Response assessment in post-transplant lymphoproliferative disorder with ^{18}F-FDG-PET/CT and minimal residual disease monitoring, a multicenter multinational feasibility study

Response assessment in post-transplant lymphoproliferative disorder with ^{18}F-FDG-PET/CT and minimal residual disease monitoring

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

^{18}F-FDG-PET/CT - ^{18}F-flurodeoxyglucose positron emission tomography/computed tomography

cfDNA - Cell-free DNA

ctDNA - Circulating tumor DNA

DICOM - Digital Imaging and Communications in Medicine

DLBCL - Diffuse large B-cell lymphoma

DSS - Disease specific survival

EBV - Epstein Barr virus

EFS - Event free survival

gDNA – [tumor] Germ line genomic DNA

HSCT - hematopoietic stem cell transplantation

MRD - Minimal residual disease

OS - Overall survival

PACS - Picture Archive and Communication System

PFS - Progression free survival

PTLD - Post-transplant lymphoproliferative disorder

R-CHOP - rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone

SAEs - Serious adverse events

SOT - Solid organ transplantation

UMCG - University Medical Center Groningen

SUMMARY

Rationale: Post-transplant lymphoproliferative disorder (PTLD) is a serious complication after solid organ (SOT) and hematopoietic stem cell transplantation (HSCT), associated with significant morbidity and mortality. Initial treatment consists of tapering immune suppression and rituximab monotherapy. ^{18}F-flurodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) has become the main tool to assess remission status, drive decisions on treatment alteration and identify relapse in patients with PTLD. In case of positive ^{18}F-FDG-PET/CT following rituximab, treatment is escalated with R-CHOP. However ^{18}F-FDG-PET/CT false positives results are commonly reported and it has limited prognostic value (positive predictive value of 38% negative predictive value of 92%). Minimal residual disease (MRD) from circulating tumor DNA (ctDNA) fragments occurs under the detection threshold of ^{18}F-FDG-PET/CT. With a blood sample one may be able to monitor MRD, thought to be responsible for disease progression and relapse. MRD may become an early response indicator used to guide treatment. We will investigate the feasibility of MRD monitoring in PTLD patients and perform an exploratory study to evaluate if MRD monitoring may be used to trace disease status during treatment and identify early responders from (non-) responders.

Objective: (1) To determine the feasibility of MRD detection using next generation sequencing (NGS) on circulating tumor DNA (ctDNA) from PTLD patients using a gene panel previously used in diffuse large B-cell lymphoma (DLBCL) (2) To explore the mutational landscape of PTLD by whole exome sequencing and validate the study's gene panel (3) To investigate the dynamics of ctDNA at diagnosis, interim and at end-of-treatment in relation to rituximab and R-CHOP treatment (4) To compare ctDNA abundance with ^{18}F-FDG-PET/CT results in patients responding to therapy vs refractory or relapsing patients.

Study design: Exploratory prospective multicenter, multinational cohort study

Study population: Patients with a histologically proven monomorphic large CD20+ B-cell PTLD, ≥ 18 years-old after SOT or HSCT treated according to standard of care (rituximab / R-CHOP)

Intervention: No intervention

Main study parameters/endpoints: To determine the feasibility of MRD detection using NGS on ctDNA from PTLD patients using a gene panel previously used in DLBCL

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Tissue biopsy and ^{18}F-FDG-PET/CT scans are part of standard diagnostic procedures Patients will receive treatment according to standard of care. For the purpose of the study patients are asked to give 60 ml of blood for a maximum of 4 times, during routine follow-up visits. Blood sampling will take place during routine laboratory assessment. As such no additional vena puncture is required and there is no additional risk.

