COVER PAGE
SOLVING RIDDLES THROUGH SEQUENCING (SIRIUS)

Long title: Testing the diagnostic supremacy of sequencing-only approaches in hematologic malignancies: an observational trial

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Testing the diagnostic supremacy of sequencing-only approaches in hematologic malignancies: an observational trial

Running Title: **S**OLV**I**NG **RI**DDLES THRO**U**GH **S**EQUENCING **(SIRIUS)**

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GCP Statement: This trial will be performed in compliance with the good clinical practice directive from the European Union (2005/28/EC)

Funding:

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SUMMARY

During the last decades hematologists have excelled at improving and refining the classification, diagnosis, and thus ultimately the therapeutic decision-making process for their patients. This continuous evolution proceeded in parallel to seminal discoveries in basic science such as FISH, PCR and NGS. So far, the current WHO classification serves as reference to diagnostic decision making and is largely based on 5 diagnostic pillars: cytomorphology of peripheral blood and/or bone marrow smears, histology and immunohistochemistry of bone marrow trephine biopsies or lymph nodes, immunophenotyping, chromosome banding analysis supplemented by FISH analysis, molecular genetics including PCR and targeted panel sequencing via NGS. This leads to a swift diagnosis in 90 % of all cases. The leftover 10 % remain a challenge for hematopathologists and clinicians alike and are resolved through interdisciplinary teams in the context of specialized boards. With the advent of high throughput sequencing (mainly WGS and WTS) the possibility of a comprehensive and detailed portrait of the genetic alterations – specifically in challenging cases – has become a realistic alternative to classical methods. In SIRIUS we will prospectively challenge this hypothesis to address the question of how often a better or final diagnosis can be delivered by WGS and/or WTS and if unclear cases can be efficiently resolved.

Type of Research: observational study, diagnostic study, hematology, leukemia, next generation sequencing

Intervention: none

APPROVAL

This study will be conducted with the utmost respect for individual patients in accordance with the requirements of this diagnostic trial protocol and especially in accordance with the following:

- Good clinical practice directive (European Union) (2005/28/EC)
- The ethical principles in accordance with the Declaration of Helsinki
- International Conference on Harmonization (ICH) E6 Good Clinical Practice: consolidated guideline
- Guidelines for Good Clinical Practice (*Deutsche Forschungsgemeinschaft* DFG)
- Standards and guidelines for the interpretation of sequence variants (PMID: 25741868)
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical trial disclosure laws, and regulations.

I. INTRODUCTION

1. Background and Rationale

Treatment of hematological diseases relies on a single cardinal prerequisite: correct classification within the broad specter of malignant diseases arising from the hematopoietic system. With the ever-expanding availability of distinct yet complementary diagnostic tools, our understanding of the landscape of hematological diseases steadily increases. As such, the current consensus classification as summarized through the WHO classification (2017) represents a compass guiding diagnostic algorithms to the correct diagnosis. Today, the gold standard of routine diagnostic process relies on five methodological pillars: cytomorphology, histology, chromosomal cytogenetics, immunophenotyping, and molecular genetic testing. This leads to a treatment enabling diagnosis in the vast majority of cases. However, approximately 10 % of cases remain unresolved from a diagnostic point of view and hence do not lead to a satisfactory diagnosis according to current WHO standards (2017). We intend to solve this issue to provide illuminating diagnostic guidance for the best possible patient´s care.

2. Objectives

To address this problem, we hypothesize that novel high throughput sequencing methods, e.g., whole genome and/or whole transcriptome sequencing are able – by virtue of painting a more delicate genetic portrait of a tumor sample – to provide a more accurate diagnosis.

To this end we generated a reference collection of 5,500 samples with the full spectrum of hematological malignancies, for which we performed whole-genome sequencing as well as whole-transcriptome sequencing. Moreover, gold standard diagnoses according to WHO classification with all needed techniques, all performed in MLL, clinical data and therapy response data are fully available for these cases. The main advantage of this reference

collection consists of the unambiguity of each diagnosis, providing a reference framework for any further classification and diagnosis especially in difficult cases.

