MLL News

September 30, 2020

Pharmacogenetic Fluorouracil Toxicity Testing at the Munich Leukemia Laboratory (MLL)

Pharmacogenetic testing for a deficiency of dihydropyrimidine dehydrogenase (DPD) contributes significantly to the prevention of fluorouracil (FU) toxicity. The analysis should be performed before commencing systemic therapy with FU-containing drugs. It is the basis for a risk-adapted algorithm for FU therapy according to the current recommendations of the German Society for Hematology and Medical Oncology (DGHO). On October 1, 2020, MLL started offering pharmacogenetic DPD testing using a CE-IVD-certified test system to all patients.

Up to 9% of the population carry a *DPYD* gene variant which encodes a DPD enzyme with a reduced activity to catalyze the fluorouracil (FU) administered during FU therapies; 0.1% to 0.5% of people have a complete DPD deficiency. Although reduced DPD enzyme activity has no clinical relevance *per se*, when combined with systemic therapy involving FU-containing drugs it leads to reduced FU breakdown, thereby putting these patients at considerable risk of serious side effects and life-threatening toxicity. Since about 30% of all severe FU toxicity reactions can be explained by an underlying genetic DPD deficiency and could be avoided, the European Medicines Agency (EMA) recommends that all patients be tested for a DPD deficiency before receiving systemic therapy with FU-containing drugs 5-fluorouracil (5-FU), capecitabine and tegafur (EMA Recommendations 2020). This recommendation was adopted by the German Federal Institute for Drugs and Medical Devices (BfArM) and included in the Summary of Product Characteristics (SmPC) of the relevant drugs.

The German Society for Hematology and Medical Oncology (DHGO) therefore recommends pharmacogenetic testing for the presence of the four most common and clinically most relevant variants of the *DPYD* gene, for which an unequivocal effect on the DPD enzyme function has been described (*DPYD*2A*, *DPYD*13*, polymorphism c.2846A> T and haplotype B3). If the genetic analysis indicates a reduced DPD enzyme function, therapy with FU-containing drugs should be carried out using a differentiated, risk-adapted algorithm, taking into account each patient's individual disease status and the existence of any alternative treatments (DGHO Position Paper 2020).

As of October 1, 2020, MLL's service offerings include a state-of-the-art genetic analysis of these four *DPYD* gene variants using a CE-IVD-certified testing system. DPD testing is requested by submitting a separate “Request for Pharmacogenetic Testing”, which also includes a Declaration of Consent in accordance with the requirements of the German Diagnostic Genetic Testing Act (GenDG). The signed Declaration of Consent must be enclosed with the Request for Testing in order to ensure that the findings are reported without delay. Swift reporting of the examination results is of utmost importance for therapy planning. For this reason, the MLL is committed to carrying out the *DPYD* gene analysis several times a week

with a processing time of 2 days. The costs of the testing to determine DPD metabolic status prior to commencing systemic therapy with FU-containing drugs are covered as a standard service by the statutory health insurance. With the introduction of the DPD analysis at MLL, all patients should benefit from a speedy and uncomplicated pharmacogenetic testing.

For further information on DPD testing at MLL, see here.

Author: PD Dr. med. Gregor Hörmann, PhD

BELUGA - A Prospective, Registered Study on the Use of Artificial Intelligence (AI) in Hematology

Even before the start of the COVID-19 pandemic, we saw how strongly digitalization is shaping our private and professional lives and will continue to do so in the future. This also applies to innovations for daily work in the paperless Munich Leukemia Laboratory (MLL), such as the complete digitalization of all medical findings in a constantly improving digital infrastructure. However, AI will mark a new era for medical diagnostic services as well.

In the last few months, many scientific publications have addressed this issue, examining the use of artificial intelligence in numerous clinical applications. In these studies, Deep Neural Networks (DNNs) are often used to test the application of machine learning for diagnostic purposes. Ranging from automated fundus examinations, to the detection of tumor foci in tissue sections and the detection of COVID-19-disease from CT scans, these systems are for the most part not only equivalent to human examiners in terms of accuracy and speed, but actually increasingly becoming superior. However, most of these studies are retrospective: A large collection of pre-annotated data (or images) is used as a training cohort to have new, previously unannotated data classified by the network.

We are now starting the prospective part of the MLL-initiated BELUGA study (Better Leukemia Diagnostics Through AI; Clinicaltrials.gov, NCT04466059). We will investigate to what extent AI-based diagnostic work-up is on a par with, or superior to, the conventional gold standard.

