**Protocol HINC-207 (OSHO #091)**

# Pheno- and genotypes of JAK2 mutated patients with and without thromboembolic events. A project of the East German Study Group (OSHO)

**Protocol**

Version 1.2, 27. December 2018

**Sponsor**

Martin-Luther-University Halle-Wittenberg, represented by the Rector, who is represented by the Dean of the Medical Faculty

Located: Magdeburger St. 8, 06120 Halle (Saale), Germany

**Executing Unit of the Institution**

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**Protocol Committee**

Al-Ali HK, Jaekel N

**Statistical consultation**

Dr. rer. medic. Andrea Schmidt-Pokrzywniak, Institute for Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University of Halle-Wittenberg, Magdeburger St. 8, 06112 Halle (Saale), Germany

**Project management / data management**

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**Financing**

Novartis GmbH, Roonstr. 25, 90429 Nürnberg, Germany

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## 1 List of abbreviations

| | |
|---|---|
| CRF | Case Report Forms |
| CSM | Central Study Management |
| ET | Essential Thrombocythemia |
| GCP | Good Clinical Practice |
| HCT | Hematocrit |
| ICF | Informed Consent Form |
| KKH | Krukenberg Cancer Center Halle |
| LDH | Lactate Dehydrogenase |
| MF | Myelofibrosis |
| MPN | Myeloproliferative Neoplasms |
| OSHO | East German Study Group |
| PMF | Primary Myelofibrosis |
| PV | Polycythemia Vera |
| WBC | White blood cells |

## 2 Background and Rationale

Philadelphia chromosome negative myeloproliferative neoplasms (MPN) comprise a group of clonal hematological malignancies that are characterized by chronic myeloproliferation, splenomegaly, bone marrow fibrosis in different degrees, and disease- related symptoms including pruritus, night sweats, fever, weight loss, cachexia, and diarrhea. Due to elevated numbers of leukocytes, erythrocytes and/or platelets, the disease course can be complicated by thromboembolic events, hemorrhage, and leukemic transformation as well as myelofibrosis (MF).

Patients with polycythemia vera (PV) typically show an increased number of blood cells from all three hematopoietic cell lineages due to clonal amplification of hematopoietic stem cells, in contrast to in patients with essential thrombocythemia (ET) showing a predominant expansion of the megakaryocytic lineage.

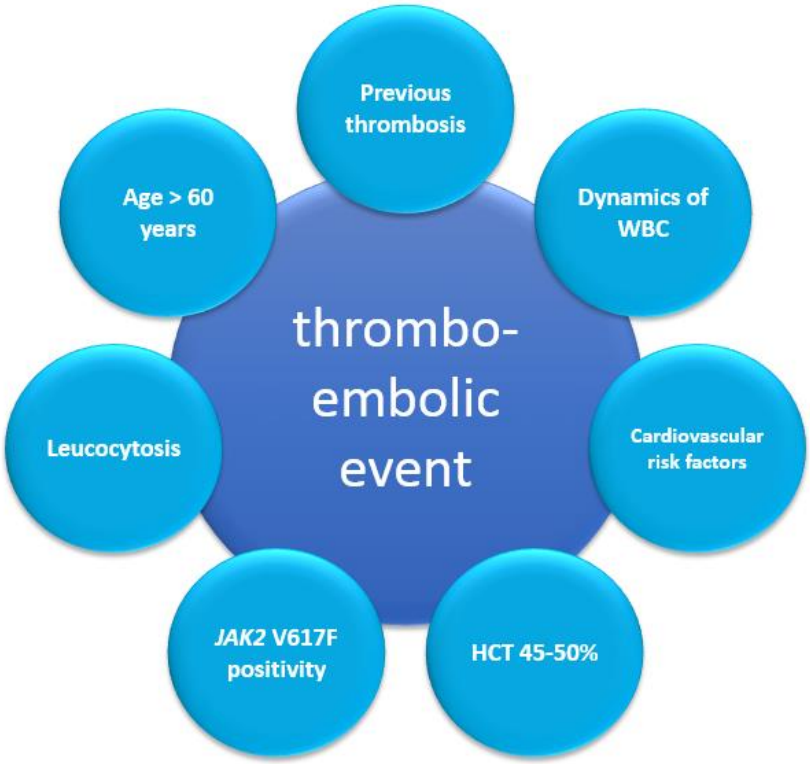
Arterial and venous thromboembolic events are the major cause of morbidity and mortality in MPN-patients particularly in JAK2 mutated PV and ET patients [1; 2].

