MLL News

December 21, 2021

We Want to Say Thank You: The MLL Annual Review for 2021

In our practice, the MLL MVZ, and in the MLL Munich Leukemia Laboratory, this year has been marked by the covid pandemic – just as it has been for everyone. This has fueled our concern for the patients treated in our practice and affected our entire team. With considerable additional efforts directed toward sanitary measures and vaccination programs both for our patients and our team, we have tried as to minimize the level of uncertainty and insecurity as much as possible. Among other things, this has gained us a vaccination rate of over 90% among our staff and enabled us to vaccinate more than 850 friends of the MLL team as part of a comprehensive vaccination program offered by the MLL MVZ.

Our primary goal at the MLL has been to offer state-of-the-art diagnostics at all times – with short diagnosis times. Along with initiating organizational measures, in part due to staff shortages related to necessary self-quarantining, we have also taken many other steps at the MLL: To the extent permitted by workplace requirements, working at home has been greatly expanded and incorporated within a regular structure. Every procedure has been assessed for its capacity to be digitalized and automated and gradually improved to make the processes even more efficient overall. We are working hard to make our digital Symptoms Query platform and Order Entry System available to all submitters. Using them is of course free of charge. We are adapting and expanding our website for all types of digital communication and information. We are also rapidly incorporating the results of our research into routine diagnostics.

Meriting special mention is the fact that the overall number of samples has increased on average by 15% in 2021 compared to the previously best year of 2020. The growth in molecular genetics was higher than normal, not only in terms of sample numbers, but also with respect to the increasingly complex requirements associated with panel sequencing. For the first time, we have been able to incorporate Whole Genome Sequencing (WGS) into the routine of our hematologically focused evaluation algorithms, especially for very complex cases. One such program ("The Difficult Case") is also available to submitters free of charge as part of our research domain.

In 2021, we have once again been able to contribute to a large number of scientific collaborations worldwide, with both presentations at international and national congresses as well as publications. The data from our routine diagnostics is therefore being used for research projects – in compliance with strict data protection requirements – and publications, resulting in leukemia diagnostics and increasingly specific therapies based on it that are effective long-term, for the benefit of the individual patient.

In routine diagnostics and pilot studies, the use of artificial intelligence (AI) is standard at the MLL for the fields of cytomorphology (peripheral blood and bone marrow differentiation), traditional cytogenetics, 24-color FISH, immunophenotyping, and genome and transcriptome sequencing. We are undertaking large research projects in these areas, including MetaSystems and AWS, which are already assisting us greatly in our daily routine and which will continue to provide a wealth of contributions in the near future. Next year, we will also be involving beta testers from other laboratories so that the products created using AI can be used outside of the MLL as well.

In 2021, we were also accredited by the CAP (College of American Pathologists) for the first time. And, for the third time in a row, we were lauded as a Top 100 Innovator among German SMEs. It continues to be our primary and also future objective to offer state-of-the-art leukemia and lymphoma diagnostics with fast turnaround times for findings reports.

The new WHO leukemia and lymphoma classification planned for next year, which we have been permitted to collaborate on for the first time as MLL authors, will greatly help all of us in hematology

to arrive at an even better understanding of hematological diseases and the therapies used to treat them. We are pursuing this objective more than ever at the MLL: to quickly make the increasingly comprehensive and specific diagnostic findings – especially from cytogenetics and molecular genetics – directly available to every single patient for classification, prognosis assessment, and targeted therapy (precision medicine). This is increasingly the case with "measurable residual disease" (MRD), which is playing an ever greater role in therapy management.

We want to thank all of our employees for their excellent work this year despite the challenging circumstances with which we all have to live right now. We also want to thank our submitters for the trust they place both in and in our diagnostics. Finally, we are grateful to all of our partners for their constructive collaboration, even in times of delivery bottlenecks.

It remains our common goal to use the results of our diagnostics to extend lives and increase healing rates.

We wish you all a peaceful Christmas season and, for all of us, a much more positive outlook for the year 2022.

