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SANGUINE

TITLE: Early Detection and Screening of Hematological Malignancies - SANGUINE

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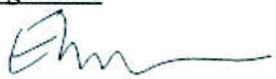
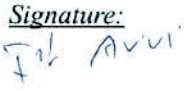
Project Coordinator, Author	Prof. Yuval Ebenstein, PhD	*Date:* 26/06/23	*Signature:* 
Author	Prof. Irit Avivi, MD, PhD	*Date:* 26/06/2023	*Signature:* 

INVESTIGATOR SIGNATURE PAGE

By my signature, I agree to personally supervise the conduct of this study in my affiliation and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, ICH Good Clinical Practices guideline, and local regulations governing the conduct of clinical non-interventional studies.

Local Site Name and Country:

Site Name	Country
Principal Investigator's Signature	Date

Printed Name:

PROTOCOL SYNOPSIS

STUDY TITLE	Early detection and screening of hematological malignancies - SANGUINE
INDICATIONS	Adult subjects with hematological malignancies: Multiple myeloma (MM), pre-MM conditions [smoldering MM (SMM) and monoclonal gammopathy of undetermined significance (MGUS)], Hodgkin lymphoma (HL), non-Hodgkin aggressive lymphoma NHL [diffuse large B cell lymphoma (DLBCL), FL, MZL, acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), subjects at risk and control subjects with no malignant disease .
STUDY DESIGN	This is a non-interventional, multi-center, open-label, controlled study to identify and characterize the epigenetic signatures for a set of hematological malignancies.
OBJECTIVES	<u>Primary Objectives:</u> 1. Biomarker discovery - define a set of differential epigenetic biomarkers that uniquely identify the following conditions: MM, pre-MM conditions (SMM and MGUS), HL, aggressive NHL (DLBCL), FL, MZL, AML, MDS subjects at risk and control subjects with no malignant disease. 2. Validation – validating the discovery platform (HemaChip) as a diagnostic tool for various hematological malignancies. 3. Towards early detection – Selected patients tested periodically to evaluate early relapse detection capability of the HemaChip. 4. Towards population screening - evaluate the sensitivity and specificity for screening in populations at risk for developing the investigated cancers: (i) elderly (>65 years old) at high risk

	to develop MM; (ii) first-degree relatives of the conditions described above. Secondary Objectives: 1. Evaluate the correlation between epigenetic status and well-known prognostic markers, including known genetic markers. 2. Predict response to therapy. Exploratory Objectives: 1. Identify new therapeutic targets based on epigenetically modified genomic loci. 2. Provide prognostic information at pre-treatment and monitoring of minimal residual disease (MRD).
METHODOLOGY	Subjects will be screened for eligibility and then, after signing an Informed Consent Form, the first peripheral blood sample will be obtained. Periodical blood samples will be obtained from the participants. Relapse patients will have their retrospective blood samples analyzed to identify early signs of disease. **The first stage** (discovery phase) will include at least 30 patients from each of the following groups: MM, pre-MM conditions (SMM and MGUS), HL, aggressive NHL (DLBCL), FL, MZL, AML, , MDS and control subjects with no malignant disease. **In the second stage**, at least 250 patients with MM and 250 patients with NHL and at least 100 patients with each of the remaining hematological malignancies mentioned above will be tested. Out of these patients, AML, lymphoma and MM patients will be followed-up at the clinical sites. Periodic sampling will be defined according to disease type and progression rate. Blood and plasma samples will

	be stored in the clinical sites until relapse diagnosis. At this stage, blood samples will be analyzed retrospectively on the HemaChip. The screening, enrollment and blood collection can begin in the first stage of the trial, in order to allow a maximum follow-up period for at-risk subjects as part of the study, and to meet the recruitment goals. **The last stage** consists of the screening of a larger group of subjects at risk to develop MM / lymphoproliferative disorder. This stage will include 400 elderly patients (>65 years old) and 500 first-degree relatives of patients (and in particular siblings). The screening, enrollment and sample collection can begin in the first stage of the trial, in order to allow a maximum follow-up period for at-risk subjects as part of the study, and to meet the recruitment goals. **In all stages**, the age and sex-matched subgroups will be considered and matched. During the follow-up period, demographic and baseline parameters including sex, age, race, height and weight, medical history, smoking status, details of initial diagnosis and treatment history, concomitant medications as well as adverse events (AEs) of special interest (see section 9.1), (serious) AEs related to study procedures, treatment for the disease, disease response and survival status will be collected (as applicable).
STUDY PERIOD	Recruitment and follow-up period: 36 months
POPULATION SIZE	First stage: at least 300 subjects Second stage: at least 1000 subjects

	Third stage: up to 900 subjects
MAIN INCLUSION CRITERIA	**General criteria for all study population :** 1. Male and female subjects ≥18 years of age 2. Ability to understand and willingness to sign a written informed consent document. **For Patients with hematological malignancies:** 1. Patients who have been diagnosed, have measurable disease and / or are being monitored / followed-up due to one of the following conditions: MM, pre-MM conditions (SMM and MGUS), HL, aggressive NHL (DLBCL), FL, MZL, AML, MDS that did not yet undergo any treatment. NOTE: Patients diagnosed with DLBCL that is transformed from FL or MZL, and patients diagnosed with AML secondary to MDS or MPN, that were treated for their primary disease (FL/MZL/MDS/MPN) prior to study enrollment, are eligible. **For subjects at risk for developing the investigated hematological malignancies:** 1. First-degree relatives; 2. Elderly subjects ≥ 65 years of age.
MAIN EXCLUSION CRITERIA	1. Patients/subjects with current co-diagnosis of another type of cancer; 2. Patients/subjects with a known active or prior cancer (other than defined as study population), occurring within the last 2 years (even if considered to be in complete remission). Patients/subjects with non-melanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection;

	3. Patients/subjects with active inflammatory autoimmune disease that requires treatment with immunosuppressive/ immunomodulation agents; 4. Patients/subjects with known human immunodeficiency virus (HIV) positive; 5. Patients/subjects with known active Hepatitis A/B/C or past hepatitis C; 6. Subjects that are likely to be noncompliant with the protocol, or felt to be unsuitable by the investigator for any other reason.
DURATION OF SUBJECT PARTICIPATION	Subjects that serve as controls, subjects at risk (from the Third stage) and a subset of patients from the First and Second stage are expected to donate blood a single time. Following this donation, their participation will end. For a subset of patients, it is expected, after signing the informed consents, that the serial samplings will be performed during the disease follow-up according to the standard clinical practice and/or recommended schedule and disease assessment plan (see Table 3). For enrolled subjects, all have the right to withdraw at any time. All follow-up patients will be followed for a period of up to 36 months. Subjects who complete the main study, may be followed up for their disease status, anti-cancer treatments, concomitant medications, new primary malignancy and survival, following a statistical review of the study results. Serial blood samplings will be performed during the long-term follow-up according to the standard clinical practice and/or recommended schedule and disease assessment plan (see Table 3).

	A detailed plan for long-term follow-up beyond the main study will be provided as an amendment to this protocol.
EVALUATIONS	Classification of a broad spectrum of haematological malignancies based on the detection of epigenetic biomarkers from genomic DNA, cell-free (cf) DNA, exosomal DNA, RNA and non-coding RNA. The identified biomarkers will include proteins, metabolites, and other characteristic biomolecules. Year 1: During the discovery phase, all tests will be conducted by JaxBio and TAU with the aid of technical service providers. At this stage, microarray measurements will be performed on a commercial platform that will be purchased from suppliers such as Agilent / Illumina. All reagents needed for the test will be either purchased or produced in-house. Years 2-3: Throughout the second phase of the project, a custom-targeted microarray, HemaChip will be developed and used for all tests. The HemaChip and custom reagents will be distributed to partners' labs and all tests will be conducted at the clinical sites and partners labs. Additional validation tests will be conducted by JaxBio and TAU, as needed.
STATISTICAL CONSIDERATIONS	All subjects meeting the eligibility criteria who signed a consent form and donated at least one blood sample will be considered evaluable for analysis. All data collected will be summarized and presented. Continuous variables will be described as the mean, median, standard deviation and range of n observations. Categorical data will be described with contingency tables including frequency and percentage. Individual patient listings will be generated and presented. We used an initial sample size determination for binomial distribution (arcsine transformation, significance level = 0.05, test power = 0.8). Based

	on previous experiments, we propose two estimates – expected target sensitivity, and minimum acceptable sensitivity. Estimate of the expected target sensitivity of the discovery chip was set to 95%. This estimate sets the minimum acceptable limits for establishing statistical tests and represents the best expected result. The second estimate sets the minimum acceptable sensitivity of the discovery chip to 89%. The value is based on reported literature and the fact that this phase is conducted on diagnosed patients with active disease. With these parameters, a minimum sample size of 30 samples/indication is needed. This should be revisited upon accumulation of data. Statistical descriptions and analyses will be carried out using SPSS and in-house statistical analysis software.
INTERIM ANALYSIS	A single formal interim comparative analysis of the epigenetic patterns in blood-derived DNA from control subjects with no malignant disease and subjects at risk for developing the investigated hematological malignancies and the patterns obtained for all malignant and premalignant hematological conditions will be conducted after approximately 24 months from the beginning of the study and will include: Comparative analysis for 250 newly diagnosed NHL samples with HemaChip Comparative analysis for 250 newly diagnosed MM samples with HemaChip Comparative analysis for 100 samples of the following conditions: SMM, MGUS, HL, AML, MDS, control subjects with no malignant disease and subjects at risk for developing the investigated hematological malignancies.

POWER AND SIGNIFICANCE LEVEL	The sample size of each stage of the clinical study will be determined in accordance with technology development requirements. Hence, three designs should be taken into account: Discovery stage performed with the discovery chip, and the validation and screening stages performed with targeted HemaChip. All the calculations have been made with optimistic assumptions that we will be able to prepare such sensitive chips that classify the studied hematological malignancies types with expected target sensitivity of above 95%. Moreover, the minimal sensitivity of 80% was set as a lower bound for all statistics to get a scale for the further Sample Size calculation. Importantly, based on incidences of particular diseases the calculated Sample Size will include significant proportions of all diseases of interest.