The pathogenesis of thrombosis results from a complex interplay of clinical and disease-related factors. Abnormalities of blood cells arising from the clonal proliferation of hematopoietic stem cells involve not only quantitative changes but also qualitative

modifications that characterize the switch of these cells from a resting to a procoagulant phenotype [1].

Although several risk factors have been identified to be associated with thrombosis, it is still unclear why some patients develop thromboembolic events and others do not (figure 1) [1-7].

Figure 1: risk factors for thromboembolic events in PV and ET




Of particular note is the fact that many young patients suffers from the thromboembolic event years prior to the haematological diagnosis independently of the presence of blood picture abnormalities [8].

In PV and ET, high risk patients are characterized by advanced age (> 60 years) and / or a history of thromboembolic or hemorrhagic events. In ET, a platelet count > 1500 x $10^9$/l is associated with an increased risk of bleeding, and thus should result in a platelet lowering treatment. In PV, in addition to the risk-score based therapy, cytoreduction is also required in patients with progressive or marked myeloproliferation (leukocytosis, thrombocytosis, symptomatic splenomegaly, increase of frequency of phlebotomy requirement), or devastating constitutional symptoms. Treatment aims are reduction of the risk of thrombosis and haemorrhage, symptoms-control and reduction of disease progression [9].

## 3 Aim of the study

The primary goal of this study is to detect differences in phenotypes (clinical/laboratory) and genotypes (allele burden of the mutated JAK2) of JAK2 mutation positive ET and PV-patients with or without thromboembolic events in there medical history, to evaluate risk factors being predictive for the risk of developing a thromboembolic event.

## 4 Study design

This study is a non-interventional multi-center study. The aim of the study is to describe the clinical and laboratory features of patients with thromboembolic events and of those without such events. By this, valuable insight and new information to the understanding of thromboembolic events in JAK2 mutated patients will be gained. The study will be started after all approvals are obtained.

The collected data of the included patients will be documented pseudonymously in the database after return of the signed and dated informed consent form (ICF).

- Routine diagnostic work-up
- Multi-center analysis
- Numbers of patients:
  - with a history of a thromboembolic event n= 60
  - without a history of a thromboembolic event n=120
- Project duration: maximum of 2 years

Please refer to section 9 for sample size calculation and statistical methods.

### 4.1 Translational analysis

The concomitant scientific project will only include patients who have signed the informed consent form for translational studies at the University Hospital Halle. It aims at the determination of further examinations such as mutational profiling as well as biomarkers such as Ybx1 and Cxcl10 as possible markers for thromboembolic event in PV and ET patients.

For the translational analysis, the following samples will be collected:

- 1x10 ml of peripheral blood in an anticoagulated tube (EDTA)
  **for all patients of the University Hospital Halle who gave consent.**

The time point of collection is described in section 7.

Responsible for the conduct of the scientific project as well as archiving address for the laboratory samples:

PD Dr. med. Haifa Kathrin Al-Ali and Dr. med. Nadja Jäkel
Universitätsklinik Halle/Saale AöR
Klinik für Innere Medizin IV
Hämatologisches Forschungslabor
Ernst-Grube-Str. 40
06120 Halle/Saale

## 5 Primary endpoint

The primary endpoint is the detection of differences in phenotypes and genotypes of JAK2 mutation positive ET and PV-patients (including post-ET and post-PV Myelofibrosis) with or without thromboembolic events according to the parameters to be collected (see section 7).

