Research in the Department of Hematology and Cell Therapy

Blood Basics

The bone marrow of a healthy adult produces around 400,000,000,000 cells every day. Half of these become red blood corpuscles responsible for transporting oxygen around the body, while the others become white cells with a variety of functions in immunity. Normal blood is deep red simply because most white cells have a life-span of just hours or days while the red corpuscles stay in the bloodstream for 3 months before being destroyed and replaced.

Leukemia (Greek “white blood”) describes an accumulation of white cells resulting from a disturbance in the balance of cell production in the marrow. In order to devise new and better treatments, we need to understand how normal blood cell production (“hematopoiesis”) is controlled and how these controls become overridden in leukemias.

More than half a century of research in this area has revealed the ground plan of hematopoiesis, with a single type of multi-potential hematopoietic stem cell giving rise to 8 major blood cell types in a hierarchical fashion. The stem cells themselves spend most of their time in a quiet “niche” in the bone marrow, dividing only rarely. The daughter cells that are produced move out and undergo multiple rounds of division to generate the large cell numbers required. As these cells divide they also undergo differentiation, becoming progressively more committed to one of the 8 major lineages before ceasing division, maturing and finally entering the blood stream.

Cell division and differentiation are influenced by signals in the form of growth factors and hormones delivered by the blood stream, the nervous system or by local bone marrow “stroma” cells. Each individual signal is recognized by a specific receptor on the surface of the hematopoietic cell. Binding of the signal to the receptor triggers a cascade of events inside the cell (“signal transduction”) that can affect survival, migration, proliferation and differentiation by changing the pattern of gene expression. Importantly, the receptors, signal transduction and gene expression components are themselves encoded by genes and it is here that we find many of the changes (mutations) that are associated with diseases such as leukemia.

The Department

Head – Prof. Dr. med. Uwe Platzbecker

The ultimate aim of all our research is to provide new knowledge and new tools that improve the lives of patients. This means translating results into applications: a process that requires close cooperation between clinical and laboratory scientists, beginning with the identification of those questions most likely to shape future clinical practice and going on to employ interdisciplinary strategies that combine our strengths and resources to maximum effect. Interaction at this level is helped by the location of our research laboratories in the José Carreras building, in direct contact to the out-patient department, diagnostic lab and GMP cell processing unit.

With the appointment of Professor Uwe Platzbecker to the directorship in 2018 and the renaming of the Department of Hematology and Cell Therapy we prepare to build on the achievements of recent years while extending our research portfolio to reflect new opportunities in the field. Specifically, Professor Platzbecker brings a fresh impulse with his internationally renowned expertise and achievements in the field of myelodysplastic disease. In addition to leading a number of high profile clinical trials in this area, he is head of the MDS centre of excellence in Dresden, Chair of the European Hematology Association MDS working group and coordinator of the European Myelodysplastic Syndromes Cooperative Group EMSCO (http://www.emsco.eu).

The myelodysplasias are now recognized to involve dysregulated interaction between developing blood cells and the stromal cells that support them. Both cell types accumulate mutations in a spiral
of events leading ultimately to imbalanced blood cell production and an associated risk of developing leukemia. Studies of MDS therefore provide a new window onto cell interactions that are relevant to normal hematopoiesis, to MDS itself and to leukemia, but have previously been very difficult to access. We look forward to a high degree of synergy between these research areas.

- **Treatment of MDS.**
  Platzbecker U.

- **Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZ2): an open-label, multicentre, phase 2 trial.**

**Cell and Gene Therapies**

The cell therapy program in Leipzig follows a tradition dating back to the first allogenic stem cell transplant in 1980. Since then, the program has grown steadily and we now perform around 150 cell therapies per year. Keeping in step with clinical and technological advances, we have adopted a range of procedures to enrich or deplete active cell populations in order to achieve the best possible results for each patient, and established the production of clinical grade mesenchymal stem cells in culture. The MSCs are the first “advanced therapeutic medicinal product” to be produced on site. All of our cell processing is carried out under strictly controlled conditions in our licensed GMP facility. The experience that we have accumulated in this area is now allowing us to interact closely with companies developing genetically manipulated cell therapy products, giving us early access to this new generation of therapies either as commercially available products with market approval, or in the context of clinical trials.

**Clinical Trials**

Clinical trials are the front line of translational research, where innovation is tested in carefully designed and controlled studies to ensure that patients receive the best available standard of care as well as access to new experimental therapies with the potential to improve results even further. As novel therapies move towards more specific targeting of individual diseases, the range of clinical trials increases accordingly. In 2018, the Department of Hematology and Cell Therapy treated patients in 30 such studies, testing a wide range of new drugs and drug combinations as well as novel cell and gene therapies. Among these was the first multinational phase 3, placebo controlled, double blinded trial of mesenchymal stem cells for Graft versus Host disease, funded by a grant from the European union (https://www.rethrim.eu).

