INFORMATION SHEET for participants in a clinical study

1. **Title of the study:**

Accurate staging of immuno-virological dynamics during acute HIV infection

2. **Aim of the study:**

Although we are able to control HIV-1 with antiretroviral therapy, we are, until this day, incapable of curing HIV and completely eradicating the virus.
The treatment, consisting of a combination of antiretroviral drugs is capable to control the virus and prevent further replication of the virus but there is still virus present in the blood and tissue as a form of latent reservoir. Research showed that this reservoir is already installed very early during primo-infection. When patients interrupt their treatment, this reservoir is responsible for the viral rebound which means the virus will start replicating again and the natural evolution of the disease strikes again. For this reason, people with HIV-1 infection are facing lifelong treatment. Starting treatment during acute infection can reduce the viral reservoir significantly. The further evolution of immunological and virological parameters in this specific patient cohort remains unclear. To better understand how to tackle the viral reservoir in patients that were treated very early during infection, we need to better understand the immune-virological dynamics. This study can help us to better define the constitution of the viral reservoir and the factors involved in this process. We need this information to be able to reduce the viral reservoir to a level where the body might be capable of controlling it without the necessity of lifelong therapy.

The goal of this study is to create a cohort of patients, like yourself, that present themselves with a recent diagnosis of HIV 1 positivity based on a positive screening test. Patients included in the study will be followed over time and we will sample blood and tissue at different time-points, before and after instauration of antiretroviral treatment.

Early treatment is an important factor in the reduction of the viral reservoir. After the first sampling, the treatment is initiated immediately. This way, we will also be able to compare this patient cohort with other cohorts where treatment was only initiated in the chronic phase of infection.

3. **Description of the study:**

The patients that can be included in this study and are interested to participate will be informed about the study and the protocol. We are aiming to include a minimum of 30 patients and max 50 patients that are consulting with a recent HIV-1 diagnosis (positive screening and staging based on Fiebig criteria).

Sampling will be organized at 3 different time-points:
1° Before initiation of antiretroviral therapy: the sampling will be organized immediately after inclusion. Preferably the sampling will take place within 72h after the first contact, in order not to delay the initiation of therapy. This sampling will consist on peripheral blood drawing (12x 9ml EDTA tubes), a lymph node excision and a left colonoscopy where 10 colon biopts will be taken. We will also collect lumbar fluid by puncture, a stool sample, urine and genital sampling (sperm or vaginal fluid).
After the sampling, treatment will be initiated in order to achieve an undetectable viral load.
2° At undetectable viral load a second sampling will take place. This sampling will consist on a leukapheresis, to collect a large amount of white blood cells. Furthermore, we will repeat the lumbar fluid collection by puncture, a stool sample, urine and genital sampling (sperm or vaginal fluid).

3° One year after the achievement of undetectable virus in the blood under antiretroviral treatment, a third sampling will be organized. This will consist on a colonoscopy, lymph node excision, stool sampling, urine and genital sampling, lumbar puncture and leukapheresis.

4° At every routine follow-up blood draw, an extra sample (3x9ml EDTA tubes) will be collected for research during the first 2 years after diagnosis.

There are 2 possibilities to be enrolled in the studyprotocol:

- Prospectively, at the diagnosis of HIV-1, in patients that are willing to participate in all aspects of the protocol. These patients will be enrolled as soon as possible after diagnosis (option A).
- Patients can also chose to participate in a limited part of the study (option B1). Patients that already initiated treatment early after infection, can participate for the second and/or third sampling (option B2). The informed consent form contains a table where you can indicate the selected option.

The leukapheresis will take place at the apheresis center. The other samples will be organized after admission at the one day clinic. The left colonoscopy will take place under sedation and the lymph node excision/ full colonoscopy will be done under short general anesthesia.

If any abnormality would be seen during one of the invasive procedures, adequate diagnostics and therapeutics will be effectuated at that time (ex. Biopsy, excision). Follow-up of these results will be guaranteed afterwards.

Explanation of the different procedures:

a: Leukapheresis

Leukapheresis is the golden standard method to obtain large volumes of peripheral cells from HIV-infected patients. Runs will last approximately three hours for the collection of > 1x108 cells. You lie on a bed or reclining chair, with a catheter into a vein in each arm. One catheter removes blood and passes it into a machine that removes white blood cells. The rest of your blood cells and normal blood fluid (plasma) go back into your body through the catheter in your other arm.

