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Deutsche Krebshilfe

PERDAM
Trial Protocol

Prospective **E**valuation of Measurable **R**esidual **D**isease in Intensively Treated Patients with **A**cute **M**yeloid Leukemia (AML) as Surrogate Endpoint for Survival

Trial Code:	PERDAM
Therapeutic Area:	Hematology/Oncology
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Summary

Acute myeloid leukemia is a genetically and phenotypically heterogeneous disorder with an incidence of 3 to 4 per 100 000 men and women per year and a median age at diagnosis of about 70 years. Prognosis, especially in older patients, has remained very poor. In patients considered suitable for intensive chemotherapy, the combination of an anthracycline and cytarabine remains the standard of care. For patients achieving a complete remission (CR), postremission therapy (PRT) ranging from chemotherapy to allogeneic hematopoietic stem cell transplantation is required; intensive PRT is still under debate in older patients.

Beyond pre-treatment genetics-based risk stratification, measurable residual disease (MRD) during treatment and follow up emerges as an important prognostic factor in first CR. Furthermore, MRD may provide a tool for a read-out of therapeutic efficacy.

In this diagnostic meta-study we intend to measure MRD using multiparameter flow cytometry across up-front randomized clinical trials which in total will accrue more than 1000 patients. MRD will be assessed early (after induction) and late (after consolidation) during treatment. The aim of the study is to analyse if levels of MRD measured early during treatment are closely related to overall survival and thus may serve as an early surrogate. There is a growing public demand that new, promising drugs are approved for therapy as rapidly as possible. Therefore, it is of great interest to obtain these approvals based on early biomarker endpoints such as MRD rather than on long-term survival endpoints.

Zusammenfassung

Die Akute Myeloische Leukämie ist eine genetisch und phänotypisch heterogene Erkrankung mit einer Inzidenz von 3-4 Fällen pro 100.000 Männer und Frauen pro Jahr. Das durchschnittliche Diagnosealter liegt bei etwa 70 Jahren. Die Prognose ist nach wie vor ungünstig, insbesondere bei älteren Patienten. Unverändert besteht die Standard-Induktionstherapie für fitte Patienten aus einer Kombination von Anthracyclinen und Cytarabin. Patienten mit Erreichen einer kompletten Remission (CR) nach der initiale Chemotherapie benötigen eine Postremissionstherapie (PRT), die aus weiterer Chemotherapiezyklen oder einer allogenen hämatopoetischer Stammzelltransplantation je nach Risikoprofil besteht. Der Nutzen der intensiven PRT ist jedoch für ältere Patienten umstritten.

Neben der etablierten Risikostratifikation anhand genetischer Merkmale nimmt die Bedeutung der „messbaren Resterkrankung“ (MRD) als prognostischer Parameter während und nach Abschluss der Behandlung insbesondere in erster CR zu. Zusätzlich kann die MRD als Indikator für therapeutische Effekte herangezogen werden.

In dieser diagnostischen Meta-Studie ist beabsichtigt, MRD mittels Multiparameter-Durchflusszytometrie im Rahmen mehrerer randomisierter Studien an insgesamt mehr als 1.000 Patienten zu bestimmen. Die MRD wird nach Induktionstherapie und nach Abschluss der Konsolidierungstherapie im Therapieverlauf gemessen. Das Ziel der Meta-Studie ist zu evaluieren, ob die MRD Messungen während der Therapie mit dem Gesamtüberleben zusammenhängen und als frühe Surrogatparameter verwendet werden können. Zunehmend besteht in der Gesellschaft der Wunsch, dass aussichtsreiche neue Therapeutika rasch zur Anwendung zugelassen werden. Deshalb besteht ein großes Interesse neue Therapeutika auf der Basis von frühen Biomarker-Endpunkten anstatt späten Überlebensendpunkten zuzulassen.

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Protocol Synopsis

Title	Prospective evaluation of measurable residual disease in intensively treated patients with acute myeloid leukemia (AML) as surrogate endpoint for survival
Therapeutic area	Hematology / Oncology
Indication	Newly diagnosed or first relapsed acute myeloid leukemia
Protocol Acronym	PERDAM
Principle Investigator	Prof. Dr. Richard F. Schlenk
Summary	Acute myeloid leukemia is a genetically and phenotypically heterogeneous disorder with an incidence of 3 to 4 per 100 000 men and women per year and a median age at diagnosis of about 70 years. Prognosis, especially in older patients, has remained very poor. In patients considered suitable for intensive chemotherapy, the combination of an anthracycline and cytarabine remains the standard of care. For patients achieving a complete remission (CR), postremission therapy (PRT) ranging from chemotherapy to allogeneic hematopoietic stem cell transplantation is required; intensive PRT is still under debate in older patients. Beyond pre-treatment genetics-based risk stratification, measurable residual disease (MRD) during treatment and follow up emerges as an important prognostic factor in first CR. Furthermore, MRD may provide a tool for a read-out of therapeutic efficacy. In this diagnostic meta-study we intend to measure MRD using multiparameter flow cytometry across up-front randomized clinical trials which in total will accrue more than 1000 patients. According to the leukemia-associated phenotype at diagnosis or the different-from-normal approach, MRD will be assessed early (after induction) and late (after consolidation) during treatment. The aim of the study is to show that levels of MRD measured early during treatment are closely related to overall survival and thus may serve as an early surrogate. There is a growing public demand that new, promising drugs are approved for therapy as rapidly as possible. Therefore, it is of great interest to obtain these approvals based on early biomarker endpoints such as MRD rather than on long-term survival endpoints.
Objectives	To demonstrate that measurable residual disease assessed by multiparameter flow cytometry during intensive treatment is a surrogate for overall survival and thus an early read-out for drug efficacy
Study design	Surrogate endpoint trial to establish that measurable residual disease assessed by multiparameter flow cytometry during intensive treatment is a surrogate for overall survival
Data protection	The rights of ownership are addressed by trial-specific contracts of conveyance. In addition, the generic concept of data protection proposed by the Telematic Platform of Medical Research Networks (Telematik Plattform Medizinischer Forschungsnetze, TMF) is in place for the network of clinical trials. Thus all samples will undergo pseudonymization before shipment to the two laboratories for flow cytometry.
Inclusion criteria	i) Acute myeloid leukemia according to the WHO classification ii) Informed consent in place for a randomized study of the Study Alliance Leukemia (SAL) including the Heidelberg Leukemia Network (HeLeNe) covering assessment of MRD by MPFC in the reference laboratories in Heidelberg and Dresden.
Exclusion criteria	No signed informed consent compliant with the requirements of PERDAM

Abbreviations

AML	Acute Myeloid Leukemia
CBF	Core-binding factor
CR	Complete remission
CRF	Case report form
CTEP	Cancer Therapy Evaluation Program
ddPCR	droplet digital Polymerase Chain Reaction
EFS	Event free Survival
ELN	European LeukemaNet
EC	executive committee
FACS	Fluorescence Activated Cell Sorter
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HeLeNe	Heidelberg Leukemia Network
HCT	hematopoietic cell transplantation
ICH	International Conference on Harmonization
IEC	Independent Ethical Committee
IPD	individual patient data
LAP	leukemia-associated phenotype
NIS	Non-Interventional Study
MPFC	multiparameter flowcytometry
MRD	Measurable residual disease
OS	Overall survival
PRT	Postremission Therapy
RFS	Relapse free survival
RQ-PCR	Real Time Quantitative Polymerase Chain Reaction
SAL	Study Alliance Leukemia
SAP	statistical analysis plan
STE	surrogate threshold effect
TMF	Telematic Plattform Medizinischer Forschungsnetze
WHO	World Health Organization

