmed. This indicates that, as opposed to CLL, telomere length is not of prognostic relevance in MCL (Jebaraj, Blood 2012).

Recently, we have aimed to elucidate the biological basis of Acute Myeloid Leukemia (AML) by means of gene expression profiling (GEP), SNP microarray analysis, DNA methylation profiling, and next generation sequencing (NGS) approaches. Currently, we are following up selected newly identified leukemia-associated gene mutations, such as cohesion factor complex aberrations, in order to investigate their functional relevance. Furthermore, by whole exome sequencing within a longitudinal study of paired diagnostic and relapse AML samples, we are focusing our efforts on genomic aberrations associated with treatment resistance and clonal evolution (PhD project Sibylle Cocciardi).

Several projects of the department are centered on the identification of molecular abnormalities in hematopoietic and epithelial malignancies that are important for the initiation and/or maintenance of the transformed phenotype Katrin Faber has used transcriptional profiling followed by functional hit validation to search for genetic vulnerabilities in specific subtypes of AML. She was able to identify the tumor suppressor KLF4 as a target of the leukemogenic oncogene CDX2, which is aberrantly expressed in 90% of patients with AML. In addition, she could show that PPARg agonists derepressed KLF4 and were preferentially toxic to $CDX2^{+}$ leukemic cells, thus opening a new route for drugging AML through modulation of PPARg signaling (Faber et al., JCI 2012). Britta Stolze



Model of CDX2 actions in AML development. Top panel: Aberrant CDX2 expression leads to downregulation of KLF4 ("OFF") through binding to distinct sites in the KLF4 regulatory region and recruitment of the H3K4 demethylase KDM5B, thereby contributing to leukemogenesis. Aberrant CDX2 expression also causes deregulated PPARγ signaling via an unknown mechanism. The bottom panel shows that PPARγ agonist treatment upregulates KLF4 expression ("ON"), thereby inhibiting the viability and proliferation of AML. Further studies are required to elucidate the mechanism whereby aberrant CDX2 expression and loss of KLF4 cause deregulated PPARγ signaling (dashed arrows).

is applying large-scale proteomic and phospho-proteomic strategies to determine the mechanism underlying the selective requirement for the serine/threonine kinase STK33 in cancers driven by mutations in KRAS, the most frequently mutated human oncogene with a high prevalence in lung, colon and pancreatic cancer.

Much evidence implicates non-coding RNAs, such as microRNAs (miRNAs), as contributing factors in the pathogenesis of hematological neoplasms. Based on a microfluidics screen and identifying characteristic miRNAs in hematopoietic subpopulations, we centered on multiple functional screens to identify the functional role of candidate miRNAs in normal and malignant hematopoiesis (PhD Project Kathrin Krowiorz). In parallel, we further investigate epigenetic mechanisms regulating the expression of miRNAs during leukemogenesis (PhD project Edith Schneider).

Recently, circulating miRNAs were discovered in blood and were found to reflect the presence of malignant and non-malignant diseases. Therefore, we explore the potential of circulating miRNAs as biomarkers in leukemias and investigate their functional role in cell-to-cell communication (PhD project Sarah Grasedieck).

Another key aspect in leukemia treatment strategies is the immune system. We have been focusing on leukemia-associated antigens such as epitopes derived from $NPM1^{mut}$, PRAME, RHAMM or WT1. We analyzed their expression in sorted cell populations of fresh AML patient cells using CD34 and CD38 markers. Gene expression analyses (Affymetrix/Taq-Man) have been performed that show significant expression differences in the leukemic stem cell, such as fraction, the hematopoietic stem cell fraction and the bulk, for different LAAs. We have been validating these results using functional assays, such as CFUs and chromium release assays, to define one target structure that might be suitable for an immunotherapeutic approach (PhD project Vanessa Schneider).

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Selected Publications:

- *Dolnik A, Engelmann JC, Scharfenberger-Schmeer M, Mauch J, Kelkenberg-Schade S, Haldemann B, Fries T, Krönke J, Kühn MWM, Paschka P, Kayser S, Wolf S, Gaidzik VI, Schlenk RF, Rücker FG, Döhner H, Lottaz C, Döhner K, Bullinger L (2012): Commonly altered genomic regions in acute myeloid leukemia are enriched for somatic mutations involved in chromatin-remodeling and splicing. Blood. 120(18):e83-92.*
- *Jebaraj BMC, Kienle D, Lechel A, Mertens D, Heuberger M, Ott D, Rosenwald A, Barth T, Möller P, Zenz T, Döhner H, Stilgenbauer S (2012): "Telomere Length in Mantle Cell Lymphoma." Blood. Epub ahead of print*
- *Faber K, Bullinger L, Ragu C, Garding A, Mertens D, Miller C, Martin D, Walcher D, Döhner K, Döhner H, Claus R, Plass C, Sykes SM, Lane SW, Scholl C, Fröhling S (2013): CDX2-driven leukemogenesis involves KLF4 repression and deregulated PPARγ signaling. J Clin Invest. 123(1):299-314.*
- *Grasedieck S, Schöler N, Bommer M, Niess JH, Tumani H, Rouhi A, Bloehdorn J, Liebisch P, Mertens D, Döhner H, Buske C, Langer C, Kuchenbauer F (2012): Impact of serum storage conditions on microRNA stability. Leukemia. 26(11):2414-6.*
- *Schneider V, Egenrieder S, Götz M, Herbst C, Greiner J, Hofmann S (2012): Specific immune responses against epitopes derived from Aurora kinase A and B in acute myeloid leukemia. Leuk Lymphoma. Epub ahead of print*
- *Hofmann S, Götz M, Schneider V, Guillaume P, Bunjes D, Döhner H, Wiesneth M, Greiner J (2013): Donor Lymphocyte Infusion Induces Polyspecific CD8+ T-Cell Responses With Concurrent Molecular Remission in Acute Myeloid Leukemia With NPM1 Mutation. J Clin Oncol. 31(3):e44-7.*