Leukapheresis is not painful, but some people find it uncomfortable to stay sitting or lying down in the same place for 2 or 3 hours.

b: Lymph node resection

Lymph node resection will be performed by the vascular surgery department. Briefly, a small incision is made in the inguinal region (triangle of scarpa) allowing a lymph node resection.

c: Colonoscopy

Colonoscopy is the endoscopic examination of the large bowel and the distal part of the small bowel with a camera on a flexible tube passed through the anus. It can provide a visual diagnosis and grants the opportunity for biopsy. We will take several samples (10) of a part of your intestine called the terminal ileum.
We will also perform collection of gut-associated lymphoid tissue via biopsy of colon mucosa. colon mucosal sampling will be obtained by left colonoscopy, collecting up to 10 mucosal biopsies. Subjects will be counseled to avoid rectal trauma and the use of anticoagulants (e.g. aspirin, NSAIDS) before and

after the procedure. Mucosal biopsies will not be taken if the participant has an increased risk for complications, including receptive anal intercourse within 3 days of the procedure, an active anal infection, or recent use of anticoagulants. Biopsies will be performed by gastroenterologists who have training and clinical certification in colposcopy.

d: Lumbar fluid

Cerebrospinal fluid (CSF) will be obtained by lumbar puncture. Lumbar puncture will not be performed if evidence of intracranial pressure is identified. Lumbar puncture (spinal tap) is performed in your lower back, in the lumbar region. During lumbar puncture, a needle is inserted between two lumbar bones (vertebrae) to remove a sample of cerebrospinal fluid — the fluid that surrounds your brain and spinal cord to protect them from injury.

e. Genital tractus access

Sperm will be collected according to the standard operating procedures from our fertility department.

Cervicovaginal secretions will be harvest during hospitalization or ambulatory at the gynaecology department.

f. Urine sample: A mid-stream urine sample will be collected. This means that you don't collect the first or last part of urine that comes out. This reduces the risk of the sample being contaminated with bacteria. Urine will be collected in a sterile container and stored in the fridge right away.

The following investigations will be performed on the samples:

Virological parameters:

- First we will isolate the immune cells out of the collected tissues. We will sort them into target subpopulations and use specific molecular tests to evaluate the amount of HIV-derived genetic material (DNA and RNA) are present.
- Specific proteins in the blood and tissues will be assessed to evaluate inflammation status.
- We will do genetic analysis of the virus and look at the relationships between the viruses found in tissue and blood to see if they are closely related to each other.
- Integration sites and methylation of the genetic material of the virus within the human genome will be investigated.
- Stimulation and immunological inhibition assays: VOA (Viral Outgrowth Assay)/TILDA (Tat/rev Induced Limiting Dilution Assay): these are specific techniques to measure HIV-1 persistence under antiretroviral therapy.

Immunological parameters –:

- Specific proteins in the blood and tissues will be assessed to evaluate inflammation status.
- The innate immune respons will be investigated by looking at complement pathways and investigating immune cells, isolated from the different tissues looking at their activation The acquired immune response will be analysed by viral inhibition assay and looking at the immune reponse against HIV infection.

Therapeutic drug monitoring: The amount of antiretroviral drugs will be assessed in the blood and the biopsies.

Microbiome analysis: the stool samples will be used to further analyse the intestinal microbiome.

Your medical records concerning your HIV infection will be assessed to enable the evaluation of the study results in combination with previous blood analysis, clinical phase of infection and Fiebig classification at treatment initiation. This will help us to assess whether the clinical classification correlates with our findings. Only medics will have access to these records in strict confidentially, and all data will be coded.

This study involves extra blood and tissue sampling. Only the laboratory experiments described will be performed on these blood and tissue samples. The study does not involve the admission of any substance. Only the experiments that are described in this information sheet will be carried out.

Patients who fulfil the criteria for participation and who agree to participate will be included.

4. What is expected from the participants?

We expect participants to this study to agree with the sampling procedure at the different time-points as described above. Patients should agree with all the investigations of viral and human markers that will be performed on the different samples, which are collected at different time-points. Patients will initiate therapy and will be followed by one of the medical doctors at our AIDS reference centre.

5. Participation and termination

Your participation in this study is voluntary. Participation at this study will probably not provide any therapeutic benefit. However, the results of this study may help patients in the future. You can refuse to participate or ask your study doctor to end your participation before the final closure of the study, at any time. Refusal to participate or early termination will not, in any way, influence your relationship with and/or your treatment by the doctor or the medical team. The team of treating physicians is working in an independent way from the investigators. You can withdraw participation in this investigation at any given time point. Your participation in this study will end when the treating physician considers that withdrawel is for your benefit.

After the study, the leftovers of the study material are completely made anonymous and stored in a bio-bank for a minimum period of 20 years. If the material is destroyed, the participants will not be informed.

Bloodsamples will be stored at the Bimetra biobank, University Hospital Ghent at a temperature of -196°C. Plasma, stool, sperm, urine and tissue samples will be stored at the HIV Cure research center lab, 120 040 MRB II building.