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LIST OF ABBREVIATIONS

AE	Adverse Event
AML	Acute Myeloid Leukemia
ASCT	Allogeneic Stem Cell Transplantation
cfDNA	Cell-Free DNA
CRF	Case Report Form
CRO	Clinical Research Organization
DLBCL	Diffuse Large B Cell Lymphoma
eCRF	Electronic CRF
FL	Follicular Lymphoma
FNOL	University Hospital Olomouc
FZMB	Forschungszentrum fur Medizintechnik und Biotechnologie
GCP	Good Clinical Practice
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IMWG	International Myeloma Work Group
IRB	Institutional Review Board
MedDRA	Medical Dictionary of Regulatory Activities
MDS	Myelodysplastic Syndrome
MGUS	Monoclonal Gammopathy of Undetermined Significance
MM	Multiple Myeloma
MRD	Minimal Residual Disease
MPN	Myeloproliferative Neoplasms
MZL	Marginal Zone Lymphoma
NHL	Non-Hodgkin Lymphoma
NKUA	National and Kapodistrian University of Athens
PCD	Plasma Cell Dyscracia
PCR	Polymerase Chain Reaction
PD	Progressive Disease
QPCR	Quantitative PCR
QA	Quality Assurance
SAE	Serious Adverse Event

SMM	Smoldering Multiple Myeloma
TAU	Tel-Aviv University
TASMC	Tel-Aviv Sourasky Medical Center
VULSK	Vilnius University Hospital Santaros Klinikos

1 BACKGROUND AND STUDY RATIONAL

Approximately 10% of cancer cases in Europe account for hematological malignancies, including leukemias, lymphomas and myelomas. In 2020, 293,775 people in Europe were diagnosed with hematological malignancies, and 148,394 people died[1]. Despite advances in drug development and emerging targeted therapies, effective and early diagnosis combined with population screening remains the most powerful tool for fighting cancer. In general, there is an urgent need to have minimally-invasive methods to screen and detect hematological malignancies. For cancer management, these tests are needed to quantify pre-treatment and residual tumor burden to provide useful prognostic information before treatment, to assess molecular response at interim time points and minimal residual disease (MRD) at the end of treatment, and to identify tumor-specific abnormalities when possible.

Bone marrow biopsy, considered the gold standard of multiple myeloma (MM) diagnosis and indicated conditions of other hematological malignancies, provides only a snapshot of the disease at the moment taken and is an invasive method, coming with its risks. However, patients diagnosed with MM and other hematological malignancies require continuous painful follow-up biopsies to monitor the condition, this may indeed lead to avoidance of follow-up visits by patients and subjects at risk of being unwilling to get regular screening. For non-Hodgkin's lymphomas (NHL) and Hodgkin's lymphomas (HL), lymph node biopsy is the gold standard for their diagnosis. Newly developed minimally-invasive diagnostic tools based on liquid biopsy may complement and possibly eventually overcome conventional biopsies. Liquid biopsy can provide real-time feedback on patient condition based on systematic and routine measurements of critical biomarkers - a characteristic that is objectively measured and evaluated as an indicator of normal biological processes or pathogenic processes. Despite extensive progress, research and development are still needed before implementation of liquid biopsy-based tests as the standard of care.

Accumulating evidence shows that epigenetic changes provide unique signatures indicating disease type and stage[2]. These modifications will be detected in genomic DNA extracted from peripheral blood cells, as well as from cell-free and exosomal DNA arising mostly from dying cells, cancer or healthy.

Cancer cells, and specifically hematological tumor cells, undergo dramatic changes in methylation patterns during the tumorigenesis process. For AML, aberration in DNA methylation has been shown to be a key factor in initiation and progression of the disease, and methylation patterns may predict disease outcome[3]. Alterations in DNA methylation were also observed at early stages of hematological cancers as well as in pre-malignant conditions such as myelodysplastic/myeloproliferative neoplasms (MPN/MDS)[4,5]. Mutations in DNA methyltransferase 3A (DNMT3A), one of the key mediators responsible for DNA methylation, were found in almost all hematological malignancies at various frequencies with up to 35%, and contribute to the epigenetic aberrations in hematological malignancies [6]. 5-hydroxymethylcytosine (5hmC), the product of demethylation process, was also found to be aberrantly expressed in hematological malignancies and other cancers, and correlate with disease stage and prognosis[7]. Furthermore, in up to 25% of hematological malignancies, the key enzyme TET2, which oxidizes 5mC to 5hmC is mutated, resulting in aberrant 5mC and 5hmC patterns[8]. These aberrant epigenetic modifications can be detected in genomic DNA extracted from peripheral blood of patients with myeloid malignancies[7]. Since cancer cells tend to proliferate at high rates, this process is also accompanied by extensive cell death and elevated representation of tumor DNA in the blood plasma[9]. Thus, methylation and hydroxymethylation patterns in cell-free DNA were shown to indicate cell-of-origin cell death by tissue specific epigenetic signal[10–12]. These facts triggered various technologies and assays aimed at probing the genetics, fragment length and methylation signatures of cell-free DNA. It has been already found that monitoring of epigenetic changes provides useful biomarkers for diagnosis and monitoring of cancer, and the first products are already appearing in the market, with Grail Inc. Currently leading the cancer diagnosis domain with a sequencing-based cell-free DNA methylation assay. Most activity in the field is focused on identifying biomarkers using existing methods (mostly sequencing, micro-arrays or QPCR for nucleic acids). Some of these methods suffer from low single-to-noise (SNR) ratio inherently due to the need to sample the entire DNA content and detect the tiny fraction of the relevant DNA population[13]. Other methods suffer from low throughput and/or high costs. SANGUINE introduces a novel platform that is capable of probing thousands of epigenetic DNA markers at high sensitivity and low cost. Preliminary results using the SANGUINE technology have shown that hundreds of loci exhibit detectable differential methylation pattern between hematological malignancies and

healthy control individuals, indicating potential for early diagnosis. Furthermore, different types of hematological malignancies showed differential methylation patterns, a fact that will be utilized for development of an accurate minimally-invasive diagnostic tool for diagnostics as well as disease monitoring.

The circulating Cell-free Genome Atlas (CCGA) study (clinical trial NCT02889978) is an active study that recruited more than 15,000 participants aiming to discover cancer-specific biomarkers in cell-free DNA. Three types of whole genome analysis were adopted, where methylation analysis (performed by whole-genome bisulfite sequencing) outperformed other methods. A mid report presented the ability to recognize multi-cancer types (cross >50 cancer types) at all stages, with specificity of 99.5%, indicating a low false- positive rate of 0.5%. Sensitivity of cancer signal detection increased with higher stages - 16.8% for stage I, 40.4% for stage II, 77% for stage III and 90.1% for stage IV[14].

The STRIVE study (Clinical Trial NCT03085888) is a similar study with ~100,000 participants aiming for biomarker discovery based on methylation analysis in cell-free DNA and included hematological malignancies. A mid report showed 99.5% specificity and increasing sensitivity with higher stage. For all cancer types, sensitivity of the detection of stages I-III was 44.2%, where in 12 high signal cancers including lymphoma, sensitivity for stages I-III was 69.8%[15].

This study aims to develop a minimally-invasive, fast, highly sensitive diagnostics tool for screening, early detection and monitoring of hematological malignancies. This test will enable classification of a broad spectrum hematological malignancies based on detection of epigenetic biomarkers from genomic DNA, cell-free (cf) DNA and exosomal DNA

1.1 HemaChip mechanism of action

It is well established that hematological tumor cells undergo dramatic changes in epigenetic patterns. Results from Tel Aviv University (TAU) show that even bulk measurement of the levels of 5mC and 5hmC in blood of patients with various types of hematological cancers show differential epigenetic pattern between healthy and cancer samples ([16] and unpublished data). Preliminary experiments aiming to map 5mC patterns in blood taken from healthy individuals, multiple myeloma (MM) patients and follicular lymphoma patients (FL) revealed 522 loci differentiating between the three states according to the methylation status. In cell-free DNA from

hematological malignancies patients, MM-associated pattern consisted of 981 loci differentially methylated between healthy and MM patients. For 5hmC, 126 differential loci were recognized between healthy and cancer. In all cases, loci with Z-score of 2 were taken into account. In regards to 5hmC, the relatively low number of differential loci is expected due to the relatively low percentage of 5hmC in genomic DNA. These results strongly support our hypothesis that epigenetic changes can be detected and indicate the existence of different cancer types, not only hematological malignancies types.

1.2 The consortium and JaxBio

The aim of SANGUINE project is to develop a novel tool for early detection and screening of hematological malignancies. The project involves the collection of blood samples from patients diagnosed with various types of hematological cancers or pre-malignant conditions, follow-up patients, individuals at-risk of developing hematological malignancy and control individuals. JaxBio Technologies exclusively licensed the technology and together with TAU developed the discovery approach utilizing commercial microarrays to generate epigenetic biomarkers from genomic as well as cfDNA. This innovative technology is at the basis of the SANGUINE project.

TAU is the coordinator of all clinical aspects of the SANGUINE project.

The SANGUINE consortium consists of four clinical partners from the following medical centers: Tel-Aviv Sourasky Medical Center (TASMC), Israel; University Hospital Olomouc (FNOL), Czech Republic; Vilnius University Hospital Santaros Klinikos (VULSK), Lithuania; National and Kapodistrian University of Athens (NKUA), Greece. Samples will be collected in all four medical centers throughout the project. Other non-clinical partners are: TEL AVIV UNIVERSITY (TAU), Israel; EUROPEAN CANCER ORGANISATION (ECO), Belgium; FZMB GMBH FORSCHUNGSZENTRUM FUR MEDIZINTECHNIK UND BIOTECHNOLOGIE (FZMB), Germany; UNIVERZITA PALACKEHO V OLOMOUCI (UP), Czechia; PREDICTBY RESEARCH AND CONSULTING S.L. (PBY), Spain; JAXBIO TECHNOLOGIES LTD (JAX), Israel and UAB ORIENTOS (Orientos), Lithuania.

The SANGUINE project was awarded by the European Commission under Horizon Europe (Project 101097026 — SANGUINE).

The results of this study may be used by JaxBio for future regulatory marketing approvals in Europe and in the US. All partners will have the access to the anonymized data.

2 STUDY OBJECTIVES

The primary and the secondary objectives of this clinical trial are defined as follows:

Primary objectives:

1. Biomarker discovery - define a set of differential epigenetic biomarkers that uniquely identify the following conditions: MM, pre-MM conditions [smoldering MM (SMM) and monoclonal gammopathy of undetermined significance (MGUS)], HL, aggressive NHL [diffuse large B cell lymphoma (DLBCL], FL, MZL, AML, MDS and control subjects with no malignant disease .
2. Validation – validating the discovery platform (HemaChip) as a diagnostic tool for various hematological malignancies.
3. Towards early detection - Patients, at risk of relapse tested periodically to evaluate early detection capability of the HemaChip.
4. Towards population screening - evaluate the sensitivity and specificity for screening in populations at risk for developing the investigated cancers: (i) elderly (>65 years old) at high risk to develop MM; (ii) first degree relatives of the conditions described above.

