ADJUVANT MUCOSAL THERAPY IN HIV-INFECTED MEN WITH INSUFFICIENT RESPONSE TO ANTIRETROVIRAL THERAPY

Protocol Identification Number: OUSHIVProbiot2

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SIGNATURE PAGE

Title Adjuvant mucosal therapy in HIV-infected men with insufficient response to antiretroviral therapy

Protocol ID no: OUSHIVProbiot2

I hereby declare that I will conduct the study in compliance with the Protocol, ICH GCP and the applicable regulatory requirements:

Name	Title	Role	Signature	Date
Dag Kvale		Sponsor Contact		
Dag Henrik Reikvam		Prinicipal Investigator		
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PROTOCOL SYNOPSIS

Title:

Adjuvant mucosal therapy in HIV-infected men with insufficient response to antiretroviral therapy

Sponsor	Oslo University Hospital
Phase and study type	Phase I single arm, open label, consecutive case series study, and subsidiary cross-sectional case control study.
Investigational product (including active comparator and placebo) :	IDOFORM®Travel No placebo/active comparator
Centers:	Oslo University Hospital (single center)
Study Period:	Estimated date of first patient enrolled: 01.12.2015 Anticipated recruitment period: 18 months Estimated date of last patient completed: 30.11.2017
Treatment Duration:	8 weeks
Follow-up:	One follow-up consultation 2 weeks after completed therapy
Objectives	Primary: Assess the safety of a probiotic compound (IDOFORM®Travel) in combined anti-retroviral therapy (cART)-treated immunologic non-responder (INR) patients with chronic HIV infection. Secondary: i) Explore the biological effects of probiotics in cART-treated INR patient with chronic HIV infection; and ii) investigate differences between cART-treated HIV-infected INR and non-INR patients with regards to gut microbiome and mucosal barrier function.
Endpoints:	Primary endpoints: i) Adverse effects; ii) Δplasma viral load; and iii) Δblood CD4 count. Secondary/Exploratory endpoints: Alterations in i) Gut microbiota composition; ii) Gene expression of gut epithelial cells; iii) Frequency and activation status of CD4+ T cell subsets in gut lamina propria, including Th17 and Th22 subsets; iv) Frequency, activation status and intracellular signaling of systemic T cells; and v) Soluble

	systemic markers of chronic inflammation and immune activation.
Study Design:	1) Single arm, open label, consecutive case series, single center intervention study on cART-treated INR patients with chronic HIV infection 2) Subsidiary cross-sectional case control, single center study comparing cART-treated INR patients with chronic HIV infection to non-INR patients with chronic HIV infection and to healthy controls.
Main Inclusion Criteria:	1) Subjects may be included as **cases** in the study if they meet all of the following criteria: • HIV seropositive >4 years. • Continuous cART >4 years. • Plasma HIV RNA <50 copies/mL >3,5 years.[1)] • CD4+ T cell count **<400 cells/µL** >3.5 years.[2)] • Male, Caucasian • Age 25-65 • Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to International Committee on Harmonization (ICH) Good Clinical Practice (GCP), and national/local regulations. 2) Subjects may be included as **control subjects** in the sub-study if they meet all of the following criteria: • HIV seropositive >4 years. • Continuous cART >4 years. • Plasma HIV RNA <50 copies/mL >3,5 years.[1)] • CD4+ T cell count **>600 cells/µL** >3.5 years. • Male, Caucasian • Age 25-65 • Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations. OR if they meet all the following criteria: • HIV seronegative. • Male, Caucasian • Age 25-65 • Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations.

	[1)]One single measurement ("blip") of HIV RNA >50 copies/ml accepted. [2)]One single measurement ("blip") of CD4 >400 cells/µl accepted.
Main Exclusion Criteria	Subjects must be excluded from participating in this study if they meet any of the following criteria: • Plasma hepatitis C (HCV) RNA positive. • Serum hepatitis B surface antigen (HBsAg) positive. • Comorbidity of inflammatory bowel disease, coeliac disease or malnutrition. • Concomitant use of non-steroid anti-inflammatory drugs (NSAID), corticosteroids, disease-modifying antirheumatic drugs, or other anti-inflammatory pharmaceutical substances. • Concomitant use of antithrombotic pharmaceutical substances • Regular (weekly) use of any probiotic substance within 3 months prior to inclusion. • Use of antibiotics within 3 months prior to inclusion. • Deranged liver function (serum albumin <25 g/L or Child-Pugh ≥10) • Renal failure (estimated glomerular filtration rate (eGFR) <30 ml/min) • Heart failure (NYHA class II-IV) • Any reason why, in the opinion of the investigator, the patient should not participate.
Sample Size:	20 INR patients for intervention study and subsidiary cross sectional study. 20 non-INR case controls and 20 HIV negative healthy volunteers for subsidiary cross sectional study.
Efficacy Assessments:	The primary objective of this study is safety assessments (see below). Efficacy assessments are exploratory secondary objectives and consist of: – Mucosa-adherent microbial composition – Gene expression of mucosal tissue with validation of protein expression – Frequency and activation status of LPMC subsets by flowcytometry

	– Alterations in systemic T cells intracellular signaling – Soluble markers of chronic inflammation and immune activation.
Safety Assessments:	– Self-reported adverse events – Physical examination – Vital signs – Medical biochemistry – HIV RNA quantification – CD4 T cell count

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
AE	adverse Event
AIDS	acquired immunodeficiency syndrome
ALAT	alanine aminotransferase
cART	combined antiretroviral therapy
CD*n*	cluster of differentiation *n*
Cfu	colony forming units
CK	creatin kinase
CRF	case record form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Event
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HBSS	Hank's Balanced Salt Solution
HCV	hepatitis C
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
INR	immunological non-responder
LPMC	lamina propria mononuclear cells
NSAID	non-steroid anti-inflammatory drug
OUH	Oslo University Hospital
PBMC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PI	principle investigator
qRT-PCR	quantitative reverse transcriptase polymerase chain reaction
R&D	research and development

REK	Regional komite for medisinsk og helsefaglig forskningsetikk, Ethics Committee
RNA	ribonucleic acid
SAE	serious adverse event
SLV	Statens legemiddelverk, Norwegian Medicines Agency
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
Th*n*	T helper cell type *n*
Tregs	regulatory T cells
UiO	University of Oslo
CPT	cell preparation tube
CTCAE	Common Terminology Criteria for Adverse Events
H_0	null hypothesis

1 INTRODUCTION

1.1 Background – Disease

Human immunodeficiency virus-1 (HIV) is a retrovirus that is transmitted between humans by sexual contact and inoculation of blood, including perinatal mother-to-child transmission. The virus infects and kills subsets of T-lymphocytes characterized by cell surface expression of cluster of differentiation (CD)4+. As CD4+ T cells are essential for cellular immunity, natural HIV infection will in most humans destruct the immune system and progress to acquired immunodeficiency syndrome (AIDS), which leads to advanced immunodeficiency, opportunistic infections and eventually death.(1) HIV infection provides a substantial disease burden to global health. More than 36 million people live with HIV infection globally and the virus annually causes 1.5 million deaths.(1, 2)

There is still no cure to eradicate the HIV virus from an infected patient. However, since late 1990s combined antiretroviral therapy (cART) has been available. A combination of at least three pharmaceutical substances prevents the virus from replicating and may subsequently allow the immune system to reconstitute.(3, 4) Patients responding to therapy will have undetectable virus in the blood, and CD4+ T cell numbers should eventually be restored to normal levels (>500 cells/µL).(4) Successful cART stops progression to AIDS and confers life expectancy comparable to the general population.(5-7)

Around 15% of cART treated patients do not achieve the treatment goal of CD4+ T cell count of >500 cells/µL despite persistent virological suppression.(8, 9) These immunological non-responders (INR) patients (aka discordant responders) have increased risk of still developing AIDS,(10, 11) increased risk of non-AIDS HIV-related morbidity such as cardiovascular disease and cancer, and increased mortality.(12-16) As both absolute and relative numbers of HIV-patients on cART increases globally,(2) efforts to improve the clinical prognosis in patients not responding to cART should be emphasized.

