MLL News

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Rethinking diagnostics and research: Our AI team introduces itself

We receive hundreds of samples daily at MLL from practices, medical care centers and clinics, which must be analyzed in a timely manner with consistently high examination quality. Artificial Intelligence and digital processes play an increasingly vital role to process the growing number of samples rapidly and accurately.

The AI (Artificial Intelligence) team is one of the newest organizational units at MLL. The team consists of Data Scientists and Engineers led by Sven Maschek. In addition to developing a unified and scalable AI platform for routine diagnostics across all diagnostic areas, the team also supports the Research & Development departments

Data, expertise and algorithms, (not) an ordinary job

At MLL, large amounts of raw data are generated every day: These range from digital image data, for example from microscopy, tabular data from measurements in other diagnostic areas and text documents to complex data from sequencing. The methodical storage of these different data types in AI models is a central task of the AI team. However, the mere collection of digital data types is not enough; this is where the real work of our data scientists and engineers begins:
data preparation and analysis, the development and training of AI models, and the continuous evaluation of results. The AI team works closely with physicians and researchers from the individual diagnostic areas to incorporate their combined expertise into the AI models. In addition, data protection aspects must be consistently observed.

So how does such an AI model help with everyday diagnostic tasks or research questions? This is where the second central focus of our work comes into play: the continuous development of our MLL AI platform. The platform runs in a cloud to be able to provide the required computing capacity at any time. The partition of the cloud used by MLL is located in Frankfurt, is accredited and complies with DSGVO standards. The creation and continuous development of a simple, web-based user interface for the use of AI-supported workflows is one of the cornerstones that the AI team takes care of. In addition, the team also coordinates closely with colleagues in application development and IT to ensure integration and automated use through technical interfaces.

But even when an AI model becomes usable by being integrated into the platform, the work is not over for us in the AI team, because there are still other interesting tasks:

- Optimizing AI models in terms of speed and resource utilization to ensure the fastest possible diagnosis for our patients and also to take into account economic aspects
- Continuous monitoring of the performance of the AI models to derive further improvements
- the development of automatic pipelines to continuously collect feedback from users in order to continuously improve the models
- the support of our colleagues with publications
- and many other tasks

It is always true that the tested and used AI models only assist us as diagnosticians. The goal is to support human diagnosticians, especially in the evaluation of large amounts of data, and thus to optimize the quality of findings. However, not a single finding is sent to senders and patients without final control by the scientists and physicians of MLL.

Possibilities of AI in leukemia diagnostics and therapy.
Digitization and automation are also playing an increasingly central role in healthcare. The COVID-19 pandemic has shown us how important it is to be able to produce findings digitally, independent of time and location, and the numerous use cases of GPT-4 have already made it clear to us what AI is already capable of achieving today.

Our ambition as an AI team at MLL is to support all our employees in the best possible way through the use of AI in their everyday and not so everyday tasks in diagnostics as well as in research & development and to establish MLL as a leading institute also in the field of artificial intelligence. To this end, we will further develop our AI platform so that it forms a central component on the path to personalized medicine and holistic diagnostic procedures through deeper integration of the diverse AI models and tools. Whatever tasks the future holds: We will continue to work to ensure that patients and physicians worldwide benefit from the latest innovations in AI, and that we can thus make our contribution to the best possible fight against leukemia.Author: Sven Maschek

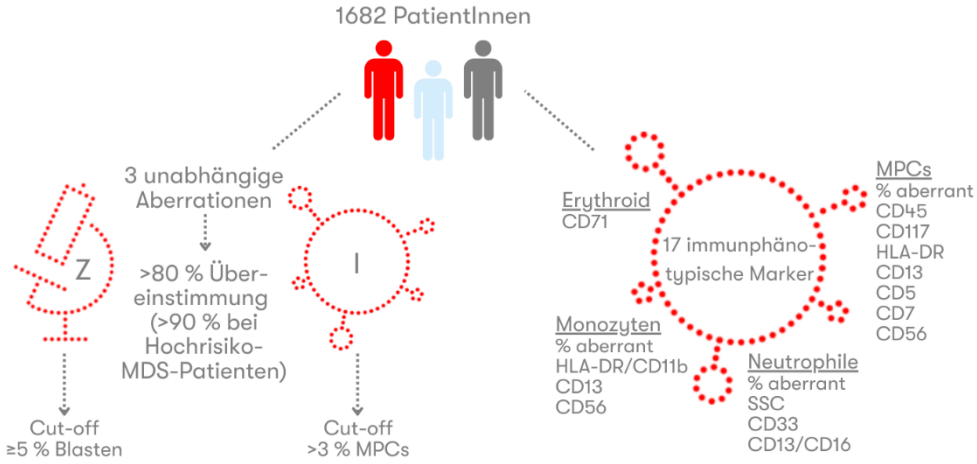
Diagnostic potential of immunophenotypic aberrations in MDS

The diagnosis of myelodysplastic neoplasms (MDS) and their classification into subgroups was based on cytomorphological features for a long time. Cytomorphology still plays a major role today, but cytogenetic criteria were added to the diagnostic criteria after the introduction of the WHO classification of tumors of hematopoietic and lymphoid tissue. It is now known that integrated diagnostics, including immunophenotyping and molecular genetics, are of great importance.

