MLL News

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Dear friends and colleagues,

As this very unusual year is now drawing to its end,we have all come to realize, both in our professional and private lives, that plans and goals are only relative. It has also become quite apparent how medicine can profoundly impact our daily lives and actions.

This is why the MLL, just like you, had to embrace the precise meaning of being "system relevant". We considered it our top priority to provide you with the very best care, as effectively as possible, and better than ever before, in the best interest of your patients.

Our team has stood by us in a truly unique way, and we are very impressed by the commitment each and every team member has demonstrated to fulfill this duty in the interests of the patients concerned.

During this process, we have come to realize more than ever before that digitalization, automation and the ability to rapidly advance projects, from data processing and transmission to the implementation of artificial intelligence in research and in routine, are key assets and will be even more so in the future.

We have also identified that there is room and there are opportunities for improvement in all of these areas of collaboration, and we are now sincerely inviting your constructive feedback, both for now and for the coming year ahead.

In the interests of all of our patients, we have also endeavored to make all of our existing science-based understanding for the future of leukemia diagnostics available in the form of lectures and publication to all potential users. With this in mind in particular, we wish to extend our thanks both to you and your patients for your great willingness to provide patient samples and, above all, information on therapy and outcome within the scope of scientific collaborations. We sincerely appreciate your support and will make every effort to be mindful of the time you are devoting to provide this resource.

The current year has not only demonstrated that science-based collaborations are of fundamental importance to us all well beyond their direct benefits to our hematology and oncology patients,but has also made us intensely aware of how vulnerable seemingly stable systems really are and an appreciation that our patients face such challenges every day as part of their diagnosis and therapy.

Our future capabilities and plans are designed to provide you with patient-centered diagnostics and targeted therapy recommendations, based on scientific principles and prognostic information in a timely manner.

We thank you for your trust in us and look forward to our ongoing collaboration!

With kind regards and all our best wishes for a peaceful holiday season and a wonderful, prosperous and as yet untold 2021

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Molecular genetics in mastocytosis and hypereosinophilia

Advanced molecular genetic testing is gaining ground in the diagnosis of mast cell diseases and hypereosinophilia. In addition to the typical mastocytosis-driver mutation KIT D816 as well as *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* rearrangements, the MLL uses advanced Next-Generation Sequencing (NGS) mutation analysis to inform the diagnosis and prognostic stratification of these diseases. These NGS gene panels are continuously updated to reflect the latest advances in scientific understanding.

Over 90% of systemic mastocytosis patients have an activating *KIT* gene mutation. In most cases this involves the *KIT D816V* mutation, which can be detected from blood or bone marrow with a very sensitive quantitative PCR test performed at the MLL. Occasionally, sequencing techniques are used to detect other *KIT* mutations. The presence of a *KIT* mutation is a diagnostic criterion for systemic mastocytosis and clonal mast cell activation syndrome (Valent et al. Blood 2017).

A proportion of mastocytosis patients have additional mutations in other genes as well as the *KIT D816V* mutation. These additional mutations are particularly common

in advanced systemic mastocytosis cases. Advanced mastocytosis patients with *SRSF2*, *ASXL1*, and *RUNX1* gene mutations have been found to have particularly unfavorable prognoses but additional mutations can also have an independent unfavorable prognostic impact in non-mastocytosis patient subgroups (indolent systemic mastocytosis patients with NRAS and *DNMT3A* mutations for instance have also been shown to be correlated with unfavorable prognoses). This has led to the inclusion of detection of mutations in these genes in prognostic scoring systems for systemic mastocytosis patients (Pardanani et al. Blood Adv. 2018, Jawhar et al. JCO 2019, Muñoz-Gonzalez et al. Blood 2019, Reiter et al. Blood 2020). This year's European Competence Network on Mastocytosis (ECNM) conference specifically highlighted the importance of advanced molecular genetic diagnostics in the prognostic stratification and management of mastocytosis patients. To address this need, the MLL now also offers a new **mastocytosis prognostic panel** that includes the molecular genetic workup of the *SRSF2*, *ASXL1*, *RUNX1*, *NRAS*, and *DNMT3A* genes.

Molecular genetic diagnostics are also essential for the diagnostic workup of hypereosinophilia. Neoplasias presenting with eosinophilia as well as *PDGFRA*-, *PDGFRB*-, *FGFR1*- or *PCM1-JAK2*-rearrangements have been included as a distinct entity in the WHO classification since 2017. The MLL can detect these typical disease gene rearrangements at the cytogenetic level with FISH as well as with PCR-based molecular techniques. The differential diagnosis of hypereosinophilia should also consider Chronic Eosinophil Leukemia (CEL). CEL is characterized by the presence of clonality, which distinguishes it from idiopathic hypereosinophilic syndrome. The MLL can test for molecular genetic clonality in CEL by using an NGS gene panel which includes the most frequently detected mutations in the *ASXL1*, *DNMT3A*, *JAK2*, *SRSF2*, and *TET2* genes, and which has recently been expanded to include mutations in the *STAT5B* gene. Recurrent *STAT5B* mutations occur in 1.6% of cases presenting with ambiguous eosinophilia. The detection of clonality allows these cases to be classified as CEL. *STAT5B* mutations have also been correlated with an unfavorable prognosis (Cross et al. Leukemia 2019, Morisa et al. Am J Hematol 2020, Reiter & Gotlib Blood 2017).