Patients with chronic HIV infection have a chronic low-grade inflammation and immune activation with hyperactivated T cells and subsequent T cell exhaustion.(17) This immune activation is considered to contribute to non-AIDS morbidity and correlates with mortality among HIV patients.(18-20) Immune activation correlates inversely with CD4 T cell counts and INR patients have augmented immune activation.(21-26)

Primary infection with the HIV virus causes massive depletion of CD4 T cells in the gut mucosa, which is only partially restored after years of cART.(27-30) These depleted CD4+ T cells, primarily of the subsets T helper cells type (Th) 17 and Th22, are crucial for maintaining the epithelial barrier and mucosal homeostasis.(31-36) Patients with chronic HIV infection has an impaired gut epithelial barrier with markers on increased epithelial apoptosis and disrupted tight junctions.(37, 38) The impaired epithelial barrier is thought to allow influx of microbial products into systemic circulation, a phenomenon called microbial translocation.(39, 40) Increased microbial translocation is a prime candidate mechanism to explain why INR cART-treated patients do not respond sufficiently to cART, have increased immune activation, and unfavorable clinical prognosis.(23, 24, 40-42) However, *in situ* mucosal assessment in order to prove that the mucosal barrier is impaired in INR patients compared to cART responders is yet to be performed. In depth focal assessments of mucosal barrier function may also disclose perturbations in the regulation of the mucosal barrier that could provide targets for adjuvant therapy to cART.(37, 43) Restoration of mucosal barrier may reduce immune activation and subsequently improve the clinical prognosis for INR HIV-infected patients.

Over the last years there has been an emerging interest in the role of the gut microbiota for health and for chronic diseases, including HIV infection.(44) Alterations in gut microbial composition have been demonstrated in chronic HIV infection.(45-48) Recently published data indicates that the composition of the microbiota correlates directly with the function of the mucosal CD4+ T cells in HIV patients (49) and also

with systemic markers of microbial translocation and immune activation.(50-54) However, the full implications for these observations with regards to epithelial barrier function are still uncertain. As these studies are all cross-sectional observations, it is not known if it is the microbial alterations that causes the altered T cell dysfunction and immune activation or vice versa. Moreover, the relevance of these observations in INR cART treated HIV-infected patients has not been assessed. On the basis of published data referred to above we hypothesize that the perturbations in the axis of gut microbiota -> epithelial barrier impairment ->Th17/Th22 disruptions ->chronic immune activation is greater in INR patients than those responding to cART.

1.2 Background - Therapeutic Information

Probiotics are bacteria that may colonize the gut lumen and provide beneficial health effects, though the mechanisms of how they confer these effects are still largely unknown. (55, 56) We have already conducted a study of administering probiotics in treatment-naïve HIV patients and demonstrated reduction in immune activation in these patients.(57) Other small, preliminary studies have shown similar results.(58, 59) As argued in section 1.1, the increasing fraction of HIV patients on cART makes the relevance of adjuvant probiotic supplement higher in INR cART-treated patients than in treatment-naïve patients, but intervention studies with probiotics in INR HIV patients have not been performed.

Probiotics are generally well tolerated and smaller studies with other probiotic formulas of lower dose have been well tolerated in HIV-infected patients.(58-60) Still, cases of septicemia in young children and adults with compromised immunity have been observed.(61, 62) As our study population has impaired immunity we find it warranted to perform a phase I safety assessment of probiotic compounds before initiating larger efficacy studies in cART-treated INR HIV-infected patients.

1.3 Rationale for the Study, Hypotheses and Purpose

Based on the scientific knowledge presented above we find it warranted to test the following hypotheses:

i) CART-treated chronic HIV-infected INR patients have an altered composition of their gut microbiota compared with HIV-infected cART-treated immunological responder patients.
ii) CART-treated chronic HIV-infected INR patients have impaired gut mucosal barrier function compared with HIV-infected cART-treated immunological responder patients, and this dysfunction relates to perturbations in mucosal Th17 and Th22 CD4+ T cells.
iii) Administration of probiotics to INR cART-treated chronic HIV-infected patients is safe and coincide with alteration of gut microbiota and restoration of mucosal barrier function and reduced systemic immune activation.

To this date there are no scientific publications answering these hypotheses. Exploration of these queries may provide targets for adjuvant therapy in substantial number of patients not responding sufficiently to cART and thereby improve their clinical prognosis.

2 STUDY OBJECTIVES AND RELATED ENDPOINTS

The **primary objective** of this study is to assess the safety of probiotics in cART-treated INR patient with chronic HIV infection.

The **secondary objectives** are to i) explore the biological effects of probiotics in cART-treated INR patient with chronic HIV infection, and ii) investigate differences between cART-treated HIV-infected INR and non-INR patients with regards to gut microbial composition and mucosal barrier function.

2.1 Primary Endpoint

i) Adverse effects; ii) Δplasma viral load; and iii) Δblood CD4 count.

2.2 Secondary Endpoints

Alterations in i) Gut microbiota composition; ii) Gene expression of gut epithelial cells; iii) Frequency and activation status of CD4+ T cell subsets in gut lamina propria, including Th17 and Th22 subsets; iv) Frequency, activation status and intracellular signaling of systemic T cells; and v) Soluble systemic markers of chronic inflammation and immune activation.

	Objectives	**Endpoints**	**Assessments**
Primary	Assess the safety of probiotics in cART-treated INR patient with chronic HIV infection.	– Adverse effects – ΔPlasma HIV viral load – ΔBlood CD4 count.	Sections 7.2
Secondary/ Exploratory	Explore the biological effects of probiotics in cART-treated INR patient with chronic HIV infection.	– Alteration in gut microbiota composition. – Alteration in gene expression of gut epithelial cells – Alteration in frequency and activation status of CD4+ T cell subsets in gut lamina propria, including Th17 and Th22 subsets. – Alteration in frequency, activation status and intracellular signaling of systemic T cells. – Alteration in soluble systemic markers of chronic inflammation	Section 7.1

		and immune activation.	
	Investigate differences between cART-treated HIV-infected INR and non-INR patients with regards to gut microbial composition and mucosal barrier function.	– Alteration in gut microbiota composition. – Alteration in gene expression of gut epithelial cells – Alteration in frequency and activation status of CD4+ T cell subsets in gut lamina propria. – Alteration in frequency, activation status and intracellular signaling of systemic CD4+ T cells. – Alteration in systemic markers of chronic inflammation and immune activation.	Section 7.1

