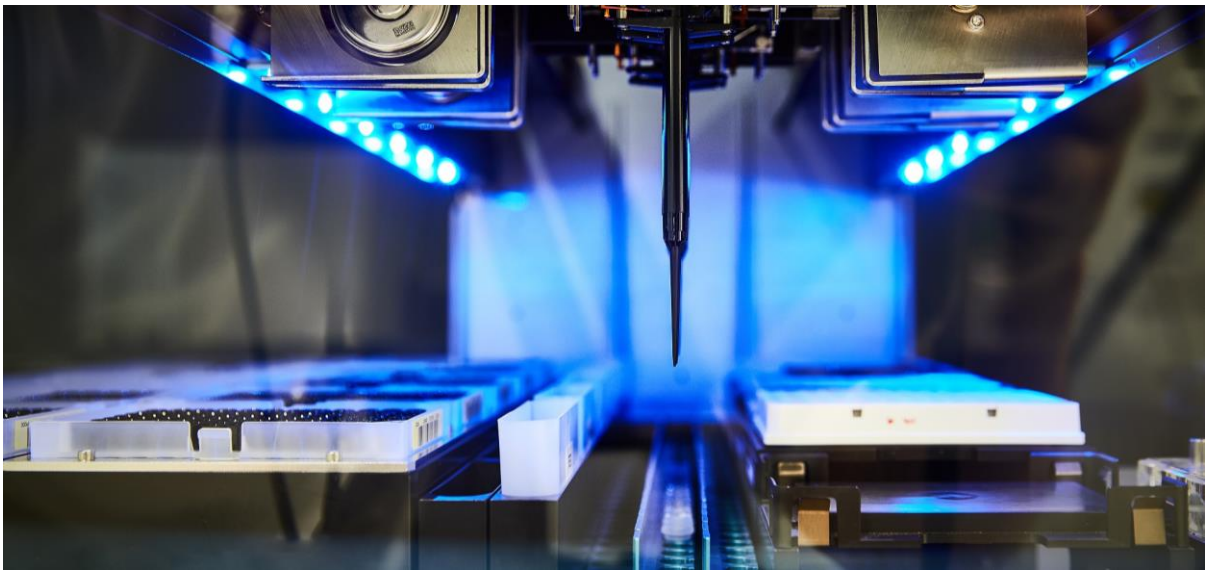
MLL News

06/15/2020



4-stage (4-tier) system for evaluating sequence variants

Sequence data arising from both diseased and healthy tissues has increased significantly in recent years. One of the main reasons for this has been the introduction of novel sequencing technologies, a.k.a. next-generation sequencing (NGS), which now allows us to sequence complete human genomes over the course of just a few days. The large number of alterations identified during this time between different healthy individuals and—of particular interest to us—between healthy and diseased tissues has posed major challenges for diagnosis regarding the classification of such alterations.

What distinguishes "*clearly pathogenic*" from "*polymorphism*"?

Sequence alterations were traditionally subdivided into the categories "*mutation*" and "*polymorphism*," whereby only the former was considered to be relevant or pathogenic. These days, however, the spectrum is much wider. The clearly pathogenic end of the spectrum comprises alterations that are functionally optimally characterized and which may entail therapeutic, diagnostic, or prognostic consequences. The other end comprises alterations termed polymorphisms, which, according to our current state of knowledge, have no disease-causing functions. In many cases, the latter can be identified from the frequency at which they occur in the population.

What alterations lie between these extremes?

A grading of the assessment becomes necessary where, in place of the typical mutation ("hotspot") in a gene for which a targeted therapy exists, another amino acid position is affected. The alterations **p.V600E** in the **BRAF** gene as well as **p.V617F** in the **JAK2** gene can be cited as therapeutic targets at this point. If positions outside the *hotspots* are affected in both genes, and if these have not yet been functionally characterized, a targeted therapy is not necessarily possible and these alterations cannot be described as "clearly," but only as "possibly pathogenic."

Unlike genes characterized by *hotspot* mutations, others are affected by alterations occurring throughout the entire gene sequence. Here again, a comparison with existing databases is important, possibly combined with *in silico* predictions, to determine whether to produce a "*clearly*" or just a "*possibly pathogenic*" assessment.

Alterations that remain (almost) unknown in the known databases and even the very large *MLL*-internal database and in which there is a suspicion of a congenital polymorphism due to the mutation burden are classified at best as *variants* (*unknown significance*). An analysis of normal tissue (e.g., oral mucosa, fingernail) provides information about the origin of an alteration (somatic or congenital).