## 6 Selection of Study Population

### 6.1 Inclusion criteria

- Age ≥ 18 years
- Signed informed consent
- Patient must fulfil WHO diagnostic criteria for either PV or ET including post PV-MF and post ET-MF
- JAK2-mutation positivity
- Male and female patients are eligible

### 6.2 Exclusion criteria

- Patients who meet criteria for Primary Myelofibrosis (PMF)
- limited legal capacity of the patient
- Age < 18 years

### 6.3 Patient Identification and Enrollment

Investigators agree to complete a patient identification log to permit easy identification of each patient enrolled in the non-interventional study.

The patient identification and enrollment log will be treated as confidential and will be filed by the investigator in the study file. To ensure patient confidentiality, no copy will be made. All reports and communications relating to the study will identify patients by patient identification number and year of birth.

### 6.4 Study Termination

The study is considered completed two years after documentation of the first patient or as soon as data of 180 eligible patients who consented to study participation have been documented, whatever comes first.

The sponsor reserves the right to close a participating site for data collection or to terminate the study at any time for any reason at the sole discretion of the sponsor. A participating site is considered closed when all required documents have been collected and the site has been officially notified by the sponsor about the site closure/study completion.

The investigator may initiate site closure at any time, provided there is a reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a participating site by the sponsor or investigator may include but are not limited to: investigator's failure to comply with the protocol, requirements of the local health authorities or the sponsor's procedures, or inadequate recruitment of patients by the investigator.

Patients may withdraw their informed consent at any time without giving reasons. A patient will be withdrawn from data collection upon withdrawal of consent. Patients who withdraw consent may be replaced within the recruitment period.

## 7 Parameters to be collected

The following standard parameters of patients with JAK2-mutation positive (post-)ET/PV will be documented for this study.

### 7.1 Data collected at time of informed consent

- Patient characteristics (age, gender, ethnicity)
- Medical history including:
  - A drug history about the use of contraception, hormone therapies, anticoagulation and aspirin, cytoreductive treatments for MPN (only drug classes will be collected) including start and end dates
  - a history of pregnancies (only for female patients)
  - cardiovascular risk factors (smoking history, diabetes, hypertension, atrial fibrillation, hyperlipidemia)
  - family history of thrombosis and/or MPN in first-degree relatives
  - History of another malignancy including date and type of diagnosis
  - Patient questionnaire for Medical History

- Most recent data of each patient gained from routine diagnostic samples at time of informed consent:
  - Blood picture, differential blood count at time of informed consent
  - Lactate Dehydrogenase (LDH), Cholesterol, HDL and LDL-Cholesterol
  - JAK2 allele burden
- Lab values for standard inherited causes of thrombophilia (These need only be collected once from the medical records if available).
  - Factor-V-Leiden (APC-Resistance)
  - Prothrombin 20210-Mutation
  - Protein C, Protein S, Antithrombin
  - Lupus-Anticoagulance, Cardiolipin-Antibodies and antibodies against Beta-2-Glycoprotein-I

### 7.2 Data collected from the time of MPN diagnosis

- Medical records data for time of MPN diagnosis:
  - Date of diagnosis of PV, ET, post-PV MF, post-ET MF, type of diagnosis
  - Blood picture, differential blood count at time of PV or ET diagnosis
  - Lactate Dehydrogenase (LDH) at time of PV or ET diagnosis
  - JAK2 mutational status and allele burden at time of PV or ET diagnosis
  - Other MPN-associated mutation at time of PV or ET diagnosis
  - for PV-patients with history of phlebotomies: start and end dates, frequency per month
  - Clinical examination (blood pressure, weight, height, spleen size, liver size) at time of PV or ET diagnosis

### 7.3 Data collected from the time of thrombosis

- For patient with thromboembolic event in medical history, the following data for the time of the first thromboembolic event (arterial and/or venous) will be collected:
  - Date of the event, type of the event (localization)
  - Blood picture, differential blood count at time of the event
  - Lactate Dehydrogenase (LDH), Cholesterol, HDL and LDL-Cholesterol at time of the event
  - History of surgical procedures at the time of the event
  - Further thromboembolic events (date, type of thrombosis (localization), treatment)

- Clinical examination (blood pressure, weight, height, spleen size, liver size, signs of thromboembolic event)