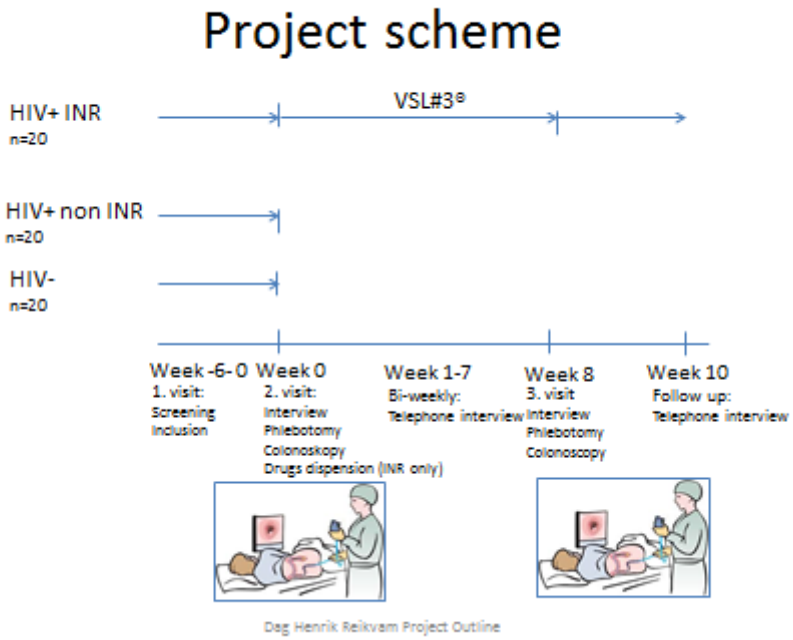
3 OVERALL STUDY DESIGN

The primary study is a phase I, single arm, open label, consecutive case series study examining the safety of IDOFORM®Travel in patients with a confirmed diagnosis of HIV infection and continuous cART treatment >4 years and concurrent HIV (ribonucleic acid) RNA <50 copies/mL and CD4+ count <400 cells/µL >3.5 years prior to inclusion. There will be no control arm to the intervention. The primary objective is safety assessment. The secondary objective is exploratory. End points at the termination of the intervention will be compared to the parameters at inclusion. (See chart below.)

Study Period	Estimated date of first patient enrolled:	01.12.2015
	Anticipated recruitment period:	18 months
	Estimated date of last patient completed:	30.11.2017
Treatment Duration:	8 weeks	
Follow-up:	Follow-up terminated at termination of treatment	

In a subsidiary cross sectional case control study, the patients included in the intervention will at baseline (week 0) be compared with individuals with chronic HIV infection and continuous cART >4 years and concurrent HIV RNA <50 copies/mL and CD4+ count >600 cells/µL >3.5 years prior to inclusion, and with

HIV uninfected individuals, both control groups matched to cases with regards to gender, ethnicity, age, and comorbidity



4 STUDY POPULATION

4.1 Selection of Study Population

Male Caucasian patients with a confirmed diagnosis of HIV infection AND continuous cART treatment >4 years AND concurrent HIV RNA <50 copies/mL >3.5 years AND **CD4+ count <400 cells/µL** >3.5 years (aka. INR patients) prior to inclusion will be recruited from the outpatient clinic at the Department of Infectious Diseases, Oslo University Hospital.

For the cross sectional sub-study, male Caucasian persons with a confirmed diagnosis of HIV infection AND continuous cART treatment >4 years AND concurrent HIV RNA <50 copies/mL >3.5 years AND **CD4+ count >600 cells/µL** >3.5 years prior to inclusion will be recruited from the outpatient clinic at the Department of Infectious Diseases, Oslo University Hospital. HIV uninfected control individuals will be recruited from men referred to the outpatient clinic at Dep. of Gastroenterology, Oslo University Hospital for cancer screening.

4.2 Number of Patients

Twenty HIV-infected INR patients (cases) will be included in this trial. In addition, 20 HIV infected non-INR patients and 20 HIV negative individuals will be included as controls for the subsidiary cross sectional study.

4.3 Inclusion Criteria

1) Subjects may be included as **cases** in the study if they meet all of the following criteria:

- HIV seropositive >4 years.
- Continuous cART >4 years.
- Plasma HIV RNA <50 copies/mL >3,5 years.[1)]
- CD4+ T cell count **<400 cells/µL** >3.5 years.[2)]
- Male, Caucasian
- Age 25-65
- Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to International Committee on Harmonization (ICH) Good Clinical Practice (GCP), and national/local regulations.

2) Subjects may be included as **control** subjects in the sub-study if they meet all of the following criteria:

- HIV seropositive >4 years.
- Continuous cART >4 years.
- Plasma HIV RNA <50 copies/mL >3,5 years.[1)]
- CD4+ T cell count **>600 cells/µL** >3.5 years.
- Male, Caucasian
- Age 25-65
- Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations.

Or if they meet all the following criteria:

- HIV seronegative.
- Male, Caucasian
- Age 25-65

Signed informed consent and expected cooperation of the patients for the treatment and follow up must be obtained and documented according to ICH GCP, and national/local regulations.

[1)]One single measurement ("blip") of HIV RNA >50 copies/ml accepted.
[2)]One single measurement ("blip") of CD4 >400 cells/µl accepted.

4.4 Exclusion Criteria

Subjects must be excluded from participating in this study if they meet any of the following criteria:

- Plasma hepatitis C (HCV) RNA positive.

- Serum hepatitis B surface antigen (HBsAg) positive.
- Comorbidity of inflammatory bowel disease, coeliac disease or malnutrition.
- Concomitant use of non-steroid anti-inflammatory drugs (NSAID), corticosteroids, disease-modifying antirheumatic drugs, or other anti-inflammatory pharmaceutical substances.
- Concomitant use of antithrombotic pharmaceutical substances
- Regular (weekly) use of any probiotic substance within 3 months prior to inclusion.
- Use of antibiotics within 3 months prior to inclusion.
- Deranged liver function (serum albumin <25 g/L or Child-Pugh ≥10)
- Renal failure (estimated glomerular filtration rate (eGFR) <30 ml/min)
- Heart failure (NYHA class II-IV)
- Intolarance to milk or phenylalanine (INR patients only)
- Any reason why, in the opinion of the investigator, the patient should not participate.

5 TREATMENT

For this study IDOFORM®Travel is the Investigational Product.

5.1 Product Identity, Supply and Storage

IDORORM®Travel capsules contain live *Lactobacillus rhamnosus GG (LGG®),- Lactobacillus acidophilus LA-5®,- Lactobacillus bulgaricus LBY-27® ,- Bifidobacterium animalis subsp. lactis BB-12®, and Streptococcus thermophilus STY-31®* at a total amount of 3 x10^9 cfu/capsula. Inactive ingredients: xylitol, aspartame, acesulfam K, microcrystalline cellulose, isomalt, mannitol, methylcellulose, inulinfatty acids, silicon dioxide, glucose, aromas, and citric acid. Storage: Room temperature (<25°C, low ambient humidity). Manufacturer: Pfizer A/S. Distributor: Alliance Healthcare Norge AS.

5.2 Dosage and Investigational Product Administration

Four capsules orally every 24 hour (12*10^9 cfu/day), with or without food or other drugs according to the subject's preference.