1. INTRODUCTION AND RATIONALE

Post-transplant lymphoproliferative disorders

Post-transplant lymphoproliferative disorder (PTLD) is a serious complication after solid organ (SOT) and hematopoietic stem cell transplantation (HSCT), associated with high morbidity and mortality. Although initially reported as a rare complication of transplantation, PTLD incidence may have been clinically underestimated (1). While lymphomas represent 4% of all cancers in an immunocompetent population, they account for 21% of all cancers cases in transplant recipients (2). Incidence of PTLD may vary from 1 to 20% depending on various risk factors such as pre-transplant Epstein Barr virus (EBV) seronegativity, the degree of immunosuppression and the transplanted organ (3,4). In allogeneic HSCT, development of PTLD predominately depends on the degree of human leukocyte antigen mismatch along with T-cell depletion methods (5). PTLD encompasses a wide morphologic spectrum ranging from polyclonal proliferation to aggressive monomorphic large B-cell proliferations. Currently, the World Health Organization categorizes PTLD into: (i) non-destructive lesions, (ii) polymorphic PTLD, (iii) monomorphic PTLD, and (iv) classical Hodgkin lymphoma type PTLD (6). The most common is the aggressive monomorphic PTLD, particularly diffuse large B-cell lymphoma (DLBCL) (7–9).

Treatment of PTLD

The current treatment for PTLD patients consists of reduction of immune suppression combined with rituximab monotherapy for 4 cycles. In case of responsive disease (complete remission / partial remission) patients receive 4 additional cycles of rituximab. In total, 20-55% of patients will achieve complete remission after initial rituximab therapy (10–12). In case of unresponsive disease (stable disease / progression) patients are treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP). This treatment regimen is associated with high treatment related toxicity (4,13–15).

Response evaluation in PTLD

Because of the risks associated with the current second-line treatment (R-CHOP), the treating physician is faced with the decision of weighting treatment benefit against the risk of treatment related complications. ^{18}F-flurodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG-PET/CT) has become the main tool to assess remission status, drive decisions on treatment alteration and identify relapse. However, incidence of false positives results from end of treatment ^{18}F-FDG-PET/CT scans reach up to 21%, mainly due to inflammatory conditions (16,17). The high incidence of false positive ^{18}F-FDG-PET/CT scans may lead to unnecessary continuation of toxic treatment Furthermore, ^{18}F-FDG-PET/CT may not be able to identify high-risk patients with a worse outcome after initial treatment with R-CHOP (18–20).

Minimal residual disease

The fraction of tumor derived from circulating tumor DNA (ctDNA) in the total amount of cell-free DNA (cfDNA) is usually limited. Minimal residual disease (MRD) may be detected through ctDNA, from malignant tumor cells undergoing apoptosis and necrosis. MRD occurs generally under the detection threshold of ^{18}F-FDG-PET/CT, but may be detectable in the ctDNA fraction using a sensitive next generation sequencing (NGS) approach. Current literature suggests that ctDNA may be used to monitor tumor dynamics throughout treatment and that a negative status after treatment is related to better outcomes in diffuse large B-cell lymphoma (DLBCL) patients. Furthermore, reaching MRD negativity within 6 weeks after treatment initiation, was related to a favorable outcome in DLBCL and thus might serve as an early response assessment (21–23). Such an early response indicator might be used to guide treatment.

DLBCL gene panel versus PTLD gene panel

MRD measurements in ctDNA may be obtained from a mutation hotspot panel using NGS (26). A panel on the mutations landscape of B-cell PTLD has been established by Menter et al. (24). To the best of our knowledge no studies have yet evaluated the feasibility of MRD detection using NGS based on a PTLD mutation panel. In personal communication with co-investigators, Dr. Rossi and Dr. Spina (IOSI-IOR), the overlap between the mutational landscape in DLBCL and PTLD indicates that the mutations identified by Menter et. al are covered by the 133 gene panel used at IOR-IOSI for DLBCL. Current results indicate a sensitivity of >90% and specificity of 100% for detecting ctDNA using this gene panel for DLBCL, using tumor gDNA genotyping as the gold standard (25). mWe expect this panel to be sensitive and specific enough to carry out MRD measurements on ctDNA in PTLD patients.

