Clinical Study Protocol

AN OPEN LABEL FEASIBILITY STUDY OF FECAL MICROBIOTA TRANSPLANTATION IN PATIENTS WITH MALIGNANCIES NOT RESPONDING TO CANCER IMMUNOTHERAPY

Short title: Malignancies FMT

Study Type:	Health-related intervention
Study Categorisation:	Other Clinical Trial Category B
Study Registration:	Swiss National Clinical Trials Portal (SNCTP): **SNCTP000004841** ClinicalTrials.gov: **NCT05273255**
Sponsor-Investigator:	Prof. Dr. med. Michael Scharl Department of Gastroenterology & Hepatology University Hospital Zürich Rämistrasse 100 CH 8091 Zürich Switzerland Phone: + 41 44 255 3419 E-Mail: michael.scharl@usz.ch
Study Intervention:	Colonoscopic administration of Fecal Microbiota
Protocol Version and Date:	v1.6 dated 27.11.2023

SIGNATURE PAGES

Study number	*Study registry and registration number* SNCTP: **SNCTP000004841**
Study Title	An open label feasibility study of fecal microbiota transplantation in patients with malignancies not responding to cancer immunotherapy

Sponsor-Investigator:

This clinical trial protocol was subject to critical review and has been approved by the Sponsor-Investigator. The information herein is consistent with:

- the current risk/benefit evaluation of the intervention
- the moral, ethical and scientific principles governing clinical research as set out in the current version of the Declaration of Helsinki, Good Clinical Practice.

Prof. Dr. med. Michael Scharl

Place/Date	Signature

Biometrician:

Dr. Yasser Morsy, PhD

Place/Date	Signature

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STUDY SYNOPSIS

Sponsor-Investigator	Prof. Dr. med. Michael Scharl
Study Title:	An open label feasibility study of fecal microbiota transplantation in patients with malignancies not responding to cancer immunotherapy
Short Title / Study ID:	Cancer FMT
Protocol Version and Date:	v1.6 of 27.11.2023
Trial registration:	**SNCTP000004841** **NCT05273255**
Study category and Rationale:	Other Clinical Study, Category B
Background and Rationale:	The intestinal microbiome forms a symbiotic relationship with the human host and continuously interacts with its immune system. Specific compositions of the intestinal microbiome in patients with cancer have been linked to the response to therapy with cancer immunotherapies (CI), such as immune checkpoint inhibitors (ICIs). We hypothesize that fecal microbiota transplantation (FMT) from patients being responsive to ICI therapy (FMT-Donor) can modulate the intestinal microbiome of patients with CI-refractory malignancies (FMT-Recipients) and render them into responders. Successful proof-of-concept studies showed that reversion from an ICI non-responsive to a responsive disease is indeed possible in melanoma patients after FMT. This trial expands the FMT intervention to patients with any malignancy treated with cancer immunotherapy as a standard of care, to demonstrate the feasibility of this FMT approach as a novel option in cancer therapy.

Objective(s):	Primary Objective: To investigate whether one-time treatment with ICI responder-derived FMT is feasible to induce a change of the intestinal microbiome after 24 weeks post FMT. Secondary Objectives: 1) To investigate the safety of FMT in cancer patients as measured by the presence of AEs. 2) To study whether FMT induces clinical response to cancer immunotherapy in patients without previous therapy success after 24 weeks as measured by objective response rate (ORR) by iRECIST, progression-free survival (PFS), and overall survival (OS). 3) To investigate whether FMT administration affects the composition and function of T-cells and innate/adaptive immune cell subsets in recipients after 6, 12 and 24 weeks. 4) To investigate whether FMT administration affects the composition of the intestinal microbiome in recipients after 6, 12 and 24 weeks. 5) To investigate whether FMT administration affects the quality of life in FMT-Recipients. Exploratory Objectives: 1) To determine whether cancer immunotherapy response in patients is associated with a distinct gut, intestinal tissue and PBMC-derived microbiota profile. 2) To determine whether cancer immunotherapy response in patients is associated with common metabolomics profile. 3) To determine whether cancer immunotherapy response in patients is associated with a distinct immune cell phenotype and T-cell subtypes (proteomics and single cell RNAseq). 4) To determine whether cancer immunotherapy response in patients is associated with a distinct immune cell composition within the tumor tissue (imaging mass cytometry). 5) To determine whether the ICI treatment type received by FMT-Donor need to match the cancer immunotherapy treatment received by FMT-Recipient with the refractory cancer (including single vs. combination and anti-CTLA-1 vs. anti-PD-1). 6) To determine whether liquid biopsy (i.e. circulating tumor cells enumeration and composition) can be

	used as a predictive biomarker for the cancer immunotherapy response after receiving FMT. 7) To determine whether cancer immunotherapy response in patients is associated with serum cytokines.

Outcome(s):	<u>Primary Study Outcome:</u> The Primary Outcome for this study is to assess the feasibility of the FMT approach to induce a change in the intestinal microbiome composition, which can bring a favorable cancer immunotherapy response in cancer patients previously not responding to cancer immunotherapy. <u>Secondary Outcomes:</u> 1) Safety outcomes will be assessed based on: • Incidence of adverse events (AEs) • Incidence of serious adverse events (SAEs) 2) Efficacy of FMT approach as measured by objective response rate (ORR) by iRECIST, progression-free survival (PFS), and overall survival (OS). 3) Evaluation of CD8+ and CD4+ T-cells, dendritic cells and innate cell composition and activity in peripheral blood of ICI responders (i.e. FMT-Donors), and additionally in intestinal and tumor tissue for FMT responders and FMT non-responders (i.e. FMT-Recipients). 4) Analysis of bacterial taxa occurrence (within-sample and between-sample differences) in ICI responders, FMT responders and FMT non-responders. 5) Quality of life based on the questionnaire. <u>Exploratory Outcomes:</u> 1) Stool samples will be collected pre-FMT (between screening visit and Baseline visit), after 6, 12 and 24 weeks using the OMNIgene GUT stool collection kits. Approximately 5g of stool per visit. Stool samples will be analyzed by 16s sequencing and shotgun metagenomics. 2) Serum and stool metabolome will be quantified by mass spectrometry at baseline and after 6, 12 and 24 weeks after FMT using 1ml of serum per visit and at baseline and after 6 and 24 weeks after FMT using app. 5g stool per visit. 3) Proteomics analyses (from 1 ml of serum collected at the baseline and after 6, 12 and 24 week and peripheral blood mononuclear cells) and single cell RNAseq from PBMCs, intestinal biopsies and tumor tissue. 4) Imaging mass cytometry (IMC) will be performed on tumor tissues collected from the Pathology and Dermatopathology Departments. 5) Metagenomic analyses will be performed on stool, PBMC and intestinal biopsy samples collected pre-FMT, after 6, 12 and 24 weeks.