IDOFORM®Travel is previously not documented administered to patients with HIV infection. Studies assessing biological effects of probiotics in other diseases strive for high-dose ranges above 10^{10} cfu/day. (63, 64)

5.3 Duration of Therapy

First dose is to be taken two days after first colonoscopy and dispensing of capsules.

Therapy shall be continued for eight weeks therapy unless serious adverse events are encountered. Last dose is to be taken at the day of the end-of-protocol colonoscopy.

5.4 Schedule Modifications

Modifications of dose or administration interval will not be allowed.

5.5 Concomitant Medication

The following medication is not allowed while the patient is included in the study:

- NSAID
- Corticosteroids
- Other anti-inflammatory pharmaceutical substances.
- Antibiotics
- Probiotic formulas other than the Investigational Product.

All concomitant medication (incl. herbal preparation and other "over-the-counter" drugs) used by the patient will be recorded in the patient's file and Case Record Form (CRF).

5.5.1 Non-investigational products

All subjects included will at screening be dispensed one packet of two sachets of Citrafleet® (ATC code: A06A B58) for bowel emptying. The participating subjects will be instructed to take the servings prior to the colonoscopy according to written information conveyed at screening visit.

5.6 Subject Compliance

Compliance will be calculated based on the number of returned capsules and the number of planned days with the interventional product.

5.7 Investigational Product Accountability

The PI will confirm receipt of study product and will use the study product only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return, and destruction (if any) of the study product must be properly documented according to the sponsor's agreed and specified procedures.

The study product will be ordered from importer/distributor by the study pharmacist. Labeling and dispension will be performed by the PI in collaboration of the study pharmacist. Study pharmacist will be responsible for product receipt. Unused returned capsules will be destructed by the pharmacist.

All investigational product containers (opened, unopened, or empty) must be returned to the sponsor after the study. An investigational product dispensing log must be prepared for each subject. At weeks 0 and 8 and at any other relevant time point the following information must be entered into the investigational product dispensing log:

- Date of visit

- Number of containers given to the patient, including individual container batch number(s)

- Number of containers returned from the patient, including individual container number(s) and whether they are unopened, opened or empty

- The number of capsules left in the containers which are opened, but not empty

- Explanation of any discrepancies

- Signature of the person distributing/collecting the container(s)

After completion of the study, the completed investigational product dispensing logs must be signed by the investigator

5.8 Product Labeling

The investigational product will have a label permanently affixed to the container outside and will be labeled according to standards originally designed for medicinal products such as ICH GCP.

In addition to manufacturer information (i.e. batch id, expiration date) labels will state (in Norwegian):

TIL UTPRØVING

Studiepasient (initialer og inklusjonsnummer): (fylles ut)

IDOFORM®Travel kapsler

4 kapsler en gang daglig (Tas sammen med andre medisiner).

Oppbevares i romtemperatur utilgjengelig for barn

Oslo universitetssykehus HF, Kirkeveien 166, 0407 Oslo
Studiekode: OUSHIVProbiot2

Kontaktperson: Dag Henrik Reikvam, Infeksjonsmedisinsk avdeling,
Telefon 22 11 98 81 / 94858939. Ta kontakt i nødstilfelle for informasjon
om IDOFORM®Travel og studien.

TA MED PAKNINGEN VED NESTE KONTROLL. IKKE KAST TOMME PAKNINGER

5.9 Subject Numbering

Each subject is identified in the study by a unique subject number that is assigned when subject signs the Informed Consent Form. Once assigned the subject number cannot be reused for any other subject

The study treatment will be administered to the subject by the investigators only.

6 STUDY PROCEDURES

6.1 Flow Chart

	Screening period	Baseline	Treatment period [1)]					Follow up [1)]
Week	**-4 to -1**	**0** ***Product start***	**1** [2)]	**3** [2)]	**5** [2)]	**7** [2)]	**8** ***Product stop***	**10** [2)]
Informed consent	X							
Inclusion/exclusion Evaluation	X	X						
Medical history and Clinical status	X	X						
Concom. med.	X	X	X	X	x	X	X	
Vital signs		X					X	
Physical exam		X					x	
Clinical chemistry	X	X					X	
Biological markers	X	X					X	
Product dispensing		X						
Product collecting							X	
Adverse events		X	X	X	X	X	X	x
Colonoscopy/Stool sample		X					X	

[1)]INR patients/intervention study only. 2) Telephone interview.

6.2 By Visit

Informed consent:

Informed consent must have been given by each subject before any study specific procedures are initiated, preferably at screening visit. The following tests will be done at screening:

Clinical status and Vital signs:

Medical history (including disease history and corresponding treatment details), physical examination (eg. cor/pulm/abdomen and peripheral lymph node status), and vital signs (weight, blood pressure, temperature and pulse).

Before vital signs are measured, the subject should be resting seated for at least 2 minutes. Any vital sign value after start of treatment that is judged by the investigator as a clinically significant worsening compared to previous exam will be considered an adverse event (AE), documented on the subject's CRF, and followed until the outcome is determined.

Concomitant medication:

All concomitant medication (incl. herbal preparation and other "over-the-counter" drugs) used by the subject within 28 days of treatment start must be recorded in the CRF. Check boxes in CRF for NSAID, glucocorticoids, disease-modifying antirheumatic drugs, and other anti-inflammatory drugs, antithrombotic drugs, antibiotics (within 3 months) and probiotics (within 3 months)

Clinical chemistry and Biological markers:

Blood samples will be taken to determine clinical chemistry analyses and biological markers.

Clinical chemistry include (one 4 ml sample tube of EDTA whole blood and one 5 ml sample Serum Separation Tube): hemoglobin, white blood cells differential count, thrombocytes, mean corpuscular hemoglobin, mean corpuscular volume, HbA1c, vitamin B12, folate, bilirubin, uric acid, C-reactive protein (CRP), sodium, potassium, creatinine, aspartate aminotransferase, alanine aminotransferase (ALAT), gamma-glutamyl transferase, lactate dehydrogenase, alkaline phosphatase, albumin, amylase, creatin kinase (CK), and lipid profile.

Biological markers include (one 2,5 ml sample tube of EDTA whole blood and one 5 ml sample Plasma Preparation Tube): CD4+ and CD8+ T cell counts and HIV RNA quantification.

In addition the following material will be collected from included subjects for exploratory assays and analyses: One 4 ml sample tube of EDTA whole blood, one 3.5 ml sample Citrate-Na tube, one 5 ml sample of Plasma Preparation Tube (CPT), and one 5 ml sample Serum Separation Tube and eight 7 ml samples Cell Preparation Tubes.

Adverse events:

Se section 8.

Colonoscopy:

Included subjects will perform bowel emptying procedure with Citrafleet® (see also section 5.5.1)starting 20-28 hours in advance of colonoscopy.

Peripheral vein cannula will be inserted in case of peri-procedural need for sedation with midazolam 5-10 mg intravenously according to routine colonoscopy procedures.

Full endoscopic examination will performed from rectum to terminal ileum. Subsequently, multiple biopsies will be collected in terminal ileum, ascending colon and sigmoid colon will be collected. See section 6.5.3 for further details.

A Standard Operating Procedure (SOP) for the colonoscopy will be produced in Norwegian to ascertain a safe, uniform and reproducible procedure in all study subjects.