### 7.4 Samples for translational analysis

At time of informed consent for all patients of the University Hospital Halle who gave informed consent to the translational analysis the following sample will be collected:

- Peripheral blood: 1x10ml in anticoagulated EDTA tube

## 8 Data collection

### 8.1 Source Documentation

At a minimum, source documentation must be available for the following to confirm data collected in the CRF: patient identification, eligibility, and study identification; study discussion and date of signed informed consent; dates of visit; relevant baseline and other medical information; date of study completion and reason for early discontinuation or withdrawal from the study, if applicable. The author of an entry in the source documents should be identifiable.

At a minimum, the type and level of detail of source data available for a patient should be consistent with that commonly recorded at the study site as a basis for standard medical care.

### 8.2 Case Report Forms (CRF)

The CRF will be prepared and provided by the CSM of the KKH in electronic form (pdf). The Sponsor receives and retains the originals of all CRF pages while the Investigator will retain a copy of all completed CRF pages.

All entries in the CRFs must be made clearly with dark ball-point pen, to ensure the legibility in self-copying or photocopied pages. Corrections are made by placing a single horizontal line through the incorrect entry, in a way that it can still be seen, and placing the revised entry beside it. The revised entry must be initialed and dated by trial staff authorized to make CRF entries. Correction fluid may not be used. If an item is not available or is not applicable, this fact should be indicated; do not leave a space blank.

The authorized trial staff will review the CRF for completeness and accuracy, sign and date all relevant CRF pages and any changes therein.

If the Investigator authorizes other personnel to enter and sign data into the CRF, the names, positions, signatures, and initials of these persons must be supplied to the Sponsor (via Signature/Personal- und Delegation-Log).

The signatures serve to attest that the information contained in the CRF is true and has not been falsified. In case of a major correction or missing data, the reason for it has to be given. The investigator or other authorized trial staff must assure completion, review and approval of all CRFs. At all times the principal investigator has final responsibility for the accuracy and authenticity of all clinical and laboratory data entered in the CRF. Even if there are no changes from a previous examination the questions which are repeated in each section of the case report forms must be answered completely.

A CRF will be provided for each patient. All protocol-required information collected during the study must be recorded by the Investigator, or designated representative, in the source documentation for the study. Source documentation is defined as any handwritten or computer-generated document that contains medical information or test results that have been collected for or is in support of the protocol specifications, e.g., laboratory reports, clinic notes, drug disbursement log, patient sign-in sheets, patient completed questionnaires, telephone logs, ECGs, etc.

The source documentation will then be used to enter the protocol required information into the CRF. The information on the CRF pages will then be entered into the study database. For data entry and analysis the completed, **original CRF forms will be sent via mail to**:

Universitätsklinik Halle/Saale AöR
Krukenberg-Krebszentrum Halle
Zentrales Studienmanagement
Ernst-Grube-Str. 40
06120 Halle/Saale

- **Registration Form: as soon as possible, latest one week after registration**
- **On-study evaluations including pseudonymous medical records, patient questionnaires and all protocol related data (see section 7): within two weeks after informed consent**

### 8.3 Data Management

Once the CRFs are transferred to CSM of the KKH, their receipt is recorded and they are filed in the TMF by the responsible data management staff for processing.

All data will be captured in the blinded project specific database by giving every patient a project specific identification number. The data will be transferred in a pseudonymous form from the CRF to the database. The collection and processing of personal data from patients enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study.

### 8.4 Archiving

All relevant trial documentation (Trial Master File), the electronically stored data, the original CRFs and the final report will be stored for at least 10 years by the sponsor at the KKH after the trial's completion. Documents will be stored at the university clinic's central archive, where they will be protected from external influences and unauthorized access, as required by legal regulations.

At the investigating sites, the investigators' files, patient identification lists, signed written consent forms, copies of all CRFs and the patients' files will be stored for at least 10 years after the trial's completion.

### 8.5 Data Protection

Investigators confirm adherence to all applicable legal conditions including the data protection law currently effective at the time of protocol signature, regulations and regulatory requirements by signing the study protocol.