	6) Enumeration and composition of circulating tumor cells (CTCs) in recipients at baseline, after 6, 12 and 24 weeks using 10 ml whole blood. 7) Serum cytokines will be quantified for FMT-Donors at Visit 1 and for FMT-Recipients at the baseline, and after 6, 12 and 24 weeks using 1ml serum per visit.
Study design:	Open label, non-randomised feasibility study

Inclusion /Exclusion criteria:	General inclusion criteria for all participants: • Patients, at minimum 18 years of age, male or female • Signed informed consent obtained from subject according to local regulations • ECOG score at the time of study enrolment 0-1 Two groups of participants will be included based on the following criteria: 1. FMT-Donor: ICI responder with stage III or IV (metastatic) solid cancer treated with immune checkpoint inhibitors who have experienced a durable partial or complete response as decided by the treating physician based on the objective response rate (ORR) by iRECIST . Patients treated recently with antibiotics and/or suffering from gastrointestinal, autoimmune, neurologic or certain infectious diseases will be excluded as potential participants. 2. FMT-Recipient: Stage IV cancer patient who has not sufficiently responded (stable disease or non-response) after at least 1 full cycle of cancer immunotherapy, as decided by the treating physician based on the objective response rate (ORR) by iRECIST. Patients with absolute contra-indications to colonoscopy, presence of brain metastases, currently treated with antibiotics and suffering from immunodeficiency, severe food-allergies or certain infectious diseases will be excludes as potential participants. For more detailed criteria please see Section 6.2.
Study Intervention:	Single-dose of fecal microbiota from FMT-Donor transplanted endoscopically to FMT-Recipient in between two cycles of cancer immunotherapy.

Number of Participants with Rationale:	Total number of enrolled patients: 30 (5 Donors and 25 Recipients) We used R package micropower to simulate a range of different effect sizes for subject groups comprising 5, 15 and 25 subjects, and show that having 25 subjects allows 90% power to detect effect size (ω^2) of 0.0025, which is less than the effect observed in other microbiome studies. Number of enrolled Donors: 5 Number of enrolled Recipients: 25 Each FMT-Donor will provide enough stool required for performing FMT in 5 FMT-Recipients.
Study Duration:	This trial duration is 36 months (3 years).
Study Schedule:	March 2022 First-Participant-In August 2024 Last-Participant-Out March 2025 Final Study Report
Study Centre:	This is a single-center study performed at: Department of Gastroenterology and Hepatology University Hospital Zürich Rämistrasse 100 8091 Zürich, Switzerland
Statistical Considerations:	Microbial taxa changes and abundances will be tested for statistical significance by using Wilcoxon rank-sum test (adjustments for multiple comparisons are done with FDR method at an alpha level of 0.05).
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements. The study will also follow the respective BRISQ (Biospecimen Reporting for Improved Study Quality) Guideline regarding the collection of human biological material (Biobanking), Appendix I.

Investigators:	**PD Dr. med. Alessandra Curioni** Center for Hematology and Oncology University Hospital Zurich Raemistrasse 100, 8091 Zürich, Switzerland Phone: +41 (43) 253 92 98 E-Mail: Alessandra.Curioni@usz.ch **Prof. Dr. med. Reinhard Dummer** Clinic for Dermatology University Hospital Zurich Raemistrasse 100, 8091 Zürich, Switzerland Phone: +41 (43) 253 09 38 E-Mail: Reinhard.Dummer@usz.ch **Dr. med. Ralph Fritsch** Clinic for Oncology and Hematology University Hospital Zurich Raemistrasse 100, CH 8091 Zürich, Switzerland Phone: +41 (44) 255 48 74 E-Mail: Ralph.Fritsch@usz.ch **Prof. Dr. med. Andreas Kremer** Department of Gastroenterology and Hepatology University Hospital Zurich Raemistrasse 100, 8091 Zürich, Switzerland Phone: + 41 44 255 85 48 E-Mail: Andreas.Kremer@usz.ch **Prof. Dr. med. Anja Lorch** Department of Medical Oncology and Hematology University Hospital Zurich Raemistrasse 100, 8091 Zürich, Switzerland Phone: +41 44 255 22 14 E-Mail: Anja.Lorch@usz.ch **PD Dr. med. Cédric Poyet** Clinic for Urology University Hospital Zurich Raemistrasse 100, 8091 Zürich, Switzerland Phone: +41 (43) 253 77 46 E-Mail: Cedric.Poyet@usz.ch

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse Event
ALAT	Alanine transaminase
ASAT	Aspartate transaminase
CEC	Competent Ethics Committee
CI	Cancer immunotherapy
ClinO	Clinical Trials Ordinance
CRC	Colorectal Cancer
CRF	Case Report Form
CRP	C-reactive protein
eCRF	Electronic Case Report Form
CTC	Circulating Tumor Cell
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
ECOG	Eastern Cooperative Oncology Group
GCP	Good Clinical Practice
H0	Null hypothesis
H1	Alternative hypothesis
ICH	International Council on Harmonization
ICI	Immune Checkpoint Inhibitor
IMP	Investigational Medicinal Product
iRECIST	Immune Response Evaluation Criteria In Solid Tumors
ISF	Investigator Site File
LHR	Law on Human Research
MSI	Microsatellite Instability
ORR	Objective Response Rate
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cell
PFS	Progression-Free Survival
PI	Principal Investigator
SAE	Serious Adverse Event
SDV	Source Data Verification
SmPC	Summary of Product Characteristics
SNCTP	Swiss National Clinical Trial Portal
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
USZ	Universitätsspital Zürich (University Hospital Zuirch)
VRE	Vancomycin-resistant Enterococcus

