MAPPING MUTATIONS
moving genetics forward

Knowledge of CLL genetics continues to increase exponentially due to new technologies. Dr. Stephan Stilgenbauer (University of Ulm, Germany) is well known for his expertise in CLL genetics. For his CLL Global Alliance project, he and his colleagues are performing whole genome sequencing of CLL cells.

Whole genome sequencing provides raw data on all six billion nucleotides in an individual's DNA. (Nucleotides are the building blocks of DNA, represented by the letters C, G, A, T.) Deletions or abnormalities in the genetic code (the sequence of the nucleotides) contribute to the development of cancer and determine its clinical course. Cells are exposed to external and toxic stress, leading to mutations or "mistakes" that change the genetic code in the cell. While most of these mistakes will be silent or repaired, some of these mistakes alter critical genes, leading to a growth of cancer cells.

Modern gene sequencing technology holds the potential to provide unprecedented insights into the mutational signatures associated with different disease courses of CLL. While mutations located in key areas of certain chromosomes are known, a precise map of mutations of the CLL genome is currently missing. Dr. Stilgenbauer hopes to draft a map of mutations to answer key clinical questions necessary to move CLL research forward.

In a recent interview, Dr. Stilgenbauer discussed the evolution of the field of genetics and work related to his CLL Global Alliance project.

WHY IS GENETICS SO IMPORTANT FOR RESEARCH?

Genetics is important for research because the genome of the cell is the primary determinant of its behavior. Changes to the genome, particularly changes that translate through RNA and proteins lead to disease and also affect the outcome of patients. Understanding genetics is one of the most important aspects in curing any disease.

ARE THERE DIFFERENCES IN HOW RESEARCH IS CONDUCTED IN GERMANY VERSUS THE UNITED STATES OR ELSEWHERE?

The differences are mostly related to the size of the university hospitals and medical centers. In the US you have

Dr. Stephan Stilgenbauer

these big medical centers like MD Anderson, Dana-Farber or Memorial Sloan-Kettering. They are, as far as I recognize, mostly single centers which encompass lab research and clinical research. In Europe, most centers are smaller. Therefore, institutions have a longer tradition of more collaborative research and cooperative trial groups. We have large medical centers such as Köln, Ulm, or Munich, but still they are not nationwide names that patients travel to from a long distance. Germany is geographically small, and sometimes within an hour's drive patients have the choice of three to five different university medical centers. I think that may be the big difference.

IT IS APPARENT THAT PATIENTS IN THE UNITED STATES ARE BECOMING REALLY INVOLVED IN THEIR CARE. DO YOU NOTICE THE SAME IN GERMANY AS WELL?

Oh yes, fortunately. Patients more and more want to understand as much as possible. There are obviously still patients that say, "Doctor, just tell me what to do." But that proportion I think is decreasing. Patients, rightly so, collect more and more information about their disease and about the different treatments available. Overall, I think we have been living in an era where there was a dramatic explosion with regard to the knowledge of the disease and development of treatment. Obviously as the doctors develop more knowledge, the patients do also.

WHY DID YOU GET INTO CLL RESEARCH?

It was a mixture of mostly good luck and a bit of dedication. I started working with Peter Lichter and Hartmut Döhner, two very important genetics and CLL scientists, just at the outset of their careers. There was limited information at the time regarding CLL genetics, but technical developments such as FISH, CGH, and DNA sequencing allowed the field to develop very rapidly. Also, when I started attending meetings such as iwCLL, I got to know the CLL authorities like Kanti Rai, Jacques-Louis Binet, Guillaume Dighiero, Daniel Catovsky, Emili Monserrat, and Michael Keating. To me these individuals are not only exceptional doctors and researchers, but most importantly they are very nice people. It was simply an inviting atmosphere and a good spirit of collaboration. They were inspiring each other. That supported my choice very much.

WHAT WAS KNOWN ABOUT BIOLOGY AND GENETICS IN CLL WHEN YOU FIRST BECAME A RESEARCHER?

When I entered CLL research, it was still considered a relatively boring disease. There were not many treatment options and the biology looked like a disease of relatively normal lymphocytes that just float around and do not do much harm. Over the years it has been recognized that there are subgroups of CLL and most importantly that certain molecular and biological findings are very closely linked to the rate of disease progression and the survival time of patients. These subgroups have become very important with regard to prognosis, and obviously we have had a dramatic development with regard to the treatment. We have opportunities not only to find out something about biology but to also use that biology to direct our therapy.

WHAT ASPECT OF CLL RESEARCH IS SHOWING THE MOST PROMISE AT THE MOMENT?

The new genome sequencing approaches will certainly bring about the next genetic revolution by identifying

multiple abnormalities associated with the clinical behavior of the disease. This will allow us, not only to predict how the disease will behave, but also to direct treatments to specific groups of patients.

The link between biology and treatment is already benefiting disease understanding and also the treatment of patients. Agents that focus on specific aspects of CLL biology such as the inhibitors of B-cell receptor signaling and apoptosis regulators clearly hold the greatest promise to bring the next revolution in CLL therapy, driven by biological disease understanding.

HOW IS WHOLE GENOME SEQUENCING PERFORMED?