The collection and processing of personal data from patients enrolled in this study will be limited to those data that are necessary to fulfill the objectives of the study. The necessary data for this non-interventional study is generated in the clinical routine of the treating physician of the patient. Patent requiring inventions are not to be expected in this study. Data of the translational study will be pseudonymized and analyzed in an anonymous form.

All data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data agree to keep the identity of patients confidential.

The sponsor will take all appropriate measures to safeguard and prevent access to confidential data by any unauthorized third party. Only the investigator, sponsor staff and (if applicable) representatives of health authorities will receive permission to view all medical data of a patient.

A Privacy Impact Assessment evaluating the current risks and safety measures has been performed. Investigators are aware of the severe impact unauthorized access and publication of sensitive data would have on the privacy and psyche of the victim and have therefore taken appropriate measures to ensure data safety. The central database will be stored on devices integrated into the network of the University Hospital Halle and therefore be protected by the general measures taken to protect sensitive date, including, but not limited to password-protected user profiles, regular backup routines (monthly, weekly and every two hours), anti-virus programs and a ban on introducing external/private electronic devices (personal computers, notebooks etc.) into the clinic's network. CRFs and other source documents will be stored in the CSM's facilities. All areas relevant to data safety can be locked and can only be accessed by study personnel. All samples collected for the translational studies will be stored at the above mentioned hematology research lab, where access is limited to the involved personnel in possession of a key card. Data concerning the samples will be stored on devices in the lab's network, which is used exclusively for research purposes and independent from the general clinic network. The members of the study team are accustomed to working with sensitive data and ensuring its confidentiality.

The informed consent obtained from the patient/the patients legal representative(s) includes explicit consent to the processing of personal data and for the investigator/institution to allow direct access to the corresponding medical records (source data/documents) for study-related audit, IEC/IRB review, and regulatory inspection. This consent also addresses the transfer of the data to other entities involved in study performance.

The patient has/the patient's legal representative(s) have the right to request through the investigator access to his or her/their wards personal data and the right to request rectification of any data that are not correct or complete. Reasonable steps will be taken to respond to such a request, considering the nature of the request, the conditions of the study, and applicable laws and regulations.

Provided the permission by the sponsor, the financing party – Novartis - will have access to the trial data. The final study report as well as following publications done by the sponsor will be available for Novartis. All personal data will be pseudonymized and anonymous as described above.

## 9 Statistics

„Statistical analysis will be done by Dr. rer. medic. Andrea Schmidt-Pokrzywniak, Institute for Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University of Halle-Wittenberg, Magdeburger St. 8, 06112 Halle (Saale), Germany. All statistical analyses will be performed with SAS, Version 9.4 (SAS Institutes, Cary, NC).

### 9.1 Justification of Trial Design

Trial design was based on the following:

a) Thromboembolic events are the major cause of morbidity and mortality in patients with PV and ET with an incidence of about 25% [10]. A one to two ratio (patients with an event to patients without an event) was chosen to adequately represent both groups and allow statistical analysis.

b) High risk patients for thromboembolic events are considered if one or both of two risk factors: Age (> 60 years) and / or a history of thromboembolic event are present. Additionally, JAK2-mutated patients have a higher incidence of thromboembolic events compared to other genetic groups [9]. To prevent heterogeneity in the study, only JAK2 mutated patients are included and patients with an event are compared to patients without an event. Thus, the study population is standardized for 2 major risk factors with age remaining as a relevant variable to be analyzed in the trial.

c) Thromboembolic events have been described often in younger patients and independent of the presence of blood picture abnormalities [9]. Again, age emerges as a significant variable to be considered in the sample size calculation.

d) In addition to the risk factors mentioned under b., several less well-defined risk factors have been further identified to be associated with thromboembolic events [1-7]. Leucocytosis, hematocrit > 45% and cardiovascular risk factors are of particular interest.