^{18}F-FDG-PET/CT versus MRD

Given the shortcomings of ^{18}F-FDG-PET/CT and the potential of a much lower detection threshold for MRD as assessed by ctDNA, we will explore the feasibility of MRD monitoring in PTLD patients being treated with rituximab / R-CHOP and compare these results with the current standard response assessments (^{18}F-FDG-PET/CT and EBV measurements)

2. OBJECTIVES

Primary Objective:

- To determine the feasibility of MRD detection using ultra-deep targeted NGS on ctDNA from PTLD patients using a gene panel previously of 133 lymphoma relevant genes previously used in DLBCL

Secondary Objective(s):

- To explore the mutational landscape of PTLD by whole exome sequencing and validate the study's gene panel
- To investigate the dynamics of ctDNA at diagnosis, interim and at end-of-treatment in relation to rituximab and/or R-CHOP treatment
- To compare ctDNA abundance with ^{18}F-FDG-PET/CT results in patients responding to therapy vs refractory or relapsing patients.

3. STUDY DESIGN

This is an exploratory prospective multicenter, multinational cohort study.

The study design is outlined in Figure 1.

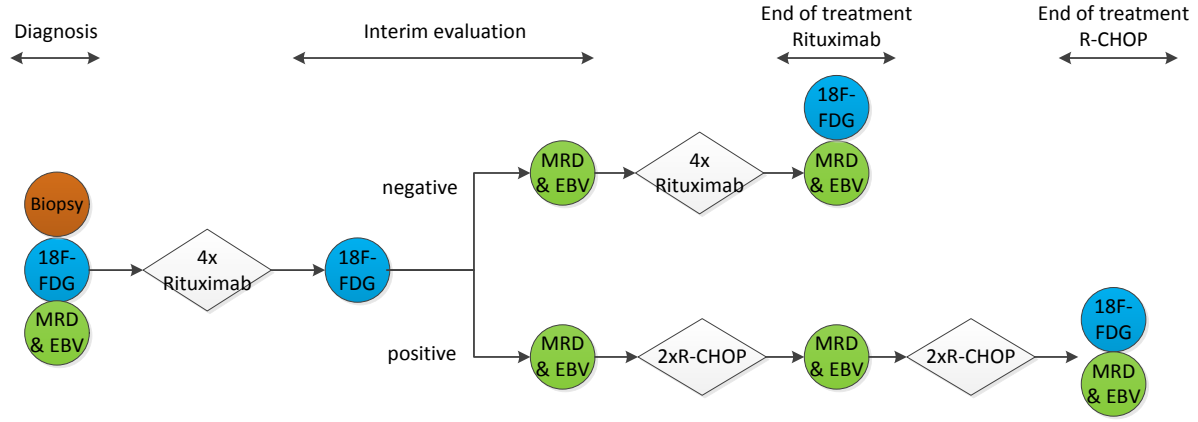

Figure 1. Study Design Outline: *Tissue biopsies are performed at diagnosis as part of routine diagnostics. ^{18}F-FDG-PET/CT scans and blood sampling for EBV measurements at diagnosis, after 4 or 8 courses with rituximab and after R-CHOP therapy are performed as standard of care. MRD measurements will be performed on blood samples obtained at diagnosis and after the 4 cycle of rituximab. In case of responsive disease MRD will be determined after the 8th cycle of rituximab. In case of unresponsive disease, MRD will be determined after cycle 2 and 4 of R-CHOP. (see appendix A voor details on plasma collection and cfDNA extraction)*

4. STUDY POPULATION

4.1 Population

Patients after SOT or HSCT with a histologically proven monomorphic large CD20+ B-cell PTLD which will be treated with the intention of curative therapy according to standard of care

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Patients having undergone a SOT or HSCT
- Histologically proven CD20+ monomorphic PTLD (with or without EBV association),
- Age > 18 years
- Intent to treat patient according to standard protocol (rituximab / R-CHOP). Clinicians are allowed to adapt protocol in the best interest of the patient
- Measurable disease on ^{18}F-FDG-PET/CT at diagnosis according to the Lugano classification 2014
- Patient's written informed consent and written consent for data collection.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- A complete surgical resection of tumor.
- Upfront treatment with external beam radiation therapy.
- Involvement of the central nervous system by the disease.
- Known to be HIV positive.
- Iatrogenic immunodeficiency lymphomas other than PTLD.