For specific virologic and immunologic analysis collabarations with other labs will take place and samples can be shipped to the lab of Lisa Frenkel at the Seattle Children's Hospital or the lab of Mathias Lichterfeld in Harvard, Boston, USA. Cerebrospinal fluid analysis will be done in collaboration with the lab of Magnus Gisslen in Goteburg, Sweden.

Samples will be anonymised for shipment. Leftover material will be send back to us or will be destroyed.

If we want to use these samples for future experiments, that are not mentioned in the described experiments above, a new informed consent will be asked to perform these experiments.

If you agree to participate you will be asked to sign the informed consent form.

6. **Risks and advantages**
   This is a basic scientific study and no benefits are to be expected.
   But, this trial may be helpful for the future development of new strategies for treatment of others with a similar illness and might provide insights on patient classification at early diagnosis. Patients that started treatment early during the course of infection have the highest potential of functional cure since they are characterised by a smaller HIV reservoir. Better characterisation of these patients will benefit future HIV cure studies.

   You have the right to ask questions about this study at any time. If during the study, information that can influence your participation becomes available, you will be informed immediately about these facts. If eventually any adverse event does occur, you will be treated appropriately.
   This study is approved by the independent Ethical Committee of the hospital and will be conducted in accordance with the guidelines for good clinical practice and the Helsinki declaration. Approval by the Ethical Committee however, may not be seen as stimulation for participation.

We are aware that at inclusion there is little time to consent to the study. This is mainly due because of the fact that we do not want to delay the initatiation of the treatment. The study protocol will therefore be rediscussed at several times during your follow-up. After diagnosis you will be followed by a multidisciplinary team at our reference centre, consisting of medical staff, psychologists and nurses. If at any point you want to withdraw from the study, or you only consent to a part of the investigations this can be adapted at an individual level. However we aim to include 2/3 of the patients for all of the investigations as described in the protocol.

The potential risk of the sampling procedures:
For all individual procedures, the individual risk will be discussed with the patient by an open communication with the responsible physician. For each procedure, the reported individual risks are low (typically 1/1000 for adverse events in all procedures), and are based on data from sick patients (e.g. lung disease for which bronchoscopy is needed).
Risk estimation per procedure is shown below.

- Possible complications from a lumbar puncture (LP) comprise of: local discomfort with pain at the puncture site, during or after the puncture, radicular pain, bleeding, infection and post-puncture headache. All the punctures within this study will be made with an atraumatic needle to decrease the risks of post-puncture headache significantly.
- Biopsy of the gastrointestinal tract:

- A colonoscopy with biopsy carries a risk of bowel perforation (an opining in the intestine) in 1/1500. If this occurs, the perforation is recognized immediately and treated during the same endoscopy. As a result of the administration of analgesics and/or narcotic drugs other side effects can occur, such as low blood pressure, nausea, or twists. These are short-lived side effects and after a colonoscopy, the patient is kept in observation overnight, with constant monitoring of the pulse and blood pressure.

- Anuscopy: Subjects will be counseled to avoid rectal trauma and the use of anticoagulants (e.g. aspirin, NSAIDS) before and after the procedure. Mucosal biopsies will not be taken if the participant has an increased risk for complications, including receptive anal intercourse within 3 days of the procedure, an active anal infection, or recent use of anticoagulants.

- Biopsy of the lymph node: Lymfocele (a cyste of lymph fluid) is the main risk in removal of lymph nodes. This complication is infrequent (<1%) and can be surgically corrected. The infectious risk is <1% and easily treatable.

- Leukapheresis: is considered a safe procedure. The most frequent side effects are numbness and tingling due to low calcium levels in the blood (1in 10patients). This could easily be managed with oral calcium supplements and/ or slower flow rates. Other reactions included hypotension and headache. We will ask participants on antihypertensive drugs to not take them the day of the leukapheresis. There is also risk of catheter problems during procedure, which can be managed by the nursing staff present. After the procedure there can be a transient increased risk of bleeding. Contra-indications for this procedure are bad peripheral venous access and disturbances of the minerals in your blood. If the procedure is contra-indicated, it will be replaced by a peripheral venous puncture (6x9ml).

- Risks due to anaesthesia: low blood pressure, nausea can occur after anaesthesia. These are short-lived side effects.

7. Costs of participating

Participation in this trial will not result in any additional cost to you. The additional tests and sampling will be payed for by the study.

8. Compensation for participation

Participants to the study will not receive any financial compensation.

9. Confidentiality

In accordance with the Belgian law concerning the private life protection (08 Dec 1992) and the patient's rights (22 Aug 2002) the information collected from your participation in this study is protected. You have the right to request information of the existence of personal data held by the study coordinator and will have the right to rectify erroneous or inaccurate data. Representatives of the study coordinators, the Independent Ethical Committee and/or Regulatory Authorities will be granted direct access to your original medical records for verification of the clinical trial procedures and/or data, without violating your confidentiality according to the laws and regulations applicable in Belgium.
By signing this informed consent form you are authorizing such access.