1 Background information and rationale

1.1 General Introduction

Acute myeloid leukemia (AML) is a genetically and phenotypically very heterogeneous disorder with an incidence of 3 to 4 per 100 000 men and women per year with a median age at diagnosis ranging from 66 to 71 years [1][2]. It is characterized by the accumulation of somatically acquired genetic changes in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation. Outcome is influenced by various factors, including patient features such as age, comorbidities, and performance status and disease characteristics of which the genetic profile of the disease is the most important. According to the recommendations from an international expert panel, on behalf of the European LeukemiaNet (ELN), AML can be grouped into 3 risk groups, favorable, intermediate and unfavorable [1]. In patients considered suitable for intensive induction therapy, the combination of an anthracycline and cytarabine remains the standard of care. Complete remission (CR) can be achieved in 65% to 75% of younger adult patients (≤60 years) and in approximately 40% to 60% of older patients (>60 years). In patients who achieve a CR after induction chemotherapy, some postremission therapy (PRT) is required to prevent relapse [1][2][3]. Although the value of PRT in the older patients continues to be debated, in younger patients, the choice for consolidation is based on genetic features and can range from high dose cytarabine to allogeneic hematopoietic cell transplantation (alloHCT), with a 5-year overall survival (OS) rate of 40% to 45%; OS in older patients still remains poor at <10% after 5 years [1][3]. In patients ineligible for intensive chemotherapy, the spectrum of treatment options is limited and includes best supportive care with hydroxyurea, low-dose cytarabine, and the hypomethylating agents decitabine or azacitidine. Using such low-dose therapy, CR can be achieved in 10% to 30% of patients and the OS at 3 years is approximately 5% [1][2].

Beyond pre-treatment risk stratification, measurement of the disease burden named measurable residual disease (MRD) during treatment and follow up emerges as one of the most important prognostic factors once a CR of the disease is achieved [4][5][6][7][8][9].

In addition to prognostic and predictive importance of MRD, the assessment of MRD may provide a tool to receive an early read-out of therapeutic efficacy. Based on the still poor outcome of AML in general, there is an increasing public demand that new, promising drugs are approved for therapy as rapidly as possible. Therefore, it is of great interest to obtain these approvals based on early biomarker endpoints such as MRD rather than on long-term clinical endpoints such as OS [6]. Exemplarily, the international CALGB 10603 (RATIFY, NCT00651261) trial evaluating midostaurin as adjunct to intensive chemotherapy in younger adults with AML and activating *FLT3* mutations recruited after screening of 3279 a total of 714 patients between 2008 and 2011. The analysis of the primary endpoint OS was planned after 507 events (deaths). However, the event rate reached a plateau with only 3 deaths in 2015 and in total 357 deaths. Thus, 507 events have been predicted to occur in the year 2025. After approval by the Data Safety Monitoring Board of the trial and the Cancer Therapy Evaluation Program (CTEP) the statistical analysis plan was amended and final adjusted statistical analysis was performed after 357 deaths [10]. Fortunately, a significant better OS in the experimental arm compared to the standard arm could be shown despite effective and diverse salvage therapy strategies in patients with refractory disease [11] or relapse AML [12]. Therefore the approval of midostaurin in AML with activating *FLT3* mutations has been achieved in US and Europe. Data from the original publication suggested that the difference in the two arms occurred early and was mainly due to midostaurin added to the first treatment cycle [10]. Unfortunately, MRD was not measured within this trial. However, MRD measured early and late during treatment would have added enormous value in interpreting the differences observed in event-free, relapse-free and OS and in better defining when the treatment effect was most pronounced (during induction, consolidation or the one year maintenance therapy).

1.2 MRD in acute myeloid leukemia

MRD can be measured and quantified by Real Time Quantitative Polymerase Chain Reaction (RQ-PCR) in AML with gene mutations (e.g. *NPM1* mutation) [7][13] and gene fusions (e.g. *RUNX1-RUNX1T1*, *CBFβ-MYH11* [7]). However, only in 30-45% of all AML cases well-

established methods are available nowadays in routine care based on this technique [4]. With newer methods such as whole genome or exon sequencing [7][14] and droplet digital Polymerase Chain Reaction (ddPCR) [7][15][16] MRD is measurable in the majority of patients and with an enormous precision. However, the different mutational patterns in individual patients and the difficulty in the interpretation of clearance or persistence of specific gene mutations after intensive chemotherapy on the background of clonal hematopoiesis of indeterminate potential [17][18][19] currently lead to limitations in the applicability of these new methods in the context of prospective multicenter clinical trials [19][20]. An alternative method is the assessment of MRD by multiparameter flowcytometry (MPFC). With this method individual pathologic leukemia-associated phenotypes (LAPs) can be defined at diagnosis in more than 90% of all AML cases and retrieved after induction and consolidation therapy with a sensitivity of 10^{-4}-10^{-5} [4][5][6][7][8][21]. In addition, MRD can be defined via a comparison to either normal or regenerating marrow by visual inspection as a cell population showing deviation from normal antigen-expression patterns seen in specific cell lineages at specific stages of maturation [22][23]. Within the studies evaluating MRD with MPFC the antibody-panel used were somewhat different including CD2, CD3, CD5, CD7, CD8, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD34, CD33, CD38, CD42b, CD45, CD56, CD61, CD64, CD65, CD71, CD90, CD117, CD123, CD133, HLA-DR measured in a four [21] and up to ten [22][23][24][25] colour flowcytometer. Of note, despite some variability of the used panels and the amount of the fluorescence channels the threshold to define a LAP was consistent across all the trials with a cut point of 0.1% of all nucleated cells providing highest prognostic information with regard to risk of relapse and overall survival. [5][21][22][23][24][25][26]. Thus from a practical point of view the LAPs and patterns different from normal defined for all patients individually resulted in MRD parameters independent of the LAPs and patterns different from normal with a value of zero in case of MRD levels below 0.1% or a continuous value between 0.1 and 100% in case of MRD levels above the cut point. So far the vast majority of analyses have been based on the comparison of MRD negative versus MRD positive patients at a certain time-point [4][5][21][22][23][24][25][26][27]. MRD levels are more and more integrated in clinical decision making after induction therapy [25] and during consolidation therapy including allogeneic HSCT [24][28]. However, so far no prospective confirmatory evaluation has been performed within randomized clinical trials defining the sensitivity and specificity MRD measurement using MPFC [3][6][7][29]. In addition, the prognostic and predictive impact of discrete values [27] of MRD and their changes over time instead of the dichotomized (positive vs. negative) approach of MRD assessment has also not been prospectively evaluated. Based on the very strong prognostic impact of dichotomized MRD (positive vs. negative) measured by MPFC in patients receiving an allogeneic HSCT, [23][24] the value of treatment intensification (e.g. with an allogeneic HSCT) to counterbalance a higher risk of relapse in MRD positive patients is still open [6][7][29].

