MLL News

August 23, 2022

Follow-up report on the training course “The new WHO classification” on July 27, 2022, in the MLL Munich Leukemia Laboratory

After the two preliminary publications on the new WHO classification (“The 5th edition of the World Health Organization Classification of Haematolymphoid Tumors”) were recently published in *Leukemia* (Khoury et al. Leukemia 2022, Alaggio et al. Leukemia 2022), the MLL Munich leukemia laboratory used this as an occasion to organize an advanced training event. There, in four expert lectures, the most important innovations in the classification were summarized and prepared for everyday diagnostic use. The event turned out to be very popular, so that in the end 47 physical and 189 virtual participants from Germany, Austria, and Switzerland participated in the hybrid event.

Increasing focus on genetics

After a brief welcome by Prof. Haferlach, Dr. Christian Pohlkamp began by reporting on the future role of cytomorphology and provided an accompanying synopsis of the changes made to the classification of myeloid neoplasms. Afterwards, Dr. Martha-Lena Müller guided us through the lymphatic component of the new WHO classification from the perspective of immunophenotyping. Then Drs. Bettina Balk and Manja Meggendorfer drew attention to the increasing importance of cytological and molecular genetic findings in the definition of hematological entities, explaining the expanded range of diagnostic procedures required for this. At the end, Prof. Haferlach led a panel discussion on the highlights. Here, both the speakers and the audience reflected on the consequences for everyday clinical practice. Some lively discussion was initiated on topics that included the new genetic definitions for MDS and AML, the management of genetically defined entities such as CHIP and CCUS, and the

importance of transcriptome analysis for diseases such as ALL or myeloid/lymphatic neoplasms with eosinophilia and tyrosine kinase gene fusions.

Mutual conclusion

Molecular genetics methods in particular have grown immensely in importance due to the new WHO classification. In this regard, reference was also made to publications on the new scores for risk stratification in MDS (IPSS-M, Bernard et al. NEJM Evidence 2022) and AML (ELN, Döhner et al. Blood 2022), where there is also a growing appreciation of molecular genetic alterations. The MLL has already adapted its diagnostic and methodological spectrum accordingly (see **test order**). Links to the publications referred to, slides from the event, and other information can be downloaded here.

What's next?

The full beta version of the new WHO classification appeared online on August 3, 2022. The final print version is expected to appear at the end of 2022. Due to the lively participation in the training, a follow-up event is planned on November 16, 2022. In hybrid format, it will delve more deeply into the most relevant aspects of the new WHO classification and also shed further light on the new scoring systems (see above). All information about the follow-up event can be found here (the event takes place in German language).

Author: Dr. Christian Pohlkamp

Save the date: MLL advanced training event on November 16, 2022

WHO, ICC, ELN/AML, and IPSS-M: The importance of genetics for myeloid entities ranging from CCUS to AML

Our advanced training event in hybrid format is entering its second round: Due to the large number of innovations in the diagnostic guidelines, we want to invite you to the event "WHO, ICC, ELN/AML, and IPSS-M: The role of genetics for myeloid entities ranging from CCUS to AML" being held on November 16, 2022, from 4 p.m. to 6 p.m. The event will take place live on site at the MLL, with virtual participation also being made possible as an alternative.

Detailed information about the program and registration will follow soon on our website.