Based on the world's most widely used 4-class system (4-tier) for assessing sequence variants[1], the MLL has now also introduced a 4-stage evaluation system. This ranges from tier 1 (clearly pathogenic), through tier 2 (possibly pathogenic), tier 3 (variant of unknown significance), and ultimately to tier 4 (polymorphism). Further information on the subject can be found on our website.

[1]Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 2017;19:4-23.

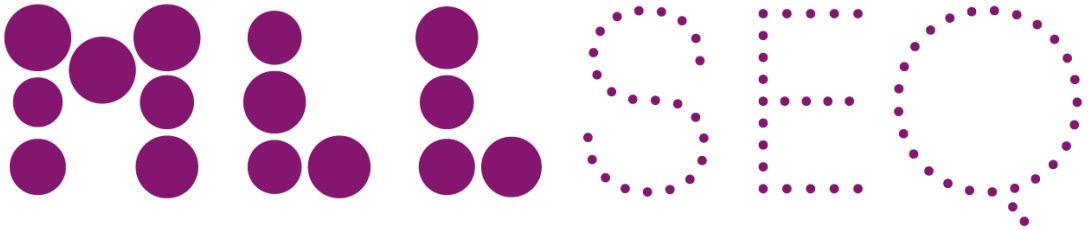
Author: Dr. Frank Dicker

MLLSEQ—our sequencing service has a new look

A year ago we introduced you to MLL Dx, the sister company of MLL, which now offers our extensive expert knowledge to submitters from abroad as well as to the pharmaceutical industry for clinical studies. In addition, the sequencing service also allows researchers and clinicians alike to make use of our sequencing know-how as well as the entire infrastructure of the MLL. This sequencing service has been very well received, and it is now our intention to expand it further. For this purpose we have launched our new MLLSEQ label and branded it with a new logo combined with a completely new website. Check it out at www.mllseq.com.

Together with the Munich design studio Blackspace, we have created a new image and website for our sequencing service, following an exciting development process for the brand's architecture: ***MLLSEQ—we are the next generation: sequencing services***. Under this label we now offer our extensive Next Generation Sequencing (NGS) knowledge extending from *Library Preparation* to *Sequencing Only*, as well as comprehensive bioinformatic processing and visualization of the data generated.

In our new *deep purple* MLLSEQ logo, the label's independence is stressed without giving up its clear association with its bigger MLL sister. Maximum quality standards, flexibility, and fast processing times are therefore all buzzwords at MLLSEQ. MLLSEQ is ISO 15189 and 17025 accredited and Illumina Propel certified.



Over the last five years we have acquired much experience in the sequencing of exomes, genomes, and transcriptomes, which we are now happy to pass on to our customers for project planning. We see ourselves as partners in science and research, and it is therefore our express wish to provide a maximum level of support in matters relating to NGS. Our workflows are quality controlled through all their individual steps. At the same time, they are also designed for a high throughput thanks to our high level of automation. In our setting, we can sequence up to 250 human genomes (WGS[1]) with 90x coverage per week and quickly process them bioinformatically via parallel processing in the private cloud. This allows us to run a complete WGS and also provide the results after just seven days. Our bioinformatics pipelines that have been set up for this purpose are also accredited and comply with all the data protection requirements set out in the GDPR[2].

In addition to the targeted NGS panel, which we use routinely at the MLL for diagnosing leukemia and lymphoma, we also offer a CHIP[3] panel, which is of interest e.g., to cardiology patients, as well as any other type of customized panel. In the latter case, we collaborate closely with IDT, namely Integrated DNA Technologies, whose panel design we can test and optimize *in silico* and then in the laboratory at short notice and within 5–6 weeks.

If you are looking for a partner in the field of sequencing, we would be more than happy to offer our support. You can see our entire range of services at www.mllseq.com.

We have also put together a number of descriptions in our website *glossary* to make it easier to understand the terminology of sequencing. In addition, under the "*Publications*" section you can find links to publications on "our" sequencing projects as well as topics such as Whole Genome Sequencing and RNASeq. We look forward to any questions you may have either via the contact form "contact us" or sent to us directly at info@mllseq.com. We look forward to your virtual visit!

[1]Whole Genome Sequencing

[2]General Data Protection Regulation

[3]Clonal hematopoiesis of indeterminate potential

Author: Dr. Manja Meggendorfer

The logic of next generation sequencing wherever myeloid neoplasia is suspected

Recently, S. Vantyghem et al (Haematologica 3/1/2021, pages 701–707)[1,2] published a "real-life study." The analyses were focused on 177 patients with suspected or already confirmed myeloid neoplasias such as MDS or MPN without a final diagnosis using cytomorphology and immunophenotyping. In a first cohort, an NGS panel with 34 genes was used either to exclude or confirm a definitive diagnosis. In a second cohort, the extent to which prognostic and especially therapeutic consequences would have to be drawn if somatic mutations were detectable was investigated. I would like to introduce this study to you in this article.