With best regards,

Prof. Dr. med. Claudia Haferlach
Prof. Dr. med. Dr. phil. Torsten Haferlach
Prof. Dr. med. Wolfgang Kern

Author: Prof. Dr. med. Dr. phil. Torsten Haferlach

Hematological Whole Genome and Transcriptome Sequencing as a New Diagnostic Tool for Acute Leukemias

Various research projects have shown that new technologies such as whole genome sequencing (WGS) and whole transcriptome sequencing (WTS) provide added value for patients with acute leukemia. In the coming year, the MLL will be offering both diagnostics using classical methods as well as WGS/WTS analysis for acute leukemia. We want to pass this added clinical value on to our patients while at the same time learning from the knowledge gained. We also want to put WGS/WTS to the test in order to validate their clinical benefit.

The Hematological Genome and Transcriptome in Routine Diagnostics

In a publication by Duncavage et al. in the New England Journal of Medicine (NEJM. 2021;384(10):924-935), it was shown that whole genome sequencing can reliably gather the information currently being collected by chromosome analysis and molecular genetic methods and that it can also identify other prognostically and therapeutically relevant alterations. In our own projects at the MLL on acute lymphoblastic leukemia (ALL; Walter et al. BMC Cancer. 2021;21(1):886), we also managed to accomplish even more detailed classification of patients using transcriptome analysis. This demonstrates the added value that these new technologies could provide to patients in the future.

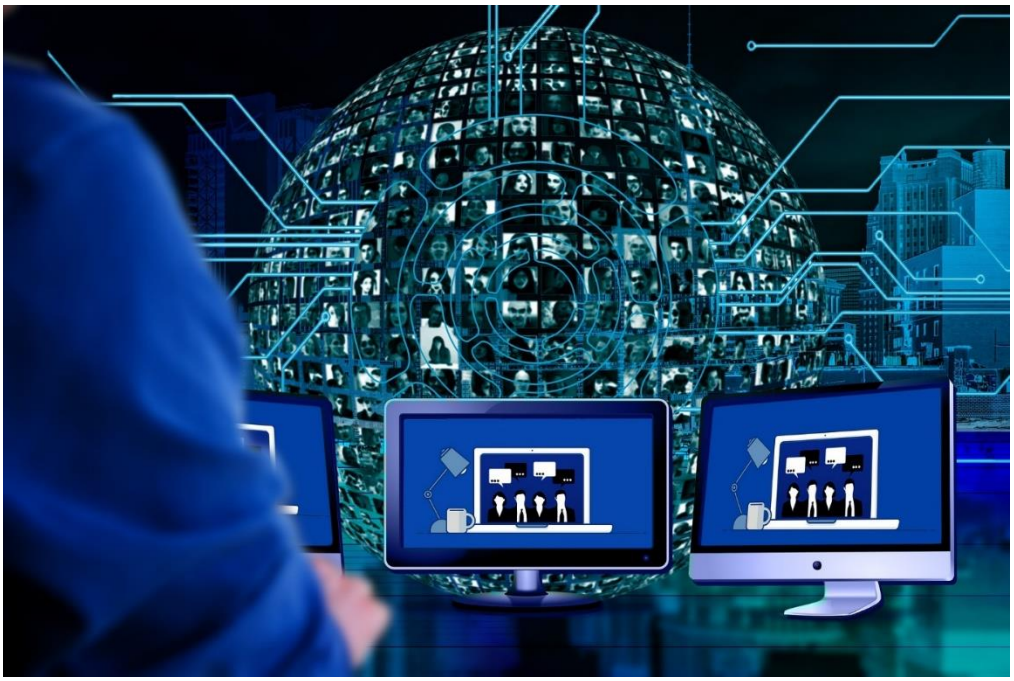
This additional diagnostic benefit for the patient is a major focus for us. We are aware that using whole genome sequencing to diagnose leukemia involves a change for both the doctor and the patient, as it is necessary to provide the patient with specific information and prepare separate declaration of consent. We want to make it very clear that we are conducting an analysis of the "hematological/clinical genome" with our approach. As with conventional molecular genetics, we use bone marrow as our starting material. At the same time, we also use peripheral blood (or separated T cells) to diagnose acute myeloid leukemia in order to eliminate any germline changes from the sequencing data, since the investigation is aimed at somatic changes within the leukemia cells. Any germline predispositions that are unrelated to the acute leukemia are therefore excluded from the investigation, being deliberately sorted out in our analytical pipeline. The preparation process for WGS/WTS diagnostics uses DNA and RNA from the corresponding sample. After sequencing, the data are specifically examined for various diagnostically and prognostically relevant alterations. On the one hand, we examine changes in copy numbers, traditionally covered by chromosome analysis. The WGS analysis produces a higher level of resolution here, also allowing for smaller alterations to be discovered that remain undetected at the resolution of normal chromosome banding analysis. In addition, we also see copy-neutral losses in heterozygosity (CN-LOH) and translocations, which usually lead to fusion transcripts. We detect them at the same time with the transcriptome analysis, the two methods providing us with an internal confirmation. For smaller alterations, such as mutations in individual genes, we study a gene set relevant for hematological neoplasia, which lets us factor in all of the mutation information necessary for the diagnostics and risk stratification. In addition to the detection of fusion genes and overexpressions of the genes involved, the transcriptome analysis also allows for the generation of an expression profile.