4.4 Sample size calculation

Proof of principle study indented to demonstrate the feasibility of MRD detection using ultra-deep targeted NGS on ctDNA from PTLD patients (see primary objective). After four courses of Rituximab administered as a single agent, complete remission rates vary between 20-55% (10,27,28). In limited PTLD studies, incidence of false positives ^{18}F-FDG-PET/CT scans varies between 4-20%, mostly due to inflammatory conditions. Incidence of false negatives ^{18}F-FDG-PET/CT scans varies between 5-10% mainly due to areas of physiological high background activity and non-destructive PTLD (15–18). Sensitivity and specificity of ultra-deep targeted next generation sequencing using the current mutation panel for DLBCL is >90% and 100% respectively (29). In personal communication with co-investigators, Dr. Rossi and Dr. Spina (IOSI-IOR), the overlap between the mutational landscape in DLBCL and PTLD indicates that the mutations identified are covered by the 133 gene panel used at IOR-

IOSI for DLBCL. There are currently no studies which have investigated the sensitivity and specificity of the mutation panel in PTLD patients. In a feasibility study such as the one proposed, it is standard to start with a limited number of patients. For the purpose of this study and its objectives inclusion of 20-30 patients will be sufficient.

5. METHODS

5.1 Study parameters/endpoints

5.1.1 Main study parameter/endpoint

1. Detection of ctDNA at diagnosis and response evaluation

5.1.2 Secondary study parameters/endpoints

2. Sensitivity and specificity of plasma ctDNA genotyping in comparison with tumor sample DNA (gDNA) as gold standard
3. Changes in ctDNA abundance throughout therapy
4. Clinical end-points: progression free survival (PFS), overall survival (OS), event free survival (EFS), disease specific survival (DSS)

5.2 Study procedures

- Tumor tissue samples are obtained during diagnostic procedures as standard of care. Tumor biopsies will be centrally reviewed at the UMCG. Tissue DNA will be sent to UZLeuven for whole exome sequencing.

- ^{18}F-FDG-PET/CT will be performed at baseline, interim and end-of-treatment as standard of care. ^{18}F-FDG-PET/CT acquisition methods should follow the EARL ^{18}F-FDG-PET/CT accreditation specifications, but the patient does not need to undergo any additional preparation. The medical images will be centrally reviewed by the UMCG according to the Deauville five-point scale.

- A maximum of 4 blood samples will be collected during a routine vena puncture as stipulated in the study design . For the purpose of MRD measurements 60ml blood will be collected using *Streck tubes*. The UMCG will serve as the central laboratory facility to for handling and storage of the blood samples. MRD analysis will be performed at the IOSI-IOR

5.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Data already obtained will be used for evaluation, unless the patient revokes the informed consent form.

5.3.1 Specific criteria for withdrawal

- Refusal of the patient to participate in any further treatment or investigations.
- If the patient cannot take part in follow-up examinations.

6. SAFETY REPORTING

No serious adverse events are expected from a routine vena puncture via which 60ml of blood will be extracted performed by specialized personnel. Therefore, no formal safety reporting will be performed.

7. STATISTICAL ANALYSIS

Clinical characteristics, pathologic findings, outcome, ^{18}F-FDG-PET/CT parameters and genotyping will be summarized with descriptive statistics. Frequencies and percentages will be used for categorical variables, and medians and ranges for continuous variables. Sensitivity and specificity of plasma ctDNA genotyping will be calculated with tumor gDNA genotyping as the gold standard. The univariate relationship between interim/end-of-treatment ^{18}F-FDG-PET/CT positive status and OS, PFS, EVF and DSS will be assessed by Kaplan-Meier curves. The univariate relationship between interim/end-of-treatment MRD positive status and OS, PFS, EVF and DSS will be assessed by Kaplan-Meier curves. Differences in the Kaplan-Meier curves between ^{18}F-FDG-PET/CT and MRD will be compared and analyzed.