Stool samples

Included subjects will immediately before starting bowel emptying sample a tea-spoon size of own stool that they will bring to the upcoming visit and colonoscopy. All included subjects will at screening visit be given a sampling kit for the collection of the stool samples.

6.2.1 Before Treatment Starts

Tentative participants (cases and controls) will be invited by letter or telephone call to participate. Screening visit agreed upon by telephone. Alternatively, tentative participants invited at routine checkup at the outpatient clinic at Dep. of Infectious Diseases at Oslo University Hospital (OUH).

Screening visit agenda:

- Recording medical history including medication
- Clinical examination
- Blood samples (se section 6.2)
- Evaluation of eligibility based on inclusion and exclusion criteria.
- Oral and written information about the treatment, colonoscopic procedure and follow up.
- If eligible and accepting participation, subjects signs informed consent. (See appendix for patient information and informed consent form).
- Information about preparations to colonoscopy and dispensing of Citrafleet® for pre-procedure bowel emptying.

6.2.2 During Treatment

Week 0: Inclusion. First visit at the endoscopy unit at outpatient clinic at Dep. of Gastroenterology, OUH.

First visit/colonoscopy agenda:

- Review of medical history
- Clinical examination
- Vital signs
- Blood samples (se section 6.2)
- Colonoscopy with biopsy collection according to SOP.
- Dispensing of study treatment (IDOFORM®Travel) for eight weeks consumption.

Week 1-7: Bi-weekly telephone interviews. Record adverse events and update concomitant medication. In case of serious adverse events or side effects, arrangement of subject visit at outpatient clinic at Dep. of Infectious Diseases

6.2.3 End of Study Visit

Week 8: End of study visit and colonoscopy at Dep. of Gastroenterology, OUH.

End of study visit/colonoscopy agenda:

- Adverse events
- Clinical examination
- Vital signs
- Blood samples (se section 6.2)
- Colonoscopy with biopsies collection according to SOP.
- Collection of residual interventional product packages and residual capsules.

6.2.4 Withdrawal Visit

Should a subject wish to withdraw (see also section 6.3) a visit for complete final evaluation at the time of the subject's withdrawal will be arranged.

A withdrawal visit will record the reason, date, and time for withdrawal in the CRF and include clinical examination and vital signs. If possible, measurements and recordings should continue according to the protocol.

The specific event or test result(s) being the reason for withdrawal will be recorded in the CRF.

6.2.5 After End of Treatment (Follow-up)

Week 12: Telephone interview recording adverse events. In case of serious adverse events or side effects, arrangement of a visit at the outpatient clinic at Dep. of Infectious Diseases will be made.

6.3 Criteria for Patient Discontinuation

Subjects may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a subject for this study are:

- Voluntary discontinuation by the subject who is at any time free to discontinue his/her participation in the study, without prejudice to further treatment. In accordance with the Declaration of Helsinki, each subject is free to withdraw from the study at any time.
- Safety reason as judged by the Principal Investigator.
- Major protocol deviation.
- Incorrect enrolment i.e., the subject does not meet the required inclusion/exclusion criteria for the study.
- Subject lost to follow-up.
- Deterioration in the subject’s condition, which in the opinion of the PI warrants study medication discontinuation (to be records as an AE).
- Subject non-compliance to study treatment and/or procedures, which in the opinion of the PI warrants study discontinuation.

6.4 Procedures for Discontinuation

6.4.1 Patient Discontinuation

Subjects who withdraw or are withdrawn from the study, will stop further treatment.

If possible, a final assessment (withdrawal visit) shall be made. The reason for discontinuation will be recorded. The investigator is obliged to follow up any significant adverse events until the outcome either is recovered or resolved, with sequela, fatal or unknown.

If a subject withdraw or are withdrawn from the study before start of treatment, it will be replaced by another individual that is found eligible according to the inclusion and exclusion criteria.

6.4.2 Trial Discontinuation

The whole trial may be discontinued at the discretion of the PI or the sponsor in the event of any of the following:

- Occurrence of AEs unknown to date in respect of their nature, severity and duration.
- Medical or ethical reasons affecting the continued performance of the trial.
- Difficulties in the recruitment of patients.
- Cancellation of investigational product development.

The sponsor and the PI will inform all investigators, the relevant Competent Authorities and Ethics Committees of the termination of the trial along with the reasons for such action. If the study is terminated early on grounds of safety, the Competent Authorities and Ethics Committees will be informed within 15 days.

6.5 Laboratory Tests

6.5.1 Blood samples

Serum: Drawn, collected and handled as routine samples and analyses by Dep. of Medical Biochemistry. Planned analyses:, vitamin B12, folat, bilirubin, uric acid, C-reactive protein (CRP), sodium, potassium, creatinine, aspartate aminotransferase, ALAT, gamma-glutamyl transferase, lactat dehydrogenase, alkaline phosphatase, albumin, amylase, CK, and lipid profile. Additional analyses may be performed.

EDTA whole blood: Drawn, collected and handled as routine samples and analyses by Dep. of Medical Biochemistry and Dep. of Immunology. Planned analyses: hemoglobin, white blood cells differential count, thrombocytes, mean corpuscular hemoglobin, mean corpuscular volume, HbA1c, and CD4+, CD8+ T cell count, and genetic analysis for presence or absence of human leukocyte antigen variants HLA-A and HLA-DR1B Additional analyses may be performed.

Plasma: Drawn, collected and handled as routine samples and analysis by Dep. of Medical Microbiology. Planned analysis: HIV RNA quantification. Additional analyses may be performed.

CPT tube, plasma and serum: Drawn, collected and handled by the Research and Development (R&D) section at Dep. of Infectious Diseases, OUH. Peripheral blood mononuclear cells (PBMC) will be isolated and frozen according to established laboratory protocols.(65) When inclusion of participants completed cells will be thawed according to established protocols and applied in functional *ex vivo* experiments and analyzed by flow cytometry. Additional analyses may be performed.

6.5.2 Colon biopsies

Forceps pinch biopsies will be collected at three different sites (terminal ileum, ascending colon and sigmoideum) during the colonoscopy. Each biopsy will be collected with a disposable biopsy forceps with a maximum insertion portion diameter of 3.3 mm (EndoJaw™, Olympus Medical Systems Corporation, Tokyo, Japan). At each site the following tissue specimens will be collected and handled in the following manner:

- Single biopsy swiftly weighted and washed in phosphate buffered saline (PBS) and then immersed in RNALater® (Quiagen). Incubated 24 hours in refrigerator (2-6°C), then frozen and stored at -20° until analyses. Four samples collected at each biopsy site. Collected and preserved for gut microbiota and gene expression analysis.

- Single biopsy swiftly washed in PBS and placed on a precut approx. 15 x3 mm carrot rod. Biopsy on carrot rod immersed in Tissue-Tek® O.C.T. Compound (Sakura Fintek Europ, Zoeterwoude, Netherlands), wrapped in aluminum foil, put in tube, and snap frozen in liquid nitrogen and stored in -70°C. One sample collected from each biopsy site. Collected and preserved for immunohistochemistry and *in situ* hybridization.

- Single biopsy swiftly washed in PBS and immediately immersed in 1.5 ml formaldehyde. Incubated 24 hours in refrigerator, then processed and embedded in paraffin. Collected and preserved for histopathology and immunohistochemistry.