Our AI approaches draw on a collection of currently over 600,000 digitized blood cells and over 300,000 digital immunophenotypic findings, which have undergone state-of-the-art interpretation and annotation at the MLL. The DNNs trained with the MLL data are now available as applications. As part of the BELUGA study, these DNNs will be used in parallel to the current gold standard of routine laboratory practices and workflows over a period of one year to prospectively evaluate new incoming patient samples. AI results will remain blinded. The two methods will then be compared at specified time points in relation to defined primary endpoints (sensitivity/specificity of the diagnoses, time to diagnosis). Other secondary endpoints will include step-by-step diagnostics focusing on molecular genetics and chromosome analyses.

For the first time, the potential of AI-guided diagnostic strategy is being investigated in a prospective study using the example of cytomorphology and

immunophenotyping. The newly collected data will be used to continuously train the algorithm. It would be exciting if, 200 years after the birth of Rudolf Virchow, the first person to describe “white” blood, the approach of the BELUGA (Russian for "white") study described here indeed helped AI-based diagnostic strategy find its way into hematologists' daily clinical routine.

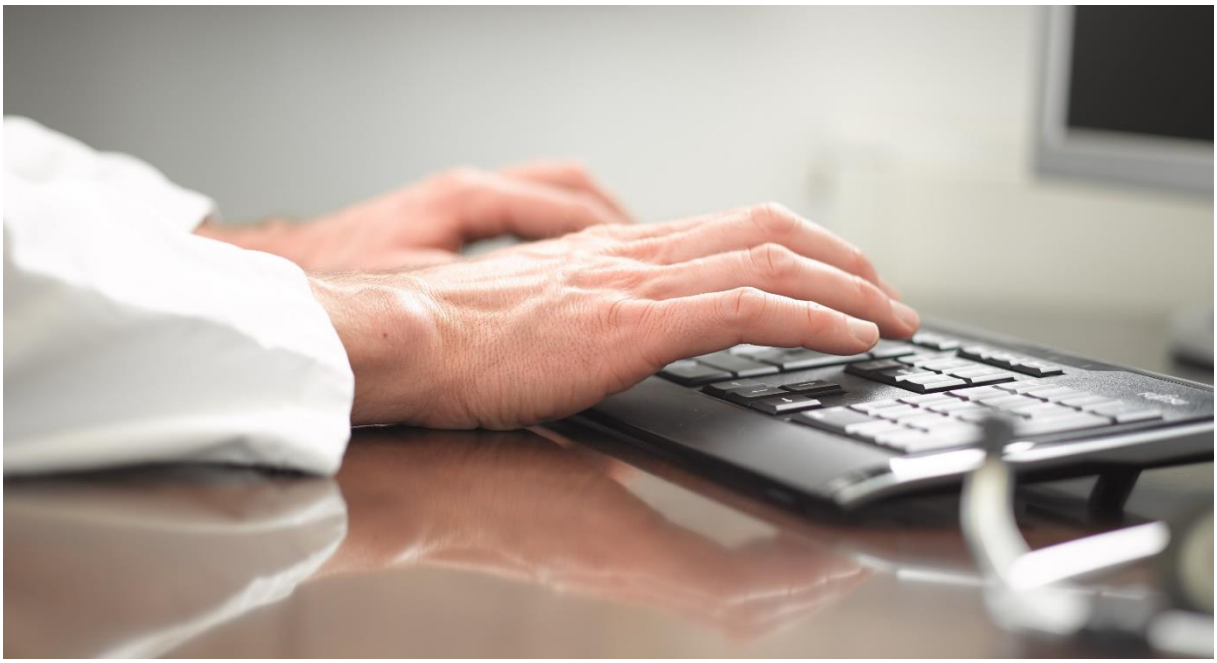
Authors: Prof. Dr. med. Torsten Haferlach, Dr. med. Christian Pohlkamp

We value your opinion!

Our goal is to offer top-notch leukemia diagnostic services to patients and strive for continuous improvement. Therefore, we would like to find out how satisfied and happy the senders of samples are with our services. We take your feedback very seriously and use it as a tool for self-improvement.

We would appreciate if you could spare 10 minutes of your time to complete our anonymous survey. Please use the opportunity to evaluate the cooperation with us and give us specific feedback. The following link will take you to the survey. The survey will be conducted until October 31, 2020.

Click here to partake in the survey.