STUDY SCHEDULE

Schedule of Assessments for the FMT-Recipients

Study Periods	Screening	FMT Intervention (Baseline)	Study visit 1	Study visit 2	End-of-study visit	Unscheduled visit
Visit	**1**	**2**	**3**	**4**	**5**	**unscheduled**
Week	**-2**	**0**	**6**	**12**	**24**	
Study Day	**-14 ±14**	**0**	**42 ±14**	**84 ±21**	**168 ±21**	
Informed Consent	x[a]					
In- /Exclusion Criteria	x	x[b]				
Demographics	x					
Medical History and Family History	x					
Concomitant Medication	x	x	x	x	x	x
Quality of Life Questionnaire	x		x	x	x	x
Dietary Questionnaire	x				x	x
Imaging results extraction[c]	x			x	x	
Physical Examination	x		x	x	x	x
Vital Signs (pulse, blood pressure, temperature, respiratory rate), Body Height[d], Body Weight	x	x	x	x	x	x
Dispense Laxative Kit	x					
Pregnancy Test from Urine	x					
Dispense Stool Collection Kit for microbiome sequencing	x[e]	x[e]	x[e]	x[e]	x[e]	x[e]
Dispense Stool Collection Kit for metabolomics	x[e]		x[e]		x[e]	
Dispense Stool Collection tube for mouse model	x					

validations (10ml 17% glycerol)						
Safety Lab from Blood (2x 10ml Blood Tube and 2x 3ml tube)	x					
Serum Collection (metabolite analyses, 1x 10ml Serum Tube)	x	x	x	x	x	x
Whole Blood Collection (immune cells analyses, 2x 10ml EDTA tube)	x	x	x	x	x	x
Sars-CoV-2 PCR	x					
Whole Blood Collection (CTC analyses in liquid biopsy 1x 10ml EDTA Tube)		x	x	x	x	x
Colonoscopy for Fecal Microbiota Transplantation		x[f]				
Colon Biopsies		x			x[g]	
Adverse Events and Serious Adverse Events		x	x	x	x	x

x[a] - ICF and Patient Information document will be provided minimum 24h before, either in person or via e-mail

x[b] - lab results assessed before the FMT is performed

x[c] - patients will undergo PET-CT/PET-MRI or CT scan before screening as well at around week 12 and week 24 of the study for clinical routine treatment outside of the frame of this study. By signing the ICF for this study, patient allows for the access to the previously stored samples and the imaging data to be used for the purpose of this study, without any further contact.

x[d] – body height to be measured only at screening

x[e] - to be returned filled with stool within 7 days after the visit

x[f] - on the day before FMT a patient is required to perform bowel cleansing using MoviPrep provided during the screening visit.

x[g] - collected during rektosigmoidoscopy

Schedule of Assessments for the FMT-Donors

Study Periods	Screening Period	Stool donation up to 170g
Visit	**1**	**2[a]**
Week	**1**	**1-3**
Study Day	**7 ±3**	**14 ±7**
Informed Consent	x	
In- /Exclusion Criteria	x	x
Demographics	x	
Medical History and Family History	x	
Concomitant medication	x	
Dietary Questionnaire	x	
Physical Examination	x	
Vital Signs (pulse, blood pressure, temperature, respiratory rate), Body Height, Body Weight	x	
Dispense Stool Collection Kit (donation container)	x	x[b]
Safety tests from Blood	x	
Whole Blood Collection (PBMCs analyses, 2x 10ml EDTA tube)	x	
Serum Collection (metabolite analyses, 1x 10ml Serum Tube)	x	
Stool donation		x
Safety tests from Stool (app. 5g parasitology, 5g virology and 5g microbiology)		x[c]
Microbiome sequencing from stool (app.5g)		x
Metabolome from stool (app. 5g)		x

Stool infusate preparation (30g stool/infusate + 5ml infusate reserve for mouse model validations)		x
Sars-CoV-2 PCR	x	

x^a – up to 2 additional visits are only required when the total stool quantity obtained on previous visit(s) was lower than 170g and therefore not sufficient to prepare 5 infusates, safety labs and microbiome sequencing

x^b – only if additional visits are necessary to bring the total obtained stool volume to 170g

x^c – will be repeated for every stool sample used for infusate preparation

1. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor-Investigator

This is an Investigator Initiated Trial where Sponsor-Investigator is Prof. Dr. Michael Scharl from the Department of Gastroenterology and Hepatology at the University Hospital Zürich. During the course of the trial, Prof. Scharl will be performing the FMT procedure. Prof. Scharl is a main contributor to this study design and will be responsible for data management and interpretation. Prof. Scharl will also oversee the report writing.

Prof. Scharl is Senior Chief Physician in the Department of Gastroenterology and Hepatology. He is Board certified (FMH) for General Internal Medicine as well as Gastroenterology and has long-standing experience in clinical trials and translational research.

Prof. Dr. med. Michael Scharl

Department of Gastroenterology and Hepatology

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1.2 Investigator(s)/ Study Sites

All investigators are employed by the University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland. They will be equally involved in the patient referral, routine clinical case of the study patients and interpretations of the results. To this end, we will established collaboration with the following clinicians.

PD Dr. med. Alessandra Curioni is responsible for the treatment of patients with lung cancers in the Center for Hematology and Oncology.

Phone: +41 (43) 253 92 98

E-Mail: Alessandra.Curioni@usz.ch

Prof. Dr. med. Reinhard Dummer is responsible for the treatment of patients with skin cancers (melanoma and squamous cell cancer) in the Clinic for Dermatology.

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Dr. med. Ralph Fritsch is responsible for the treatment of patients with CRC, stomach cancer, esophageal cancer, pancreatic cancer and HCC in the Clinic for Oncology and Hematology.

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Prof. Dr. med. Andreas Kremer is responsible for the treatment of patients with liver cancers in the Clinic of Gastroenterology and Hepatology.

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Prof. Dr. med. Anja Lorch is responsible for the treatment of patients with kidney, prostate, bladder and testical cancer.