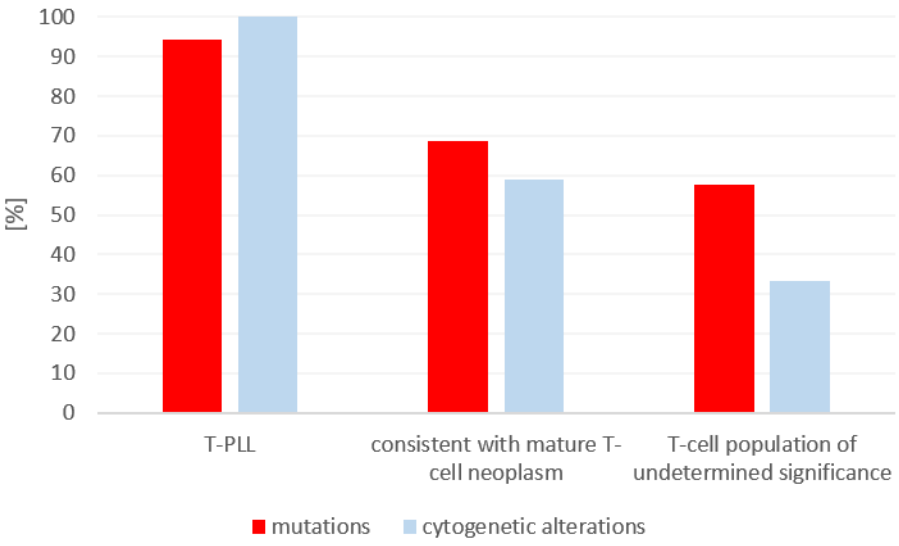
Reactive versus neoplastic – New panel to clarify clonal T-cell populations

Due to their clinical, histopathological, and molecular heterogeneity, mature T-cell neoplasms pose a major diagnostic and therapeutic challenge. Unlike the more common mature B-cell neoplasms, in which clonality is evidenced by immunoglobulin light chain restriction, most mature T-cell neoplasms lack a specific immunophenotypic signature. Wherever a suspect mature T-cell population is detected by immunophenotyping, a molecular genetic test to determine the clonality of T-cell receptor rearrangements is currently recommended for further diagnostic clarification and for differentiation from reactive changes. However, a positive finding is not a reliable criterion for malignancy in this case, since non-malignant clonal expansion of T cells can occur as an excessive immune reaction, e.g., with infections,

autoimmunity, or after administration of certain drugs. In routine diagnostics, additional genetic analyses of more meaningful markers are therefore required to enable a clear distinction between benign and malignant clonal T-cell populations and thus shorten the time to diagnosis.

Next generation sequencing in patients with a clonal T-cell population

To evaluate the potential benefit of genetic analysis in patients with a suspected mature T-cell population, NGS was performed in 83 patients using a lymphatic gene panel. All patients had previously been tested using immunophenotyping, and cytogenetic data was available for 52 patients. Based on the results of immunophenotyping, 18 patients had T-PLL, 32 patients had findings consistent with another mature T-cell neoplasm (TCL), and 33 patients had a T-cell population of undetermined significance (TPUS). In all patients, it was possible to detect a clonal T-cell receptor rearrangement in the molecular genetic analysis. Due to its specific immune phenotype and its entity-defining cytogenetic changes (*TRAD::TCL1A* rearrangement or *TRAD::MTCP1* rearrangement), T-PLL is comparatively easy to diagnose – all 18 patients showed the T-PLL-typical cytogenetic changes and 94% of the cases also had mutations. Structural changes and/or copy number changes were also detected by chromosome analysis and FISH in 59% of TCL and 33% of TPUS patients. As many as 69% of the TCL and 58% of the TPUS patients showed mutations. While mutations in *JAK3*, *ATM*, *JAK1*, and *STAT5B* primarily occurred in T-PLL, mutations in *STAT3*, *TET2*, and *DNMT3A* were mainly observed in TCL and TPUS. *STAT3* mutations commonly occur in approximately 30-40% of T-LGLs, but they also occur in other mature T-cell neoplasms. It is worthy of note that 60% of the TPUS patients exhibited cytogenetic and/or molecular genetic markers.



In the meantime, there have been numerous publications that describe in detail the molecular landscape of the various T-cell neoplasms. Thanks to larger data sets and expanded sequencing approaches, the diagnostic and prognostic value of certain molecular changes is becoming increasingly apparent. Most notable is the high number of overlapping mutations in epigenetic modifiers in angioimmunoblastic T-cell lymphoma (AITL), which is referred to as nodal follicular T-cell lymphoma of the angioimmunoblastic type (nTFHL-AI) according to the WHO classification of 2022 (Aleggio et al. Leukemia 2022). These include *TET2* (50%-80%), *DNMT3A* (20%-30%), and *IDH2*-R172 (20%-30%). The *RHOA*-G17V mutation is also observed in up to 70% of nTFHL-AI cases. However, none of these mutations are specific for nTFHL-AI, as they can also be observed in other entities, particularly in nodal PTCL with a TFH phenotype

(nTFHL-NOS according to the new WHO classification). Cytogenetic changes with diagnostic value include *ALK* rearrangements in ALK-positive anaplastic large cell lymphoma (ALCL), rearrangements of *DUSP22* or *TP63* in ALK-negative ALCL, and *ITK::SYK* fusions in follicular T-cell lymphoma (nTFHL-F according to the new WHO classification).