- Twenty biopsies from each site pooled in 25 ml ice cold Hank's Balanced Salt Solution (HBSS. Washed twice in ice cold HBSS. Transferred to tube with ice cold RPMI culture medium with 10% fetal calf serum and 1% penicillin/streptomycin and brought to R&D section at Dep. of Infectious Diseases for collagenase digestion and filtering for isolation of single-cell suspension of lamina propria mononuclear cells (LPMC). Cells frozen according to the same established laboratory protocols as applied for PBMCs (See section 6.5.1). When inclusion of participants completed cells will be thawed according to established protocols and applied in functional *ex vivo* experiments and analyzed by flow cytometry.

- Single biopsy swiftly weighted and washed in PBS and put in clean tube and snap frozen in liquid nitrogen. Two samples collected at each biopsy site. Collected and preserved for HIV RNA quantification, Western blot and other protein and nucleic acid assays.

6.5.3 Stool samples

Stool will be sampled by the individual subjects immediately before he starts bowel emptying. Stool sample will be put in buffer-containing. *PSP® Spin Stool DNA sampling tube (Invitek) v01*. The sample tube will be collected by the investigator at colonoscopy visit and stored in -80°C for later nucleic acid-*baced assays for analysis of microbial composition and function.*

7 ASSESSMENTS

7.1 Assessment of Efficacy

The primary objective of the intervention study is safety and is described in section 7.2.

The secondary objectives of the study are exploratory efficacy assessments. Efficacy of intervention and observed differences between groups will be evaluated by analyses of biologic material collected as described in section 6.5. Key *ex vivo* mucosal barrier assessments at weeks 0 and 8 are:

- Mucosa-adherent microbial composition
- Gene expression of mucosal tissue with validation of protein expression
- Frequency and activation status of LPMC subsets by flowcytometry
- Alterations in systemic T cells intracellular signaling
- Soluble markers of chronic inflammation and immune activation.

Efficacy assessments will be performed at the R&D section of the Department of Infectious Diseases, at the Norwegian PSC Research Centre, at the Department of Pathology, all OUH, and at the Biotechnology Centre at the UiO. These research labs have state of the art facilities for biomedical research and are committed to close scientific collaboration through the virtual K.G. Jebsen Inflammation Research Centre hosted by UiO

7.2 Safety and Tolerability Assessments

Safety will be monitored by the assessments described below as well as the collection of AEs at every visit. Significant findings that are present prior to the signing of informed consent must be included in the relevant medical history/ current medical condition page of the CRF. For details on AE collection and reporting, refer to Section 8.3.

For the assessment schedule refer to Flow chart in Section 6.1.

At inclusion (week 0) and at interventional product stop (week 8) patients will go through physical examination, vital signs evaluation, and blood samples for medical biochemistry analysis.

- Physical examination will include an examination of general appearance, skin, eyes, heart, abdomen, and peripheral lymph nodes.

- Vital signs include body temperature, blood pressure, pulse, and respiratory rate. Seated blood pressure and respiratory rate assessments will be performed after 2 min seated rest in a chair next to colonoscopy bench prior to colonoscopy.

- Medical biochemistry analysis consists of: Hemoglobin, white blood cells count, Thrombocytes, CRP, sodium, potassium, creatinine, ALAT, Bilirubin, Albumin, Amylase, and CK.

At weeks 1, 3, 5, and 7, participants will be telephoned and asked for side effects or adverse symptoms. Symptoms will be categorized as:

- General (fever, wellbeing/sense of illness, fatigue).

- Circulatory (palpitations, thoracic pain/discomfort, cold/painful extremities)

- Respiratory (shortness of breath, coughing, bronchial discharge)

- Dermal (rash, pruritus).

- Gastrointestinal (diarrhea, bloating, nausea, abdominal pain/discomfort.

- Other.

In case of side effects or adverse symptoms, participants will be called in for a visit for physical examination and vital signs and evaluation of need for discontinuation of study.

8 SAFETY MONITORING AND REPORTING

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE). Each patient will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

The methods for collection of safety data are described below.

8.1 Definitions

8.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

The term AE is used to include both serious and non-serious AEs.

If an abnormal laboratory value/vital sign are associated with clinical signs and symptoms, the sign/symptom should be reported as an AE and the associated laboratory result/vital sign should be considered additional information that must be collected on the relevant CRF.

8.1.2 Serious Adverse Event (SAE)

Any untoward medical occurrence that at any dose:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Medical and scientific judgment is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Hospitalization for administrative reason (for observation or social reasons) is allowed at the investigator's discretion and will not qualify as serious unless there is an associated adverse event warranting hospitalization.

8.1.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Adverse Reaction: all untoward and unintended responses to an investigational medicinal product related to any dose administered;

Unexpected Adverse Reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Suspected Unexpected Serious Adverse Reaction: SAE (see section 8.1.2) that is unexpected as defined in section 8.2 and possibly related to the investigational medicinal product(s).

8.2 Time Period for Reporting AE and SAE

For each patient the standard time period for collecting and recording AE and SAEs will begin at start of study treatment (week 0) and will continue 14 days following the last dose of study treatment, for each patient.

During the course of the study all AEs and SAEs will be proactively followed up for each patient; events should be followed up to resolution, unless the event is considered by the investigator to be unlikely to resolve due to the underlying disease. Every effort should be made to obtain a resolution for all events, even if the events continue after discontinuation/study completion.

8.3 Recording of Adverse Events

If the patient has experienced adverse event(s), the investigator will record the following information in the CRF:

- The nature of the event(s) will be described by the investigator in precise standard medical terminology (i.e. not necessarily the exact words used by the patient). ICD-10 diagnose applied if possible
- The duration of the event will be described in terms of event onset date and event ended data.
- The intensity of the adverse event will be graded on a 1-5 scale (1: mild; 2: moderate; 3: severe or medically significant; 4: life threatening; 5: death) according to Common Terminology Criteria for Adverse Events version 4.0 (CTCAE).(66)
- The Causal relationship of the event to the study medication will be assessed as one of the following:

  Unrelated:

  There is not a temporal relationship to investigational product administration (too early, or late, or investigational product not taken), or there is a reasonable causal relationship between non-investigational product, concurrent disease, or circumstance and the AE.

  Unlikely:

  There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.

  Possible:

  There is reasonable causal relationship between the investigational product and the AE. De-challenge information is lacking or unclear.

  Probable:

  There is a reasonable causal relationship between the investigational product and the AE. The event responds to de-challenge. Re-challenge is not required.

Definite:

There is a reasonable causal relationship between the investigational product and the AE.

- Action taken, including withdrawal from the study.
- The outcome of the adverse event – whether the event is resolved or still ongoing.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.1. An AE of severe intensity need not necessarily be considered serious. For example, nausea that persists for several hours may be considered severe nausea, but is not an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4 Reporting Procedure

8.4.1 AEs and SAEs

All adverse events and serious adverse events that should be reported as defined in section 8.1.1 will be recorded in the patient's CRF.

SAEs must be reported by the investigator to the sponsor, Oslo University Hospital by contact Dag Kvale, within 24 hours after the site has gained knowledge of the SAE. Every SAE must be documented by the investigator on the SAE pages (to be found in the CRF). The Serious Adverse Event Report Form must be completed, signed and sent to the Regional Ethics Committee (REK) and the Norwegian Medicine Agency (SLV). The initial report shall promptly be followed by detailed, written reports if necessary. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter.