Immunophenotyping can be used to detect aberrant antigen expression patterns in suspected or confirmed MDS. This provides valuable information for diagnosis and prognosis. In a recent prospective and multicenter study, the International MDS-Flow (iMDS-Flow) working group of the European LeukemiaNet (ELN) validated the significance of different immunophenotypic markers and also identified important parameters that contribute to the clinical utility of immunophenotyping in MDS diagnostics (Kern et al. Cytometry B Clin Cytom 2022).

Immunophenotypic detection of MDS depends on a combination of MDS-associated aberrations and, to date, no flow cytometric score has been able to establish itself as the gold standard. The study described here has now identified 17 different immunophenotypic parameters independently associated with MDS or CMML in at least one of the subgroups (low-risk MDS, high-risk MDS, CMML) or the overall cohort. In addition, using a cut-off value of three independently occurring MDS/CMML-related aberrations, flow cytometric results showed greater than 80% concordance with cytomorphology, regardless of the number of cell compartments affected. In high-risk MDS patients, the agreement was even above 90%.

Furthermore, the percentage of myeloid progenitor cells (MPCs) was identified as a strong indicator for MDS, correlating with disease risk. A cut-off of >3% MPCs detected by flow cytometry was strongly associated with MDS/CMML (98% of cases with more than 3% MPCs had MDS by cytomorphology) and can therefore be used - analogous to the value of 5% blasts in cytomorphology - in the diagnostic decision.



The iMDS Flow Working Group proposes that the 17 identified parameters be mandatorily incorporated into routine diagnostics as a core set of markers for immunophenotypic evaluation of suspected MDS cases. Wherever possible, additional markers should be investigated, as listed in the ELN recommendations. This will ensure the most comprehensive evaluation possible. The high concordance of immunophenotyping and cytomorphology results across risk groups and subgroups also demonstrates the importance of an integrated diagnostic approach.

References:

Kern W et al. Multicenter prospective evaluation of diagnostic potential of flow cytometric aberrancies in myelodysplastic syndromes by the ELN iMDS flow working group. Cytometry B Clin Cytom 2023;104(1):51-65.

Author: Prof. Dr. med. Wolfgang Kern

BAX mutations mediate venetoclax resistance in AML

The development of targeted therapies for the treatment of hematological neoplasms has yielded a large number of promising drugs in recent years. Among these, substances that induce programmed cell death (apoptosis) by inhibiting anti-apoptotic proteins, e.g. BCL2, deserve special mention. Currently, the best-known representative of this substance class is venetoclax. However, as with many other targeted therapies, the problem of resistance formation during the course of treatment frequently arises.

Use of venetoclax in the treatment of AML

The use of venetoclax has particularly changed the therapy of AML patients who are not eligible for treatment with intensive high-dose chemotherapy. These patients are mostly elderly or unfit patients. The combination of venetoclax with hyomethylating agents or low-dose cytarabine has emerged as the standard treatment option for newly diagnosed AML.[1,2]. Since resistance and disease progression often occur during the course of treatment, the elucidation of the underlying mechanisms is the subject of intensive research.