Clinical relevance of molecular genetic changes

While an effective targeted therapy with brentuximab vedotin is only available for CD30-positive anaplastic large cell lymphoma (ALCL), clinical trials are primarily testing histone deacetylase inhibitors (HDACi) at present. In particular, R/R T-cell neoplasms with the TFH phenotype showed a significantly better response compared to T-cell neoplasms without the TFH phenotype (HR: 0.322; p = 0.009). They also exhibited a typical mutation pattern from *TET2* and/or *DNMT3A* and/or *RHOA* mutations more frequently (83% vs 40%; p = 0.034) (Ghione et al. Blood Adv 2020).

Based on this data and the clinical relevance, an extended genetic investigation of clonal T-cell populations looks promising. We have therefore expanded our range of tests and are offering a new T-cell-specific panel in molecular genetics, including the genes ***TET2, DNMT3A, RHOA*-G17V, *IDH2*-R172, *CD28, FYN, PLCG1*, and *VAV1*** (see our current test order as well as our digital order entry system (only available in German language)).

Author: Dr. Janine Mueller

TOP100 elite group visiting the MLL

On July 22, 2022, the MLL hosted the TOP 100 elite circle of German medium-sized companies. We exchanged views on the topics of digitization, automation, cloud computing, and artificial intelligence with 15 CEOs whose companies – like us at MLL – had been recognized for outstanding innovative achievements. MLL was able to show how the use of these technologies drives the optimization of leukemia diagnostics in order to achieve the goal of personalized precision medicine.

IPSS-M: Molecular International Prognostic Scoring System

Risk scoring for patients with myelodysplastic neoplasms (MDS) has been based on clinical variables and cytogenetic aberrations as part of the Revised-International Prognostic Scoring System (IPSS-R). A decade of molecular genetic analysis emphasized the impact of mutations (Bejar et al. NEJM 2011, Papaemmanuil et al. Blood 2013, Haferlach et al. Leukemia 2014). The Molecular International Prognostic Scoring System (IPSS-M) now takes mutations in 31 genes in account, as well as cytogenetics, bone marrow blasts, hemoglobin and platelet count (Bernard *et al*. NEJM Evid 2022).

What is the IPSS-M?

An international research team (The International Working Group for the Prognosis of MDS) analyzed 2,957 MDS patients and developed an improved prognostic score using the following parameters:

- Hemoglobin
- Platelet counts
- Bone marrow blasts
- IPSS-R cytogenetic risk category
- Molecular genetic information on 31 genes

The authors particularly emphasize the importance of *FLT3* alterations (TKD or ITD) and *KMT2A*-PTD (MLL^{PTD}). While very rare in MDS, they have a highly adverse impact on the prognosis. For *TP53*, the status "multihit" plays a significant role; this is referred to as the presence of two or more mutations, a mutation and a deletion (del(17p)) or a mutation with a copy-neutral loss of heterozygosity (cnLOH).There are also new results regarding *SF3B1*. The positive prognostic significance is lost, if present with a del(5q) or a mutation in one of the following genes: *BCOR*, *BCORL1*, *NRAS*, *RUNX1*, *SRSF2*, or *STAG2*.

How can the IPSS-M be calculated?