The sponsor keeps detailed records of all SAEs reported by the investigators and performs an evaluation with respect to seriousness, causality and expectedness.

8.4.2 SUSARs

SUSARs will be reported to the Competent Authority. The following timelines should be followed:

The sponsor will ensure that all relevant information about suspected serious unexpected adverse reactions that are fatal or life-threatening is recorded and reported as soon as possible to the Competent Authority (i.e. SLV) and Ethics Committee (i.e. REK) in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information is subsequently communicated within an additional eight (8) days.

All other suspected serious unexpected adverse reactions will be reported to the Competent Authority (i.e. SLV) concerned and to the Ethics Committee (i.e. REK) concerned as soon as possible but within a maximum of fifteen (15) days of first knowledge by the sponsor.

SUSARs will be reported using the CIOMS form scanned sent by e-mail.

8.4.3 Annual Safety Report

Once a year throughout the clinical trial, the sponsor will provide the Competent Authority with an annual safety report. The format will comply with national requirements.

8.4.4 Clinical Study Report

The adverse events and serious adverse events occurring during the study will be discussed in the safety evaluation part of the Clinical Study Report.

8.5 Procedures in Case of Emergency

The investigator is responsible for assuring that there are procedures and expertise available to cope with emergencies during the study.

9 DATA MANAGEMENT AND MONITORING

9.1 Case Report Forms (CRFs)

The investigator will enter the data required by the protocol into the Case report forms (CRF) kept in paper format. The PI is responsible for assuring that data entered into the CRF is complete, accurate, and that entry is performed in a timely manner. The signature of the investigator will attest the accuracy of the data on each CRF. If any assessments are omitted, the reason for such omissions will be noted on the CRFs. Corrections, with the reason for the corrections will also be recorded.

The CRF will be completed legibly in black ink, with reasons given for any missing data. Any errors should be corrected by a single line strike-through, and annotated with the current date and initials of the person correcting the error. Under no circumstances should data be permanently obliterated with ink or correcting fluid.

The PI will sign the last page of the CRF. Any corrections to the data will be made in a manner that does not obscure the original entry and will be dated and initialed by the investigator or assigned designee.

9.2 Source Data

The medical records for each included subject should contain information which is important for the patient's safety and continued care, and to fulfill the requirement that critical study data should be verifiable.

To achieve this, the medical records of each patient should clearly describe at least:

- That the patient is participating in the study, e.g. by including the enrollment number and the study code or other study identification;
- Date when Informed Consent was obtained from the patient and statement that patient received a copy of the signed and dated Informed Consent;
- Results of all assessments confirming a patient's eligibility for the study;
- Diseases (past and current; both the disease studied and others, as relevant);
- Surgical history, as relevant;
- Treatments withdrawn/withheld due to participation in the study;
- Results of assessments performed during the study;
- Treatments given, changes in treatments during the study and the time points for the changes;
- Visits to the clinic / telephone contacts during the study, including those for study purposes only;
- Non-Serious Adverse Events and Serious Adverse Events (if any) including causality assessments;
- Date of, and reason for, discontinuation from study treatment;
- Date of, and reason for, withdrawal from study;
- Date of death and cause of death, if available;

Source data will be recorded directly into the CRF in addition to the patient's medical record.

9.3 Study Monitoring

The protocol and study conductance will be reviewed by a Clinical Study Monitor, who will check the following:

- Informed consent process
- Reporting of adverse events and all other safety data
- Adherence to protocol
- Maintenance of required regulatory documents
- Study Supply accountability
- Facilities and equipment (example: laboratory, pharmacy, ECG machine, etc…)
- Data completion on the CRFs including source data verification (SDV).

The monitor will review the relevant CRFs for accuracy and completeness and will ask the site staff to adjust any discrepancies as required.

When the responsible study monitor has checked and verified the CRFs, the data will be entered into a computer database at the OUH's scientific server (i.e. K:\Forskning\Forskningsstudier) for further handling and statistical evaluation. For verification the data will be entered twice at two separate sessions.

Sponsor's representatives (e.g. monitors, auditors) and/or competent authorities will be allowed access to source data for source data verification in which case a review of those parts of the hospital records relevant to the study may be required.

9.4 Confidentiality

The investigator will arrange for the secure retention of the patient identification and the code list. Code list and signed informed consent forms will be kept in a locked cabinet at the principle investigator's office. Patient files shall be kept for the maximum period of time permitted by each hospital. The study documentation (CRFs, Site File etc.) shall be retained and stored during the study and for 15 years after study closure. All information concerning the study will be stored in a safe place inaccessible to unauthorized personnel.

9.5 Database management

All included subject will be assigned a unique study number which will be used along with initials in all further data handling. The code list will be kept in paper only and stored in a locked cabinet at the PI's office.

During the study the CRFs will be stored at the technician's office at the R&D section at Dep. of Infectious Diseases, OUH. CRFs from subjects that have completed the study will be stored in the office in an open book shelf in the PI's office at OUH.

To promote data quality, data from CRF and results from assessments will be entered doubly into two separate Excel spreadsheets at two separate sessions. The double entries will subsequently be compared and merged in order to avoid data inaccuracies resulting from data entry errors. All entries and changes in the CRF, data files or database will be logged by signature (initials) of entrant and date of entry.

Data entered electronically will be kept at the OUH's scientific server (i.e. K:\Forskning\Forskningsstudier) inaccessible for unauthorized personnel.

All data both electronically entered and in paper format, will be stored for 15 years after the last subject has completed the study. After 15 years all data will then be deleted or anonymized.

10 STATISTICAL METHODS AND DATA ANALYSIS

10.1 Determination of Sample Size

The study probiotic (IDOFORM®Travel) has previously not been tested in HIV-infected patients. Other probiotics have been well tolerated in other samples of HIV-infected patients, but has not been tested in INR patients.(58-60) We expect the probiotic to have few side effects and adverse events occurring at a low frequency. The study design for assessment of the primary objective is an uncontrolled intervention for detection of adverse events occurring at a frequency and with a severity that disqualify further efficacy studies of the investigational product.

There are no published data providing an expected frequency or variability of adverse events that could be applied in power or sample size calculations for the primary objective of this study. For the safety study, 20 patients will be observed for serious adverse events (SAE) for 8 weeks. If we observe 0/20 adverse events then we can be 95% certain that the true population proportion of SAE in 2 weeks fall within the range (0%, 17%). If we observe 1/20 adverse events then we can be 95% certain that the true population proportion of SAE in 8 weeks fall within the range (0.1%, 25%). Here the confidence intervals have been calculated using the Clopper-Pearson method.(67)

The secondary objectives of the study are exploratory. There are no relevant published data that can provide expected efficacy size or variability. Sample size calculations for the secondary objectives are therefore not feasible.

The sample size in this study is also influence by queries in our HIV database on number of patients eligible for inclusion and by available resources at the departments participating in the study.

Expert statistician at Regional Research Support at OUH has been consulted.

10.2 Population for Analysis

The following populations will be considered for the analyses:

Primary objective:

Includes all subjects who received at least one dose of the investigational product. Subjects who withdrew from the study will be included in the safety analysis. A list of withdrawn subjects, preferably with the reasons for withdrawal, will be made.