Therefore, SIRIUS will compare the diagnostic superiority of WGS or WTS to the combined approach with gold standard results and by matching the obtained results to the nearest “digital sibling” within our reference cohort of more than 5,500 WGS and WTS (both in 93% of cases). To this end, we will use an inhouse developed matching algorithm, which is able to match genomic or transcriptomic profiles to a group of similar cases and gold standard results from timepoint of this study. Current workflows intended to generate WTS/WGS data from patient samples – all while fulfilling state of the art accreditation (ISO 15189) – require up to 5 – 7 days. This is largely comparable to classical methods but holds the promise to replace error prone and arduous iterations in the methodological work up. The objective is to test whether WTS and/or WGS based approaches can surpass classical methods regarding diagnostic precision and routine reliability. Here we will test this hypothesis in a prospective real-world setting under diagnostically difficult circumstances.

II. STUDY DESIGN

1. Type of Study

SIRIUS is conducted as a monocentric prospective case-control study. The study population consists of carefully chosen patients with potential hematological malignancy, for which current diagnostic methods were not sufficient to provide clear-cut diagnosis and definitive clinical guidance. SIRIUS is entirely a non-interventional study without therapeutic consequences for direct patient care. Data will be used as research study and micro-cost analysis will be provided.

2. Duration of Study

SIRIUS will be conducted for a total number of 110 patients with inconclusive diagnosis by gold standard techniques for a total of up to nine months after the first enrollment. Patients to qualify for study will be selected by referring center and PI before material is send to MLL.

3. Quality Control

Due to the heterogeneity in quality of blood and bone marrow samples arriving at MLL, SIRIUS is preceded by a rigorous quality assessment of every patient history, data already available and sample source and quality to be potentially included in the present study. The minimal requirements are listed in III.

4. Primary Study Endpoints

Overall, the efficacy and supremacy of WGS/WTS analysis will be subjected to current standard procedures. The primary endpoint will be assessed as follows: unclear cases will be subjected to three diagnostic algorithms:

(1) Inhouse at referring site by histopathological diagnosis in the context of a hematological tumor board according to current standards (“best practice”)

(2) Current gold-standard diagnostic workup as performed routinely by the MLL, consisting of

a. Cytomorphological diagnosis

b. Immunophenotypic diagnosis

c. Chromosomal banding analysis

d. Molecular testing

e. NGS based Targeted panel sequencing

(3) WGS and WTS sequencing plus matching to nearest digital sibling in 5,5k cohort

The results of (2) and (3) arms will be first compared to the result obtained in (1) since this is the therapy guiding approach *in domo*. Clinical follow-up observations will be made to assess the success of this first diagnosis.

Next results from (2) and (3) will be compared against each other to obtain an assessment of replaceability of current gold-standard methods to either WGS or WTS and WGS/WTS combined. If both approaches do not yield a similar conclusion, specific data from (2) will be added to results in (3) until a definitive assessment can be made.

5. Secondary Study Endpoints

- Turn-over time until potential therapy guiding diagnosis
- Cost-effectiveness until timepoint of diagnosis and therapy
- Compare WES to WGS data in SIRIUS cohort
- Identify number of potential actionable targets for which a therapy has been approved but identification was missed in (1) and (2)
- Identify putative disease stage in comparison to (2) and clinical history

III. SUBJECT SELECTION AND WITHDRAWAL

1. Number Of Subjects

SIRIUS intends to enroll a total number of 110 cases provided by approximately 50 certified hematological centers.

2. Gender, Age

Patients' samples from both sexes will be used (male and female). Only samples from adult patients (18 years or older) will be used.

3. Inclusion Criteria

- Patients having been investigated with a suspected hematological disorder and:
  - Having unclear diagnosis after internal routine diagnosis
  - Unusual clinical course
  - Unusual r/r status or non-responder
  - Multiple parallel hematological conditions
  - Difficult/rare therapy associated/secondary neoplasms
- Current diagnostic work-up is not satisfactory in terms of (1) accuracy (2) clinical behavior
- Only samples of patients min. 18 years of age will be used
- Material with a minimum of 20% tumor content in bone-marrow or peripheral blood sample
- Samples must suffice quality attributes control which are denoted in (4.)
- Patient´s informed consent

4. Exclusion Criteria

- Sample is not fit for state-of-the-art diagnosis, fails initial quality control. For quality insurance we will exclude samples with wrong anticoagulant sent. Samples with damage due to meteorological reasons (freeze-thaw damage or elevated temperature) will be excluded.
- Samples with to scarce material jeopardizing routine gold-standard diagnosis will be excluded (tumor content < 20 %).