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PD Dr. med. Cédric Poyet is responsible for the treatment of patients with urological cancers (prostate cancer, bladder cancer, renal cancer) in the Clinic for Urology

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Prof Dr. med. Achim Weber is a Deputy Director of the Institute for Pathology and Molecular Pathology.

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1.3 Statistician (“Biometrician”)

Dr. Yasser Morsy, PhD

Biometrician

University Hospital Zurich c/o University of Zurich

Department of Gastroenterology and Hepatology

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1.4 Laboratory

The analysis of study-relevant samples will be performed mainly at the University Hospital Zurich. Depending on the sample type, multiple diagnostic laboratories will be involved. This includes:

Institute of Clinical Chemistry

University Hospital Zurich

Raemistrasse 100, CH 8091 Zurich, Switzerland

Tel: +41 44 255 22 60

E-mail: studies.ikc@usz.ch

Clinical sample analyses will be performed at the Institute of Medical Microbiology.

Institute of Medical Microbiology

University Hospital Zurich

Gloriastrasse 28, CH 8006 Zurich, Switzerland

Tel: +41 44 634 26 85

E-mail: labor_imm@imm.ch

Clinical stool sample analyses (i.e. detection of infectious agents except parasites) will be performed at the Institute of Medical Microbiology.

Institute of Parasitology

University of Zurich

Winterthurerstrasse 266a, CH 8057 Zürich, Switzerland

Tel. +41 (044) 635 8501

E-mail: info.dzp@uzh.ch

Stool parasites will be analyzed by the Institute of Parasitology.

Institute of Medical Virology

University Hospital Zurich, Switzerland

Winterthurerstr. 190

CH 8057 Zurich

Tel. +41 44 634 26 53

E-mail: virusdiagnostig@virology.usz.ch

Virology samples (SARS-CoV-2) will be performed at the institute of Medical Virology.

Central Laboratory of Clinic for Hematology

University Hospital Zurich

Raemistrasse 100, CH 8091 Zurich, Switzerland

Tel: +41 44 634 38 99

E-mail haematologielabor@usz.ch

Clinical blood sample analyses (except the detection of infectious agents) will be performed at the Institute of Medical Microbiology.

Clinic for Immunology

University Hospital Zurich

Raemistrasse 100, CH 8091 Zurich, Switzerland

Tel: +41 44 634 27 00

E-mail labor.immunologie@usz.ch

Detection of infectious agents (HIV, HAV, HBV, HCV) from blood will be performed at the laboratory of the Clinic for Immunology.

Clinic for Dermatology

Raemistrasse 100, CH 8091 Zurich, Switzerland

Tel +41 44 25 53155

E-mail dermatologie@usz.ch

Detection of Syphilis from blood will be performed at the laboratory of the Clinic for Dermatology.

1.5 Radiology Study Team

Information about objective response rate (ORR) by iRECIST will be provided by the Institute of Diagnostic and Interventional Radiology at USZ.

Institute of Diagnostic and Interventional Radiology

University Hospital Zurich

Raemistrasse 100, CH 8091 Zurich, Switzerland

Tel: +41 43 254 41 10

E-mail : radiologie@usz.ch

1.6 Monitoring Institution

Monitoring of this study will be performed externally by the Clinical Trials Center (CTC) from the University Hospital Zurich. To this end, the following actions will be taken by CTC:

1) Site Qualification Visits (SQV): Review of study feasibility according to protocol, assessment of resources, discussion of responsibilities.
2) Site Initiation Visits (SIV): safety management and GCP refresher, recapitulation of project workflow and correct documentation.
3) Routine Monitoring Visits (RMV): Logging of project progress, quality control of collected data and study documentation, optimization of processes, monitoring of safety management.
4) Close-out visits (COV): Control of documentation, final meeting for reporting and archiving.

A study-specific risk assessment will be conducted in order to design a risk-based monitoring plan. First 2 FMT-Donors and 2 FMT-Recipients enrolled in the study will be fully monitored and ICF-eligibility will be assessed (100%). Next, random patients will be chosen to cover between 50% of enrolled study cohort. Additionally, CTC will provide the support to close-out the study and properly archive all necessary documentation. Additional information will be included in the Monitoring Agreement.

A clinical research associate (CRA) will be assigned from:

Clinical Trial Center

University Hospital Zurich

Rämistrasse 100/MOU2, 8091 Zurich

Telefon +41 43 253 01 17

www.ctc-zkf.usz.ch

2. ETHICS AND REGULATORY ASPECTS

Before this study will be conducted, the protocol, the proposed participant information and consent form as well as other study-specific documents will be submitted to a properly constituted Competent Ethics Committee (CEC) in agreement with local legal requirements, for formal approval.

The decision of the CEC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from the CEC has been received.

2.1 Study Registration

The study will be registered in the SNCTP (Swiss National Clinical Trials Portal („Portal“) and in the international trial registry ClinicalTrials.gov.

2.2 Categorisation of Study

This study is classified as Other Clinical Study Category B.

This categorisation is adequate since we do not intend to use any drug or organ transplant, however, an invasive procedure (i.e. endoscopy with sedation) is being performed.

Overall, this health-related intervention entails more than minimal risks and burdens and is not recognized as standard in guidelines prepared in accordance with internationally accepted quality criteria.

2.3 Competent Ethics Committee (CEC)

Approval from the appropriate constituted Competent Ethics Committee is sought for each study site in the clinical trial. The reporting duties and allowed time frame are respected. No substantial amendments are made to the protocol without prior Sponsor and CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

Premature study end or interruption of the study is reported within 15 days. The regular end of the study is reported to the CEC within 90 days, the final study report shall be submitted within one year after study end. Amendments are reported according to chapter 9.4.

2.4 Ethical Conduct of the Study

The study will be carried out in accordance with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss competent authority's requirements.

CEC will receive annual safety and interim reports and be informed about non-substantial amendments, the course of the study, and the study stop/ end in agreement with local requirements.

2.5 Declaration of Interest

This study personnel claims no conflict of interests, including intellectual, financial or proprietary.

2.6 Participant Information and Informed Consent

The investigator will explain to each Participant (both FMT-Donor and FMT-Recipient) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment.

Each participant will be informed that his/her medical records may be examined by authorized individuals other than their treating physician.