Secondary objectives:

Includes all subjects who completed biopsy sampling at the site of interest during colonoscopy.

10.3 Planned analyses

The statistical analysis is planned to be performed when all subjects have been included and completed the study treatment. No interim analyses will be made.

Deviation from the original statistical plan will be described and justified in the Clinical Study Report. Amendments to plan can be done until day of DB lock.

10.3.1 Study population variables

A table will be provided with the following information:

- number of subjects, both screened and enrolled, included in the study
- number of subjects included in the safety analysis (primary objective)
- number of subjects included in the efficacy analysis (secondary objective)
- number of subjects withdrawn from the study and the reason for withdrawal

Demographic information will be summarized using descriptive statistics.

Medical histories will be summarized by counts. Concurrent medications will be recorded and coded using a standard classification system and grouped by primary and secondary classes if applicable.

Vital signs

Categorical data will be summarized by treatment and as a total using count and percentages of patients. Continuous data will be summarized by treatment and as a total using medians, quartiles and ranges.

Physical examination

Categorical data will be summarized by treatment and as a total using count and percentages of patients. Continuous data will be summarized by treatment and as a total using median, quartiles and ranges.

Clinical Laboratory Measurements

Categorical data will be summarized by treatment and as a total using count and percentages of patients. Continuous data will be summarized by treatment and as a total using median, quartiles and ranges.

10.4 Statistical Analysis

Non-parametrical statistics will be applied for all analyses, two-sided tests only. Level of statistical significance defined as $p<0.05$. Patients with missing values will be excluded from analysis of the parameter where value is missing. This study does not have any additional separate statistical analysis plan.

Primary endpoints: (see section 2.1)

i) Adverse events:

   a. Descriptive presentation of frequencies concurrent to study treatment. No comparative statistical analysis.

ii) Δplasma HIV RNA load:

a. Δ defined by changes between end of study (week 8) and baseline (week 0).

b. Assessed by routine HIV RNA quantification at Dep. of Medical Microbiology, OUH.

c. H_0: Eight weeks treatment with probiotics does not change HIV RNA load in INR cART-treated chronic HIV-infected patients.

d. Analyzed by Wilcoxon signed-rank test.

iii) Δblood CD4 count:

a. Δ defined by changes between end of study (week 8) and baseline (week 0).

b. Assessed by routine CD4 cell quantification at Dep. of Immunology, OUH.

c. H_0: Eight weeks treatment with probiotics does not change blood CD4 cell count in INR cART-treated chronic HIV-infected patients.

d. Analyzed by Wilcoxon signed-rank test.

Secondary endpoints: (see section 2.2)

The statistical analysis strategy is described below. As the efficacy assessments of this study are of exploratory nature, the exact parameters assessed and variable to be analyzed are difficult to predict. The conductance of the statistics may diverge from the planned analyses.

- Alterations are defined by:
    - Changes in *variable* between end of study (week 8) and baseline (week 0) in INR cART-treated chronic HIV-infected patients.

  or

    - Differences in *variable* between INR cART-treated chronic HIV-infected patients (week 0) (cases) and cART-treated immunological responding chronic HIV-infected patients (controls) and HIV negative subjects (healthy controls).

- Null hypothesis (H_0) will be formulated as:
    - H_0-1: Eight weeks treatment with probiotics does not alter the v*ariable* in INR cART-treated chronic HIV-infected patients.

      or

    - H_0-2: INR cART-treated chronic HIV-infected patients (week 0) (cases), cART-treated immunological responding chronic HIV-infected patients (controls) and HIV negative subjects (healthy controls) have equal *variable*.

- Statistical tests will consist of:

- Composition of microbiota tentatively analyzed by principle components analysis and Monte Carlo permutation test. Microbiota diversity tentatively analyzed by Shannon index and Simpson reciprocal index.

- For single variable analyses H_0-1s analyzed by Wilcoxon signed-rank test and H_0-2s analyzed by One-way ANOVA with Bonferroni posttests.

11 STUDY MANAGEMENT

11.1 Investigator Delegation Procedure

The PI is responsible for making and updating a "delegation of tasks" listing all the involved co-workers and their role in the project. He will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

11.2 Protocol Adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations.
All significant protocol deviations will be recorded and reported in the Clinical Study Report (CSR).

11.3 Study Amendments

If it is necessary for the study protocol to be amended, the amendment and/or a new version of the study protocol (Amended Protocol) must be notified to and approved by the Competent Authority and the Ethics Committee according to EU and national regulations.

No changes from the final approved and signed protocol will be made without the Competent Authority's and the Ethics Committee's prior written approval of an amendment. Exceptions are when i) necessary to eliminate immediate hazards to the study subjects, or ii) when the change involves only logistics or administration. The investigator will sign the protocol amendment.

11.4 Audit and Inspections

Authorized representatives of a Competent Authority and Ethics Committee may visit the centre to perform inspections, including source data verification. Likewise the representatives from sponsor may visit the center to perform an audit. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP, and any applicable regulatory requirements. The PI will ensure that the inspectors and auditors will be provided with access to source data/documents.

12 ETHICAL AND REGULATORY REQUIREMENTS

The study will be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements. Registration of patient data will be carried out in accordance with national personal data laws.

12.1 Ethics Committee Approval

The study protocol, including the patient information and informed consent form to be used, must be approved by the regional ethics committee before enrolment of any patients into the study.

The investigator is responsible for informing the ethics committee of any serious and unexpected adverse events and/or major amendments to the protocol as per national requirements.

12.2 Other Regulatory Approvals

The protocol will be submitted and approved by the applicable competent authorities before commencement of the study.

The protocol will also be registered in www.clinicaltrials.gov before inclusion of the first patient.

12.3 Informed Consent Procedure

The investigator is responsible for giving the patients full and adequate verbal and written information about the nature, purpose, anticipated benefit, potential hazard, and insurance arrangements of the study. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever she/he wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements. The investigator is responsible for obtaining signed informed consent.

A copy of the patient information and consent will be given to the patients. The signed and dated patient consent forms will be filed in the Investigator Site File binder.

12.4 Subject Identification

The investigator is responsible for keeping a list of all patients (who have received study treatment or undergone any study specific procedure) including patient's date of birth and personal number, full names and last known addresses.

The patients will be identified in the CRFs by patient number, initials, and date of.

13 TRIAL SPONSORSHIP AND FINANCING

This study is initiated and conducted by the investigators. The study is funded by the K.G. Jebsen Inflammation Research, Centre which is sponsored by the University of Oslo and Oslo University Hospital in addition to the gross funding from the K.G. Jebsen Foundation.

Additional research grant applications for the study may be submitted to the regional health authorities (Helse Sør-Øst RHF).

Proposal for Investigator Initiated Trial programs at the company manufacturing the investigational product may be submitted.

14 TRIAL INSURANCE

The PI has insurance coverage for this study through membership of the Drug Liability Association (see http://www.laf.no for more details).

15 PUBLICATION POLICY

Upon study completion and finalization of the study report the results of this study will either be submitted for publication and/or posted in a publicly assessable database of clinical study results.

The results of this study will also be submitted to the Competent Authority and the Ethics Committee according to EU and national regulations.

All personnel who have contributed significantly with the planning and performance of the study (Vancouver convention 1988) may be included in the list of authors.

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