Depending on the subtype, this produces a different pattern, particularly for ALL, making classification into specific groups possible. From our research projects, we know that a larger proportion of patients can be assigned to a specific group than is possible using traditional diagnostic methods. If you, the treating doctor, are interested in participating in our study, please send a declaration of consent signed by your patient at the time when you explained AML or ALL, after which the patient can be included and tested using WGS/WTS along with the current gold standard diagnostic method.

In this case, of course, you will also receive a corresponding supplementary report on the results of the WGS/WTS analysis. Naturally, we will cover the costs for the WGS/WTS analysis ourselves.

Author: Dr. rer. nat. Manja Meggendorfer

63rd ASH Annual Meeting & Exposition – a Postscript



The 63rd Annual Meeting & Exposition of the American Society of Hematology was held on December 11-14 – this year as a hybrid event for the first time. The MLL team attended virtually with a total of 13 contributions. "The ASH Annual Meeting is an absolute highlight for us every year. This is where the latest findings from our basic and clinical research are presented. After the congress, we sit down and discuss what adjustments we need to make to our diagnostic offers and to the interpretation of our findings based on the data presented. It is also exciting to see what kind of reactions we get to the data presented, all of which greatly encourages future scientific projects and collaborations," said Prof. Dr. med. Claudia Haferlach, MLL founder and managing director.

The main topics were the continuous improvement of today's gold standard and the diagnostics of tomorrow, including the use of artificial intelligence (AI) in the clinical routine and research, along with genome-wide methods of WGS (whole genome sequencing) and WTS (whole transcriptome sequencing). We have summarized for you all of this year's ASH presentations with MLL participation.

Improvement of Diagnostic Workflows in Molecular Genetics and Immunophenotyping

In molecular genetics, Walter et al. show how the diagnostic spectrum of next-generation sequencing (NGS) can be expanded using a target enrichment process to allow losses in heterozygosity (CN-LOH, copy number neutral loss of heterozygosity) to be detected as well. The prognostic importance of this is gaining in recognition. A commercially available panel for detecting changes in chromosome copy numbers was used for the purposes of enrichment. By combining target enrichment and NGS, CN-LOH was able to be detected with sensitivity and cost-effectively at a shallow depth of sequencing. In a patient cohort of 1,196 patients, 10% had at least one instance of CN-LOH, with 4q, 7q, 9p, and 11q being affected with particular frequency. Another investigation by Hörmann et al. made it clear that expanding the NGS panel for myeloid neoplasia to include the *PIGA* gene also has diagnostic and clinical relevance in cases where PNH is not suspected. In a cohort of 20,320 patients examined using this panel, a *PIGA* mutation was found in 67 patients. Of the 37 patients without a history of PNH, 20 patients were also examined by immunophenotyping, showing that a PNH clone was detectable in the

vast majority of cases (85%). Truncating *PIGA* mutations were highly specific for the presence of a PNH clone.