All Participants for this study will be provided a participant information sheet and a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study.

The participant information sheet and the consent form will be submitted with the protocol for review and approval for the study by the CEC. The formal consent of a participant, using the

approved consent form, will be obtained before that participant is submitted to any study procedure.

The participant will be having an opportunity to read and consider the study participation before signing and dating the informed consent form, and will be given a copy of the signed document. The consent form will also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

2.7 Participant Privacy and Confidentiality

The investigators are liable to treat the entire information related to the study and the compiled data strictly confidentially. Any passing-on of information to persons that are not directly involved in the study must be approved by the owner of the information.

Data generation, transmission, archiving and analysis of personal data within this study, strictly follows the current Swiss legal requirements for data protection. Prerequisite is the voluntary approval of the Participant given by signing the informed consent prior start of participation of the clinical trial.

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Participant's confidentiality will be further ensured by utilizing participant identification code numbers to correspond to treatment data in the computer files.

Data generated as a result of this study are to be available for inspection on request by the monitors and by the CEC.

3. INTRODUCTION

3.1 Background and Rationale

Cancer is one of the most challenging medical conditions worldwide. Despite all improvements in screening and therapy, millions of people still die per year due to cancer [1]. Particularly in the metastatic setting, therapy options are limited and cure is almost never achieved. Surgical approaches are not highly successful or even recommended in the metastatic setting. Chemotherapy, targeted therapies or radiotherapy provide only a short-term benefit, while exerting tremendous side effects [2]. This further limits the quality of life of the cancer patients. Overall, many patients do not respond to such established therapeutic approaches. Recently, cancer immunotherapies (checkpoint inhibitor therapies) have been established and show a striking benefit in some patients [3,4]. However, though some patients have outstanding long-term benefits, cancer immunotherapies are only efficient in a minority of cancer entities, such as melanoma, MSI^{high} colorectal carcinoma, lung cancer or breast cancer. Also in those entities, response rates are limited to below 50% and long-term survival of a broad number of patients is still poor [5].

Thus, new and more efficient, but also safer therapeutic approaches for cancer treatment are urgently needed. Biomedical research has revealed that the response to cancer immunotherapies is critically dependent on the intestinal microbiome [6-11]. Thus, strategies to modulate the microbiome might offer a new avenue for the treatment of cancer patients. A clinically well-established way to modulate the microbiome is fecal microbiota transplantation (FMT), which is routinely used to treat refractory *Clostridioides difficile* infection [12-14]. Two small proof-of-concept studies have now provided evidence that FMT could also be useful in improving response to cancer immunotherapies [15,16]. Those FMT approaches were based on the fact that they

utilized stool from cancer patients responding to cancer immunotherapy (checkpoint inhibitor therapy) and transferred the stool via FMT to patients not responding to checkpoint inhibitor therapy.

The use of checkpoint inhibitor therapy has significantly increased in oncology and virtually all solid tumor entities are currently treated with checkpoint inhibitors in the frame of clinical trials. However, outcome is often poor, so strategies to improve results of checkpoint inhibitor therapy are needed. The principle of ICI therapies lies in the fact that they target immune checkpoint molecules that limit the antitumor immune response. Inhibition of those checkpoint molecules releases the break of the anti-tumor immune system and the tumor cells can be attacked again [17-18].

Immune checkpoint proteins (ICP), such as programmed cell death–1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) receptor-ligand interactions are commonly hijacked by tumors to suppress the anti-immune response. This process involves the blockade of cytotoxic CD8+ T cells from attacking the cancer cells. Accumulating evidence shows a strong correlation between tumor-infiltrating lymphocytes (TILs) and favorable patient outcome in various cancer types. In particular, the presence of CD8+ T cells correlates with improved prognosis and long-term survival, especially in solid tumors [19, 20].

Under healthy conditions, PD-1 and CTLA-4 are expressed on the cell surface of activated T cells in order to down-modulate unwanted or excessive immune responses, including autoimmune reactions. The mechanism by which PD-1 and CTLA-4 down modulate T-cell responses are comparable, as both molecules regulate an overlapping set of signaling proteins.

Although tissues lack the surface presence of PD-L and CTLA-4 ligands, multiple cancers types were shown to express significant levels of these T cell inhibitory molecules. Immune checkpoint inhibition (ICI) can be achieved with monoclonal antibodies targeting ICPs. This approach has been shown to be highly beneficial in almost 40% of patients with advanced melanoma [3,4,17, 21,22].

CTLA-4 was the first immune checkpoint with inhibitory functions to be described in detail [23]. Two targeted therapies with monoclonal antibodies (mAbs) against CTLA-4, ipilimumab (IgG1) and tremelimumab (IgG2), have demonstrated clinical activity in patients with advanced solid malignancies by augmenting effector T-cell–mediated immune responses. Mouse-based data suggest that these mAbs may also selectively deplete intratumoral FOXP3+ regulatory T cells via an Fc-dependent mechanism [18].

Pembrolizumab and Nivolumab are two examples of FDA-approved monoclonal antibody therapies targeting PD1 and interfering with ICPs allowing the activation of anti-tumor T cells. Both medicinal products are made up from a highly selective humanized mAb of the IgG4/kappa isotype. Pembrolizumab was first approved in the United States by the Food & Drug Administration (FDA) on September 4, 2014, for the treatment of patients with unresectable or metastatic melanoma and disease progression. In December 2014, Nivolumab received the approval for the same use cases. Few months later, both medicines were approved as monotherapy for the treatment of unresectable or metastatic melanoma in adults and for broader indications including squamous cell lung cancer, renal cell carcinoma and Hodgkin’s lymphoma.

Anti-PD1/PDL1 antibodies have become some of the most widely prescribed anticancer therapies. A combination therapy including both, anti-PD-1 and anti-CTLA-4 antibodies is currently widely used and the list of cancer-related indications continues to increase. This list includes, among many others, melanoma, lung cancer, breast cancer, renal cell cancer, bladder cancer, colorectal cancer and squamous cell cancer.