7.1 Primary study parameter(s)

1. Detection of ctDNA at diagnosis and response evaluation

Descriptive statistics

7.2 Secondary study parameter(s)

2. Sensitivity and specificity of plasma ctDNA genotyping in comparison with tumor sample DNA (gDNA) as gold standard

Sensitivity and specificity of plasma ctDNA genotyping mutation detection will be calculated based on the t=0 ctDNA sample in comparison to with the profile obtained for the tumor gDNA genotyping which will serve as the gold standard

3. Changes in ctDNA abundance throughout therapy

Descriptive statistics

4. Clinical end-points: progression free survival (PFS), overall survival (OS), event free survival (EFS), disease specific survival (DSS)

PFS will be defined as the length of time from the end of therapy to the first objective evidence of relapse/progression or end point of our study (2 years). OS will be defined as the length of time from end of therapy until death or end point of our study (2 years after diagnosis). EFS will be defined as the length of time between end of therapy and onset of certain complications or events that the treatment was intended to prevent or delay. DSS will be defined as the length of time between end of therapy and death as a consequence of PTLD or treatment toxicity. Progress events will be determined on the basis of imaging findings, results of physical examination, patient-reported symptoms and/or biopsy. ^{18}F-FDG-PET/CT and MRD measurements will be considered as dichotomous variables. Drop out will be excluded from final results.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

8.2 Recruitment and consent

Patients will be informed about the study by their treating physician. After being informed by their treating physician about the study, patients are allowed to participate or drop-out until initiation of therapy. A patient information brochure and informed consent form (ICF) will be available for participants.

8.3 Compensation for injury

UMCG is the sponsor. There is no/minimal risk for the participating patient. The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

9.1 Handling and storage of data and documents

Patient Data

Demographic information as well as biomedical parameters will be collected by the coordinator investigator from the UMCG. Demographic information includes: gender, age, date of transplant, date of diagnosis, type of transplanted organ. Biomedical parameters related to lymphoma include: morphological PTLD type, biopsy location, EBV measurements, LDH measurements, international, prognostic index, stating and treatment response evaluation with ^{18}F-FDG-PET/CT according to Lugano classification 2014, clinical end-points: PFS, OS, EFS, DSS and ctDNA levels. All data will be collected from the (electronic) patient dossier and recorded in REDCap. This data storage environment allows for careful access management and is only accessible with username and password. We will minimize the collection of possible identifiable information; names and addresses will not be collected. All participants will receive a research ID number in the dataset, using a subject identification code. Research ID number will be coordinated with other participating hospitals via the research coordinator. The subject identification code list will be maintained by research coordinator and principal investigator in a password protected master file. The raw data containing identifiable information will be kept strictly separate from the processed data and can only be accessed by the data manager, research coordinator and principal investigator. Data analyses will take place on the cleaned data that does not contain the (in)direct identifiable data, using programme IBM SPSS Statistics for Windows or R Statistical Software. All the syntaxes describing data processing and analyses will contain or be accompanied by the comments explaining the code and the decisions made. The syntaxes will be checked by a data quality controller.

The digital data will not exceed 15 GB and will be stored for at least 15 years after publication of the main results. The basic research and storage infrastructure is accounted for by the UMCG. A description of the data collection will be documented in the UMCG Research Register and in the UMCG data catalogue (containing the Dublin Core). An UMCG Research Register Identifier and a DOI (digital object identifier) code will be assigned. The DOI will be included in the publications deriving from the dataset.

Access permissions are with the data manager, research coordinator and principal investigator. After publication of the main results, the processed, pseudo-anonymized data will be made available for re-use. Requests for re-use of data will be evaluated by the principal Investigator who will check whether the research question falls within the scope of the informed consent. (Adapted from Data Management Plan, UMCG, TPL 80H1.01, version 2).

^{18}F-FDG-PET/CT

We will collect and analyze imaging data from prospectively selected patients who underwent whole-body ^{18}F-FDG-PET/CT for tumor assessment. Only the original Digital Imaging and Communications in Medicine (DICOM) series will be selected, all derived data such as secondary captures, structured reports, etc. will be excluded from the selection to avoid identity breach since they may contain identifiable information even after de-identification of the DICOM header. The collected data will be stored in DICOM format. The user-identifiable information from the DICOM header will be removed or de-identified upon extraction from the institutional Picture Archive and Communication System (PACS). All participants will receive a research ID number in the dataset, using a subject identification code. The subject identification code list will be maintained by research coordinator and principal investigator. Suggested coding: <research ID number> <baseline/interim/EOT> <Name Hospital>. Research ID number will be coordinated with other participating hospitals via de research coordinator. The subject identification code list will be maintained by research coordinator and principal investigator in a password protected master file. Visualization of the data will be performed using Syngo.via.