Drug development in patients with AML is currently somewhat more focussed on older patients not eligible for intensive chemotherapy because clinical endpoints event-free and overall survival can be observed within a short time period due to the overall very unfavourable prognosis of this patient population [30]. In contrast, drug development in intensively treated younger patients is mainly hampered by confounding therapeutic interventions such as allogeneic HSCT during first line therapy or after AML relapse. Current examples are i) the CALGB 10603 international randomized double blinded placebo-controlled phase-III study in newly diagnosed *FLT3*-mutated AML evaluating the FLT3 inhibitor midostaurin (NCT00651261) in which the event-driven final analysis on the primary endpoint OS was reported at the annual meeting 2015 of the American Society of Hematology after amendment of the statistical analysis plan [10] as well as ii) the double blinded placebo-controlled phase-III VALOR study evaluating vosaroxin in relapsed or refractory AML in combination with cytarabine in which results were overall not significant (p=0.06) for the endpoint OS due to the not significant (p=0.60) results in younger patients with nearly superimposable survival curves [31]. In contrast, results were significant (p=0.02) if patients receiving an allogeneic HSCT were censored at the time of transplant and in the subgroup of patients above the age of 60 years (p=0.003). These examples illustrate that surrogate early biomarker endpoints are of high interest to provide an early read-out of clinical efficacy. Such early read-outs will allow designing reasonable adaptive drug development plans with the aim to integrate new drugs as early as possible in routine

patient care. Quantifying MRD by MPFC has the potential to serve as an early biomarker surrogate for survival endpoints but has so far not been evaluated in a confirmatory prospective manner [32]. In addition, the direct comparison of quantified MRD evaluated by MPFC, by RQ-PCR [33] (or ddPCR) for gene mutations and the gene fusions, and by next generation sequencing (NGS) within prospectively randomized trials offers the unique opportunity of a pivotal reference cross validation of the different methods for recent and future studies.

2 Objectives and Endpoints

2.1 Objectives

- To assess quantitatively measurable residual disease by multiparameter flow cytometry in patients with acute myeloid leukemia who are intensively treated after induction (newly diagnosed AML) or salvage chemotherapy (relapse/refractory AML) and at the end of consolidation therapy (newly diagnosed AML and relapse/refractory AML)
- To evaluate the association of measurable residual disease at two different time points with survival endpoints, primarily overall survival, secondarily event-free and relapse-free survival.

2.2 Endpoints

2.2.1 Primary Surrogate Parameter Endpoint

- Measurable residual disease assessed after induction therapy (newly diagnosed AML) or salvage chemotherapy (relapse/refractory AML)

2.2.2 Secondary Surrogate Parameter Endpoint

- Measurable residual disease assessed at the end of consolidation therapy (newly diagnosed AML and relapse/refractory AML)

2.2.3 Primary Clinical Endpoint

- Overall survival

2.2.4 Secondary Clinical Endpoints

- Event-free survival
- Relapse-free survival

3 Trial design and rationale

3.1 Detailed Design

Patients with AML participating in interventional prospective randomized trials of the Study Alliance Leukemia including interventional prospective randomized trials initiated from the University Hospital Heidelberg are aimed to be included into this diagnostic meta-study on MRD. In all evaluable patients initial diagnostic flow cytometric analysis and assessment of MRD after induction (newly diagnosed AML) or salvage chemotherapy (relapse/refractory AML) and at the end of consolidation therapy (newly diagnosed AML and relapse/refractory AML) are intended.

Within the PERDAM study no additional clinical data is collected. All data is collected within the activities of the participating trials in the randomized clinical trials. MRD measurement with MPFC is done in the two laboratories named in chapter 5).

3.1.1 Study Alliance Leukemia (SAL) and Heidelberg Leukemia Network (HeLeNe) trials

Independently of the PERDAM trial, patients with either newly diagnosed or relapsed AML in the centers of the Study Alliance Leukemia (SAL) including the Heidelberg Leukemia Network (HeLeNe) are registered in the SAL-registry (NCT03188874) after informed consent is obtained. Samples including peripheral blood and bone morrow are sent to the reference labs

and analyzed for cyto- and molecular-genetics, immunophenotype and cryopreserved for biobanking. All patients with either newly diagnosed or relapsed AML are screened for eligibility for up-front randomized clinical trials. If patients are eligible and informed consent is obtained, patients may be included in these trials.

Currently, 2 studies are open for recruitment, the BLAST trial (ClinicalTrials.gov Identifiers: NCT02502968) and the DaunoDouble trial (ClinicalTrials.gov Identifier: NCT02140242). New studies may be added without need for an amendment of the PERDAM trial protocol.

3.1.2 Inclusion of additional studies during the run-time of PERDAM

During the run-time of PERDAM new studies are integrated into PERDAM via a uniform procedure. Prerequisites for eligibility are

1. Investigator-initiated study (Pharma-sponsored studies are not eligible)
2. The IC of the respective study fulfils the minimum requirements (see chapter 12.2)
3. Access to study data guaranteed by the principle investigator of the respective study.
4. Either i) centralized assessment of flow-cytometry for evaluation of leukemia-associated phenotypes (LAPs) at diagnosis/relapse or ii) the different from normal approach
5. Centralized assessment of flow-cytometry for evaluation of MRD based on predefined LAPs from diagnosis/relapse or the different from normal approach
6. Studies of the SAL including those of the Heidelberg Leukemia Network
7. Access to the central web-based PERDAM database

The aim of the PERDAM study is only achievable on the basis of academic research. Therefore, the primary prerequisite for inclusion of additional studies is that the new study is an Investigator-initiated trial with an academic sponsorship.

Furthermore, a new study will be included upon completion and signature of the data access form by the principle investigator of the new study (Appendix 15.2) to guarantee access to the study data comprising baseline characteristics including a minimal set of laboratory and genetic data, unblinded randomization (after unblinding of the study), response to induction therapy, type of induction and consolidation treatment including date of alloHCT if applicable, event-free, relapse-free and overall survival.

The primary outcome measure in this study is MRD assessed by multiparameter flow cytometry (MPFC). MPFC is assessed at the time of diagnosis/relapse and subsequently after induction therapy (newly diagnosed AML) or salvage therapy (relapse/refractory AML) and completion of consolidation therapy (newly diagnosed AML and relapse/refractory AML) at the laboratory for flow cytometry in Heidelberg and Dresden, respectively. Assessments in other laboratories of MRD with MPFC may be into the PERDAM study after standardization.

Documentation of MPFC is performed in the web-based PERDAM database. Lab data of MRD assessed by MPFC from diagnosis, after induction/salvage and completion of consolidation therapy are uploaded via a file import procedure or manually. Based on the documented data within the PERDAM database regular virtual meetings are performed to discuss results for continuous harmonization of laboratory procedures and MRD evaluations between the two involved laboratories.

4 Patient selection

4.1 Patient Inclusion Criteria

- Acute myeloid leukemia according to the WHO classification
- Informed consent in place for a registry study and/or a randomized study of the Study Alliance Leukemia (SAL) including the Heidelberg Leukemia Network (HeLeNe) covering assessment of MRD by MPFC in the reference laboratories in Heidelberg and Dresden.

4.2 Patient Exclusion Criteria

- No signed informed consent compliant with the requirements of PERDAM

4.3 Withdrawal of Patients

A patient must be withdrawn from the study for the following reasons:

- At any time at their own request.
- Withdrawal of patient's consent to use their data

If the patient withdraws consent for disclosure of information, no additional data can be collected. Data already being transferred to the PERDAM database before the withdrawal may still be uses unless, the signed Informed Consent of the respective clinical trials imposes a deletion.

4.4 Premature Closure of the study and exclusion of participating studies

The trial can be prematurely closed or suspended by the PI. Participating studies can be excluded. Furthermore, the IEC themselves may decide to stop or suspend the study.

Reasons for premature closure or exclusion of participating studies:

- Non-compliance with the protocol
- Poor data quality
- No recruitment in 6 months
- Non-adequate IC

The PIs of all participating and affected studies have to be informed immediately about a cessation/suspension of the study. No further data will be collected.