As said, response to those medications seems to be dependent on the composition of the intestinal microbiota. The intestinal flora is comprised of the entire composition of microorganisms located in the gastrointestinal tract. This microbiome forms a symbiotic relationship with the human host, and actively contributes to intestinal health and disease. Intestinal dysbiosis has been implicated in a wide range of disease states including inflammatory bowel disease (IBD), obesity, allergic disorders, Type 1 diabetes mellitus, autism, obesity, and cancer in both human

and animal studies. Specifically in cancer, intestinal dysbiosis has been linked to both colorectal carcinogenesis [12] and response to anti-cancer therapies [8] including chemotherapy [24], radiotherapy and immunotherapy [9]. Mechanisms implicated in the role of intestinal microbiota in anti-cancer immune responses are complex and likely involve many interlinked pathways. Published data suggests that commensal microbes promote crosstalk between myeloid or lymphoid cells with dendritic cells, which can sensitize the tumor to anti-cancer immune therapies [25].

Several studies have shown the role of the gut microbiome in influencing therapy responses to CTLA-4 and PD-1/PD-L1 blockade in mouse melanoma models [6, 10]. Additionally, investigations of intestinal microbiome samples from patients with non-small cell lung cancer, renal cancers, and melanoma patients, have shown that the presence of certain bacteria species correlated with improved clinical outcome upon PD1 blockade [6, 7,11]. Metagenomics studies of stools from 100 lung and renal cancer patients revealed correlations between better clinical outcome upon PD-1 blockade and the relative abundance of Akkermansia muciniphila [7]. Importantly, oral administration of these microorganisms to non-responding tumor-bearing germ-free mice restored the efficacy of PD-1 blockade. In addition, 16S RNA sequencing of stools obtained from a limited number of melanoma patients showed increased alpha diversity and relative abundance of Ruminicoccaceae/Faecalobacterium bacteria in PD-1 responders and lower Bacteroidales abundance in PD-1 non-responders [11]. The above data suggest that ICI responders and ICI non-responders have a clearly distinct intestinal microbiota and this feature is common across different cancer types. The fact that these differences are present even before the treatment start provides an opportunity to modulate the composition of intestinal flora in order to increase the chances of successful therapy outcomes.

Importantly, most of the studies investigating the role of microbial composition in cancer therapy response have been performed in mice, which is challenging to translate directly into the clinical settings. Murine models, although helpful to dissect the underlying mechanisms of carcinogenesis, cancer progression and its interactions with the immune system, often fail to completely recapitulate the features of spontaneous human tumors. Moreover, intestinal microbiota can vary between mice with identical genetic backgrounds housed in similar conditions - with profound physiologic effects. Taking these factors together, the complexity and variability in human microbiota relative to mice, indicates the need of a detailed characterization of ICI-response in clinical settings.

Two recent proof-of-concept studies showed that transfer of stool-derived bacteria by fecal microbiota transplantation (FMT) can be successfully utilized to convert advanced melanoma patients from ICI non-responders to responders [15,16]. In both studies, including a total of 26 patients, response to continued checkpoint inhibitor therapy could be induced by responder FMT in about 30 % of patients. This represents a strong signal and a very encouraging outcome in this very difficult-to-treat patient collective. On a molecular level, the authors found that the FMT of responder stool induced a strong anti-tumor CD8 T-cell response in the new responders, which is what caused the clinical effect.