Mechanisms of resistance formation

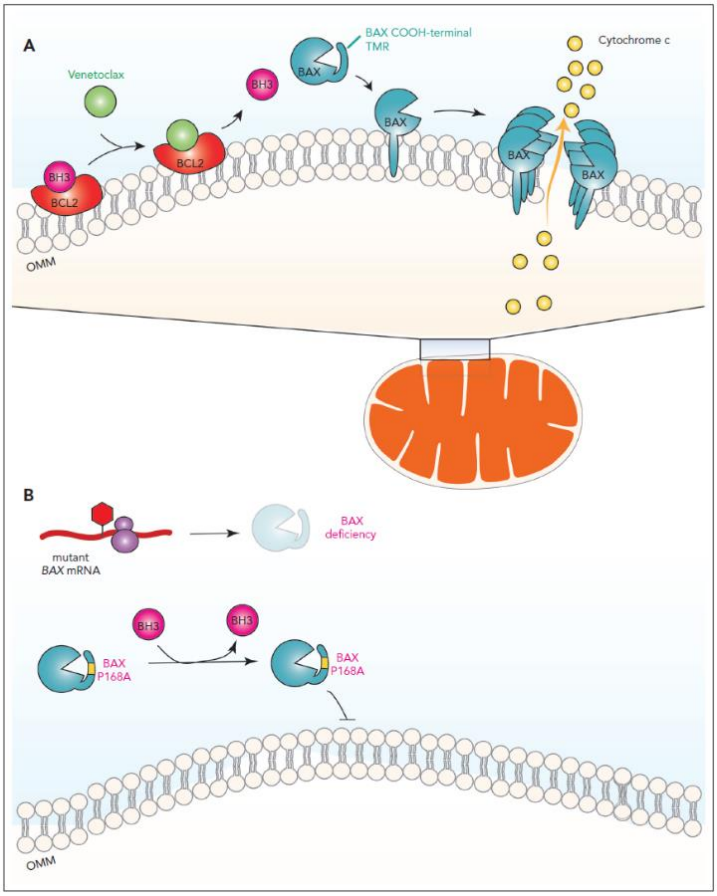
Already known mechanisms of resistance to venetoclax treatment include mutations in genes such as *TP53* as well as *K/NRAS*. *In addition*, *FLT3-ITD* is also a corresponding risk factor.[3,4]. In contrast, mutations in *BCL2*, one of the "targets" of venetoclax, which are already known from studies on CLL, do not play a role.
A newly discovered candidate gene involved in AML is the proapoptotic *BAX* gene. This is reported in a recent publication in *Blood* by the group of Moujalled et al.[5].

Apoptosis induction by venetoclax and inhibition of apoptosis by *BAX* mutations.

The mechanisms of apoptosis induction are shown in the figure (taken from Kim WJ et al, Blood 2023, Comment on Moujalled et al). Here, apoptosis is ultimately induced by the binding of *BAX* to the outer mitochondrial membrane. BAX-mediated permeabilization of the membrane occurs, which in turn leads to the release of cytochrome c from inside the mitochondria into the cytosol of the cell. Two steps are critical for the activation of *BAX* to bind to the outer mitochondrial membrane:

1. BCL2 must be bound by a so-called proapoptotic "BH3 only" protein or by the BH3 mimetic venetoclax as an alternative ligand.
2. Excess venetoclax, in addition to binding BCL2, simultaneously results in increased release of "BH3-only" proteins, which have as a second function the activation of *BAX*.

The activation of BAX can now be prevented by two types of *BAX* mutations. First, by acquired frameshift or nonsense mutations that reduce BAX protein levels or by missense mutations that prevent permeabilization of the outer mitochondrial membrane by *BAX*.
In the study by Moujalled et al, corresponding *BAX* mutations can be detected in 17% of patients who relapse on venetoclax therapy.



References:

1. DiNardo CD, Jonas BA, Pullarkat V, et al. Azacitidine and venetoclax in Previously Untreated Acute Myeloid Leukemia. *N Engl J Med*. 2020;383(7):617-629.
2. Wei AH, Montesinos P, Ivanov V, et al. Venetoclax plus LDAC for newly diagnosed AML ineligible for intensive chemotherapy: a phase 3 randomized placebo-controlled trial. *Blood*. 2020;135(24):2137-2145.
3. DiNardo CD, Tiong IS, Quaglieri A, et al. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood*. 2020;135(11):791-803.
4. Thijssen R, Diepstraten ST, Moujalled D, et al. Intact TP-53 function is essential for sustaining durable responses to BH3-mimetic drugs in leukemias. *Blood*. 2021;137(20):2721-2735.
5. Moujalled DM, Brown FC, Chua CC, et al. Acquired mutations in BAX confer resistance to BH3-mimetic therapy in acute myeloid leukemia. *Blood*. 2023;141(6):634-644.

Author: Dr. rer. nat. Frank Dicker

Update: the Onkopedia recommendations on chronic lymphocytic leukemia (CLL)

Under the scientific leadership of Prof. Dr. Clemens Wendtner, the Onkopedia recommendations on CLL have been updated. Relevant changes are summarized here with a focus on the associated diagnostics:

1) Adaptation to the current WHO classification: This continues to describe CLL as indolent (lymphocytic) B-cell lymphoma characterized by a leukemic course with at least 5,000 clonal B-lymphocytes per µl in the peripheral blood. In contrast to previous versions, B prolymphocytic leukemia (B-PLL) is no longer included as a distinct entity in the current version of the WHO classification, but is absorbed into other entities, including

prolymphocytic progression of CLL (defined as having >15% prolymphocytes). Thus, the diagnosis of CLL can be made unchanged by blood count and multiparametric immunophenotyping in the majority of cases; the genetic risk profile is an additional factor.