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Advances in the targeted therapy of hematological neoplasias

Research into the underlying pathogenetic mechanisms of hematological neoplasias has led in recent years to remarkable advances in our understanding of these diseases. Cytogenetic and molecular aberrations are the most pertinent factors to consider when determining response to chemotherapy. They also help to predict long-term outcome and are also potentially promising therapeutic targets. An improved understanding of the pathogenesis of these diseases, particularly through Next-Generation Sequencing (NGS), has contributed to drive the development of novel therapeutic algorithms for the treatment of leukemia and lymphoma. Recent clinical trials have investigated several new agents, in particular tyrosine kinase inhibitors (TKIs), immune checkpoint inhibitors, monoclonal or bispecific T-cell engager antibodies, metabolic and proapoptotic agents, that have now been approved as novel targeted therapies. The best response rates are often achieved when novel molecular targeted therapies are combined with standard chemotherapy. In this current issue of the newsletter, we would like to start by giving an overview of novel AML therapies.

FLT3 Tyrosine Kinase Inhibitors (TKIs)

Approximately one third of AML patients are found to have mutations in the FLT3 kinase coding sequences. **Midostaurin** (Rydapt®, Novartis Pharmaceuticals, Inc.) is the first TKI to be approved as part of a combination therapy with standard 7+3 chemotherapy in newly diagnosed AML patients with *FLT3*-mutations, independently of patient age.

Midostaurin was approved for AML with *FLT3*-mutations based on the positive results of a large international randomized phase III trial (Cancer and Leukemia Group B [CALGB] 10603 / RATIFY). As both a concurrent *NPM1* mutation and the *FLT3*-ITD to wild type allele ratio affects the prognosis of an otherwise cytogenetically normal

FLT3-mutated AML, these two characteristics need be established. The MLL has the capacity to monitor the mutation as well as the allelic ratio at the initial diagnosis and during the course of the disease to allow as early an individually tailored therapeutic intervention as possible.

Other TKI studies in phase II/III trials include:

- **Quizartinib,** a more specific and effective second-generation TKI compared to midostaurin. **Quizartinib** inhibits both wild type *FLT3* and *FLT3*-ITD activity, but not *FLT3*-TKD (Kayser & Levis 2014).
- **Crenolanib** is another selective *FLT3* inhibitor that inhibits both *FLT3*-ITD and *FLT3*-TKD mutations (Galanis et al. 2014)
- **Gilteritinib** is, compared to crenolanib, a more novel, more highly selective, and more potent oral *FLT3* inhibitor which inhibits both ITD and TKD mutations (Lee et al. 2017)

Inhibitors of isocitrate dehydrogenase (*IDH*) *IDH1/IDH2*

IDH1 and *IDH2* mutations are respectively detected in approximately 8% and 12% of AML patients (Papaemmanuil et al. 2016). *IDH1* mutations occur almost exclusively at R132, whereas *IDH2* substitutions are observed at R140 or R172 (Stein 2015). In terms of function, *IDH* mutations block the differentiation of hematopoietic cells by increasing levels of the oncometabolite 2-hydroxyglutarate, which indirectly results in DNA hypermethylation by inhibiting histone demethylation (Stein 2015).

A dose-finding study in a cohort of predominantly relapsed/refractory AML patients with the selective and potent *IDH2* inhibitor enasidenib (AG-221 / CC-90007; Celgene Corp.) showed promising results when used as a single agent treatment in patients with *IDH2* mutations
Based on these results, enasidenib was approved in the U.S.A. by the FDA (August 1, 2017) for the treatment of relapsed/refractory AML with an *IDH2* mutation.

The initial data on a combination therapy with the *IDH1* inhibitor ivosidenib and azacytidine in newly diagnosed *IDH1*-mutated AML in elderly patients, also appears promising, with a reported complete remission rate of 61% (DiNardo 2020). But approval of these active substances in Europe is currently not imminent.