In immunophenotyping, it was possible for findings from a prospective, multicenter study to lead to the optimization of MDS diagnostics. MDS patients exhibit aberrant immune phenotypes, which, however, are not defined by a single characteristic marker and can instead involve a large number of markers in different cell lines. Only by looking at it as a whole can it be assessed whether the findings of the immunophenotyping are consistent with a diagnosis of MDS. As Kern et al. have shown, only 17 markers contribute significantly to flow cytometry findings of MDS. If an aberrant expression profile can be determined for three of these markers, the correspondence with the cytomorphological diagnosis is already 80%. The data also indicates that the blast cell threshold – currently > 5% and also an important indicator for an immunophenotypic diagnosis of MDS – can be corrected to > 3%.

Using AI to Reach a Diagnosis Quickly and Reliably

The use of AI remains an important topic at the MLL and was once again a major emphasis at the annual ASH conference this year. A total of four presentations on AI were given there, all of which were groundbreaking with regard to the application of AI in routine clinical diagnostics.

Concerning cytomorphology, two projects involving the automatization of blood and bone marrow smear differentiation were presented this year. In a prospective study, presented by Haferlach, T. et al., 10,082 blood smears were obtained both by highly qualified medical technicians as well as by using a tool fully automated from scanning to analysis that had been developed together with MetaSystems and Amazon Sagemaker. The correspondence between the two methods was a stunning 95% for pathogenic cases and will be significantly improved in the future through further training, including with rare cell types. The automated imaging of individual cells from bone marrow smears was also successfully established this year by Pohlkamp et al. – a major step toward automated single-cell image classification, remote diagnosis, and the generation of image galleries for human review and revision. The automatic classification of bone marrow smears will continue to develop rapidly through real-time training based on dynamic data sets.
With immunophenotyping, it has now become possible to analyze and evaluate raw data from flow cytometry matrices using AI, as the project presented by Bellos et al. confirmed. Various machine learning models have already been employed for this purpose: XGBoost, weighted SVC and LinearSVC, hierarchical model, and AutoGluon. This has made it possible for impressive R and P values to be achieved. In the future, the focus will not only be on improving existing models but also on identifying further sub-entities and applying transferred learning to enable the universal use of the tool for flow cytometry data.
One exciting project in the area of molecular genetics was presented by Nadarajah et al., in which WGS and WTS data is interpreted using an AI tool. The goal here is to initially predict a final diagnosis without any human input. The complexity of such data exceeds the capabilities of purely manual analysis, meaning that an AI tool is able to compensate for any loss of data. It was possible for an overall precision of 85% to be achieved, with hard-to-distinguish entities such as MGUS/MM or MDS-EB-2/AML/CMML playing a major role. In independent tests, a precision of up to 100% was able to be achieved for clearly defined entities. The tool will be made available via a web application. The results of the automated decisions are presented transparently in the form of a clear visualization, thus ensuring comprehensibility for users.

WGS and WTS – Increasing Knowledge and the Path to Routine Diagnostics

WGS and WTS combine the diagnostic disciplines of chromosome banding analysis (CBA), fluorescence in situ hybridization (FISH), and molecular genetics. Like CBA, both WGS and WTS allow

genome-wide insights – albeit at a nucleotide base size resolution. As the previously presented study by Nadarajah et al. underscores, genetic characterization using genome-wide methods is so comprehensive that it has already enabled AI-assisted classification of diseases even today. Along with this, research on genome-wide data is also increasing our knowledge of pathogenetic mechanisms.