Secondary objectives:

1. Evaluate the correlation between epigenetic status and well-known prognostic markers, including known genetic markers.
2. Predict response to therapy.

Exploratory objectives:

1. Identify new therapeutic targets based on epigenetically modified genomic loci.
2. Provide prognostic information at pre-treatment and monitoring of MRD.

3 ENDPOINTS

Primary endpoints:

1. Define a set of biomarkers for each condition. Determine efficiency, sensitivity, specificity, limit of detection.
2. Determine assay efficiency, sensitivity, specificity, limit of detection for each stage.

Secondary endpoints:

1. Determine prognostic value of discovered epigenetic markers.
2. Determine metrics for response to therapy.

Exploratory endpoints:

1. Identify therapeutic targets base on differential epigenetic loci.
2. Determine an MRD protocol for epigenetic follow-up.

4 STUDY DESIGN

This is a multi-center, open-label, non-interventional controlled study to identify and characterize the epigenetic signatures for a set of hematological malignancies.

Subjects will be screened for eligibility and then, after signing an Informed Consent Form, the first peripheral blood will be obtained.

Periodical blood samples will be obtained from the participants. Relapse patients will have their retrospective blood samples analyzed to identify early signs of disease.

4.1 First stage (discovery phase)

The first stage (discovery phase) will include at least 30 patients from each of the following groups: MM, pre-MM conditions (SMM and MGUS), HL, aggressive NHL (DLBCL), FL, MZL, AML, MDS and control subjects with no malignant disease.

4.2 Second stage

In the second stage, at least 250 patients with MM and 250 patients with NHL and at least 100 patients with each of the remaining hematological malignancies mentioned above will be tested.

Out of these patients, AML, lymphoma and MM patients will be followed-up at the clinical sites. Periodic sampling will be defined according to disease type and progression rate. Blood and plasma samples will be stored in the clinical sites until relapse diagnosis. At this stage, blood samples will be analyzed retrospectively on the HemaChip. The screening, enrollment and samples collection can begin in the first stage of the trial, in order to allow a maximum follow-up period for at-risk subjects as part of the study, and to meet the recruitment goals.

4.3 Third stage

The last stage consists of screening of a larger group of subjects at risk to develop MM / lymphoproliferative disorder. This stage will include 400 elderly patients (>65 years old) and 500 first-degree relatives of patients (and in particular siblings). The screening, enrollment and sample collection can begin in the first stage of the trial, in order to meet the recruitment goals.

In all stages, the age and sex-matched subgroups will be considered and matched.

4.4 Clinical follow-up

Subjects that serve as controls, subjects at risk (from the Third stage) and a subset of patients from the First and Second stage are expected to donate blood a single time. Following this donation, their participation will end.

For a subset of patients from the Second stage it is expected, after signing the informed consent forms , that serial samplings will be performed during the disease follow-up according to the standard clinical practice and/or recommended schedule and disease assessment plan (see Table 3).

All follow-up patients will be followed for a period of up to 36 months.

For this clinical trial, demographic and baseline parameters including sex, age, race, height and weight, medical history, smoking history and status, details of initial diagnosis and treatment history concomitant medications as well as adverse events (AEs) of special interest (see section 9.1), (serious) AEs related to study procedure, treatment for the disease, disease response and survival status will be collected (as applicable).

Subjects who complete the main study, may be followed up for their disease status, anti-cancer

treatments, concomitant medications, new primary malignancy and survival, following statistical review of the study results. Serial blood samplings will be performed during the long-term follow-up according to the standard clinical practice and/or recommended schedule and disease assessment plan (see Table 3).

A detailed plan for long-term follow-up beyond the main study will be provided as an amendment to this protocol.

4.4.1 Recommended follow-up schedule

Follow-up patients will donate blood periodically according to the following recommendations described in Table 3.

5 STUDY POPULATION

5.1 Number of subjects

First stage: at least 300 subjects.

Second stage: at least 1000 subjects.

Third stage: up to -900 subjects.

The sample size calculations and consideration are presented in section 10.4 below.

The number of participants in the study is described in Table 1 below.

Table 1. Number of participants

	1st Stage No. of Patients/Subjects (at least)	2nd Stage* No. of Patients/Subjects (at least)	3rd Stage* No. of Patients/Subjects (up to)
MM	30	250	-
MGUS	30	100	-
SMM	30	100	-
HL	30	100	-
DLBCL	30	250	-
FL	30		-
MZL	30		-
AML	30	100	-
MDS	30	100	-
Control (with no malignant disease)	30		
Subjects at risk	-	-	400 Elderly >65 500 First degree relatives

* The screening, enrollment and sample collection can begin in the first stage of the trial, in order to allow a maximum follow-up period for at-risk subjects as part of the study and to meet the recruitment goals.

5.2 Eligibility

5.2.1 Inclusion criteria

Subjects must meet all of the following inclusion criteria to be enrolled in the trial:

General criteria for all study populations:

1. Male and female subjects ≥ 18 years of age;
2. Ability to understand and willingness to sign a written informed consent document.

For patients with hematological malignancies:

1. Patients who have been diagnosed, have measurable disease and / or are being monitored / followed-up due to one of the following conditions: MM, pre-MM conditions (SMM and MGUS), HL, aggressive NHL (DLBCL), FL, MZL, AML, MDS that did not yet undergo any treatment.

NOTE: Patients diagnosed with DLBCL that is transformed from FL or MZL, and patients diagnosed with AML secondary to MDS or MPN, that were treated for their primary disease (FL/MZL/MDS/MPN) prior to study enrollment, are eligible. .

For subjects at risk for developing the investigated hematological malignancies:

1. First degree relatives;
2. Elderly subjects ≥ 65 years of age.

5.2.2 Exclusion criteria

Subjects meeting any of the following exclusion criteria will not be enrolled in the trial:

1. Patients/subjects with current co-diagnosis of another type of cancer;
2. Patients/subjects with a known active or prior cancer (other than defined as study population), occurring within the last 2 years (even if considered to be in complete remission). Patients/subjects with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection;
3. Patients/subjects with active inflammatory autoimmune disease that requires treatment with immunosuppressive/ immunomodulation agents;

4. Patients/subjects with known human immunodeficiency virus (HIV) positive;
5. Patients/subjects with known active Hepatitis A/B/C or past Hepatitis C
6. Subjects that are likely to be noncompliant with the protocol, or felt to be unsuitable by the investigator for any other reason.

5.3 Subject screening and registration

Subjects willing to participate in the study will provide written informed consent according to Good Clinical Practice (GCP). Written informed consent must be obtained before any study-related activity.

Upon signing the informed consent, the subject will be registered and assigned a unique subject number. Once a subject number has been assigned, it cannot be reused, and the number stays with the subject even if the subject is subsequently determined to be ineligible for the study.

5.4 Subject withdrawal criteria

Subjects will be withdrawn from the study for the following reasons:

1. The subject withdraws consent at his/her own request or at the request of his/her legally acceptable representative. A subject may decline to participate in the study at any time during the study and without giving particular reasons for his/her decision. In this case, the investigator should make reasonable efforts to ascertain the reason(s), while fully respecting the subject's rights. The subject will not suffer any disadvantage consequently.
2. If, in the investigator’s opinion, continuation of study would be harmful to the subject's well-being.
3. In case of unexpected new malignancy (excluding: FL and MZL transformed to lymphoma, MDS transformed to AML and MGUS or SMM transformed to MM) or active inflammatory disease that requires intervention.
4. Lost to follow-up.
5. The consortium discontinues the study.

A subject who discontinues study participation prematurely for any reason after enrolment but before providing the first blood sample will be replaced.

5.5 Enrollment restrictions

The consortium may increase the number of samples and/or stop analysis of a specific subgroup depending on the analysis results throughout the trial conduct period.

6 DESIGN & TREATMENT PLAN

6.1 Duration of participation

Follow-up patients with hematological cancers will be followed for treatment for the disease, disease response and survival status for up to 36 months, unless they terminate early due to meeting one of the withdrawal criteria.

Subjects that serve as controls, subjects at risk (from the Third stage) and a subset of patients from the First and Second stage are expected to donate blood a single time. Following this donation, their participation will end.

Subjects who complete the main study, may be followed up for their disease status, anti-cancer treatments, concomitant medications, new primary malignancy and survival, following statistical review of the study results. A serial blood samplings will be performed during the long term follow-up according to the standard clinical practice and/or recommended schedule and disease assessment plan (see Table 3).

A detailed plan for long term follow-up beyond the main study will be provided as an amendment to this protocol.

6.2 Schedule of events

The detailed summary of the evaluations and procedures planned in this study is presented in Tables 2 and 3.

Table 2. Schedule of events

Evaluation	Screening visit[1] (Day -28 to -1)	Baseline sample (Day 1 ± 28 days)	Follow-up visit[2] (sub-set of patients in Stage 2)
Signed consent form	X		
Assessment of eligibility criteria	X		
Assigning patient ID	X		
Assigning patient barcode	X		
Record medical history[3]	X	X	
Record concomitant medications	X	X	X
Record demographics[4]	X		
Record smoking history / status	X	X	X
Record prior anti-cancer treatments[5]	X	X	
Record weight	X	X	X
Record heights	X		
Record baseline disease characteristics including prognostic profile[2]	X		
MM lab profile for subjects at risk to develop the investigated hematological malignancies[6]	X		
Blood sampling for central lab (JaxBio)[7]		X[2]	X[2]
Disease response evaluation			X[2]
Survival status			X
Record adverse events		X	X

[1] Screening visit can be performed at the same day as baseline sample.
[2] Refer to Table 3 for recommended schedule and disease assessment plan for follow-up patients in Stage 2.
[3] Medical history will include details of initial diagnosis.
[4] Demographics include sex, age and race.
[5] Only for Secondary-AML, transformed FL and MZL conditions.
[6] **In Stage 3**. MM lab profile will include SPEP and FLC, full chemistry and CBC.
[7] Two (2) blood tubes for each sample will be collected and shipped. Refer to Study Manual.

HemaChip tests at the clinical sites[8]		X[9]	X[9]

[8] **In stages 2 and 3 of the clinical trial only**. The HemaChip and custom reagents will be distributed to partners' labs and all tests will be conducted at the clinical sites.
[9] Two (2) blood tubes for each sample will be collected. One (1) for test at the clinical sites and the second as a backup for additional validation tests at JaxBio and TAU, as needed. Refer to Study Manual.