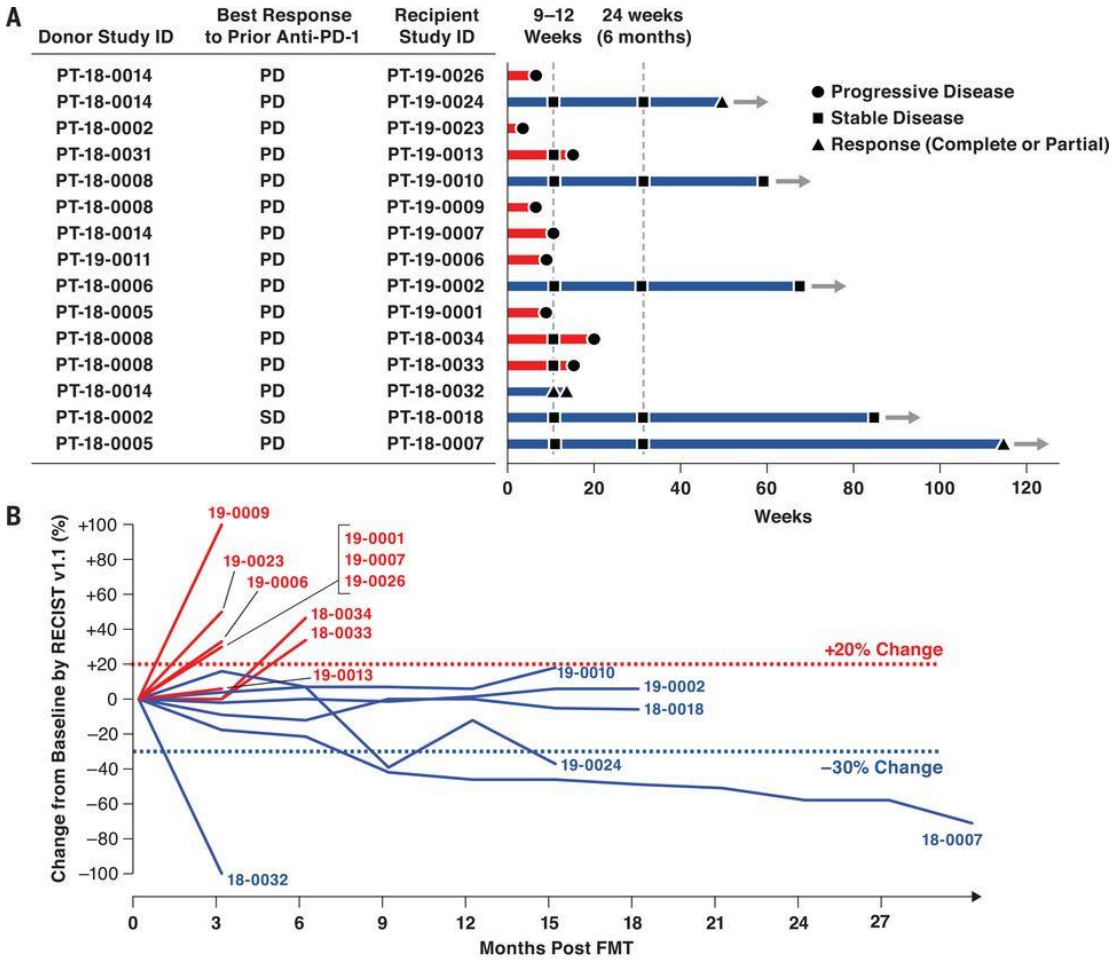


Fig. 1. Radiographic responses from a phase 2 study of anti–PD-1 responder–derived FMT and pembrolizumab in PD-1–refractory melanoma. Adapted from Davar et. al. Science 2021. (A) *FMT donor and best response to prior line(s) of anti–PD-(L)1 therapy singly or in combination are shown for each FMT-recipient patient. The length of each bar corresponds to the duration of time that patients received treatment (in weeks). Response status is color coded (R, blue; NR, red). Response symbols represent status at first restaging scan (9 to 12 weeks) and at most recent review. Patients with ongoing response in the study are depicted with horizontal arrows.* ***(B)*** *Radiographic change of tumor burden from baseline (investigator assessed per RECIST v1.1; n = 15).*

Based on those data, we propose to investigate whether the FMT approach could be successfully adapted to any cancer type when patients receiving cancer immunotherapy lack a significant treatment response. We will also study molecular mechanisms, which characterize therapy response, such as composition of transplanted microbiome in donor stool and changes in systemic and local response in stool recipients. For this reason, we will include patients with different cancer types and undergoing different therapies, rather than focusing only on 1 cancer type (eg. melanoma receiving anti-PD1 treatment). This will allow us to identify more general

mechanisms common across patients that do not respond to cancer immunotherapies. Our overarching goal is to identify specific bacteria strains that can be widely used as a cancer therapy.

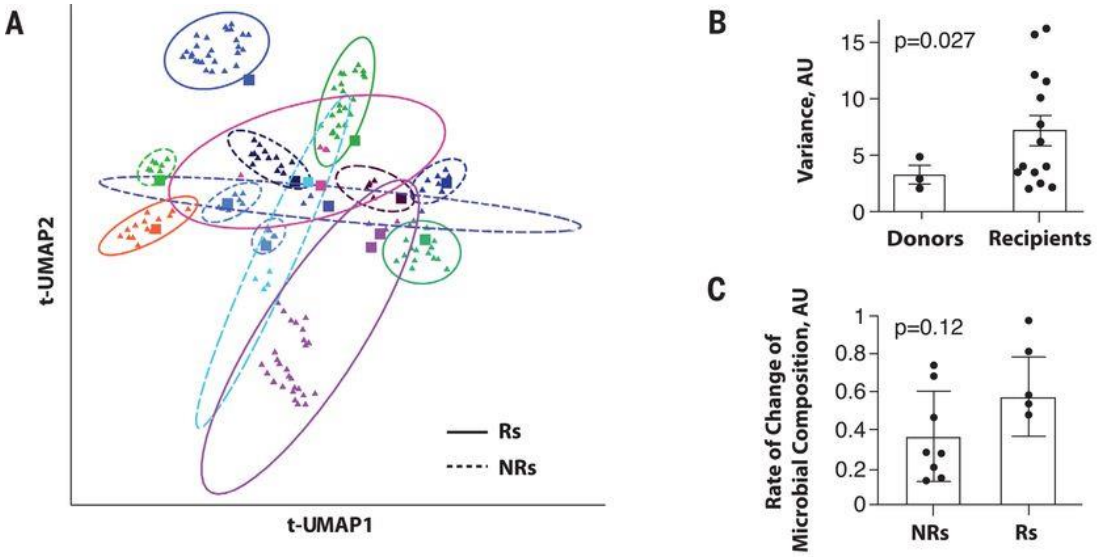


Fig. 2. Microbiome analyses before and after FMT in melanoma patients. Adapted from Davar et. al. Science 2021. (A) *Dimensionality reduction plot of microbial taxa abundances by last known taxon of FMT recipients at different time points. Each color corresponds to a different FMT-treated patient. Pre-FMT stool samples are depicted as squares and post-FMT stool samples as triangles. Ellipses encapsulate each recipient's pre- and post-FMT samples, and the size of the ellipse spans two standard deviations from the centroid. Rs and NRs are distinguished by solid and dashed lines, respectively.* ***(B)*** *Intrapatient variance of stool samples from donors and recipients after standardization and dimensionality reduction. Donors (n = 3) and recipients (n = 15) who contributed at least three fecal samples are depicted.* ***(C)*** *Rate of taxonomic change of stool samples sequentially obtained from treated patients.*

3.2 Study Intervention and Indication

Fecal Microbiota Transplantation (FMT) involves the transfer of stool from a carefully screened donor into the colon of a diseased patient that can benefit from the compositional change of the intestinal flora. The administration routes can vary from naso-enteric tube, orally taken capsules to the most direct method - colonoscopy. The method has gained rapid acceptance since the first modern use in 1958 [26].

The mode of action has been described as an establishment of a new gut microbiota community replacing the previous, dysbiotic bacterial community in the intestine of the recipient (patient). Repopulation of the gut with a healthy microbiome by FMT is well established in the treatment of antibiotic-refractory *Clostridioides difficile* infection. However, promising results suggest that FMT could also play an important role in the treatment of many other disorders, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), autoimmune disorders, allergic diseases, metabolic disorders such as obesity or even neuropsychiatric disorders such as autism. Recently,

new data strongly indicated that FMT could also be beneficial in patients suffering from immunotherapy-resistant melanoma [15,16].

3.3 Clinical Evidence to Date

Currently available clinical data for using FMT in ICI-resistant cancers are limited to two small feasibility studies in advanced melanoma patients by Davar, 2021 and Baruch, 2021. In these studies, a successful FMT contributed to conversion from ICI-refractory to ICI-responsive disease in 37.5% and 30% of patients, respectively. Notably, these early proof-of-concept studies included a limited number of patients as both donors (n=7 and n=6), as well as recipients (n=16 and n=10). Nevertheless, the patient safety and therapeutic efficacy of the FMT-based approach has been clearly confirmed, marking a way for larger-scale trials. In fact, in both trials even a complete response had been observed in a single patient each. Further, both trials showed significant molecular changes in the intestinal microbiome and in the immune cell compartments in the intestine as well as in the tumor tissue, providing further rationale for the potential efficacy of the FMT approach in the treatment of human cancer.