For example, a prognostically negative influence of *TP53* aberrations (due to mutations, deletions, or CN-LOH) has been described and validated for various forms of neoplasia. In one large cohort of 4,646 patients, Stengel et al. systematically evaluated the frequency and composition of *TP53* alterations and their clinical impact on 29 cases of neoplasia. It was detected in 13% of these patients, revealing a general association between lymphatic entities and *TP53* aberrations. In addition, a clear association between *TP53* alterations and a complex karyotype was detectable for the entire cohort as well. For HGBL, MZL, and T-NHL, a *TP53* aberration did not affect overall survival. There was a negative prognostic influence in patients with MCL and MPAL, but this was independent of whether one or both alleles ("double hit") were affected. For the remaining entities, the presence of a *TP53* alteration shortened overall survival, an effect that was increased for cases with a double hit. The study by Wahida et al. furnishes an interesting insight into the biological impacts of *TP53* mutations. In this study, telomere length and telomerase activity were studied using WGS and WTS in patients with AML, MDS, and PNH and compared with data from healthy subjects. Due to the high level of replication stress to which strongly proliferating tumor cells are exposed, shortened telomeres usually represent a characteristic feature of cancer. Contrary to this expectation, elongated telomeres were found in one subgroup of the AML cohort in which an association with *TP53* mutations was discovered. Moreover, there was also a correlation between telomere length and the *TP53* mutation burden as well as telomerase activity and the *TP53* mRNA level.

A diagnostic gap was able to be filled by WGS. Up until now, it was not possible to detect microdeletions either by sequencing or by chromosome analysis. Only the use of FISH probes permits a targeted detection, although not comprehensive screening. The abstract by Baer et al., for which various myeloid entities were tested for microdeletions, showed that this can be achieved by means of WGS. *RUNX1* and *TET2* were the genes most frequently affected. Other deletion mutations were found mainly in genes for which a loss-of-function profile is known from myeloid neoplasia. In addition to mutations, microdeletions represent yet another mechanism by which functional gene loss can occur.

Can WGS and WTS measure up to the current gold standard in order to establish themselves in clinical routine? The abstracts by Haferlach, C. et al., Truger et al., and Hörmann et al. provide a clear answer for a range of different entities. For acute leukemia and multiple myeloma, there is a high level of correspondence between findings from routine diagnostics and WGS/WTS. In the vast majority of cases, alterations not detected by genome-wide methods were attributable to small clone sizes or a low mutation burden. In addition, duplications of the entire set of chromosomes (e.g., tetraploidy) cannot be identified using WGS. Against this stand the benefits of genome-wide methods. In the case of ALL, this already starts during the processing of samples – while the low *in vitro* proliferation of the ALL cells is a limiting factor for cytogenetics, only purified nucleic acids are needed for WGS and WTS. In addition, the ALL subtypes of Philadelphia-like ALL and of ALL with *DUX4* rearrangement can be identified easily using WTS, while they are difficult to map with the current gold standard. What is more, rare and cytogenetically cryptic rearrangements along with numerous chromosomal changes can also be detected using WGS/WTS. Last but not least, by providing information on the mutation status, WGS also enables the search for molecular genetic markers relevant to diagnosis, prognosis, and therapy. For mastocytosis, detecting the characteristic *KIT* D816V mutation using WGS was only possible in 21% of the patients. Here, too, the generally low mutation burden is assumed to be the cause. Even for the example of mastocytosis, however, the clear advantage of the genome-wide methods was the comprehensive genetic characterization they provide; at least one non-*KIT* mutation

was able to be detected in 46% of the patients, while cytogenetic aberrations were detected in 21%. The detected alterations also had an impact on the prognosis; patients with non-*KIT* mutations and chromosomal changes exhibited the shortest overall survival. If there was only one type of aberration (mutation or chromosomal change), survival was already reduced compared to patients without any aberrations.

Author: Dr. rer. nat. Ines Schmidts

MLL Dx Successfully Accredited by the College of American Pathologists (CAP)

MLL Dx, the sister company of the Munich Leukemia Laboratory (MLL) with its MLLSEQ brand, was appraised by the College of American Pathologists (CAP) and successfully accredited according to their requirements. Since 2019, MLL Dx has been accredited according to the international standards DIN EN ISO 15189 "Medical Laboratories – Requirements for Quality and Competence" and DIN EN ISO/IEC 17025 "General Requirements for the Competence of Testing and Calibration Laboratories" by the Deutsche Akkreditierungsstelle (German Accreditation Body – DAkkS).