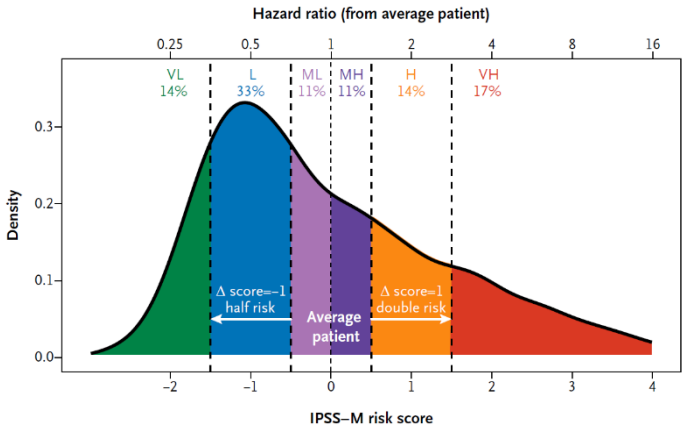
The following is needed in addition to the blood counts:

- Cytomorphology (bone marrow blast count)
- Cytogenetics/chromosome analysis
- The IPSS-M panel according to Bernard et al. in molecular genetics

The result of the IPSS-M is a number, which is classified into six risk categories (see Fig. 1):

- Very Low (VL)
- Low (L)
- Moderate Low (ML)
- Moderate High (MH)
- High (H)
- Very High (VH)

Fig 1. Shows the classification of numerical values to the risk groups and their relative frequency in MDS (Bernard et al. NEJM Evidence 2022).



In the publication, the risk groups show strong prognostic separation in terms of overall survival, leukemia-free survival, and AML transformation.

The IPSS-M can be calculated on the MDS Foundation website: A few practical tips for use:

- At the end of our report, you will find the table *Conducted analysis*. Enter a tier 1 and 2 type mutation as “Mutated.”
- The original gene name *MLL* is used in the WebTool. We use the current name *KMT2A*.
- *TP53:* You can find the number of mutations in the *Mutations Changes*. In addition, the “Maximum *TP53*VAF” must be entered. This can be found in the VAF column in the same table. In the future, we will also provide information on the “Loss of heterozygosity”

We are currently updating our reports in accordance with the IPSS-M. If you send us blood counts and ask for cytomorphology, cytogenetics and molecular genetic diagnostics, we will soon also be able to provide you with the IPSS-M as part of our integrated report.

Author: Dr. Constance Bär

Events

Oncology Symposium 2022

For the fourth time now, the Trillium Academy is cordially inviting you to the oncological symposium with the motto "From biomarkers to therapy." The event will take place on Friday, October 21, 2022 – live on site at MLL or virtually via livestream. The symposium series offers an insight into modern oncological precision medicine, which combines innovative diagnostic methods and therapeutic strategies to form a greater whole. Our newsletter subscribers benefit from a discount code on the basic ticket price.

Click here to register and enter your discount code

Most Recent Publications with MLL Involvement

- Burkhardt B et al. Clinical relevance of molecular characteristics in Burkitt lymphoma differs according to age. Nat Commun. 2022. Open publication
- Hoermann G. Clinical Significance of Clonal Hematopoiesis of Indeterminate Potential in Hematology and Cardiovascular Disease. Diagnostics (Basel). 2022. Open publication
- Peter B et al. BRD4 Degradation Blocks Expression of MYC and Multiple Forms of Stem Cell Resistance in Ph+ Chronic Myeloid Leukemia. Am J Hematol. 2022. Open publication
- Ramos-Campoy S et al. TP53 Abnormalities Are Underlying the Poor Outcome Associated with Chromothripsis in Chronic Lymphocytic Leukemia Patients with Complex Karyotype. Cancers (Basel). 2022. Open publication
- Schneeweiss-Gleixner M et al. CDK4/CDK6 Inhibitors Synergize with Midostaurin, Avapritinib, and Nintedanib in Inducing Growth Inhibition in KIT D816V+ Neoplastic Mast Cells. Cancers (Basel). 2022. Open publication
- Smiljkovic D. et al. Expression and Regulation of Siglec-6 (CD327) on Human Mast Cells and Basophils. J Allergy Clin Immunol. 2022. Open publication
- Wang BA et al. Alternatively spliced CSF3R isoforms in SRSF2 P95H mutated myeloid neoplasms. Leukemia. 2022. Open publication

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MLL Münchner Leukämielabor GmbH
Max-Lebsche-Platz 31
81377 Munich, Germany

Phone: +49 89 990 17 0
Fax: +49 89 990 17 111
E-mail: info@mll.com
Website: www.mll.com