The starting constellations were e.g., anemia, thrombocytopenia, neutropenia, or pancytopenia in general, but also thrombocytosis or monocytosis in other cases. Until the molecular analyses were evaluated, the ultimate diagnoses were e.g., ICUS, suspected MDS/MPN, or suspected aplastic anemia. Debatable MPNs were normal for the classic 3 driver mutations. For the patients in the second group, diagnoses based on morphology and cytogenetics were already at hand, mostly classified as low-risk by a relevant scoring system.

NGS revealed clonal hematopoiesis with at least one somatic mutation in a third of the patients of the first group. The genes *ASXL1*, *TET2*, and *DNMT3A* were the most frequently affected. These are of course also found in clonal hematopoiesis of indeterminate potential (CHIP). Detection of already noticeable blood changes should lead to more closely-knit follow-ups. However, a lack of evidence of any somatic mutations could also represent a valuable gain in clinical information. In 33% of cases the diagnosis was confirmed by positive findings, while in a further 50% malignant disease was almost completely excluded by negative findings. If the constellation is unclear, the authors suggest including such results in clinical decision-making in order to mitigate any diagnostic or therapeutic uncertainties.

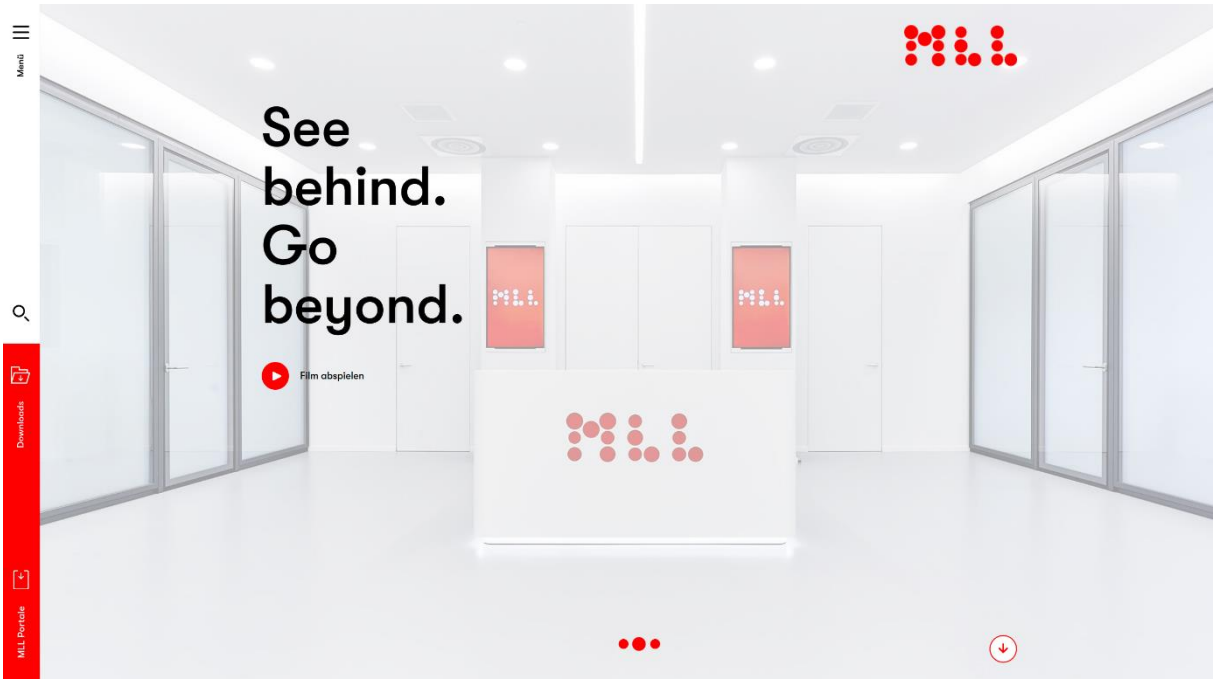
For the second cohort, in addition to prognostically relevant mutations such as *ASXL1* or *RUNX1*, therapeutically important mutations such as those in *SF3B1* could be demonstrated. Overall, the authors therefore assume that where the constellation is unclear, the clinical reliability of the diagnosis and the informing of patients will be improved in 83% of the cases examined, while in 19% of the investigated cases (cohort 2), prognostically and/or therapeutically relevant information becomes available. The publication also deals with the question of the extent to which the information contained here from the myeloid NGS panel can be relevant not only clinically and therapeutically, but also for overall cost calculations. Repeated and further diagnostic measures or sometimes even unnecessary treatment (e.g., allogeneic transplantation) could be avoided by excluding clonal alterations. Further comparative analyses were suggested. In view of the falling costs for sequencing, it can already be assumed that not only the clinical gain for patients but also the economic benefit will make an earlier use of NGS-assisted molecular screening feasible in the future.