2) In first-line therapy, age is no longer used as a stratification parameter. Therapy is predominantly based on specific therapy-limiting comorbidity and genetic risk profile rather than on calendar age. Chemotherapy-free treatment with BTK inhibitors, anti-CD20 antibodies, and the BCL2 inhibitor venetoclax is the first priority. Analysis of major genetic risk factors is recommended for treatment selection:

- del(17p13) or *TP53 mutation* in FISH and molecular genetics.
- complex karyotype (≥3 aberrations) in cytogenetics
- unmutated IGHV status in molecular genetics

3) Second-line therapy also focuses on chemotherapy-free treatments. Second-generation BTK inhibitors have been included at major changes. In addition, the option for allogeneic hematopoietic stem cell transplantation arises in case of unfavorable prognosis and loss of efficacy of BTK inhibitors and venetoclax. The choice of relapse therapy depends on clinical parameters such as the type of primary therapy and the remission duration achieved with it, in addition to the patient's age and comorbidity. In addition, altered biology of CLL based on clonal evolution should be considered. From a diagnostic point of view, this includes in particular:

- Acquisition of a del(17p13) or *TP53 mutation* or a complex karyotype. In case of clinical suspicion of a relapse of CLL, it is therefore explicitly recommended to initiate molecular (cyto)genetic testing again in order to be able to exclude newly occurred and therapy-relevant high-risk aberrations (in particular del(17p13), *TP53 mutation* and complex karyotype) with certainty.
- After therapy with BTK or BCL2 inhibitors, specific resistance mutations (including in the *BTK*, *PLCG2*, *and BCL2 genes*, respectively) may also occur, the detection of which makes it inappropriate to repeat the corresponding therapy. These resistance mutations are detected by sequencing the corresponding genes in molecular genetics.

Author: PD Gregor Hörmann, MD, PhD

Advanced Training of the Genome Network Hematology: From Myeloma to MDS

Together with the Comprehensive Cancer Center Klinikum Rechts der Isar TUM and the Comprehensive Cancer Center Mainfranken/Universitätsklinikum Würzburg, MLL has founded the Genome Network Hematology. Now the Genomnet is hosting its first educational event "From Myeloma to MDS" and invites to the MLL Munich Leukemia Laboratory on June 22 from 4 pm to 6 pm. Virtual participation is also possible.

Click here for registration and program

Recent publications with MLL participation

- Bahaj W et al. Novel Scheme for Defining the Clinical Implications of TP53 Mutations in Myeloid Neoplasia. Res Sq. 2023. Open publication
- Degenfeld-Schonburg L et al. Antineoplastic efficacy profiles of avapritinib and nintedanib in KIT D816V+ systemic mastocytosis: a preclinical study. Am J Cancer Res. 2023. Open publication.
- Durmaz A, et al. A multimodal analysis of genomic and RNA splicing features in myeloid malignancies. iScience. 2023. Open publication
- Hehr M et al. Explainable AI identifies diagnostic cells of genetic AML subtypes. PLOS Digit Health. 2023. Open publication
- Huber S et al. MDS subclassification-do we still have to count blasts? Leukemia. 2023. Open publication
- Ivanov D, et al. Phenotypic Characterization of Disease-Initiating Stem Cells in JAK2- or CALR-mutated Myeloproliferative Neoplasms. Am J Hematol. 2023. Open publication.
- Mayerhofer E et al. Prevalence and Therapeutic Implications of Clonal Hematopoiesis of Indeterminate Potential in Young Patients With Stroke. Stroke. 2023. Open publication
- Sakuma M et al. Novel causative variants of VEXAS in UBA1 detected through whole genome transcriptome sequencing in a large cohort of hematological malignancies. Leukemia. 2023. Open publication
- Sauta E. Real-world validation of Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. J Clin Oncol. 2023. Open publication.
- Valent P et al. European Competence Network on Mastocytosis (ECNM): 20-Year Jubilee, Updates, and Future Perspectives. J Allergy Clin Immunol Pract. 2023. Open publication

- Vlachonikola E et al. T cell receptor gene repertoire profiles in subgroups of patients with chronic lymphocytic leukemia bearing distinct genomic aberrations. Front Oncol. 2023. Open publication.
- Willmann M et al. Engraftment in NSGSCF mice correlates with the WHO category and prognosis in systemic mastocytosis. Leukemia. 2023. Open publication

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