### 9.2 Justification for Sample Size

Based on the main variable age, the unpaired Student t-test was used to determine sample size. With an expected clinically relevant mean difference of 3 years in age between the two groups and an expected standard deviation of 5 years and considering a 1:2 matching, a sample size of a minimum of 41 patients in the thromboembolic group and 82 patients in the group without such an event will be needed with a level of significance of 0,025 and a power

of 80% to detect this difference. Thus, the calculated sample size of 60:120 patients (thromboembolic group: non thromboembolic group) is statistically adequate and would compensate the assumption of adequate data cannot be collected in 15-20% of cases.

### 9.3 Statistical Methods

A complete statistical analysis of all parameters will be performed at the completion of the study (a maximum of two years after the start of the study. The study will be terminated earlier if 60 patients with a history of thromboembolic event (venous and/or arterial) and 120 patients without such a history were included prior to the end of the two years). All statistical analyses in this study will be exploratory and descriptive only. Data will be appropriately summarized and analyzed using tabulation and graphs with respect to demographic/baseline characteristics and diagnostic results. Standard descriptive summary statistics (including number of patients [n], mean, standard deviation, median, minimum, maximum, and quartiles) will be calculated for continuous variables. Categorical and dichotomous data will be presented in frequency tables using counts and percentages.

Thrombosis-free time will be estimated by Kaplan-Meier survival analysis. We will use crude and multivariable Cox proportional hazards regression to estimate unadjusted and adjusted hazard ratios (HR) and corresponding 95% confidence intervals (95%CIs) to determine the independent effect of the variables on thrombosis risk. We will check the assumption of proportional hazards by using the Schoenfeld residual plot. We would use the Cox model to estimate the independent effect of these variables on thrombosis risk.
We will identify minimally sufficient adjustment sets depending on the outcome using causal diagrams that represent the presumed associations between exposure, outcome, and other variables [11].

## 10 Milestones

| | |
|---|---|
| 10/2018 | Final version of the protocol |
| 12/2018 | Vote from the Ethics Committee of the Medical Faculty of the Martin-Luther-University of Halle-Wittenberg. Comments from the Ethics Committee to be answered. |
| 01/2019 | Second version of the protocol submitted to Ethics Committee |
| 02/2019 | Final vote from the Ethics Committee |
| 03/2019 - 03/2021 | Inclusion of patients |
| 04/2021 - 09/2021 | Finalization of the database, statistical analysis |

## 11 Patients' consent and vote from the ethics committee

- All patients have to give a written informed consent to this study after being informed in detail about the study by an investigator in a timely manner.
- The present study will be performed in accordance to all applicable laws and regulations and abiding to ICH GCP principles concerning conduct, evaluation and documentation of clinical investigations according to the declaration of Helsinki (2013).
- A vote from the Ethics Committee of the University of Halle-Wittenberg will be obtained for the specific analyses as part of this descriptive study.

## 12 References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9 Vannucchi AM, Barbui T, Cervantes F, et al. Philadelphia chromosome-negative chronic myeloproliferative neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015:v85–v99.

10 Geyer H, Scherber R, Kosiorek H, et al. Symptomatic Profiles of Patients With Polycythemia Vera: Implications of Inadequately Controlled Disease *J Clin Oncol.* 2016:34(2):151-9.

11 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* 1999:10:37–48.

Protocol Signature Page

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**Protocol**

Version 1.2, 03.Januar 2019

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|---|---|---|
| PD Dr. med. Haifa Kathrin Al-Ali<br>Coordinating Investigator | Signature | 14/Januar/2019<br>Date |
| Dr. med. Nadja Jäkel<br>Coordinating Investigator | Signature | 14.1.19<br>Date |
| *Dr. rer. medic. Andrea Schmidt-Pokrzywniak*<br>Statistical consultation | Signature | 11.1.19<br>Date |

## 12.1 Investigator's Agreement

I have read the attached protocol entitled "Protocol HINC-207 (OSHO #091) - Pheno- and genotypes of JAK2 mutated patients with and without thromboembolic events. A project of the East German Study Group (OSHO)" dated 27. December 2018, and agree to abide by all provisions set forth therein.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the sponsor.

| Name of Investigator | Signature | Date |
|---|---|---|