5 Sample procurement & shipment

5.1 Sample shipment to the University Hospital Heidelberg

Send biosamples and their accompanying routing forms at room temperature, Monday to Friday, carrier for next day delivery before 9 a.m., to

Universitätsklinikum Heidelberg
Medizinische Klinik, I. OG, Zimmernummer 01.136 ,
Weiterleitung an die **Hämatologische Diagnostik**/Leitung PD. Dr. Hundemer.
INF 410,
69120 Heidelberg
Phone: 06221-56 36955 (Frau Ute Bauer)

e-mail: Katharina.Kriegsmann@med.uni-heidelberg.de
Michael.Hundemer @med.uni-heidelberg.de

5.2 Sample shipment to the University Hospital Dresden

Send biosamples and their accompanying routing forms at room temperature, Monday to Friday, via overnight carrier for next day, delivery before 9 a.m., to:

Hämatologisches Labor Haus 65, EG
Medizinische Klinik und Poliklinik I
Universitätsklinikum Carl Gustav Carus
Fetscherstr. 74
01307 Dresden
Germany
Tel.: +49(0)351 458-5621
Email: malte.bonin@uniklinikum-dresden.de

6 MRD assessment by MPFC

6.1 Sample preparation and measurement

In order to obtain comparable results, instrument set up, sample preparation and surface staining will be performed in the same manner in both laboratories. The procedures are based on the HAROMIZE initiative [34] and listed in detail in the lab manual.

6.2 Regular MRD assessment conferences and cross validation

In terms of cross validation and harmonization the same SOPs for MRD assessment by MPFC are used in the two laboratories. In addition, regular MRD assessment phone/web conferences are hold every three months to discuss all cases of the three month time period and achieve agreement. A cross validation between the two laboratories is performed at the beginning and thereafter every 6 months during the PERDAM study conduct with divided samples send out to the two laboratories and measured in both laboratories at the same time-points, at diagnosis/relapse and after induction/salvage therapy (see lab manual).

7 Perdam Database

Data management is performed by the NCT trial center. The Web-based registration platform for the PERDAM study is based on the generic data protection concept of the Telematics Platform for Medical Research Networks (Telematikplattform für Medizinische Forschungsnetze, TMF) using appropriate measures for data protection and disaster recovery.

7.1 Registration process and data input

During the registration process every patient already identified via a unique pseudonym consisting of short name of the randomized study and the study-specific patient-identifier receives a second unique second pseudonym, the PERDAM identifier. Data obtained in the two laboratories are handled in certified lab-specific IT-systems and transferred to the PERDAM databases via secured file-upload or are manually documented. The PERDAM database fulfills EudraLex Volume 4 Annex 11 and FDA Title 21 CFR Part 11 requirements. MRD assessed by MPFC is documented for patients who at least have one MPFC result at diagnosis/relapse describing the LAPs for an individual patient and one MRD assessment by MPFC after induction/salvage therapy. Patients responding to induction/salvage therapy but without

evaluable LAPs are documented with the different to normal approach in the PERDAM database. In addition, the MRD assessment after completion of consolidation therapy is included as third assessment time point. The registration process, file-upload and manual input are described in detail in the PERDAM database manual (separates document).

8 Clinical data

The demographic, laboratory, clinical and outcome data of the interventional randomized clinical trials are documented in trial-specific case report forms and after validation stored in trial-specific databases (Trial-databases) fulfilling EudraLex Volume 4 Annex 11 and FDA Title 21 CFR Part 11. Linkage between data of the Trial-databases and the PERDAM database are achieved via the uniform patient-specific pseudonym. Regular data exports from the Trial-databases including a core dataset (excluding explicitly the randomization) including data assessed at diagnosis/relapse (age at diagnosis/relapse, sex, date of diagnosis/relapse, WBC, bone marrow blast percentage, AML-entity according to WHO-2016), induction/salvage treatment (start of induction/salvage treatment, dose-reduction (yes/no) of induction- and/or consolidation therapy, response to induction/salvage treatment, date of assessment, date of response), consolidation treatment data (start of consolidation treatment, date of allogeneic HCT) and outcome data (date of relapse, date of last follow-up or date of death, vital status) are implemented on a three monthly basis. These data are imported into the PERDAM database to provide clinical background for the regular MRD-assessment conferences.

9 Statistical analyses

9.1 Design aspects

Clinical development of new drugs in hematology/oncology is an increasingly challenging endeavor. With the number of new compounds that can potentially be tested in the clinic, and the need to reach conclusions about the safety and efficacy of new treatments as quickly as possible, the search for early clinical endpoints and biomarkers that can be used as surrogate endpoints for long-term clinical endpoints has emerged as a new discipline in its own right [35]. Prentice's definition of a surrogate endpoint was that of a response variable for which the test of the null hypothesis of no relationship to the treatment groups under comparison was also a valid test of the corresponding null hypothesis based on the true endpoint [36]. Subsequently, Freedman et al. and Buyse and Molenberghs emphasized estimation and prediction rather than hypothesis testing in the process of endpoint validation [37][38]. The measures proposed using data from a single trial, such as the proportion of treatment effect explained, have however been shown to be insufficient to establish surrogacy [39]. The focus therefore shifted to methods based on a meta-analytic approach using data from several randomized trials [40][41]. When data from several trials are available, a surrogate endpoint can be assessed both at the individual level and at the trial level for its ability to predict the effect of treatment on the true endpoint. Using the trial level association between the effects of treatment on the surrogate and on the true endpoint, a calculation of the surrogate threshold effect (STE), which is the minimum effect required on the surrogate to predict a significant effect on the true endpoint in a future trial can be performed [42]. Hence, if a surrogate was accepted as valid (qualified), a future trial could be designed to show that the treatment effect on the surrogate exceeds the surrogate threshold effect, rather than to show a treatment effect on the true endpoint. Therefore, our aim is to benefit from our established trial infrastructure with randomized interventional clinical trials running in parallel and to evaluate MPFC as a general approach to measure MRD for surrogacy in respect to survival endpoints.

9.1.1 Experimental assessment:

MRD assessed by flow- cytometry as described previously [5][6][7][8] [21][22][23][24][25][26][27] is assessed in patients after induction (newly diagnosed AML) or salvage chemotherapy (relapsed/refractory AML) and after completion of consolidation therapy (newly diagnosed AML and relapse/refractory AML) in the two laboratories at the University Hospital Heidelberg and the University Hospital Dresden. MRD assessed by MPFC will be evaluated first at the same time as the remission status based on morphology after induction/salvage therapy. This MRD assessment by MPFC after induction/salvage therapy is used to test the primary hypothesis. A second MRD assessment by MPFC will be performed after completion of consolidation therapy including high-dose chemotherapy and alloHCT. MRD positive values are documented as discrete numbers up to 100%.