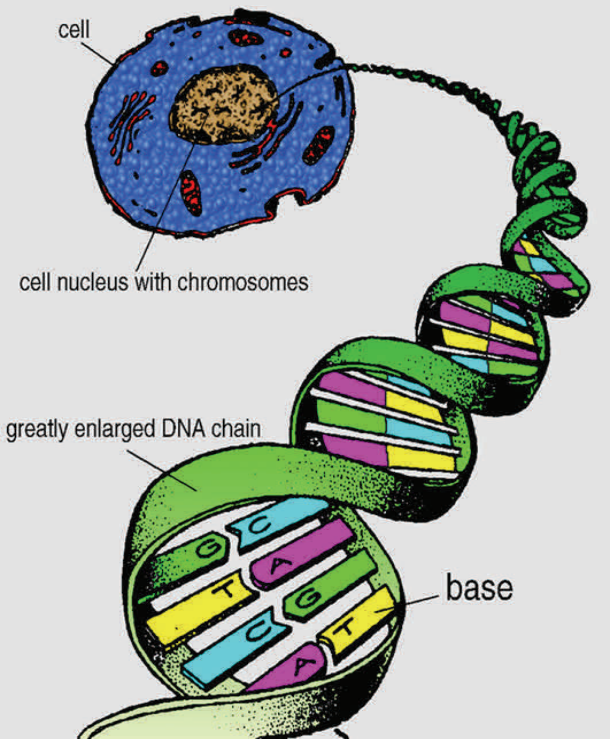
Blood samples are taken from patients, and the CLL cells are separated out. The chromosomes of a cell are unraveled, and the entire content of the DNA is examined. Sophisticated technology determines the nucleotide sequence of every gene. To analyze and understand which genes are mutated, we are sequencing both CLL and normal, healthy cells of patients. We then look intra-individually at what changes are present in the tumor DNA compared to the normal cells to determine mutations. This technique will be performed in several patients, comparing different disease courses and repsonses to various treatments. There will be a significant amount of data to analyze and to compare to establish which genetic mutations are related to CLL.

IS WHOLE GENOME SEQUENCING BETTER FOR CLL RESEARCH THAN OTHER TECHNOLOGIES LIKE FISH?

We hope so, and initial evidence is supporting this. With FISH we know that we are detecting important abnormalities, but we detect only what we look for. With these new approaches we can identify new abnormalities that we cannot detect with FISH. The best approach is not to focus on the genes that we know of, but to take an unbiased approach toward looking at all genomic regions.

SOME PEOPLE HAVE GENETIC CHANGES OVER THE COURSE OF THEIR DISEASE. ARE THERE SPECIFIC CHANGES YOU SEE MORE FREQUENTLY THAN OTHERS?

Genomic abnormalities of the CLL cells can evolve during the disease course or during treatment, something that we call clonal evolution. Among the most frequently acquired new abnormalities is 17p deletion (17p-) or TP53 mutation, often times arriving as a result of DNA



Nucleotides are the building blocks of DNA (represented by C, G, A, T). Scientists are examining every nucleotide in the DNA of CLL cells in hopes of finding small, but potentially significant mutations. Image: myweb.tiscali.co.uktaxia.pages/index.htm.

damage caused during the selective pressure of chemotherapy. Clonal evolution can happen in all subgroups of CLL, but does happen more frequently among patients with unmutated IGVH gene and ZAP70 positivity who appear to be genetically unstable.

IT SEEMS THAT YOU HAVE AN INTEREST IN PATIENTS WITH 17P-. IS THIS TRUE?
17p- or TP53 mutation is probably the strongest factor that we know of that predicts the failure of our current treatment approaches. And certainly I think these patients are the ones who are in most urgent need of improvement. Therefore I think to focus on this patient population will be very rewarding.

82% OF CLL PATIENTS HAVE ABNORMALITIES DETECTED BY FISH (11Q-, 13Q-, T12 AND 17P-). DO YOU THINK THAT THE OTHER 18% HAVE ABNORMALITIES WHICH ARE CURRENTLY UNKNOWN?
One of the thoughts is that among the "normal" cases we will find novel mutations that are emerging from the next generation sequencing. In essence, I think that all cancer cells have mutations; we just have to look sensitively enough for them. Cancer is a genetic disease. It is a bold statement to make, but there is compelling evidence from very different areas of research that argue in that direction.

HOW IS RESEARCH IN GERMANY BENEFITTING ALL CLL PATIENTS?
We have the habit of being structured and organized. The German CLL Study Group offers a very dedicated structure of setting up clinical trials which are basically open to all sites, not only in Germany, but in other countries as well. What has to me always been very important in running strong, quality science projects is bio-banking [the storing of human biological samples for research purposes] within the clinical trials. This allows easy analysis of biological factors of patients. For example, once we have established a list of abnormalities from gene sequencing, we can use banked samples to verify our work. Or we can validate correlations between certain mutations and patients' response to treatment. This benefits all patients. We must go both ways for our patients: from bench to bedside and from bedside to bench.

WHAT MAJOR CHANGES WILL COME IN CLL IN THE NEXT FEW YEARS?
I think the coming years will be full of dramatic developments with regard to biology and treatment. The increasing spectrum of biologically directed therapies will really be the way forward and hopefully one day make classical chemotherapy much less frequently used or even obsolete. Chemotherapy currently is the backbone of our treatment. However, our hope is that within a couple of years at least some patient subgroups get away from chemotherapy and strong candidates are patients with TP53 mutations, patients who do not tolerate chemotherapy or patients with comorbidities.
Many of us believe that new immunological approaches offer tremendous potential. Modified T-cells such as the CAR [chimeric antigen receptor] technology hold great promise; clinical agents such as lenalidomide are very useful drugs through their actions on the immune system; and last but not least, monoclonal antibodies have already changed the treatment of CLL and will continue to do so in the future with the development of new antibodies.