If you agree to participate in this study, your personal data and clinical information will be collected and coded. No reports containing your personal data will be publicly available. When the results of this study will be published your identity will remain confidential. If reference to you is made, this will only be done by using code numbers.

10. Insurance

The coordinators of this study are liable, even without fault, for damage that you or your legal successor has sustained and that has direct or indirect link with the trial. To that end the study has provided insurance coverage.

11. Contact person

If lesions would occur as consequence of this study, or if you would want extra information about this study, or about your rights and duties, you can at any time during this study contact Prof. Dr. VANDEKERCKHOVE LINOS (09/3323398) or Dr. De Scheerder Marie-Angélique (09/3321349)

Informed consent form

I,_____ , declare that I received and read a copy of "information sheet for participants in a clinical study" Page 1 to 7. I fully agree with the content of the document and am willing to participate in this study as a volunteer.

I have been informed about the different options to enroll in this study. Based on these explanations, I understand the content of the table and I selected the option that is valid for me.

		Option A (the complete study protocol)	Option B1 and Option B2 (adapted to personal preferences and depending on timing of inclusion)
Selected option		☐	Option B1 ☐ Option B2 ☐
Sampling To	Blooddraw, lumbar puncture, left colonoscopy, lymph node excision, urine, genital and stool sampling	Blooddraw 9x10ml Lumbar puncture Urine/genital sample Stool sample Lymph node excision Left colonoscopy	Blooddraw 9x10ml ☐ Lumbar puncture ☐ Urine/genital sample ☐ Stool sample ☐ Lymph node excision ☐ Left colonoscopy ☐
Sampling at undetectable viral load	Leukapheresis, lumbar puncture, urine, genital and stool sampling	Leukapheresis Lumbar puncture Urine/genital sampling Stool sample	Leukapheresis ☐ lumbar puncture ☐ Urine/genital sampling ☐ stool sample ☐
Sampling 1 year after undetectable viral load	Leukapheresis, lumbar puncture, colonoscopy, lymph node excision, urine, genital and stool sampling	Leukapheresis Lumbar puncture Urine/ genital sampling Stool sample Lymph node excision Colonoscopy	Leukapheresis ☐ lumbar puncture ☐ Urine/genital sampling ☐ stool sampling ☐ lymph node excision ☐ colonoscopy ☐
M1, M3, M6, M12, M18, M24	3x 9ml EDTA blood samples	Extra 3x 9ml at routine blooddraws	☐

I received a copy of the signed and dated approval form. I received explanations on the nature, the aim, the duration and the expected effects of the study. I received information on my specific contribution to this investigation and on the potential risks and advantages of the study. I am aware

that my participation in this study will not lead to direct benefits. I was able to ask questions and remarks about this study, and I received satisfying answers on all the questions and remarks, including the medical issues.

I fully agree to collaborate with the researcher/medical doctor in this scientific investigation. In case of unexpected symptoms, I will immediately report to the responsible medical doctor.

I was fully informed about the existence of an assurance in case of any physical damage related to the present research procedure.

I am aware this investigations have been approved by an independent Medical and Ethical Committee, and I know this investigation will be performed according to the guidelines of Good Clinical Practice (ICH/GCP) and to the declaration of Helsinki, which was formulated to protect participants in experimental investigations. This approval was in no case the main motive to participate in this investigation.

I am aware that I can withdraw my participation in this investigation at any given time point. I will not be asked to state a reason for this withdrawal and this will not influence the current patient relation with the treating physician.

I have been informed that both personal information as well as individual health information will be processed and archived for at least 20 years. I agree with, and am aware of my rights to access and correct this information. Since this information will be processed in the framework of the clinical and medical goals of this investigation, I am aware that access to this information may be withheld from me until the end of the investigation. In case I want access to my personal information, I will contact the medical doctor who is responsible for data processing.

I am aware that samples can be shipped to internationally collaborating laboratories for assay development.

I understood that auditors, representatives of the principal investigator, and the Medical and Ethical Committee are authorized to inspect my personal data. By signing this document, I approve this control, as my privacy will be respected at all times.

I understand that my samples will be preserved in the Bimetra Biobank of the Ghent University and at the biobank of the HIV Cure Research Center at the MRB II building, located on the UZ Ghent Campus. The experiments that will be done on the samples, are the ones described in the information letter.

I am fully prepared to voluntarily participate in this scientific investigation

Name of the participant: _____

Date: _____

Signature:

I confirm that I informed the participant about the nature the objective and the anticipated effects of the study to the above-mentioned participant.

The participant agreed to participate to the study by signing and dating this document.

Name of the person who provided information _____

Date: _____
Signature: