Single-cell sequencing of leukemia therapy: Shared genetic program, patient-specific execution

Researchers at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases in Vienna, the University Medical Center of Regensburg, and the National Institute of Hematology and Infectious Diseases and the Semmelweis University in Budapest have studied the response to targeted leukemia therapy in unprecedented detail, using single-cell sequencing and epigenetic analysis. The paper published in the journal *Nature Communications* uncovers a precise molecular program in patients with chronic lymphocytic leukemia (CLL) who start treatment with the targeted cancer drug ibrutinib. While this program was shared by all patients, the speed of its execution differed widely. These results will help develop personalized strategies for managing CLL as a chronic disease, which is particularly relevant for CLL as a disease of the elderly.

(Vienna, 29 January 2020) Chronic lymphocytic leukemia (CLL) is the most common form of blood cancer (leukemia) in the Western world, affecting approximately 1.2% of all cancer patients.[1] This type of cancer starts with the lymphocytes (a type of white blood cells) that are produced in the bone marrow. CLL is characterized by the proliferation of abnormal lymphocytes (B cells) that fail to mature and grow out of control. These abnormal cells accumulate in the bone marrow and lymph nodes, taking the place of other healthy cell types and impeding their normal development. Finding the most suitable therapy for each patient poses a challenge due to the clinical and molecular heterogeneity of this disease, with some patients facing slow disease progression, whereas others face rapid progression and require quick medical response.

The cancer drug ibrutinib, a Bruton tyrosine kinase (BTK) inhibitor, has remarkable efficacy in most patients with CLL. It is becoming the standard of care for most patients requiring treatment due to its clinical efficacy and mostly tolerable side effects. However, it does not cure the disease, and patients must undergo prolonged periods of treatment. Christoph Bock and his group at CeMM investigated the molecular program with which CLL cells and other immune cells response to ibrutinib treatment in patients with CLL. Their goal was to learn the epigenetic and transcriptional patterns that predict how swiftly the treatment is having an effect on the CLL cells and how long it takes for the disease to respond in each individual patient.

In previous studies, scientists had investigated only specific aspects of the molecular response to ibrutinib, focusing largely on genetic drug resistance or the transcriptome response of cancer cells. For the first time, CeMM researchers provide a comprehensive genome-scale, time-resolved analysis of the regulatory

[1] Data provided by the National Cancer Institute (NIH): https://seer.cancer.gov/statfacts/html/clyl.html

response to this drug in primary patient samples. The authors used a combination of immunophenotyping, single-cell transcriptome profiling (scRNA-seq) and chromatin mapping (ATAC-seq) to jointly monitor the activity, regulation and expression of the CLL cells and other cell types of the immune system. Importantly, they performed this analysis at eight pre-defined time points during the ibrutinib therapy, following seven individual patients over a standardized 240-day period after the start of the treatment.

Through integrative bioinformatic analysis of the resulting dataset, the authors were able to describe at high resolution how ibrutinib induces a very consistent chain of events on cancer cells over time across all patients. They found that ibrutinib first acts right at the center of the CLL cells' activity, causing the genes that establish the cancer cell identity of the CLL cells to shut down, and then puts them in a dormant state. This means that the cancer cells stop dividing but quiescently survive, waiting for the right environment conditions to begin proliferation once again.

The present study by André Rendeiro, Thomas Krausgruber and colleagues is the result of cross-disciplinary collaborations with researchers from the Department of Hematology and Stem Cell Transplantation of the National Institute of Hematology and Infectious Diseases at the Central Hospital of Southern Pest, and the Department of Pathology and Experimental Cancer Research of the Semmelweis University in Budapest (Hungary). It constitutes one of the first high-resolution, multi-omics time series of the molecular response to targeted therapy in cancer patients, and it establishes a broadly applicable approach for analyzing drug-induced regulatory programs, identifying molecular response markers for targeted therapy. Finally, the study could help stratify patients into fast and slow responders based on characteristic molecular markers and open up new directions for the development of ibrutinib-based combination therapies for CLL.

Attached pictures:

1. Co-first authors André Rendeiro and Thomas Krausgruber with co-last author Christoph Bock (© Klaus Pichler/CeMM)
2. Leukemia cells in the course of therapy (computer graphics). Leukemia cells are depicted in green, normal blood cells in red, and the intricate bundles in each cell visualize the complex arrangement of DNA and chromatin in the cell nucleus (© Ella Marushchenko)
3. Visualization of the chromatin structure that folds ~2 meters of DNA into the cell's micrometer-scale nucleus.

The study "Chromatin mapping and single-cell immune profiling define the temporal dynamics of ibrutinib response in chronic lymphocytic leukemia" was published in *Nature Communications* on 29 January 2020 DOI: 10.1038/s41467-019-14081-6

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Christoph Bock is a principal investigator at CeMM. His research focuses on epigenetics and gene regulation in cancer, to help develop better diagnostics and therapies. He is also a guest professor at the Medical University of Vienna, scientific coordinator of the Biomedical Sequencing Facility at CeMM, group leader at the Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, elected member of the Young Academy of the Austrian Academy of Sciences, and co-founder of the Vienna-based startup company Aelian Biotechnology.

The mission of **CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences** is to achieve maximum scientific innovation in molecular medicine to improve healthcare. At CeMM, an international and creative team of scientists and medical doctors pursues free-minded basic life science research in a large and vibrant hospital environment of outstanding medical tradition and practice. CeMM's research is based on post-genomic technologies and focuses on societally important diseases, such as immune disorders and infections, cancer and metabolic disorders. CeMM operates in a unique mode of super-cooperation, connecting biology with medicine, experiments with computation, discovery with translation, and science with society and the arts. The goal of CeMM is to pioneer the science that nurtures the precise, personalized, predictive and preventive medicine of the future. CeMM trains a modern blend of biomedical scientists and is located at the campus of the General Hospital and the Medical University of Vienna. www.cemm.oeaw.ac.at

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