The CAP is a medical organization founded in 1946, to which around 18,000 certified pathologists belong. The task of the CAP is to promote and represent the best practices in pathology and laboratory medicine. The CAP offers medical laboratories accreditations and suitability tests and also publishes checklists with requirements for conducting laboratory tests. Their standards are accepted as the strictest and most demanding in the medical industry. The CAP accreditation system for laboratories is based on globally, objectively verifiable quality standards, thereby generating both comparability and mutual trust.

"The fact that MLL Dx has been successfully accredited by the CAP is very important to us. Quality and constant improvement are a high priority for us and play an enormous role in our everyday work. As with our DakkS accreditations, the CAP accreditation confirms that we adhere to very high quality standards," said Prof. Dr. med. Wolfgang Kern, founder and managing director of the MLL.

MLL Dx was founded in 2017 as a sister company of the MLL and offers comprehensive leukemia and lymphoma diagnostics for foreign patients and as part of clinical studies. The services and knowledge of the MLL and MLL Dx are directly interconnected, guaranteeing patients the best possible diagnostics according to the current state of the art. Both companies have embodied high quality and constant improvement since they were founded.

The CAP accreditation certificate can be found here. Click here to access the CAP website. More information on the MLL Dx or MLLSEQ services, methods, and offers can be found at www.mllseq.com.

Author: Dr. rer. nat. Christine Käppel

Important Dates

MLL Academy 2022

The next MLL Academy will take place from April 25 to 29, 2022, as a virtual event. At the five-day workshop on the topic "State-of-the-art diagnostics in hematological malignancies," the 15 participants can expect a mixture of theoretical and practical content, along with joint discussions on leukemia and lymphoma diagnostics. Registration is still possible up until February 28, 2022.

More information about registration can be found here.

Most Recent Publications with MLL Involvement

- Baer C et al. Detection of ABL1 kinase domain mutations in therapy naïve BCR-ABL1 positive acute lymphoblastic leukemia. Haematologica. 2021. Open publication
- Bendig S et al. Diagnostic challenge of identifying cases with recurrent t(8;14)(q24.21;q32.2) Involving BCL11B in acute leukemias of ambiguous lineage: an analysis of eight patients. Leuk Lymphoma. 2021. Open publication
- Gurnari C et al. TET2 mutations as a part of DNA dioxygenase deficiency in myelodysplastic syndromes. Blood Adv. 2021. Open publication
- Heuser et al. 2021 Update Measurable Residual Disease in Acute Myeloid Leukemia: European LeukemiaNet Working Party Consensus Document. Blood. 2021. Open publication
- Kongkiatkamon S et al. Molecular characterization of the histone acetyltransferase CREBBP/EP300 genes in myeloid neoplasia. Leukemia. 2021. Open publication
- Lin WY at al. Genome-wide association study identifies susceptibility loci for acute myeloid leukemia. Nat Commun. 2021;12(1):6233. Open publication
- Mallesh N et al. Knowledge transfer to enhance the performance of deep learning models for automated classification of B cell neoplasms. Patterns (N Y). 2021;2(10):100351. Open publication
- Marcault C et al. Prognostic of Core Binding Factor (CBF) Acute Myeloid Leukemia With Complex Karyotype. Clin Lymphoma Myeloma Leuk. 2021. Open publication
- Matek C et al. Highly accurate differentiation of bone marrow cell morphologies using deep neural networks on a large image data set Blood. 2021;138(20):1917-1927. Open publication
- van de Loosdrecht et al. Clinical application of flow cytometry in patients with unexplained cytopenia and suspected myelodysplastic syndrome: A report of the European LeukemiaNet International MDS-Flow Cytometry Working Group. Cytometry B Clin Cytom. 2021. Open publication
- van der Velden et. Flow cytometric analysis of myelodysplasia: Pre-analytical and technical issues-Recommendations from the European LeukemiaNet. Cytometry B Clin Cytom. 2021. Open publication

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