Total data size will be of less than 500 GB. The imaging data and the measurement results will both be stored for at least 15 years. The de-identification key will be managed by a trusted third party. All imaging data will be made available through the XNAT environment of the dept. of Nuclear Medicine and be kept there with restricted access for only participants of the study. The basic research and storage infrastructure is accounted for by the UMCG. The data collection will be documented in the UMCG Research Register and a UMCG Research Register Identifier will be assigned. Measurements and meta data will be collected in REDCap. For further analyses exports will be made from this information system that will be explored using the statistical package IBM SPSS Statistics for Windows or R Statistical Software.

The data can be accessed by the data manager, research coordinator and the principal investigator. After the project is completed, all data go the principal investigator. Access to the data is arranged and evaluated by the principal investigator. If allowed on the research project agreement and the informed consent, data can be shared. Before data sharing the shared data will be fully anonymized as a new dataset before transfer to a third party. External imaging data will be exchanged in DICOM format and via SURFfilesender. After the data is obtained it will be transferred to UMCG servers and erased from SURFfilesender. (Adapted from Data Management Plan, UMCG, TPL 80H1.01, version 2).

Tissue biopsies & Blood Samples

Biopsies and blood samples will be collected from prospectively PTLD patients who underwent tissue biopsies/blood sampling during standard diagnostic procedures. All participants will receive a research ID number in the dataset, using a subject identification code. The subject identification code list will be maintained by research coordinator and principal investigator. Suggested coding for tumor tissue

samples/blood sampling: <research ID number> <baseline/interim/2-RCHOP/EOT > <Name Hospital>. The subject identification code list will be maintained by the research coordinator and principal investigator in a password protected master file. Research ID number will be coordinated with other participating hospitals via de research coordinator. Afterwards, collected tissue biopsies will be centrally reviewed at the UMCG, department of Hematology. Then, tumor blocks will be sent to UZLeuven for whole exome sequencing. The blood samples from the patients will be processed after reception. DNA samples will be stored at -80 degrees at the department of Hematology. DNA samples collected for the study will be shipped to the Institute of Oncology Research, Switzerland (IOSI-IOR) in batches of 10 patients using existent protocols. All measurement results will be collected in REDCap. The basic research and storage infrastructure is accounted for by the UMCG. The data can be accessed by the data manager, research coordinator and the principal investigator. After the project is completed, all data go the principal investigator. Access to the data is arranged and evaluated by the principal investigator. If allowed on the research project agreement and the informed consent, data can be shared. Before data sharing the shared data will be fully anonymized as a new dataset before transfer to a third party. (Adapted from Data Management Plan, UMCG, TPL 80H1.01, version 2).

9.2 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

9.4 Temporary halt and (prematurely) end of study report

- The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

  The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
- In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

9.5 Public disclosure and publication policy

This study will be conducted according to the "CCMO statement on publication policy" (27).

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Appendix A

Plasma collection

Keep always peripheral blood samples in Cell-Free DNA BCT tube at room temperature until plasma collection.

Peripheral blood samples in EDTA tubes must be processed within 1-2 hours from blood sampling.

- Peripheral blood sample from Cell-Free DNA BCT tube or from EDTA tube will be centrifuged at 820 g for 10 min at 4˚C to separate plasma from cells.

- After centrifugation, Plasma will be collected (as many as possible) in 2ml Eppendorf tubes (2ml/tube).

- Plasma collected in Eppendorf tubes will be further centrifuged at 14,000 rpm for 10 min at 4˚C, to pellet and remove any remaining cells, splitted into 1 ml vials and stored at -80°C.

cfDNA extraction

cfDNA from 1 ml of Plasma will be extracted with the QIAamp® Circulating Nucleic Acid Kit (Qiagen) following the manufacturer's instructions (pag.22-25).

Quality control and concentration will be obtained by running 1µl of cfDNA extacted on a Bioanalyzer DNA High sensitivity chip. A successfully cfDNA extracted will have an average fragment size of ~170 bp.