Proapoptotic agents (*BCL2* inhibitor)

The anti-apoptotic B-cell lymphoma 2 (*BCL2*) protein, which is frequently expressed in hematological neoplasias, plays an essential role in AML cell survival (Adams & Cory 2007). Overexpression of *BCL2* has been correlated with AML chemoresistance (Pan et al. 2014). **Venetoclax** (Venclexta®, AbbVie Inc.) is a highly selective oral *BCL2*-inhibitor that has shown activity in *BCL2*-dependent leukemia and lymphoma cell lines (Andreeff et al. 1999, Pan et al. 2014, Souers et al. 2013, Vogler et al. 2013). The

inhibition of *BCL2* induces cell death in progenitor and stem cells of AML patients (Konopleva et al. 2006, Lagadinou et al. 2013).

BCL2 inhibition may thus have the potential to eliminate chemotherapy-resistant leukemia stem cells while at the same time sparing normal hematopoietic stem cells (Konopleva et al. 2006, Lagadinou et al. 2013). In clinical trials, combination treatments with HypoMethylating Agents (HAM) and the *BCL2*-inhibitor venetoclax showed a significantly higher overall CR/CRi rate of 67%, with the highest complete remission rate of 91% in patients with *NPM1*-mutated disease and the lowest complete remission rate of 47% in patients with a *TP53* mutation. The median overall survival was 17.5 months (DiNardo et al. 2019). Similarly, an early clinical trial evaluating the combination treatment of low-dose cytarabine (LDAC) and venetoclax reported a CR/CRi rate of 54% with a median OS of 10.1 months (Wei et al. 2019).

The combination treatment of venetoclax and azacytidine has already been approved by the FDA, and an application for its approval in Europe was submitted to the EMA in the summer of 2020. A very recent publication also reported that this combination improves the treatment response of diseases which include an additional mutation, either in the *IDH1* (but not in *IDH2*), *NPM1*, or the *SRSF2* gene (Chua et al. 2020).

The advanced clinical development of the *BCL2*-inhibitor venetoclax, the *IDH1* inhibitor ivosidenib, the selective *FLT3* inhibitors quizartinib and gilteritinib, the hedgehog inhibitor glasdegib, the *MDM2* inhibitor idasanutlin, the oral azacitidine, the histone deacetylase inhibitor pracinostat, and the hypomethylating agent guadecitabine, give reason to hope that the therapeutic options and thereby also the improved prospects for curing AML will increase significantly in the near future.

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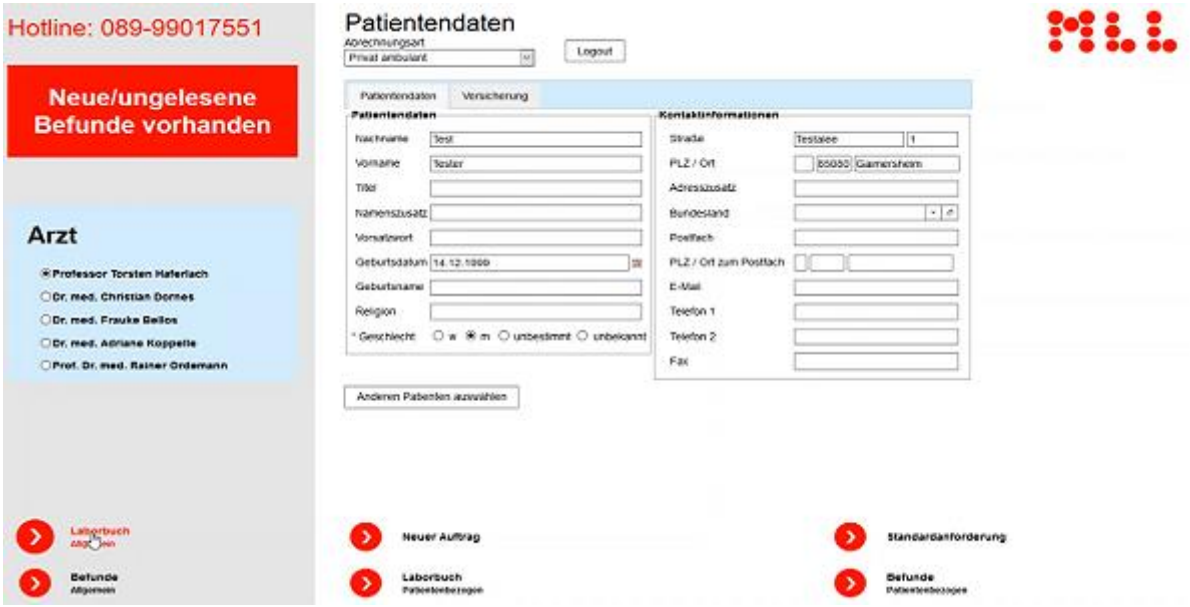
Digital order entry platform enables online order submission

In an era where administrative processes are increasingly being digitized, you can now utilize our web-based portal for digital order submission. Apart from the reliable transmission of all the necessary data, it also offers, for example, the ability to post-edit orders (even after material submission) and to view findings online.

Click here to submit a registration request.



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