1) Vantyghem S, Peterlin P, Thepot S, Menard A, Dubruille V, Debord C, Guillaume T, Garnier A, Le Bourgeois A, Wuilleme S, Godon C, Theisen O, Eveillard M, Delaunay J, Maisonneuve H, Morineau N, Villemagne B, Vigouroux S, Subiger F, Lestang E, Loirat M, Parcelier A, Godmer P, Mercier M, Trebouet A, Luque Paz

D, Le Calloch R, Le Clech L, Bossard C, Moreau A, Ugo V, Hunault M, Moreau P, Le Gouill S, Chevallier P, Bene MC, Le Bris Y. Diagnosis and prognosis are supported by integrated assessment of next-generation sequencing in chronic myeloid malignancies. A real-life study. *Haematologica*. 2021;106(3):701-707. https://haematologica.org/article/view/9704

2) Haferlach T. The time has come for next-generation sequencing in routine diagnostic workup in hematology. *Haematologica*. 2021;106(3):659-661. https://haematologica.org/article/view/10189

Author: Prof. Dr. Dr. Torsten Haferlach



Novel aspects of the MLL website in the area "Diseases/Diagnostics"

The "Diseases/Diagnostics" page on the website of the Munich Leukemia Laboratory describes, among other things, the detailed diagnostics, classifications, and prognosis of hematological diseases. The website has been updated over the past few months. A number of new hematological entities have been added. And as part of a further internationalization drive for our company, all pages are now also available in English.

Highest scientific standards and interdisciplinary cooperation are resulting in an ever expanding range of services offered by the Munich Leukemia Laboratory. The MLL team, consisting of experienced doctors, biologists, bioinformaticians, and medical-technical assistants, is working intensively on further optimizing the diagnosis of hematological diseases, thereby enabling more targeted and more efficient therapies for the patient quicker than ever.

The website also strives to fulfill these demands by providing extensive information. For example, you can find detailed and up-to-date articles on a variety of hematological diseases and their classifications there. The diagnostic possibilities of cytomorphology and immunophenotyping to define the phenotype as well as

chromosome analysis, fluorescence in situ hybridization (FISH), and molecular genetics are described for each hematological entity. Molecular genetics in particular is leading us to new insights into the pathogenesis of hematological diseases. This directly impacts on therapeutic decisions, therapy management, and ultimately the prognosis for the patient.

Over the past few weeks, a number of new entities have been added to the website. These include, for example, mastocytosis, a heterogeneous disease which back in 2017 formed its own separate chapter in the new WHO classification. Since May 2020, the website of the Munich Leukemia Laboratory has been offering all the relevant information regarding mastocytosis diagnostics.

There is also a new, detailed information sheet relating to "clonal hematopoiesis of indeterminate potential" (CHIP) in cardiovascular diseases. Over the last few years, it has been revealed that mutations in genes influencing epigenetic regulation are also found in hematologically healthy people of an advanced age. These individuals seem to have a higher risk of developing not only hematological, but also cardiac diseases. The detection of certain somatic mutations is associated with an increased rate of atherosclerosis, aortic valve stenosis, and heart failure. However, no evidence-based recommendations or therapies exist for specifically lowering the CHIP-associated cardiovascular risk.

The internationalization of the company also remains a main focus of our activities. The entire website of the MLL is offered not only in German, but also in English. Diseases, of course, do not respect national boundaries. For this reason it is only logical to provide the expertise and diagnostic possibilities of the Munich Leukemia Laboratory across national boundaries. The aim is to better meet the needs of international submitters and partners alike, and to make the expertise of the Munich Leukemia Laboratory much more accessible to them.

Author: Prof. Dr. Rainer Ordemann

Important dates

Oncological Symposium 2021

Save the date: The successful symposium series "Oncological Symposium 2021— From Biomarkers to Therapy" will enter its third round on November 5, 2021. The event offers insights into modern oncological precision medicine, which combines innovative diagnostic methods and therapeutic strategies into a greater whole. The symposium will take place virtually and, if circumstances allow, also as a face-to-face event at the MLL Munich Leukemia Laboratory. You can now reserve your seat.

Further information as well as a link for registration can be found here.

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