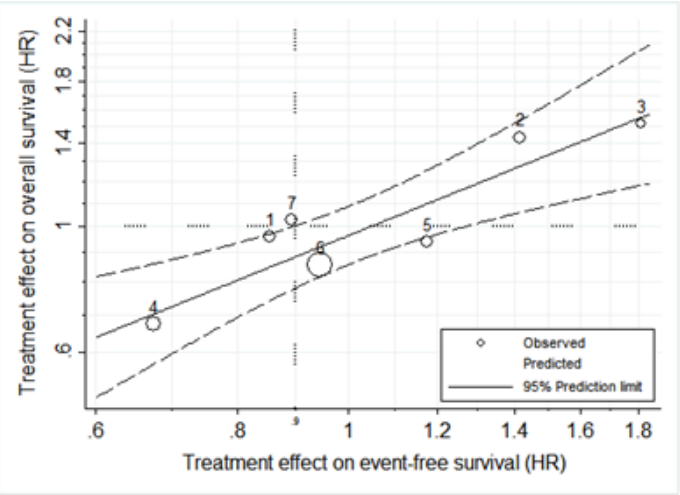
9.1.2 Standard assessment:

Overall survival (OS) is defined as time from entry into the study until death from any cause. Event-free survival (EFS) is measured from entry into the study until one of the following events, whichever comes first, i) no response to induction therapy, ii) relapse, iii) death from any cause. For assessment of response to induction therapy and relapse, morphological evaluation of peripheral blood and bone marrow smears is the standard assessment. Relapse-free survival (RFS) is measured in patients who achieve a first CR. RFS is measured from achievement of first CR until one of the following events, whichever comes first i) relapse, ii) death from any cause. For assessment of relapse morphological evaluation of peripheral blood and bone marrow smears is the standard assessment. OS, EFS, and RFS are censored at the date of last follow up in case of no event. The survival endpoints OS, EFS, and RFS defined according to the ELN recommendations [1] are accepted standards. Within the proposed study the primary survival endpoint is OS, EFS and RFS are included as secondary survival endpoints.

9.1.3 Proposed sample size/Power calculations

For validation of MRD assessed by MPFC as surrogate endpoint for OS, it is critical that the effect of treatment on MRD assessed by MPFC is closely correlated with the effect of treatment on OS. In our previous work [43] evaluating EFS as a surrogate endpoint for OS in AML we were able to show in an individual-patient data approach of 1,811 patients from four randomized trials (AMLHD 98B [44] AMLSG 06-04, [45] AMLSG 07-04, [46] and AMLSG 12-09, [47]) with 7 independent treatment comparisons due to multiple therapeutic questions that although EFS is only moderately correlated with OS (Spearman's rho = 0.76, 95% CI: [0.73 , 0.79]) the treatment effects on EFS were strongly correlated with treatment effects on OS (correlation coefficient R = 0.99, for log hazard ratios). A two-level modelling approach was used to estimate the association between EFS and OS, and between treatment effects on EFS and OS [48]. At the individual level, a bivariate (Hougaard-copula-based) survival model with Weibull marginal hazards was fitted to model the joint distribution of EFS and OS, and Spearman's rank correlation coefficient (rho) was used to quantify the association between the endpoints. At the trial-level, an error-in-variables linear regression model was fitted to the estimated treatment effects (Weibull‑model‑based log hazard ratios) on EFS and OS, taking into account the estimation error. The correlation coefficient (R) was used to quantify the strength of association between the treatment effects.

Study	Comparison	Control arm Experimental arm	N
AMLSG 06-04	1	Valproic acid All-trans-retinoic acid + Valproic acid	96 93
AMLSG 12-09	2	Standard Azacitidine prior/concurrent/after	36 103
	3	Standard Azacitidine after	64 65
AMLSG HD98B	4	Standard All-trans-retinoic acid	128 126
AMLSG 07-04	5	Standard All-trans-retinoic acid	92 94
	6	Standard All-trans-retinoic acid	369 359
	7	Valproic acid All-trans-retinoic acid + Valproic acid	95 91



The figure illustrates the correlation of the treatment effects on EFS and OS from 7 independent treatment comparisons as outlined and numbered in the table on the left. In this example clearly a substantial treatment effect could be demonstrated in 4 of the 7 treatment comparisons. Based on this experience we also expect treatment effects in most of the trials being part of the application. The sample size of the proposed study is fixed by the limited number of randomized trials and the trial-individual sample size calculation. We assume that 1000 patients are eligible for MRD assessment by MPFC after induction/salvage therapy. Based on i) our previous experience showing a high correlation of treatment effects on EFS and OS (R=0.99 for log hazard ratios with lower 95% confidence limit of 0.85), ii) the compelling data available on the prognostic value of MRD assessed by MPFC [5][21][22][23][24][25][26][27] and iii) the growing body of evidence indicating efficacy of new drugs especially in combination with induction/salvage therapy and not or to a much lesser extend later on in the treatment course (consolidation, maintenance) [10][32][49][50] the primary hypothesis is that MRD assessment by MPFC after induction/salvage therapy is a good surrogate for OS defined by trial level correlation R_T of the treatment effects on MRD assessed by MPFC and OS with $R_T > 0.8$.

We aim to test the one-sided null hypothesis H_0: $R_T \leq 0.8$ against the alternative hypothesis H_1: $R_T > 0.8$ at a significance level of $\alpha = 2.5\%$. To this aim we will use an individual patient data (IPD) meta-analytic approach and focus on the coefficient of determination $(R_T)^2$ to quantify the strength of association between treatment effects on MRD assessed by MPFC (log odds ratios, derived by logistic regression models) and treatment effects on OS (log hazard ratios, using Cox regression models) at the trial level. Based on the given sample size of at least 1000 patients within the included randomized clinical trials and considering a true R_T of 0.9 the power to identify this effect will be larger than 85%.

9.1.4 Statistical Analysis

With respect to Prentice's original proposal [36] current consensus is that for surrogate validation a correlation-based approach can be applied, with estimation of correlation measures on a trial level and an individual patient level [51][52][53]. We will apply the correlation approach according to Buyse et al. [40] to investigate the validity of MRD assessed by MPFC as surrogate endpoint for the long-term time to event endpoints OS (primary endpoint), RFS, and EFS (secondary endoints). Two levels of surrogacy will be considered. On an individual patient level we will assess how well the surrogate predicts the survival endpoint. This is done by evaluating the prognostic value of MRD assessed by MPFC for the survival endpoint after adjustment for treatment. We will use the R^2 measure of Schemper and Henderson as well as Brier Score to assess the predictive accuracy of MRD assessed by MPFC [54]. Within the trial-level evaluation an individual patient data (IPD) meta-analytical approach will be used to test if the treatment effect on MRD assessed by MPFC allows reliable prediction of the treatment effect on the survival endpoints. We consider logistic regression for the surrogate endpoint MRD assessed by MPFC to achieve odds ratios (OR) and the proportional hazards model of Cox for the survival endpoints resulting in per trial hazard ratios (HR) and an error-in-variables linear

regression model of the log transformed HRs on the log transformed ORs. The trial-level coefficient of determination R_T^2 is then used to quantify the strength of association between the treatment effects on MRD assessed by MPFC and on the survival endpoints at the trial level.

In addition the surrogate threshold effect (STE) [42] will be estimated as the minimum effect of MRD assessed by MPFC that predicts a statistically significant effect on EFS, RFS and OS.

In addition, exploratory analyses will be performed on

- MRD assessed by MPFC after consolidation as a surrogate for OS, EFS and RFS
- Correlation of MRD assessed by MPFC after induction therapy and after consolidation therapy with respect to applied postremission treatment

A detailed statistical analysis plan (SAP) will be finalized before database closure.

10 Quality assurance and monitoring

Quality within the randomized interventional studies is assured by central and by on-site monitoring according to German law. On-site monitoring is implemented via each randomized interventional clinical trial including source data verification. Quality of laboratory procedures and analyses is assured by a quality-management system in place and regular MRD conferences and cross validation described above (see 6.2).

11 Executive committee

Tasks and responsibilities:

The executive committee (EC) is a collaborative operative board for the PERDAM study. Its overall roles are to review the results and outcomes collected in the PERDAM database, to define, foster and lead all multiparty publications of the PERDAM database. It has to assure that investigators of all study sites contributing to the PERDAM study will be appropriately involved in writing committees and authorship of publications. For number of authors and order, number of patients per site and the specific contributions must be taken into account. Details of operation and the interfaces to the other parties involved will be defined in a separate manual. Members of the EC are all collaborators listed on signature page-I and – II.