Table 3. Recommended schedule and disease assessment plan – Only for follow-up patients

Condition / Visit	Baseline Characteristics and Assessments[1]	Follow-up for Disease Evaluation		Blood Sample for Central Lab/HemaChip at site[2,3]
		Disease Evaluation[1]	Recommended Schedule	
MM	1. PCD diagnosis[4] 2. Chemistry[5] 3. Blood count[6] 4. Virology[7] 5. MM lab profile[8] 6. Other baseline assessments[9]	1. Response will be evaluated according to IMWG criteria[10] 2. MM lab profile[8]	MGUS: Once every 6 months SMM: Once every 3-6 months MM: Once every 2 months	X
HL, DLBCL, FL, MZL	1. Disease diagnosis[11] 2. Chemistry[12] 3. Blood count[13] 4. Virology[7] 5. Disease evaluation[14] 6. Other baseline assessments[15]	1. Response will be evaluated according to Lugano 2014 criteria 2. Assessment of response[14,16] 3. Assessment of B Symptoms	1. First 2 Years: every 3 months 2. From the 3rd year: every 6 months	X
AML	1. AML diagnosis[17] 2. Chemistry[18] 3. Blood count and smear[19] 4. Coagulation tests[20] 5. Virology[21] 6. Other baseline assessments[22]	1. Response will be evaluated according to ELN guidelines 2017/2022[23] 2. Molecular MRD when evaluable[23] 3. Blood count and chemistry	1. Intensive chemotherapy a. Day14 assessment[24] b. 3-5 weeks after induction therapy upon hematologic recovery c. Optional – upon hematologic recovery following each consolidation cycle d. If ASCT: 1 month after therapy	X

[1] Will be performed locally at the clinical site.
[2] **In stage 1 of the clinical trial only**, Two (2) blood tubes for each sample will be collected in STRECK tube and sent to central lab (JaxBio). Refer to Study Manual. **In stages 2 and 3 of the clinical trial,** the HemaChip and custom reagents will be distributed to partners' labs and all tests will be conducted at the clinical sites. Two (2) blood tube for each sample will be collected, one (1) for test at the clinical sits and the second as a backup for additional validation tests at JaxBio and TAU, as needed.
[3] Blood samples for Blood Sample for Central Lab/HemaChip at site – at diagnosis (baseline) and at all disease evaluation follow-up visits.
[4] PCD diagnosis – date and age at diagnosos, type, ISS, R-ISS, BM Aspirate/Biopsy %PCs, FISH
[5] Chemistry includes: albumin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, calcium, chloride, phosphorus, potassium, sodium, lactate dehydrogenase (LDH), total protein, magnesium, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, β2 microglobulin.
[6] Blood Count: Hemoglobin, hematocrit, white blood cells (WBCs) with complete manual or automated differential (total neutrophils, lymphocytes, monocytes, eosinophils, basophils; absolute or percentage will be acceptable), red blood cells (RBCs), platelet count.
[7] Virology: Anti HCV, HBs Antigen, HBs ab, Anti-HBc, HIV.
[8] MM lab profile: M-protein by serum protein electrophoresis (SPEP) and immunofixation (immunofixation is required at baseline and to confirm CR regardless of whether measurable M-protein was present at baseline), Urine M -protein electrophoresis (UPEP) and urine immunofixation (requires 24-hour urine collection) only if patient has disease measurable by UPEP at baseline (Screening value ≥ 200 mg/24 hours) but not by serum free light chain (sFLC), sFLC (regardless if the patient had measurable disease at baseline), qIgA levels in patients with immunoglobulin A (IgA) type MM only.
[9] Other baseline assessments – bone marrow involvement, extramedullary disease.
[10] Refer to Appendix 2
[11] Disease diagnosis – date, age, stage (per to Ann Arbor, see Appendix 3), **for HL only** – early favorable/early unfavorable/advanced (including IPS), **for FL only** – FLIPI score
[12] Chemistry**:** Albumin, lactate dehydrogenase (LDH), β2 microglobulin.
[13] Blood count: Hemoglobin, hematocrit, white blood cells (WBCs) with complete manual or automated differential (total neutrophils, lymphocytes, monocytes, eosinophils, basophils; absolute or percentage will be acceptable), red blood cells (RBCs), platelet count
[14] Assessments to include disease burden and symptoms, staging, CT, FDG PET, PE, bone marrow aspirate + biopsy (in the event of CR for participants who do not have PETFDG avid lymphoma). No further PET scans are required for NHL, which are not FDG-avid at baseline, unless clinically indicated. For FDG-avid NHL, PET is required at baseline, Weeks 12 and 24, to confirm CR or as clinically indicated.
[15] Other baseline assessments – bone marrow involvement, extranodal disease, nodal sites, B-symptoms.
[16] Refer to Appendix 4.
[17] AML diagnosis – date and age at diagnosis, AML type (de novo VS secondary to MDS/MPN/therapy related), AML risk stratification (ELN 2022), BM Aspirate/Biopsy – % blast cells, cytogenetics, FISH, FACS, molecular testing.
[18] Chemistry includes: albumin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, calcium, chloride, phosphorus, potassium, sodium, lactate dehydrogenase (LDH), total protein, magnesium, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin.
[19] Blood Count and smear: Hemoglobin, hematocrit, white blood cells (WBCs) with complete manual or automated differential (total neutrophils, lymphocytes, monocytes, eosinophils, basophils; absolute or percentage will be acceptable), red blood cells (RBCs), platelet count. % peripheral blast cells on smear.
[20] Coagulation tests: PT, PTT, INR, fibrinogen.
[21] Virology: Anti HCV, HBs Antigen, HBs ab, Anti-HBc, HIV.
[22] Other baseline assessments – extramedullary disease.

			e. After completing therapy: every 3 months for 2 years, and every 6 months from 3rd year, or as guided in selected treatment protocol 2. Low-intensity chemotherapy: a. First response assessment as guided in selected protocol b. Every 3-6 months	

[23] Refer to Appendix 5.
[24] Day 14 assessment: according to routine per site, if assessed mention %blasts or MLFS (morphologic leukemia free state).

6.2.1 Unscheduled visits

Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or as deemed necessary by the Investigator.

Evaluations and procedures to be performed at unscheduled visits, will be as clinically indicated at the Investigator's discretion, and may be based on those listed in Section 6.2, Schedule of events.

6.2.2 Response assessment

For MM patients, response assessments will be performed according to the International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma[20] (See Appendix 2).

For HL and DLBCL patients, response assessments will be performed according to the 2014 Lugano criteria assessing FDG-PET/CT[21] (See Appendix 4).

For AML patients, response assessments will be performed according to ELN guidelines 2022, NCCN guidelines 2021[22] (See Appendix 5).

7 STUDY ASSESSMENTS

This section details the tests and the assessments that will be performed during the study (36 months).

AEs of special interest, (serious) AEs related to study procedures, concomitant medications, second primary malignancies and anti-cancer treatments will be collected from the time of signing of ICF until completion of the clinical trial.

7.1 Screening period

- Signed consent form
- Assessment of eligibility criteria
- Assigning patient ID
- Record medical history - Medical history will include details of initial diagnosis
- Record concomitant medications
- Record demographics - sex, age and race
- Record smoking history

- Record prior anti-cancer treatments only for Secondary AML, transformed FL and MZL conditions
- Record weight
- Record height
- Record baseline disease characteristics including prognostic profile - Refer to Table 3 for recommended schedule and disease assessment plan
- MM lab profile - will be performed for subjects at-risk in Stage 3.

7.2 Baseline sample

- Review medical history
- Review concomitant medications
- Review prior anti-cancer treatments
- Record weight
- Record smoking status
- Blood sampling for central lab (JaxBio). Two (2) blood tubes for each sample will be collected and shipped. Refer to study manual.
- Record AEs
- Hemachip tests at the clinical sites - **in stages 2 and 3 of the clinical trial only**. Two (2) blood samples will be collected. One (1) for testing at the clinical sites and the second as a backup for additional validation tests at JaxBio and TAU, as needed. Refer to Study Manual.

7.3 Clinical follow-up – sub-set of patients in Stage 2

- Review concomitant medications
- Record weight
- Record smoking status
- Blood sampling for central lab (JaxBio) - refer to Table 3 for recommended schedule and disease assessment plan. Refer to study manual.

- Record AEs
- Disease response evaluation (for follow-up patients in Stage 2) - refer to Table 3 for recommended schedule and disease assessment plan
- Survival status
- Hemachip tests at the clinical sites - **in stages 2 and 3 of the clinical trial only**. Two (2) blood samples will be collected. One (1) for testing at the clinical sites and the second as a backup for additional validation tests at JaxBio and TAU, as needed. Refer to Study Manual.

8 HEMACHIP

Operational requirements are divided to two periods:

Year 1: During the discovery phase, all tests will be conducted by JaxBio and TAU, which developed and optimized and routinely perform the procedure. At this stage, microarray measurements will be performed on a commercial platform that will be purchased from suppliers such as Agilent / Illumina. All reagents needed for the test will be either purchased or produced in-house.

Blood will be collected by all medical centers in cell-free DNA preservative tubes (STRECK) which stabilizes cell-free DNA for up to 14 days at room temperature. If needed, one ml of the blood will be immediately frozen for genomic DNA extraction. The required blood samples for the discovery phase will be shipped to JaxBio via FedEx. Cell-free tubes will be shipped at room temperature, while frozen blood will be shipped with ice packs to avoid thawing of the sample which may lead to DNA degradation. Import/export issues will be handled by JaxBio or TAU which holds a dedicated import department and has extensive expertise in all importing aspects. At JaxBio/TAU, plasma, serum and buffy coat will be separated, and cell-free DNA and genomic DNA will be extracted. Subsequently, methylation / hydroxymethylation labeling, microarray hybridization, detection and analysis will be performed.

Years 2-3: Throughout the second phase of the project, a custom targeted microarray, HemaChip will be developed and used for all tests. The HemaChip will be developed by Forschungszentrum fur Medizintechnik und biotechnologie (FZMB) who specializes in microarray development, manufacturing and distribution. (FZMB) will be responsible for all aspects of distributing the

HemaChip to partners' labs including manufacturing, labeling, shipping etc. At this stage, all partners will purchase the reagents needed for performing the methylation labeling and HemaChip assays. Reagents that are produced at JaxBio/TAU will be validated in-house and shipped to partners' labs along with a validation kit to ensure performance. Quality control measures will be set up to ensure the same analytic condition for all laboratory partners. All tests will be conducted at the clinical sites. Additional validation tests will be conducted by JaxBio and TAU, as needed.