On our own behalf: Optimizing the quality of service

It is our job to offer you the current repertoire of state-of-the-art leukemia diagnostic services in the Munich Leukemia Laboratory (MLL). To fully optimize the diagnostic process, the integrity of the sample collected is of great importance, both to provide reliable test results and to apply the requisite methods within the operating workflow. So that we may always be able to best serve you as the sender of the sample and the

affected patients, we would like to explain some important aspects of the pre-analytical phase, which includes blood and bone marrow sample collection, handling and transport to the laboratory.

The quality of our diagnostic process depends on our diagnostic methods as well as the integrity of the forwarded sample.

Provision of certain basic information is very important for creating the workflow (i.e. order control) and for the final interpretation of results (particularly for initial diagnoses). In addition to a clearly formulated (suspected) diagnosis (ICD codes on their own are rarely helpful) and a clinical diagnostic question, it is essential to provide blood count details (preferably a differential hematology diagnosis). In the case of follow-up examinations, information on previous therapy is helpful. Unambiguous identification of sample material and any accompanying material (name, date of birth, EDTA or heparin) is essential.

Prolonged sample transit times create obstacles for cytomorphology (if samples need to be plated), cytogenetics (requires viable cells) and immunophenotyping. Samples for these types of tests should ideally reach us within 1-2 days. Samples for molecular genetic analyses also deteriorate if transport takes longer than 3 days.

In urgent cases, a courier service guarantees next-day delivery for biological samples. Please note that shortly before the weekends the "Saturday delivery" comment box is ticked. Our laboratory also accepts samples on Saturdays and on all public holidays (including Bavarian public holidays). It should also be noted that DHL does not currently deliver on public holidays.

When sending bone marrow, the focus is of course on obtaining an aspirate containing tissue fragments (please be sure to provide at least 5 mL in EDTA and, additionally, 5 mL in heparin). In the case of a dry tap ("punctio sicca"), a bone marrow trephine suspended in 0.9% NaCl (not formalin!) with 1,000 IU heparin is a suitable alternative; please also provide 20 mL of peripheral blood in this case.

As for anticoagulation, it must be taken into account that EDTA (or citrate) is necessary for a cytomorphologic diagnostic work-up, whereas heparin (500 IU per mL) is required for cytogenetic analyses. For a cytomorphologic analysis, it is recommended to use the first aspirate, which is most likely to contain tissue fragments. If you make smears for the cytomorphology analysis yourself, we ask you to avoid including too much blood, to avoid the smear being too "thick" and producing squeeze artifacts, and to allow for sufficient air drying time (optimally 30-60 min) before packaging and shipping.

We hope that this information will contribute to the optimal use and optimization of our diagnostic services in the best interest of your patients.

Author: Dr. med. Christian Pohlkamp

MDS-Associated Aberrant Phenotypes in Multiple Myeloma

It has been known for about a decade that in some patients with multiple myeloma (MM), MDS-associated changes in the bone marrow can be detected at the time of diagnosis or later during the course of disease. These include genetic alterations, such as evidence of clonal hematopoiesis and/or MDS-associated cytogenetic anomalies*, as well as aberrant immunophenotypes typical of MDS **. A recent study underlines the clinical relevance of such MDS-associated (immuno)phenotypic anomalies (MDS-PAs) and indicates