12 Ethical and legal aspects

12.1 Good Clinical Practice & Declaration of Helsinki

The procedures set out in this trial protocol, pertaining to the conduct, evaluation, and documentation of this trial, are designed to ensure that all persons involved in the trial abide by Good Clinical Practice (GCP) and the ethical principles described in the current version of the Declaration of Helsinki. The trial will be carried out in keeping with local legal and regulatory requirements.

12.2 Patient Information and Informed Consent

Prior to using data from any individual, the respective patient must be informed about the potential use of the data and consent in written form to the usage of his data.

For the PERDAM study no study specific patient information or study specific informed consent is needed, as long as the IC used in the respective clinical trial matches the following **minimum requirements**:

- Witten consent to transfer pseudonymized data for other research projects
- Consent to use the data is voluntary and does not influence the possibility of participation in the respective clinical trial

- Duration of data storage must be addressed
- Appropriate data protection has to be ensured
- The IC has to be approved by the responsible ethical review board.

If the IC of the respective study does not match the needed minimum requirements, the IC must be changed per amendment or an additional IC must be handed to the patient. Recommended phrases for informed consent and patient information can be found in appendix 15.1.

12.3 Confidentiality

The data obtained in the course of the trial will be treated pursuant to the Federal Data Protection Law (Bundesdatenschutz- bzw. Landesdatenschutzgesetz, BDSG, LDSG), as well as the General Data Protection Regulation (GDPR, EU 2016/679).

During the study, patients will be identified solely by means of an individual identification code (PERDAM identifier). Only pseudonymized data will be used, name and address will neither be stored nor transferred.

Storage of study data on a computer will be done in accordance with local data protection law and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of local data legislation will be fulfilled in its entirety.

The patient consents in writing to relieve the investigator from his/her professional discretion ("ärztliche Schweigepflicht") in so far as to allow inspection of original data for monitoring purposes by health authorities and authorized persons (inspectors, monitors, auditors). Authorized persons (clinical monitors, auditors, inspectors) may inspect the patient-related data collected during the trial, ensuring the data protection law.

No unauthorized person will have insight to the data.

Only pseudonymized data may be transferred.

12.4 Approval of Trial Protocol and Amendments

Before the start of the trial, the trial protocol, informed consent document and any other appropriate documents will be submitted to the independent Ethics Committee (EC). A written favorable vote of the EC is a prerequisite for initiation of the trial.

All substantial changes will be submitted to EC.

12.5 Legal status

The PERDAM trial is a non-interventional study (NIS). According to §4 Section 23 AMG this meta-study is classified as other study ("Sonstige Studie").

12.6 Financial status

The study is exclusively funded by intuitional funds and supplemented by a research grant from the German Research Foundation (DFG grant number: Schl 2118/2-1).

12.7 Patient incentives

No patient incentives or compensations will be paid.

12.8 Registration of the Trial

The coordinating / principal investigator will register the trial at the public accessible clinical trial register www.clinicaltrials.gov.

13 Signatures

13.1 Signatures Heidelberg

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The undersigning persons

- agree that moral, ethical, and scientific principles as set out in the applicable version of Declaration of Helsinki are met
- are willing to support this study and will conduct the study according to the protocol
- will enrol patients only after all ethical requirements are fulfilled

Principle Investigator

Date	Prof. Dr. Richard F. Schlenk National Center for Tumor Diseases

Trial Coordination (HD)

Date	Dr. Lucian Le Cornet National Center for Tumor Diseases

Biometry

Date	Axel Benner German Cancer Research Center Heidelberg
Date	Dr. Christina Kunz German Cancer Research Center Heidelberg

Leading Clinical Site (HD)

Date	Prof. Dr. Carsten Müller-Tidow Klinik für Innere Medizin V

Flow cytometry (HD)

Date	PD Dr. M. Hundemer, Labor für Durchflusszytometrie
Date	Dr. K. Kriegsmann Labor für Durchflusszytometrie

13.2 Signatures Dresden

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The undersigning persons

- agree that moral, ethical, and scientific principles as set out in the applicable version of Declaration of Helsinki are met
- are willing to support this study and will conduct the study according to the protocol
- will enrol patients only after all ethical requirements are fulfilled

Trial Coordination (DD)

Date	PD Dr. Christoph Röllig Medizinischen Klinik und Poliklinik I

Leading Clinical Site (DD)

Date	Prof. Dr. med. Gerhard Ehninger Medizinischen Klinik und Poliklinik I
Date	Prof. Dr. med. Martin Bornhäuser Medizinischen Klinik und Poliklinik I

Flow cytometry (DD)

Date	Dr. rer. Medic Uta Oelschlägel MK1-L06, Labor Durchflusszytometrie
Date	Dr. med. Malte von Bonin MK1-L06, Labor Durchflusszytometrie

14 References

[1] Döhner H, Estey E, Grimwade D, *et al.* Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129(4):424–447.

[2] Schlenk RF, Döhner H. Genomic applications in the clinic: use in treatment paradigm of acute myeloid leukemia. Hematol. Am Soc Hematol Educ Progr. 2013;2013(1):324–330.

[3] Schlenk RF. Post-remission therapy for acute myeloid leukemia. Haematologica 2014;99(11):1663–70.

[4] Buccisano F, Maurillo L, Del Principe MI, *et al.* Prognostic and therapeutic implications of minimal residual disease detection in acute myeloid leukemia. Blood 2012;119(2):332–41.

[5] Terwijn M, van Putten WLJ, Kelder A, *et al.* High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. J. Clin. Oncol. 2013;31(31):3889–97.

[6] Kayser S, Walter RB, Stock W, *et al.* Minimal residual disease in acute myeloid leukemia-current status and future perspectives. Curr. Hematol. Malig. Rep. 2015;10(2):132–44.

[7] Ossenkoppele G, Schuurhuis GJ. MRD in AML: does it already guide therapy decision-making? Hematology 2016;2016(1):356–365.

[8] Del Principe MI, Buccisano F, Maurillo L, *et al.* Minimal Residual Disease in Acute Myeloid Leukemia of Adults: Determination, Prognostic Impact and Clinical Applications. Mediterr. J. Hematol. Infect. Dis. 2016;8(1):e2016052.

[9] Schuurhuis GJ, Heuser M, Freeman S, *et al.* Minimal/measurable residual disease in AML: consensus document from ELN MRD Working Party. Blood 2018;blood-2017-09-801498.

[10] Stone RM, Mandrekar SJ, Sanford BL, *et al.* Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N. Engl. J. Med. 2017;377(5):454–464.

[11] Wattad M, Weber D, Döhner K, *et al.* Impact of salvage regimens on response and overall survival in acute myeloid leukemia with induction failure. Leukemia 2017;31(6):1306–1313.

[12] Schlenk RF, Frech P, Weber D, *et al.* Impact of pretreatment characteristics and salvage strategy on outcome in patients with relapsed acute myeloid leukemia. Leukemia 2017;31(5):1217–1220.

[13] Krönke J, Schlenk RF, Jensen K-O, *et al.* Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. J. Clin. Oncol. 2011;29(19):2709–16.

[14] Klco JM, Miller CA, Griffith M, *et al.* Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. JAMA 2015;314(8):811–22.

[15] Hindson CM, Chevillet JR, Briggs HA, *et al.* Absolute quantification by droplet digital PCR versus analog real-time PCR. Nat. Methods 2013;10(10):1003–5.