9 REGULATORY AND REPORTING REQUIREMENTS

9.1 Adverse event definition

An AE is any change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical trial. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease related to study procedure. Disease progression will not be recorded as an AE. Progressive disease (PD) will be captured in a disease evaluation form in the CRF and not in the AE form.

9.2 Adverse events of special interest

Any new malignancy (excluding: FL and MZL transformed to lymphoma, MDS transformed to AML and MGUS or SMM transformed to MM) or active inflammatory disease that requires intervention will require data collection in the eCRF and be recorded in a timely manner, irrespective of seriousness (ie, serious and non-serious AEs)or the time of occurrence during the clinical trial.

9.3 Serious adverse event definition

A serious adverse event (SAE) is any AE occurring that:

- Results in death.
- Is life-threatening. Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires in subject hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease

existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned). Planned hospitalizations for treatments or evaluations or that are related and expected as the natural course of the underlying hematological condition are also not considered SAEs.

- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event.

- An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in the definitions for SAE.

9.4 Adverse events reporting

All (serious) AEs occurred within 24 hours after study procedure and deemed to be related to study procedure(s) and AEs of special interest (see section 9.2) will be recorded in the e-CRF.
Study Medical Monitor must be notified of the occurrence of any SAE within 24 hours of the investigator, designee, or site personnel's knowledge of the event. SAEs will be reported by completing a Serious Adverse Event Form and e-mailing it to the assigned e-mail distribution list within 24 hours of their knowledge of the event, with any supporting documentation (results of relevant investigations, list of concomitant medications etc.). Follow-up must be submitted in a timely fashion as additional information becomes available.

SAE Reporting Contact Information

Email: lenagrin@gmail.com **AND** mariagabria@gmail.com

The Investigator, designee or site's personnel will also update the SAE information within the eCRF as part of eCRF data entry requirements.

The study investigator of each study site is responsible for notifying the Institutional Review Board (IRB) or Local Ethics Committee (LEC) in accordance with local regulations, of all SAEs.

9.5 Medical monitoring and safety management

A Medical Monitor will be assigned to oversee the safety of the trial and will be responsible for review of AEs and SAEs and for causality assessment. The Medical Monitor will be a hematology specialist with expertise in clinical trials. The Medical Monitor will not serve as a principal investigator in any of the participating clinical sites.

10 STATISTICAL CONSIDERATIONS

10.1 Study design

Controlled study - This study aims to identify methylation patterns indicating the presence of cancer. Comparison to control participants with no malignant diseases is essential for identifying tumor-specific methylation patterns.

Open study - The current study is an open study where all participants are divided into known groups. For the first stage of the study, the group identification is essential for discovery of biomarkers for each disease type. Discovery stage will be based on empirical comparison between controls and patients diagnosed with various hematological cancers. Therefore, group identification is required for validation, comparison with state-of-the-art diagnosis and disease status.

The current study contains only one type of intervention and therefore randomized/crossover or parallel groups are inapplicable.

10.2 Statistical methods

All data collected will be summarized and presented. Continuous variables will be described as the mean, median, standard deviation, minimum and maximum. Categorical data will be described with contingency tables including frequency and percentage. Individual patient listings of all data will be generated and presented.

Statistical descriptions and analyses will be carried out using a statistical analysis software.

10.2.1 Study populations

The analyses of the study will be on those patients who were eligible, who have signed a consent form and donated at least one blood sample.

Gender: If available, a similar number of males and females participants will be employed for all stages. Gender differences may be reflected by differential epigenetic patterns. Our mission is to develop a screening and diagnostic tool that will be employed by all genders.

Age group: In general, all participants will be over 18 years old. As epigenetic patterns undergo changes with increasing age, the study will focus on (but not limited to) participants at an age close to the median age at diagnosis, i.e. 55-80 years old. This is applicable with all groups including patients, control, follow-up patients, at risk screening populations and first degree relatives. Aged matched controls will account for age-related changes.

All samples will be anonymized.

10.2.2 Demographic and baseline parameters

Demographic and baseline parameters including sex, age, race, ethnicity, height and weight, medical history, smoking history, details of initial diagnosis and treatment history will be summarized overall and by group. All continuous variables will be summarized by descriptive statistics. All discrete variables will be summarized by frequencies and percentages.

10.3 Interim analysis

A single formal interim comparative analysis of the epigenetic patterns in blood-derived DNA from control subjects with no malignant disease and subjects at risk for developing the investigated hematological malignancies and the patterns obtained for all malignant and premalignant hematological conditions will be conducted after approximately 24 months from the beginning of the study and will include:

Comparative analysis for 250 newly diagnosed NHL samples with HemaChip

Comparative analysis for 250 newly diagnosed MM samples with HemaChip

Comparative analysis for 100 samples of the following conditions: SMM, MGUS, HL, AML, MDS, control subjects with no malignant disease and subjects at risk for developing the investigated hematological malignancies.

10.4 Sample size and power calculation

The sample size of each stage of the clinical study will be determined in accordance with technology development requirements. Hence, three designs should be taken into account: Discovery stage performed with high density discovery chip, and validation and screening stages performed with targeted HemaChip. All the calculations below have been made with optimistic assumptions that we will be able to prepare such sensitive chips that classify the studied hematological malignancies types with expected target sensitivity of above 95%. Moreover, the minimal sensitivity 80% was set as a lower bound for all statistics to get a scale for the further Sample Size calculation. Importantly, based on incidences of particular diseases the calculated Sample Size will include significant proportions of all diseases of interest.

Discovery stage:

The method Proportion sample size determination for binomial distribution (arcsine transformation, significance level = 0.05, test power = 0.8) was used to analyze the required sample size. Based on previous experiments, we propose two estimates – expected target sensitivity, and minimum acceptable sensitivity. Estimate of the expected target sensitivity of the discovery chip was set to 95%. This estimate is needed to set the minimum acceptable limits for establishing statistical tests and represents the best expected result that could theoretically be achieved with the current state of technology and knowledge. The estimate of the minimum acceptable sensitivity of the discovery chip was set to 89%. The value is based on reported literature and the fact that this phase is conducted on diagnosed patients with active disease.

Expected Sensitivity	Sample Size
0.84	569
0.85	356
0.86	241
0.87	173
0.88	128
0.89	99
0.90	77
0.91	62
0.92	50
0.93	41
0.94	34
0.95	30
0.96	23
0.97	19

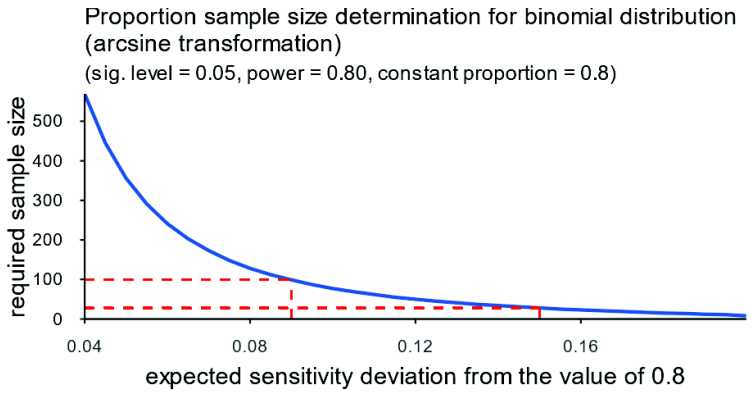

Figure 1. Required sample size for discovery microarray, analyzed by Proportion sample size determination for binomial distribution (arcsine transformation, significance level = 0.05, test power = 0.8).

As evident from Figure 1, the larger the deviation of the observed sensitivity from the 80% value, the smaller the sampling size required to prove that the actual sensitivity of the tested discovery array is statistically significantly higher than 80%. In case of the optimistic estimate, it is expected the sensitivity of the chip designed for the disease detection to be 95%, which corresponds to a minimum sample size of 30 subjects for each disease subtype. In the context of the mentioned numbers, and the confidence level of 95%, the confidence interval will be 0.817, and 0.999. In case of the minimum acceptable estimate, the sensitivity of the discovery array is set to be 89%, which corresponds to a sample size range of about 99 subjects per disease. In the context of the mentioned numbers, and the confidence level 95%, the confidence interval will be 0.810, and 0.943.

Since the real sensitivity of the discovery is unknown and based on experience with 60,000 pixel arrays, we have decided to start with a set of 30 subjects and use the data for more precise and evidence based power calculation. The selection of the biomarker panel may be performed even under this uncertainty by including also more variable markers in the HemaChip panel and further tune the array once sufficient data has been accumulated.

Validation stage:

The large discovery chip serves as a matrix for selecting significant markers and as an experimental platform for the final targeted HemaChip design with a reduced number of spots/markers. Here, the same statistics need to be performed to prove that the HemaChip will work with a high statistical accuracy. Since there has been a reduction in markers, we will assume that the sensitivity of the chip will be at a lower level, yet still above the minimum threshold of 80% With the same test setup (arcsine transformation, significance level = 0.05, test power = 0.8) and the expected specificity of 85% (lower bound), approximately 356 subjects (Sample size) will be needed to verify the results. In the context of the mentioned numbers, and the confidence level 95%, the confidence interval will be 0.810, 0.887 (Figure 12).

The research program is designed to test diagnosed ~250 subjects of MM and NHL, and 100 subjects of the remaining conditions in the validation stage. If the selection of biomarkers was sufficiently good, the expected sensitivity of the HemaChip will not dramatically decile relative to

the discovery array and this number of tests should suffice. Accumulation of additional data is expected in the next phases of the project in order to accurately tune the statistical analysis according to the data.

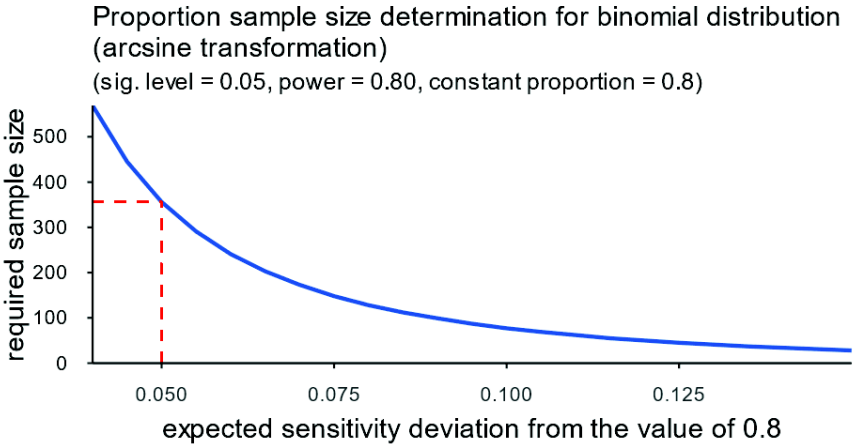

Figure 2. Required sample size for HemaChip, analyzed by Proportion sample size determination for binomial distribution (arcsine transformation, significance level = 0.05, test power = 0.8).