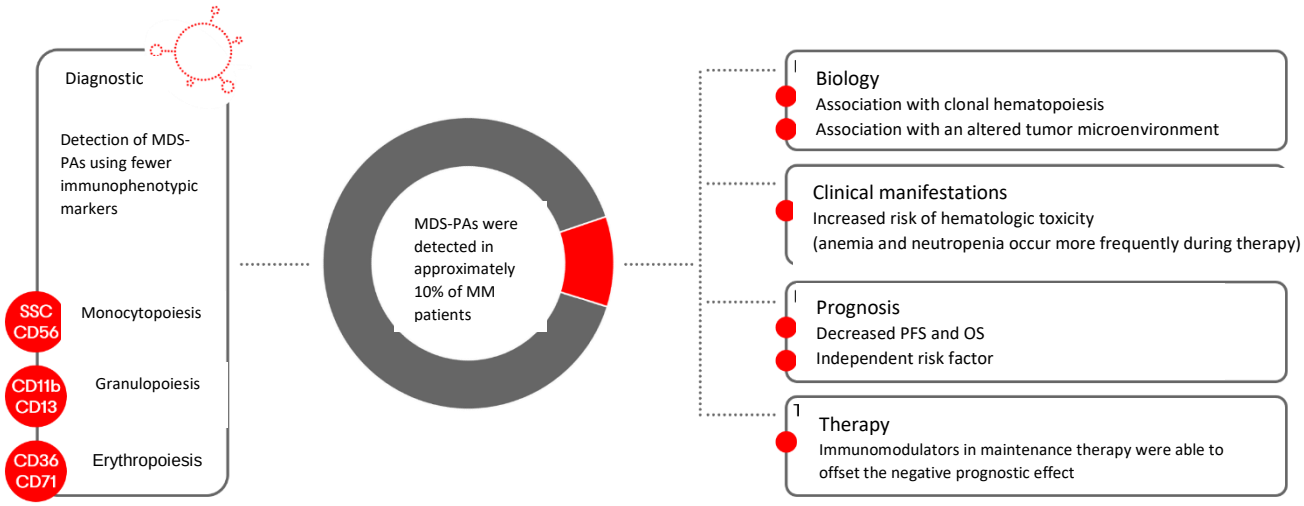


Figure 1: Incidence and clinical relevance of MDS-associated Phenotype Alterations (MDS-PAs) in multiple myeloma in the study by Maia et al. (Blood 2020).

Following the initial literature reports** on the use of flow cytometry to detect MDS-associated Phenotype Alterations (MDS-PAs) in multiple myeloma, a current study (Maia et al. Blood 2020) now deals with the biological, clinical, prognostic and therapy implications of MDS-PAs. Data were collected at the time of diagnosis and after high-dose therapy and autologous stem cell transplantation (HDT/ASCT).

Immunophenotypic characterization at the time of diagnosis was carried out in a group of 285 patients who participated in a therapeutic study. Characteristic MDS alterations were detected in bone marrow aspirates of 33 cases (11.6%), which most frequently included granulocytic dysplasia (22 patients), followed by erythroid dysplasia (10 patients) and monocytic dysplasia (7 patients). More than one lineage was affected in only 5 cases. However, even in cases with MDS-PAs, bone marrow smears displayed unremarkable morphology. In terms of biology, there was an association with an altered tumor microenvironment as well as clonal hematopoiesis. Molecular genetic analyses of 67 patients detected clonal hematopoiesis in 50% of MDS-PAs cases, but only in 22% of cases without MDS-PAs. Phenotype alterations and/or clonal hematopoiesis detected at diagnosis persisted in the majority of patients during the course of the disease; *de novo* MDS-PAs or somatic mutations after HDT/ASCT appeared in only a small percent of cases.

To evaluate the clinical consequences of MDS-PAs, a larger group of 1,252 patients selected from a total of four studies was examined. Since only data on CD56 expression were collected across all study cohorts, MDS-PA detection was restricted to the monocyte lineage. MDS-PAs could be detected at the time of diagnosis in 70 patients (5.6%). Patients with MDS-PAs had an increased risk of treatment-related hematologic toxicity and displayed anemia and neutropenia significantly more frequently than patients without MDS-PAs. MDS-PAs were also associated with decreased progression-free survival and overall survival. MDS-PAs turned out to be a risk factor that was independent of established risk parameters such as ISS stage III, high LDH and high-risk chromosome anomalies (cytogenetics). The negative prognostic effect on survival could, however, be counteracted by post-ASCT maintenance therapy with immunomodulatory drugs.

In summary, screening by flow cytometry proposed by Maia et al. is a cost-effective and rapid method for identifying patients with dysplastic hematopoiesis as early as the time of diagnosis. According to the authors, this may be particularly useful for MM patients with cytopenia of unknown etiology. The increased risk of therapy-related hematologic toxicity and the negative prognostic effect, which can be counteracted therapeutically by administering immunomodulators, make MDS-PA diagnostics clinically relevant.

Additional references:
*Barlogie et al. Blood 2008, Usmani et al. Blood 2013, Chitre et al. Leukemia 2018
**Matarraz et al. Haematologica 2012, Matarraz et al. Leukemia 2014

Author: Dr. Ines Schmidts

Digital order entry platform enables online order submission

In an era where administrative processes are increasingly being digitized, you can use the options of our web-based portal for digital order submissions. 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.

Important dates

Oncology Symposium 2020

Given the success of the Oncology Symposium 2019 "From Biomarkers to Therapy Recommendations", the event will take place again on November 13, 2020 in a digital format. Experts from the field of diagnostic medicine met in 2019 to report on the important role of biomarkers as a guide for personalized medical approaches and to give insights into their experiences of diagnostic oncology.

Click here to submit a registration request.

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