[16] Brambati C, Galbiati S, Xue E, *et al.* Droplet digital polymerase chain reaction for DNMT3A and IDH1/2 mutations to improve early detection of acute myeloid leukemia relapse after allogeneic hematopoietic stem cell transplantation. Haematologica 2016;101(4):e157-61.

[17] Jaiswal S, Fontanillas P, Flannick J, *et al.* Age-related clonal hematopoiesis associated with adverse outcomes. N. Engl. J. Med. 2014;371(26):2488–98.

[18] Genovese G, Kähler AK, Handsaker RE, *et al.* Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N. Engl. J. Med. 2014;371(26):2477–87.

[19] Thol F, Klesse S, Köhler L, *et al.* Acute myeloid leukemia derived from lympho-myeloid clonal hematopoiesis. Leukemia 2017;31(6):1286–1295.

[20] Thol F, Köhler L, Klesse S, *et al.* Characteristics and Prognosis of AML Patients with or without a History of Clonal Hematopoiesis, in *Bood*, 2015, 126:224 (abstract).

[21] Freeman SD, Virgo P, Couzens S, *et al.* Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J. Clin. Oncol. 2013;31(32):4123–31.

[22] Walter RB, Buckley SA, Pagel JM, *et al.* Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. Blood 2013;122(10):1813–21.

[23] Walter RB, Gyurkocza B, Storer BE, *et al.* Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. Leukemia 2015;29(1):137–44.

[24] Zhou Y, Othus M, Araki D, *et al.* Pre- and post-transplant quantification of measurable ('minimal') residual disease via multiparameter flow cytometry in adult acute myeloid leukemia. Leukemia 2016;30(7):1456–64.

[25] Araki D, Wood BL, Othus M, *et al.* Allogeneic Hematopoietic Cell Transplantation for Acute Myeloid Leukemia: Time to Move Toward a Minimal Residual Disease-Based Definition of Complete Remission? J. Clin. Oncol. 2016;34(4):329–36.

[26] Anthias C, Dignan FL, Morilla R, *et al.* Pre-transplant MRD predicts outcome following reduced-intensity and myeloablative allogeneic hemopoietic SCT in AML. Bone Marrow Transplant. 2014;49(5):679–83.

[27] Chen X, Xie H, Wood BL, *et al.* Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. J. Clin. Oncol. 2015;33(11):1258–64.

[28] Bassan R. Using Minimal Residual Disease to Improve Treatment Response Definitions and Hematopoietic Cell Transplantation Strategy in Acute Leukemia. J. Clin. Oncol. 2016;34(4):300–2.

[29] Kayser S, Schlenk RF, Grimwade D, *et al.* Minimal residual disease-directed therapy in acute myeloid leukemia. Blood 2015;125(15):2331–5.

[30] Döhner H, Lübbert M, Fiedler W, *et al.* Randomized, phase 2 trial of low-dose cytarabine with or without volasertib in AML patients not suitable for induction therapy. Blood 2014;124(9):1426–33.

[31] Ravandi F, Ritchie EK, Sayar H, *et al.* Vosaroxin plus cytarabine versus placebo plus cytarabine in patients with first relapsed or refractory acute myeloid leukaemia (VALOR): a randomised, controlled, double-blind, multinational, phase 3 study. Lancet. Oncol. 2015;16(9):1025–1036.

[32] Ravandi F, Jorgensen J, Borthakur G, *et al.* Persistence of minimal residual disease assessed by multiparameter flow cytometry is highly prognostic in younger patients with acute myeloid leukemia. Cancer 2017;123(3):426–435.

[33] Ouyang J, Goswami M, Peng J, *et al.* Comparison of Multiparameter Flow Cytometry Immunophenotypic Analysis and Quantitative RT-PCR for the Detection of Minimal Residual Disease of Core Binding Factor Acute Myeloid Leukemia. Am. J. Clin. Pathol. 2016;145(6):769–77.

[34] Bücklein V, Krause S, Oelschlägel U, *et al.* Defining minimal residual disease by flow cytometry in acute myeloid leukemia: the HARMONIZE initiative. Oncol Res Treat 2017;40((suppl 3)):43 (P192).

[35] Burzykowski T, Molenberghs G, Buyse M. *The Evaluation of Surrogate Endpoints*. New York: Springer-Verlag, 2005.

[36] Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. Stat. Med. 1989;8(4):431–40.

[37] Freedman LS, Graubard BI, Schatzkin A. Statistical validation of intermediate endpoints for chronic diseases. Stat. Med. 1992;11(2):167–78.

[38] Buyse M, Molenberghs G. Criteria for the validation of surrogate endpoints in randomized experiments. Biometrics 1998;541014–29.

[39] Molenberghs G, Buyse M, Geys H, *et al.* Statistical challenges in the evaluation of

surrogate endpoints in randomized trials. Control. Clin. Trials 2002;23(6):607–25.

[40] Buyse M, Molenberghs G, Burzykowski T, *et al.* The validation of surrogate endpoints in meta-analyses of randomized experiments. Biostatistics 2000;1(1):49–67.

[41] Daniels M, Hughes M. Meta-analysis for the evaluation of potential surrogate markers. Statist Med. Stat. Med 1997;161965–82.

[42] Burzykowski T, Buyse M. Surrogate threshold effect: an alternative measure for meta-analytic surrogate endpoint validation. Pharm. Stat. 5(3):173–86.

[43] Schlenk R, Döhner H, Döhner K, *et al.* Event-Free Survival Is a Surrogate for Overall Survival in Patients Treated for Acute Myeloid Leukemia, in *Blood*, 2015, 126:3744 (abstract).

[44] Schlenk RF, Fröhling S, Hartmann F, *et al.* Phase III study of all-trans retinoic acid in previously untreated patients 61 years or older with acute myeloid leukemia. Leukemia 2004;18(11):1798–803.

[45] Tassara M, Döhner K, Brossart P, *et al.* Valproic acid in combination with all-trans retinoic acid and intensive therapy for acute myeloid leukemia in older patients. Blood 2014;123(26):4027–36.

[46] Schlenk RF, Lübbert M, Benner A, *et al.* All-trans retinoic acid as adjunct to intensive treatment in younger adult patients with acute myeloid leukemia: results of the randomized AMLSG 07-04 study. Ann. Hematol. 2016;95(12):1931–1942.

[47] Schlenk R, Herr W, Wulf G, *et al.* Azacitidine-Containing Induction Regimens Followed by Azacitidine Maintenance Therapy in High Risk Acute Myeloid Leukemia: First Results of the Randomized Phase-II AMLSG 12-09 Study (ClinicalTrials.gov No. NCT01180322), in *Blood (ASH Annual Meeting Abstracts)*, 120AD, 120:412 (abstract).

[48] Burzykowski T, Molenberghs G, Buyse M, *et al.* Validation of surrogate endpoints in multiple randomized clinical trials with failure-time endpoints. J R. Stat Soc C 2001;50405–22.

[49] Hills RK, Castaigne S, Appelbaum FR, *et al.* Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet. Oncol. 2014;15(9):986–96.

[50] Thol F, Schlenk RF. Gemtuzumab ozogamicin in acute myeloid leukemia revisited. Expert Opin. Biol. Ther. 2014;14(8):1185–95.

[51] Buyse M, Sargent D, Grothey A, *et al.* Biomarkers and surrogate endpoints - the challenge of validation. Nat. Rev. Clin. Oncol. 2010;7309–317.

[52] Buyse M, Michiels S, Squifflet P, *et al.* Leukemia-free survival as a surrogate end point for overall survival in the evaluation of maintenance therapy for patients with acute myeloid leukemia in complete remission. Haematologica 2011;96(8):1106–12.