Stage 3: Screening at-risk cohorts

When calculating the sample size for the screening stage, the incidences of individual types of hematological malignancies were considered. The assumption is that the individual types will occur in the same proportion also in the risk cohorts of patients. We also consider the minimum number of samples with respect to the HemaChip and its expected sensitivity (as detailed in stage 2), and the known fact that the incidence of the hematological malignancies is about five percent in the risk cohort. Then, the sample population was determined to be approximately 7120. Such a screen is beyond the scope of this project but given that the tests performed side by side with standard diagnosis, the incidence rate is sufficient to derive initial performance metrics based on several hundred tests. Positive results will allow performing a large scale clinical study needed for screening purposes.

10.5 Criteria for termination of the trial

The consortium reserves the right to terminate the study early for administrative (e.g. center not complying with GCP) or other reasons (see section 13.6)

10.6 Procedure for accounting for missing, unused and spurious data

Missing data will be indicated in the listings, but excluded from all descriptive analyses. All data will be listed, including otherwise unused data. Spurious data will be identified as such, wherever possible. Further details will be provided in the Statistical Analysis Plan.

11 ADMINISTRATIVE ASPECTS

11.1 Contract research organization

A Contract Research Organization (CRO) will be utilized for this trial. The CRO will be responsible for study and site management, communication, study monitoring, medical monitoring, safety management and data management and quality assurance for this trial. The CRO will be responsible for preparing all the documentation required for the ethics committees across all medical centers involved in the SANGUINE project according to the Commission Directive 2005/28/EC guidelines

11.1.1 Communication between sites and CRO

Regular contact will be made between the CRO and all participating sites in order to discuss study progress and updates.

11.2 Study conduct

The present international clinical trial project will be conducted in accordance with the ICH/Good Clinical Practice guideline.

The Clinical and Regulatory Teams will systematically control the essential documents generated during this trial. All phases of the trial will be monitored by the CRO representative. All monitoring visits and inspections by Quality Assurance (QA) will be followed by internal reports and corrective actions, if needed. Follow-up letters will be forwarded to sites after all clinical visits.

11.3 Quality control by the monitoring team

The clinical site will be visited periodically by a monitor to ensure compliance with the study protocol, international and local guidelines and legal aspects. Monitoring and auditing procedures defined/agreed by the Sponsor/Investigator will be followed, in order to comply with ICH/GCP

guidelines and will be described in the Monitoring Plan and the Quality Assurance and Project Management Plan. This will include on-site checking of the electronic CRF (eCRF) for completeness and clarity, cross-checking with source documents, clarification of administrative matters and providing additional guidance where appropriate.

The Investigator must make himself available for study monitors during their visits and ensure that they have access to all documents that they require, including to the patient's files (direct access). The Investigator agrees to cooperate with the trial monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

The Investigator is responsible for maintaining adequate case histories in the source records of each patient. Source data should be preserved for the maximum period of time permitted by local regulations and made available by the Investigator in the cases described above

The anonymity of the patient/subject must be safeguarded and data checked during these monitoring visits remain confidential.

11.4 Quality assurance by an audit team

This study may be selected for audit at any moment by an audit team originating from the coordinator or its designee.

The Investigator agrees to cooperate with the auditor to ensure that any problems detected in the course of these audit visits are resolved.

The anonymity of the patient/subject must be safeguarded and data checked during these monitoring visits remain confidential.

11.5 Data handling and data management

The data generated in the study will be recorded by the site staff in electronic case report forms (e-CRFs). The electronic system keeps track of all data entries/changes, including the original entry, the date of entry/change and the identity of the person making the entry/change.

Data management services for the purposes of this trial will be arranged by the coordinator or designee, including database establishment, data entry, review and coding as written in the Data Management Plan.

Coding of AEs will be performed using the MedDRA dictionary. A suitably qualified person will perform a periodic medical review of the coding.

In case the patient/subject discontinued the study for any reason after donation of blood sample(s), the consortium may only use samples and/or data collected until the date of discontinuation.

12 ETHICS

12.1 Independent ethics committee or institutional review board

Approval from the appropriate Ethics Committee(s)/Institutional Review Board(s) will be obtained and documented for the participating center prior to study start, according to the ICH/GCP guidelines, local laws and applicable regulations. When necessary, an extension, amendment or renewal of the Ethics Committee approval must be obtained and also forwarded to the Sponsor/Investigator. The Ethics Committees will be requested to supply to the Sponsor/Investigator, a list of the Ethics Committee members involved in the vote and a statement to confirm that the Ethics Committee is organized and operates according to ICH/GCP guidelines and applicable laws and regulations.

12.2 Regulatory approvals

All required regulatory authority approvals/authorizations/notifications must be in place and fully documented prior to study start.

All clinical studies will comply with the regulatory guidelines as described in Regulation (EU) No 536/2014 and Good Clinical Practice according to ICH E6.

12.3 Patient/subject information and consent

An informed consent form in layman's language will be provided to each subject. Prior to the beginning of the study, the investigator must have the IEC/IRB written approval for the written informed consent form and any other written information to be provided to subjects. Before enrollment in the study the investigator, or an authorized member of the study-site personnel, shall explain orally, in a language that the patient understands, the aims, methods, reasonably anticipated benefits, and potential hazards of the study, and any discomfort participation in the study may entail.

Signed informed consent must be obtained before any study-specific procedure takes place. This signed and dated informed consent form will be kept by the investigator as part of the source documents, and a copy will be provided to the study subject. Participation in the study and date of informed consent given by the subject should be documented appropriately in the subject's files.

The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and sponsor policy.

Subjects will be informed that their participation is voluntary and that they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care the subject will receive for the treatment of his or her disease.

12.4 Confidentiality

All records identifying the subject are confidential and, to the extent permitted by the applicable laws and/or regulations, will remain so.

A coding system in accordance with regulatory requirements will be applied for recording data in the eCRF. Study findings stored electronically will be in accordance with local data protection laws and with international guidelines where applicable. The patients will be informed in writing (informed consent form) that representatives of the Sponsor/Investigator, IEC/IRB, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection laws.

If the results of the study are published, the subject's identity will remain confidential.

The investigator will maintain a list to enable subjects' records to be identified.

12.5 Changes to the protocol

The consortium will agree to any change to this protocol, prior to its implementation. Any protocol amendment is required to be submitted for information/consideration to the IRB.

The duration of the clinical trial may be extended depending on the amount of samples collected and the results of the analysis throughout the trial subject to all required regulatory approvals.

12.6 Study termination

The Consortium has the right to close the study site or the entire study, at any time, after discussion between the involved parties. The site's Ethics Committee must be informed about the study termination. Should the study/site be closed prematurely, all study materials (except documentation that must remain stored at site) must be returned to the study Sponsor/PI. The investigator will retain all other documents until a notification is given by the Sponsor/PI for destruction or after 15 years, whichever occurs first. In any case, in accordance with the local guidelines.

13 FINANCE AND INSURANCE

Participating clinical sites will maintain appropriate insurance coverage, in accordance with the applicable local laws and/or regulations, to provide cover for all patients participating in the study. The Sponsor/Investigator will not provide any financial incentives to patients eligible to participate in the study.

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APPENDIX 1 – ECOG Evaluation

Score	ECOG Performance Status Scale Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work)
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carryout any work activities. Up and about more than 50% of waking hours
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chairmore than 50% of waking hours
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

APPENDIX 2: International Myeloma Work Group (IMWG) criteria for disease response evaluation

MM International Uniform Response Criteria Consensus Recommendations

Response	Response Criteria
Stringent complete response	• CR as defined below, *plus* • Normal FLC ratio, *and* • Absence of clonal PCs by immunohistochemistry or negative 2-4 color flow cytometry
Complete response[a]	• Negative immunofixation of serum and urine, *and* • Disappearance of any soft tissue plasmacytomas, *and* • <5% PCs in bone marrow • No evidence of initial monoclonal protein isotype(s) on immunofixation of the serum and urine[b]
Very good partial response[a]	• Serum and urine M-component detectable by immunofixation but not on electrophoresis, *or* • ≥90% reduction in serum M-component plus urine M-component <100 mg/24 hours
Partial response	• ≥50% reduction of serum M-protein and reduction in 24-hour urinary M-protein by ≥90% or to < 200 mg/24 hours • If serum and urine M-protein are not measurable, a decrease ≥50% in the difference between involved and uninvolved FLC levels is require in place of the M-protein criteria • If serum and urine M-protein are not measurable, and serum free light assay is also not measurable, ≥50% reduction in bone marrow PCs is required in place of M-protein, provided baseline percentage was ≥30% • In addition to the above criteria, if present at baseline, ≥50% reduction in the size of soft tissue plasmacytomas is also required.
Minimal response[c]	• ≥25% but ≤49% reduction of serum M-protein *and* reduction in 24-hour urine M-protein by 50% to 89% • In addition to the above criteria, if present at baseline, ≥50% reduction in the size of soft tissue plasmacytomas is also required
Stable disease	• Not meeting criteria for sCR, CR, VGPR, PR, MR, or progressive disease
Progressive disease[d]	Any one or more of the following criteria: • Increase of 25% from lowest response value in any of the following: – Serum M-component (absolute increase must be ≥0.5 g/dL), *and/or* – Urine M-component (absolute increase must be ≥200 mg/24 hours), *and/or* – Only in subjects without measurable serum and urine M-protein levels: the difference between involved and uninvolved FLC levels (absolute increase must be > 10 mg/dL) – Only in subjects without measurable serum and urine M-protein levels and without measurable disease by FLC levels, bone marrow PC percentage (absolute increase must be ≥10%) • Appearance of a new lesion(s), ≥50% increase from nadir in SPD of >1 lesion, or ≥50% increase in the longest diameter of a previous lesion >1 cm in short axis • Definite development of new bone lesions or definite increase in the size of existing bone lesions • ≥50% increase in circulating plasma cells (minimum of 200 cells per μL) if this is the only measure of disease

Key: CR=complete response; FLC=free light chain; PC=plasma cell; PR=partial response; sCR=stringent complete response; SPD=sum of the products of the maximal perpendicular diameters of measured lesions; VGPR=very good partial response.

aClarifications to the criteria for coding CR and VGPR in subjects in whom the only measurable disease is by serum FLC levels: CR in such subjects indicates a normal FLC ratio of 0.26 to 1.65 (or reference range in testing laboratory) in addition to CR criteria listed above. VGPR in such subjects requires a ≥90% decrease in the difference between involved and uninvolved FLC levels. For subjects achieving very good partial response by other criteria, a soft tissue plasmacytoma must decrease by more than 90% in the sum of the maximal perpendicular diameter (SPD) compared with baseline.

bIn some cases it is possible that the original M protein light-chain isotype is still detected on immunofixation but the accompanying heavy-chain component has disappeared; this would not be considered as a CR even though the heavy-chain component is not detectable, since it is possible that the clone evolved to one that secreted only light chains. Thus, if a subject has IgA lambda myeloma, then to qualify as CR there should be no IgA detectable on serum or urine immunofixation; if free lambda is detected without IgA, then it must be accompanied by a different heavy chain isotype (IgG, IgM, etc.).

cPatients with 0.5-1.0 g/dL at baseline cannot be assessed for minimal response.

dClarifications to the criteria for coding progressive disease: bone marrow criteria for progressive disease are to be used only in subjects without measurable disease by M-protein and by FLC levels; “25% increase” refers to M-protein, and FLC, and does not refer to bone lesions, or soft tissue plasmacytomas and the “lowest response value” does not need to be a confirmed value.