**Detecting and Monitoring Disease**

AML - Dr. med. Sebastian Schwind
CML - Prof. Dr. med. Thoralf Lange / Dr. med. Georg Franke / Dr. rer.nat. Jacqueline Maier

Hematological diseases are diverse and heterogeneous, their characteristics reflecting both the cells type affected and the lesions (usually genetic mutations) responsible. As new therapeutic options become available, it is increasingly important to define and characterize each patient’s disease precisely in order to then select the best possible treatment. While the established techniques of
microscopy, FACS analysis of cell surface markers and high resolution cytogenetics continue to play an essential role in this respect, more recent technological advances are allowing us to look even deeper into the genetic material and to uncover new information relevant to diagnosis and therapy. We are developing and using these techniques to provide a high resolution profile of the disease before treatment, but also enable us to monitor the response very closely during and after treatment.

In the group of Sebastian Schwind, a systematic analysis of acute myeloid leukemia patients receiving a stem cell transplantation has shown that those patients in whom remnant leukemic cells are detectable by a characteristic mutation in the npm1 gene immediately prior to transplantation are at a higher risk of experiencing a recurrence of the disease afterwards, suggesting that these patients should be monitored particularly closely. Further work has shown that previously described associations between specific mutations and the results of therapy may not apply to all patient groups, with mutations in the dnmt3a gene being relevant for younger, but not older patients.

In the group of Georg Franke and Jacqueline Maier working in the area of chronic myeloid leukemia, we continue to work with a commercial partner to establish a “digital PCR” test that is not only sensitive enough to detect a single leukemic cell in 100,000 normal ones, but also sufficiently precise to register the slightest increase over time. The aim here is to pick up potential relapses long before the patient notices any symptoms and to allow the clinician to adjust the therapy in order to avoid, rather than treat, an impending relapse.

- Digital droplet PCR-based absolute quantification of pre-transplant NPM1 mutation burden predicts relapse in acute myeloid leukemia patients.  

- Prognostic relevance of DNMT3A R882 mutations in AML patients undergoing non-myeloablative conditioning hematopoietic stem cell transplantation.  
  Schmalbrock LK1, Bonifacio L1, Bill M1, Jentzsch M1, Schubert K1, Grimm J1, Cross M1, Lange T1, Vucinic V1, Pönisch W1, Behre G1, Franke GN1, Niederwieser D1, Schwind S1.  

- Optimized Digital Droplet PCR for BCR-ABL.  
  Maier J, Lange T, Cross M, Wildenberger K, Niederwieser D, Franke GN.  

**Molecular mechanisms**  
*Prof. Dr. med. Gerhard Behre*

While advances in molecular diagnostics are already improving our ability to classify leukemias and to monitor the response to therapy, the development of novel therapies targeted towards specific diseases is a long term aim that requires an understanding of the mechanisms by which leukemias arise. It is therefore important to determine how changes in the genetic material change cell behavior, for instance by increasing survival and proliferation, blocking differentiation or enabling abnormal cells to escape recognition by the immune system. Among the regulators of these processes, much attention is currently focused on microRNAs (miRNAs). These small RNA molecules are themselves gene products that, once made, can regulate the production of functional proteins from whole groups of other genes. In this way miRNAs coordinate many cellular processes, including the survival, proliferation and differentiation programs that are disrupted in leukemia. The group of Gerhard Behre has identified miRNAs that are expressed only by particular types of leukemic cells and others that can be found only in their non-leukemic counterparts. On the one hand, this
information can now be used to further improve the power of molecular diagnostics. On the other hand, the miRNAs themselves are potential targets for leukemia-specific therapies and we are currently working on strategies by which the activity of key mRNAs can be reduced or increased in leukemic cells.

- **miR-451a abrogates treatment resistance in FLT3-ITD-positive acute myeloid leukemia.**

- **Disruption of the C/EBPα-miR-182 balance impairs granulocytic differentiation.**

**Metabolism**

*PD Dr. Michael Cross*

Blood cell production has long been known to be regulated by growth factor signals that influence the survival, proliferation and differentiation of specific cell types. However, recent evidence suggests that hematopoiesis requires not just on specific signals, but also particular metabolic environments. Furthermore, the metabolism of leukemic cells tends to differ from that of normal cells, making cell metabolism a target for selective therapies. The group of **Michael Cross** is therefore combining specialized cell culture and analytical biochemistry techniques to characterize the metabolic demands and preferences of normal hematopoietic stem and progenitor cells and of their leukemic counterparts. By mass spectrometric analysis of sera used for stem cell culture, we have identified metabolites that positively or negatively affect the ability of stem cells to self-renew (produce more stem cells), while a proteomic analysis of mitochondria isolated from differentiated cells has revealed key differences in the metabolism used by progenitors destined to make red or white cells. Our current work is exploring the ways in which metabolic and signaling pathways interact to determine cell fate and examining in more detail the metabolic characteristics of leukemic cells. The potential applications of this work include the identification of metabolic targets for therapy, the development of culture-based diagnostic tests and the optimisation of culture conditions for blood cell production in bioreactors.

- **Phospholipase A2 products predict the hematopoietic support capacity of horse serum.**
  Ditz T, Schnapka-Hille L, Noack N, Dorow J, Ceglarek U, Niederwieser D, Schiller J, Fuchs B, Cross M.

- **Features of lineage-specific hematopoietic metabolism revealed by mitochondrial proteomics.**