[53] Buyse M, Molenberghs G, Paoletti X, *et al.* Statistical evaluation of surrogate endpoints with examples from cancer clinical trials. Biom. J. 2016;58(1):104–32.

[54] Hielscher T, Zucknick M, Werft W, *et al.* On the prognostic value of survival models with application to gene expression signatures. Stat Med 2010;29818–829.

15 Appendices

15.1 Templates for patient info and informed consent

The following passages are recommended to be implemented in the patient information and written informed consent of the respective, participating trials. The passages do not need to be implemented literally but should match by content. The study specific informed consent and patient information have to fulfil the key requirements laid down in chapter 12.2.

As the studies will be performed in Germany and thus require ICs in German lay language the templates will be in German too.

15.1.1 Patienteninformation

Datennutzung außerhalb der Studie

Außerhalb der klinischen Studie XYZ möchten wir Ihre pseudonymisierten Daten gern für weitere Forschungsprojekte wie im Folgenden beschrieben verwenden:

- Zum jetzigen Zeitpunkt können wir noch nicht genau sagen, in welche medizinischen Forschungsvorhaben Ihre Daten einfließen werden und **welche Ziele diese Projekte** verfolgen. Die Forschungsvorhaben müssen jedoch folgende Kriterien erfüllen:
  - Positives Votum der zuständigen Ethikkommission
  - Ausschließlich medizinisch-wissenschaftliche, krankheitsbezogene Forschung die direkt oder indirekt der Verbesserung von Diagnose, Therapie und/oder Lebensqualität dient
  - keine direkte kommerzielle Nutzung

- Ihre Einwilligung in die Weiterverwendung Ihrer Proben und Daten für weitere Forschungsprojekte ist **freiwillig**. Wenn Sie der Weiterverwendung nicht zustimmen oder Ihre Einwilligung später zurückziehen, entstehen Ihnen keine Nachteile. Ihre Entscheidung über die Weiterverwendung Ihrer Daten hat **keinerlei Einfluss auf Ihre Teilnahme an der Studie XYZ**. Ihre Einwilligung zur Studie und zur Weiterverwendung können Sie im Anschluss an diese Patienteninformation gesondert schriftlich erteilen und später auch gesondert widerrufen.

- Sollten Sie Ihre Einwilligung in die Weiterverwendung später **widerrufen**, werden Ihre Daten ab Ihrem Widerruf für keine weiteren Forschungsprojekte verwendet. Sind Ihre Daten zu diesem Zeitpunkt bereits in andere Forschungsprojekte eingeflossen, werden sie in diesen allerdings weiter verwendet, sofern dies für die Güte des Forschungsprojekts notwendig ist. Bis zum Rückzug Ihrer Einwilligung an Ihren Daten generierte Daten werden ebenfalls weiter verwendet, sofern diese für die Güte des jeweiligen Forschungsprojekts notwendig sind.

- Jedem weiterführenden Forschungsprojekt und damit jeder Weiterverwendung Ihrer Daten muss vorab eine zuständige **Ethikkommission** zustimmen. Die Ethikkommissionen schützen im Rahmen von Forschungsvorhaben die Interessen der teilnehmenden Patienten, und vertreten deren Rechte gegenüber den forschenden Ärzten und Wissenschaftlern.

- Die weitere Speicherung sowie die Verwendung Ihrer Daten erfolgt in **pseudonymisierter** Form. **Pseudonymisiert** bedeutet, dass keine Angaben von Namen oder Initialen verwendet werden, sondern nur ein Nummern- und/oder Buchstabencode. Eine Entschlüsselung der Pseudonymisierung ist nur durch eine an

Ihrer Prüfstelle hinterlegte Identifizierungs-Liste, welche die Sie identifizierenden persönlichen Daten (z.B. Name und Geburtsdatum) enthält, möglich.

- Möglicherweise ergeben sich in Zukunft neue wissenschaftliche Fragestellungen, die mithilfe Ihrer Daten beantwortet werden können. Daher kann heute noch nicht festgelegt werden, **wie lange Ihre Daten aufbewahrt werden**. Ggf. werden die Daten nicht gelöscht.

- Unter Umständen möchten wir Ihre Daten auch Forschern außerhalb der Studie XYZ (sogenannte berechtigte Dritte) für Untersuchungen zur Verfügung stellen. Jede **Weitergabe an Dritte** erfolgt in pseudonymisierter Form. Die Identifizierungs-Liste verbleibt in jedem Fall an Ihrem Prüfzentrum, so dass ein Rückschluss auf Ihre Person allein aus diesen Daten nicht möglich ist.

- Aus Ihren Daten gewonnene Erkenntnis und Ergebnisse werden nur in pseudonymisierter Form in **wissenschaftliche Veröffentlichungen** verwendet.

15.1.2 Einwilligungserklärung

- Ich willige ein, dass meine Daten nach Beendigung oder Abbruch der Prüfung **mindestens zehn Jahre aufbewahrt** werden, wie es die Vorschriften über die klinische Prüfung von Arzneimitteln bestimmen.

- Ich willige ein, dass meine im Rahmen der klinischen Studie XYZ erhobenen personenbezogenen Daten pseudonymisiert (verschlüsselt) und ohne zeitliche Begrenzung **für weitere Forschungsprojekte verwendet** werden dürfen und im Rahmen dessen zweckgebunden **an Dritte weitergegeben** werden dürfen Die Forschungsvorhaben müssen jedoch folgende Kriterien erfüllen:
  - Positives Votum der Zuständigen Ethikkommission
  - Ausschließlich medizinisch-wissenschaftliche, krankheitsbezogene Forschung die direkt oder indirekt der Verbesserung von Diagnose, Therapie und/oder Lebensqualität dient
  - keine direkte kommerzielle Nutzung

- Die Einwilligung zur Weiterverwendung meiner Daten in weiteren Forschungsprojekten **kann ich jederzeit widerrufen**, ohne dass dies Einfluss auf meine Teilnahme an der Studie XYZ hat. Ich habe das Recht, jederzeit **Auskunft** über die Weitergabe bzw. Verwendung meiner Daten zu erhalten.

- Ich willige ein, dass ich **nicht** über Forschungsergebnisse und Zufallsbefunde **informiert** werde, auch wenn diese mit meiner Erkrankung in Zusammenhang stehen sollten. Zufallsbefunde können Erkenntnisse über weitere Erkrankungen oder Veranlagungen zu Erkrankungen sein.

Ich willige in diese **Weiterverwendung** meiner Daten **außerhalb der klinischen Studie XYZ** ein:

☐ ja ☐ nein

15.2 Declaration of commitment to the PERDAM diagnostic meta-study

Interventional trial principle investigator's declaration of commitment to the PERDAM diagnostic study

Information on the interventional clinical trial:

Title:_____

_____

EudraCT No:_____

Clincaltrials.gov identifier:_____

Sponsor:_____

As principle investigator of the above mentioned interventional clinical trial I hereby agree to include this trial into the PERDAM diagnostic study with diagnostic- and MRD-assessment by multiparameter flow cytometry at diagnosis and after induction as well as consolidation therapy, respectively, and I confirm unreserved access to clinical data (defined in chapter 8 of the PERDAM protocol) during and after completion of the above mentioned trial and/or the PERDAM study for single patients and cumulative evaluation as described in the PERDAM protocol.

I confirm that individual data will only be transferred if the respective patient has consented the transfer and use of his data outside of the above mentioned trial.

Date/Signature

Title:_____

First name:_____

Family name:_____

Affiliation:_____