Notes: All response categories (CR, sCR, VGPR, PR, MR, and progressive disease) require 2 consecutive assessments made at any time before the institution of any new therapy; CR, sCR, VGPR, PR, MR, and stable disease categories also require no known evidence of progressive or new bone lesions if radiographic studies were performed. VGPR and CR categories require serum and urine studies regardless of whether disease at baseline was measurable on serum, urine, both, or neither.

Radiographic studies are not required to satisfy these response requirements. Bone marrow assessments need not be confirmed. For progressive disease, serum M-component increases of ≥1 g/dL are sufficient to define relapse if lowest M- component is ≥5 g/dL.

Source: Adapted from Durie (2015) Rajkumar (2011), and Kumar (2016).

APPENDIX 3 – Ann Arbor staging scale

Main Staging criteria

Stage	Description
Stage I	involvement of a single lymph node region or of a single extralymphatic organ or site
Stage II	involvement of two or more lymph node regions on the same side of the diaphragm or localized involvement of an extralymphatic organ or site
Stage III	involvement of lymph node regions or structures on both sides of the diaphragm
Stage IV	diffuse or disseminated involvement of one or more extralymphatic organs, or either: • isolated extralymphatic organ involvement without adjacent regional lymph node involvement, but with disease in distant sites • involvement of the liver, bone marrow, pleura or cerebrospinal flui

Sub-staging Variables

Grade	Description
A	asymptomatic
B	presence of B symptoms (including fever, night sweats and weight loss of ≥10% of body weight over 6 months)
E	involvement of a single, extranodal site, contiguous or proximal to a known nodal site (stages I to III only; additional extranodal involvement is stage IV)
S	splenic involvement
X	bulky nodal disease: nodal mass >1/3 of intrathoracic diameter or 10 cm in dimension

APPENDIX 4 – Lugano criteria for disease response evaluation

Complete Response

Complete Response	PET-Based Response – CMR	CT/MRI-Based Response - CR
Target lesions	Score 1, 2, or 3	Target nodes/nodal masses regress to <1.5 cm in LDi; no extranodal sites of disease remain
Non-target lesions	Not applicable	Absent
Organ enlargement	Not applicable	Regress to normal
New lesions	None	None
Bone marrow	No evidence of FDG-avid disease inmarrow	Normal by morphology; if indeterminate, negative by immunohistochemistry

Abbreviations: CMR=complete metabolic response; CR=complete response; CT=computed tomography; FDG=2 fluorodeoxyglucose; LDi=longest diameter; MRI=magnetic resonance imaging; PET=positron emission tomography

Partial Response

Partial Response	PET-Based Response – PMR	CT/MRI-Based Response - PR
Target lesions	Score 4 or 5 without new lesions Reduced overall uptake (extent and/orintensity) compared with baseline	≥50% decrease from baseline in SPD of target lymph nodes and extranodal sites (up to 6)
Non-target lesions	Not applicable	Anything other than progression
Organ enlargement	Not applicable	Spleen must have regressed by ≥50% in excess length
New lesions	None	None
Bone marrow	Residual uptake higher than uptake in normal marrow but reduced comparedwith baseline. If there are persistent focal changes in themarrow in the context of a nodal responseand without recent growth factor use, perform biopsy, or consider MRI, or an interval scan.	Not applicable

Abbreviations: CT=computed tomography; MRI=magnetic resonance imaging; PET=positron emission tomography PMR=partial metabolic response; PR=partial response; SPD=sum of products of diameters

Stable Disease

Stable disease	PET- Based Response – SMD	CT/MRI-Based Response - SD
Target lesions	Score 4 or 5 (without new lesions) No significant change in FDG uptakefrom baseline or nadir	<50% decrease from baseline in SPD of target lesions. No lesion shows progression
Non-target lesions	Not applicable	No increase consistent with progression
Organ enlargement	Not applicable	No increase consistent with progression
New lesions	None	None
Bone marrow	No change from baseline	Not applicable

Abbreviations: CT=computed tomography; FDG=2-fluorodeoxyglucose; MRI=magnetic resonance imaging; PET=positron emission tomography; SD=stable disease; SMD=stable metabolic disease; SPD=sum of products of diameters.

Progressive Disease

Progressive disease	PET-Based Response – PMD	CT/MRI-Based Response - PD
Target lesions	Score 4 or 5 with an increase in overall uptake (extent and/or intensity) compared to nadir	Growth of any target lesion: Increase ≥50% from nadir PPD and Increase in LDi or SDi from nadir of: ≥0.5 cm for lesions <2 cm ≥1.0 cm for lesions ≥2 cm and Current LDi >1.5 cm for a lymph node or ≥1.0 cm for an extranodal lesion
Non-target lesions	Not applicable	Clear progression of pre-existing non-target lesions
New lesions	New FDG-avid foci consistent with lymphoma rather than another cause (eg, infection, inflammation). If uncertain regarding cause of new lesions, biopsy or interval scan may be considered.	Regrowth of previously resolved lesions A new node >1.5 cm in LDi A new extranodal site of any size, as long as its presence is unequivocal and attributable to lymphoma
Organ enlargement	Not applicable	When splenomegaly was already present, the excess length must increase by ≥50% (≥1 cm absolute increase) from nadir. If no prior splenomegaly, or prior splenomegaly had resolved, spleen length must increase by ≥2 cm to >13 cm.
Bone marrow	New or recurrent FDG-avid foci, confirmed by biopsy	New or recurrent involvement

Abbreviations: CT=computed tomography; FDG=2-fluorodeoxyglucose; LDi=longest diameter; MRI=magnetic resonance

imaging; PET=positron emission tomography; PD=progressive disease; PMD=progressive metabolic disease; PPD=product of perpendicular diameters; SDi=short axis diameter.

APPENDIX 5 – European Leukema Net (ELN) Criteria

Table 5. 2017 ELN risk stratification by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}† Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡ Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2,MECOM(EVI1)* 25 or del(5q); 27; 217/abn(17p) Complex karyotype,§ monosomal karyotype|| Wild-type *NPM1* and *FLT3*-ITD^{high}† Mutated *RUNX1*{Mutated *ASXL1*{Mutated *TP53*#

Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

*Prognostic impact of a marker is treatment-dependent and may change with new therapies.

†Low, low allelic ratio (,0.5); high, high allelic ratio ($0.5); semiquantitative

assessment of FLT3-ITD allelic ratio (using DNA fragment analysis) is determined as ratio of the area under the curve "FLT3-ITD" divided by area under the curve "FLT3- wild type"; recent studies indicate that AML with NPM1 mutation and FLT3-ITD low allelic ratio may also have a more favorable prognosis and patients should not routinely be assigned to allogeneic HCT.57-59,77

‡The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

§Three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring translocations or inversions, that is, t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23.3), t(6;9), inv(3) or t(3;3); AML with BCR-ABL1.

||Defined by the presence of 1 single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality (excluding core-binding factor AML).

{These markers should not be used as an adverse prognostic marker if they co- occur with favorable-risk AML subtypes.

#TP53 mutations are significantly associated with AML with complex and monosomal karyotype

Table 6. 2017 ELN response criteria in AML

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)	MRD* or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine (see Table 8) are generally considered as the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	Deaths occurring ≥7 d following completion of initial treatment while cytopenic, with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 d following its completion; or deaths occurring ≥7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 mo
Progressive disease (PD)*,†	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused); or • >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)†; or • New extramedullary disease	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 wk apart; the date of progression should then be defined as of the first observation date Some protocols may allow transient addition of hydroxyurea to lower blast counts *Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

ANC, absolute neutrophil count; IDH, isocitrate dehydrogenase; MLFS, morphologic leukemia-free state; WBC, white blood cell.

*The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.

†Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the

percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily

indicate PD.

Table 6 - 2022 ELN risk classification by genetics at initial diagnosis

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/ *CBFB::MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK::NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR::ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A::CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/ *GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2*‡‡ • Mutated *TP53*[a]

*Frequencies, response rates and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.

†Mainly based on results observed in intensively treated patients. Initial risk assignment may change during the treatment course based on the results from analyses of measurable residual disease.

‡Concurrent KIT and/or FLT3 gene mutation does not alter risk categorization.

§AML with NPM1 mutation and adverse-risk cytogenetic abnormalities are categorized as adverse-risk.

‖Only in-frame mutations affecting the basic leucine zipper (bZIP) region of CEBPA, irrespective whether they occur as monoallelic or biallelic mutations, have been associated with favorable outcome.

¶The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse-risk gene mutations.

#Excluding KMT2A partial tandem duplication (PTD).

**Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities.

††Monosomal karyotype: presence of two or more distinct monosomies (excluding loss of X or Y), or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding

factor AML).

‡‡For the time being, these markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.

a TP53 mutation at a variant allele fraction of at least 10%, irrespective of the TP53 allelic status (mono- or biallelic mutation); TP53 mutations are significantly associated with AML with complex and monosomal karyotype.