5. Location

SIRIUS will be conducted as monocentric trial at the MLL.

IV. STATISTICAL PLAN

To determine the statistical significance within the endpoint analysis we will assess both quantitative as well as qualitative variables. Quantitative variables will be described with the number of non-missing values, mean, standard deviation, median, and minimum/maximum values. Qualitative variables will be expressed as a number and percentage of patients with each qualitative characteristic. The missing values are not intended to be included in the calculation of percentages. Sensitivity and specificity will be assessed specific to each method, with respect to internal gold standard diagnostic work-up.

V. RISKS AND BENEFITS

Potential Direct Benefits to Subject

Conducting SIRIUS will not bear any risks for patients enrolled in the study. Normal State-of-the-art diagnosis is provided for each sample and prioritization of sample material in favor current diagnostic material and reporting is performed to prevent jeopardization of gold standard diagnosis.

VI. DATA HANDLING AND RECORD

Data management documents will be generated under the responsibility of the Sponsor. A management plan will be issued before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation. The data management documents will describe analysis methods and individuals who are authorized to enter the data, decisions about ownership of data, source data storage, the origin and destination of the data and who will always get access to the data. Data Management Responsibilities are

primarily handled by co-investigator Wolfgang Kern (WK). Per request of external researchers, the Sponsor will provide these investigators with additional data relating to the trial, duly anonymized and protected in accordance with applicable requirements.

VII. STUDY MONITORING, AUDITING, AND INSPECTION

The Investigator will make all the trial-related source data and records available at any time to quality assurance auditor(s) mandated by the Sponsor, or to domestic/foreign regulatory inspectors or representatives who may audit/inspect the trial. The main purposes of an audit or inspection are to assess compliance with the trial protocol and the principles of ICH-GCP including the Declaration of Helsinki and all other relevant regulations.

VIII. FINANCIAL CONSIDERATIONS

The MLL is the main sponsor of this trial. Reagents for gold standard investigations will be provided by MLL. Reagents for sequencing will be provided for by Illumina®, Inc. San Diego, CA. All Data generated are fully owned by MLL. MLL will share aggregated and anonymized data with Illumina®, i.e., without revealing patient sensitive data such as germline mutations.

IX. CONFLICT OF INTEREST

Prof. Dr. phil. Dr. med. Torsten Haferlach and Prof. Dr. med. Wolfgang Kern are part owners of the MLL Munich Leukemia Laboratory.

PUBLICATION PLAN

The results obtained from SIRIUS will analyzed according to the guidelines of Good scientific Practice of the German Science funding agency (DFG). Results that will be interesting for the

scientific community will be submitted and subsequently published in peer-reviewed academic journals, according to the appropriate scope and audience.

At the end of the trial, one or more manuscripts for joint publication may be prepared in collaboration between the Investigator(s) and financial sponsors. The PI reserves the right to be last author(s) in all publications related to this trial. In the event of any disagreement in the content of any publication, both the Investigator's and the Sponsor's opinion will be fairly and sufficiently weighed and represented in the publication.

X. ARCHIVING

The PI is fully responsible for maintaining all the records (protocol and protocol amendments, relevant correspondence, and all other supporting documentation), which enable the conduct of the trial at the site to be fully understood, in compliance with ICH-GCP.

The study site should plan on retaining such documents for 10 years after study completion. These documents should be retained for a longer period if required by the applicable regulatory requirements or the hospital, institution, or private practice in which the study is being conducted. Patient identification codes (patient names and corresponding study numbers) will be retained for this same period.

Trial Master File

The Sponsor will archive the Trial Master File in accordance with ICH-GCP and applicable regulatory requirements.