Table 8 – 2022 Response criteria in AML

Category	Definition	Comment
Response CR*,†,‡	Bone marrow blasts , 5%; absence of circulating blasts; absence of extramedullary disease; ANC $ 1.0 3 10^9/L (1,000/mL); platelet count $ 100 3 10^9/L (100 000/mL)	
CRh*,†,‡	ANC $ 0.5 3 10^9/L (500/mL) and platelet count $ 50 3 10^9/L (50 000/mL), otherwise all other CR criteria met	If CRh used, CRi should only include patients notmeeting the definition of CRh
CRi*,†,‡	All CR criteria except for residual neutropenia , 1.0 3 10^9/L (1,000/mL) or thrombocytopenia , 100 3 10^9/L (100 000/mL)	
MLFS	Bone marrow blasts , 5%; absence of circulating blasts; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be “aplastic”; bone marrow spicules should be present; at least 200 cells should be enumerated in the aspirate or cellularity should be at least 10% in the biopsy. Mainly used in the context of phase 1-2 clinical trials
PR	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pre-treatment bone marrow blast percentage by at least 50%	Mainly used in the context of phase 1-2 clinical trials
No response	Patients evaluable for response but not meeting thecriteria for CR, CRh, CRi, MLFS or PR are categorized as having no response prior to the response landmark. Patients failing to achieve response by the designated landmark are	

Nonevaluable for response	designated as having refractory disease Nonevaluable for response	
Response (if including assessment of MRD)§ CR, CRh, or CRi without MRD‡ (CR_{MRD-},CRh_{MRD-},or CRi_{MRD-})	CR, CRh or CRi with MRD below a defined threshold for a genetic marker by qPCR, or by MFC. Response without MRD should be confirmed with a subsequentassessment at least 4 wk apart. The date of responsewithout MRD is the first date in which the MRD was below the defined threshold Response with MRD detection at low-level (CR_{MRD-LL}) is included in this category of CR, CRh or CRi without MRD. CR_{MRD-LL} is currently only defined for *NPM1*- mutant and CBF-AML	Sensitivities vary by marker tested, and by method used; therefore, test used, tissue source and minimum assay sensitivity for evaluability should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Treatment failure Refractory disease Relapsed disease (after CR, CRh or CRi	No CR, CRh or CRi at the response landmark, ie, after 2 courses of intensive induction treatment or a defined landmark, eg, 180 d after commencing less-intensive therapy Bone marrow blasts $ 5%; or reappearance of blasts inthe blood in at least 2 peripheral blood samples at least one week apart; or development of extramedullary disease	Patients not responding to a first cycle of 7 1 3 should be considered for a regimen containing higher doses of cytarabine Patients not responding to a first cycle of 7 1 3 should be considered for a regimen containing higher doses of cytarabine
Treatment failure(if including assessment of MRD)§ MRD relapse (after CR, CRh or CRi without MRD)	1. Conversion from MRD negativity to MRDpositivity, independent of method, or 2. Increase of MRD copy numbers $ 1 $\log_{10}$ between any two positive samples in patients with CR_{MRD-LL}, CRh_{MRD-LL} or CRi_{MRD-LL} by qPCR The result of 1. or 2. should be rapidly	Test methodology, sensitivity of the assay, and cutoffvalues used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

	confirmed in asecond consecutive sample from the same tissue source	

ANC, absolute neutrophil count; CBF, core-binding factor; VAF, variant allele frequency.

*To recognize the potential for continuing improvements in blood counts after myelosuppressive therapy, response definitions for patients with marrow blast clearance (, 5%) may be adjusted to reflect the best hematologic response achieved prior to commencement of the next treatment cycle. Aspirate reports that include MLFS, CRh, or CRi should note the potential for post-marrow blood counts to alter the final response designation. Patients should not have received G-CSF, nor platelet transfusions within 7 d prior to hematologic response determination.

†For patients with CR, CRh, or CRi, the presence of a low percentage of circulating blasts in the blood may represent a regenerating marrow and should not be interpreted as persistent disease. In such cases the blasts generally disappear within a week.

‡A response landmark for CR, CRh, or CRi should be stated, eg, after 2 cycles of intensive therapy; this landmark may be longer for nonintensive based treatment options, eg, 180 days.

§MFC-MRD positivity is defined as $ 0.1% of CD45 expressing cells with the target immunophenotype. MRD test positivity by qPCR is defined as cycling threshold (Ct) , 40 and is negative if Ct $ 40 in $ 2 of 3 replicates. In NPM1-mutated and CBF-AML, CR with molecular MRD detectable at low-level (CRMRD-LL) defined as , 2% is designated as negative for MRD, because when measured at the end of consolidation treatment, is associated with a very low relapse rate

APPENDIX 6 – Proposed modified International Working Group response criteria for altering natural history of MDS

Category	Response criteria (responses must last at least 4 wk)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines*
	Persistent dysplasia will be noted*†
	Peripheral blood‡
	Hgb ≥ 11 g/dL
	Platelets ≥ 100 × 10^9/L
	Neutrophils ≥ 1.0 × 10^9/L†
	Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except:
	Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	Cellularity and morphology not relevant
Marrow CR†	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment†
	Peripheral blood: if HI responses, they will be noted in addition to marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following:
	Return to pretreatment bone marrow blast percentage
	Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
	Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete
	Disappearance of the chromosomal abnormality without appearance of new ones
	Partial
	At least 50% reduction of the chromosomal abnormality
Disease progression	For patients with:
	Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts
	5%-10% blasts: ≥ 50% increase to > 10% blasts
	10%-20% blasts: ≥ 50% increase to > 20% blasts
	20%-30% blasts: ≥ 50% increase to > 30% blasts
	Any of the following:
	At least 50% decrement from maximum remission/response in granulocytes or platelets
	Reduction in Hgb by ≥ 2 g/dL
	Transfusion dependence
Survival	Endpoints:
	Overall: death from any cause
	Event free: failure or death from any cause
	PFS: disease progression or death from MDS
	DFS: time to relapse
	Cause-specific death: death related to MDS

Deletions to IWG response criteria are not shown. To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10. MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-AmericanBritish; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival. *Dysplastic changes should consider the normal range of dysplastic changes (modification).41 †Modification to IWG response criteria. ‡In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

DOCUMENT HISTORY	
Document	**Date**
Amendment 2	25 June 2023
Amendment 1	02 February 2023
Original Protocol	01 January 2023

Amendment 1 (02 February 2023)

Section number and name	Description of change	Brief rationale
Date and version of protocol	The date and version was updated	Administrative
Protocol synopsis, “TYPE OF INTERVENTION.”	Deleted	Correcting typo to clarify that this study is not classified as "in-vitro diagnostics medical device"

Amendment 2 (25 June 2023)

Section number or name	Description of change	Brief rationale
Header	The date was added and the version was updated	Administrative
Title page	The Protocol registration number was added. The version and the date were updated	Administrative
Throughout the protocol	Administrative and typo corrections	-
Confidentiality Statement	Removing the sentence regarding study drug	Correction of typo
Table "STUDY ADMINISTRATIVE INFORMAITON AND INVESTIGATORS"	Correction the TASMC PI's name and addition of her email address. Removing of other SANGUINE Partners	Administrative Information regarding SANGUINE partners added to section 1.2
INVESTIGATOR SIGNATURE PAGE, Protocol synopsis “STUDY DESIGN”, Section 4	The classification of the study is elaborated	Clarifies that this study is a “Non-interventional” study
Section 1.2	Adding the identity of all non-clinical partners	Administrative
Protocol synopsis and throughout the document	TASMC BM sub-study was removed from the protocol	Additional and separated protocol will be written for this sub-study
Protocol synopsis and throughout the document	The indication “Blood cancer” was replaced by “Hematological malignancies"	Administrative

Protocol synopsis and throughout the document	Few Investigational hematological malignancies were grouped	Investigational and scientific reason
Protocol synopsis and throughout the document	The indication “Healthy” was replaced by “Subjects at risk and control subjects with no malignant disease”	Clarification. To have a more accurate definition
Protocol synopsis "OBJECTIVES", Section 2, Section 3	Objectives and endpoints re-defined as exploratory. OBJECTIVES: 1. Identify new therapeutic targets based on epigenetically modified genomic loci. 2. Provide prognostic information at pre-treatment and monitoring of MRD. ENDPOINTS: 1. Identify therapeutic targets base on differential epigenetic loci. 2. Determine an MRD protocol for epigenetic follow-up.	To be in line with the clinical plan of SANGUINE project
Protocol synopsis “METHODOLOGY”, Section 4, Table 1 - footnote	Allowing the collection of samples for stages 2 and 3 from the time of stage 1	To meet the recruitment goal and to allow maximum period of follow-up
Protocol synopsis “METHODOLOGY", Section 4.2, Table 3 and Section 6.2.2.	Follow-up population has changed to patients with MM, lymphoma (HL, DLBCL, FL, MZL) and AML	To be in line with the clinical plan of SANGUINE project
Protocol synopsis "METHODOLGY", Section 4.4, Section 6.1 (only for survival status), Table 2, Section 7, Section 10.2.2 (only for smoking status)	Addition of smoking history and smoking status during the trial and survival status for follow-up patients	Collection of relevant clinical data
Protocol synopsis “POPULATION SIZE”, Section 5.1, Table 1	Numbers of subjects from each indication and for each stage were updated	To be in line with the clinical plan of SANGUINE project
Protocol synopsis “MAIN INCLUSION CRITERIA”, Section 5.2.1	Inclusion criterion for patients with hematological malignancies: removal of surgery as previous treatment, NOTE was re-phrased.	Clarification
Protocol synopsis “MAIN INCLUSION CRITERIA”, Section 5.2.1	Inclusion criterion #1 for subjects at risk: removal of spouses and/or escorts of patients	Clarification. To be in line with the clinical plan of SANGUINE project
Protocol synopsis, “MAIN EXCLUSION CRITERIA”, Section 5.2.2	Exclusion criterion #3: only inflammatory autoimmune diseases that require treatment will be excluded	To allow recruitment of wider population

Protocol synopsis "MAIN EXCLUSION CRITERIA", Section 5.2.2	Addition of exclusion criterion # 5: Patients/subjects with known active Hepatitis A/B/C or past Hepatitis C	Exclusion of patients/subjects meeting this criterion
Protocol synopsis “EVALUATIONS”	Year 2-3: tests will be conducted also at the partners labs	Clarification
Table 2 Table 2, Section 7.1	Allowed time window for Baseline sample – extended to 28 days Subjects in Stage 3: will be tested for partial myeloma profile, CBC and chemistry	Adherence to clinical practice and standards To be in line with the clinical plan of SANGUINE project. To have a better evaluation of the sensitivity and specificity for screening
Section 9.4	AEs/SAEs definition and reporting requirements was redefined as deemed to be related to study procedure and occurred within 24 hours.	To clarify the reporting requirements for AEs/SAEs
Section 9.4	Updating SAE reporting contact information	Adding the study Medical Monitor as the recipient of SAE reports