3.4 Justification of Study Intervention

Metastatic cancer is generally associated with a worse prognosis for the affected patients. Immune checkpoint inhibitors have been shown to be useful in a number of cases; however, the treatment failure is still frequent. Such patients have very limited additional treatment options, and regularly subsequent cycles of immunotherapy are being prescribed. Thus, new therapy approaches for such patients are urgently needed.

We will perform a one-time FMT with ICI-Responder stool via colonoscopy in patients that have not sufficiently responded to cancer immunotherapy or have not shown a significant improvement in response to the therapy (either stable diseases or non-response). This is the approach that had been performed by Davar et al. Importantly, the patients will continue with their regular cancer immunotherapy based on best medical judgement as per the treating physician's opinion as their standard medical therapy based upon accepted treatment guidelines. This will give us the opportunity to study the impact of responder stool FMT on CI-responsiveness when FMT is applied in between regular therapy cycles. Thus, the FMT approach will be performed as part of the regular cancer immunotherapy of the cancer patients. Alternative therapies would include already approved medications, such as chemotherapeutics, that have been extensively shown to have very limited efficacy, particularly in advanced stage cancer patients, as well as tremendous side effects.

3.5 Explanation for Choice of Comparator

No Comparator will be used for the study.

3.6 Risk/ Benefits and Ethical Considerations

According to the circumstances mentioned above, we believe that the Risk/Benefit ratio for any subject willing to participate in this trial would be extraordinarily good. As far as benefit, CI-refractory patients with metastatic cancer may potentially profit by clinical response or even remission, which may be expected to be of a comparable magnitude as in the two small feasibility trials [15,16]. A positive response might render a step up in medical therapy towards options with a worse side effect profiles and limited efficacy unnecessary. However, if cancer still progresses after FMT, a preliminary withdrawal from the study including increase of medical therapy according to the direction of the treating study physician would, of course, always be possible.

Regarding potential risks, we believe that the FMT procedure renders an excellent safety profile. To the best of our knowledge, there are no reports of serious or noteworthy side effects of FMT in the literature when a proper donor safety screening has occurred.

Thus, the risk to subjects in this trial will be minimized by compliance with inclusion/exclusion criteria, careful donor safety testing to avoid transmission of diseases/infections via the FMT, careful clinical monitoring, regular measurement of safety parameters, vital signs, strict adverse event documentation, and possibility for unscheduled visits and adherence to investigator guidance regarding specific safety areas. Screening, selection and inclusion of appropriate stool donors will be based on the European Consensus Guidelines for Fecal Microbiota Transplantation from 2019 [27].

FMT is generally considered as a safe and well-tolerable intervention. Thus, participating patients will most likely not have to expect any (serious) safety issues caused by the FMT. Nevertheless, the benefit for the participating patients experiencing the potential efficacy of the FMT would be enormous. Considering the fact that our study participants will be patients with metastasized, advanced cancer non-responding to standard therapy, demonstrating efficacy of our intended intervention offers the prospect of a significant benefit for those patients with malignancies not responding to immune checkpoint inhibitors. Inducing a response to immune checkpoint inhibitors by our FMT intervention would result in a considerable tumor response in the treated patients that might lead to improved overall survival, which would obviously be a great achievement for the respective patients. Considering this safety/anticipated benefit ratio, from an ethical perspective it is well plausible that this trial should be performed.

3.6.1 Risk of blood drawing

During blood withdrawal, swellings and itching can occur at the puncture of the needle. In rare cases infection as well as dizziness or faint can occur during the procedure. Blood draws are a part of clinical routine and carry minimal risk.

3.6.2 Risk of endoscopy

Colonoscopy, in which an endoscope is inserted into a colon through the anus, is in general considered a safe procedure. Colonoscopy is done routinely without major risk-related concerns, particularly if no interventions, such as polyp removal or stenting are performed. Occasionally, it can cause bleeding, tears in the colon, inflammation or infection. In most cases, colonoscopy will be performed under sedation, routinely using propofol. Propofol is considered a safe mean of sedation. Rarely, side effects such as a decline in oxygen saturation or hypotonia can occur which are in most cases self-limiting after stopping the propofol sedation. Colonoscopy with propofol sedation is performed about 2’500 times per year in our department, demonstrating that it is a highly standardized and safe procedure.

3.6.3 Risk of FMT

FMT via colonoscopy is characterized as a dispersion of a stool-derived solution directly inside/into the colon. FMT has no major risks and is generally well accepted as a safe and well tolerable approach. FMT does also not raise significant safety concerns after careful donor testing [27, 14]. Particularly, when comparing the safety of the proposed FMT approach with the safety profile of cancer therapies, e.g. chemotherapies or immunotherapies, our FMT approach will be clearly superior with respect to safety issues.

In addition to its overall safety in the general population, FMT has been shown to be relatively safe in immunocompromised adult patients, including solid organ transplant recipients and patients with IBD [13, 28-30]. In a recent multicenter pediatric FMT review analyzing the safety of FMT in 336 patients, the overall occurrence of serious AEs was only 5%. The most serious complications involved aspiration pneumonia following upper GI delivery of FMT (in 1 patient) and

worsening IBD symptoms requiring hospitalization following FMT. No death has been reported following FMT in pediatric patients.

Reported serious AEs in adults only have been related to aspiration occurring during colonoscopy for FMT delivery and aspiration of fecal content after nasoduodenal tube delivery [31]. A case of post-FMT colitis leading to death occurred in a 68 year-old man who developed pneumoperitoneum and sepsis within 3 days of FMT. Clear causality, however, is difficult to establish based on the case description [32]. A recent pediatric case series (42 patients, 47 FMTs, median age of 9 years) utilizing a nurse-led intragastric FMT procedure only reported vomiting as postprocedural complication (13%). In all cases, the vomiting was a single, self-limited episode that did not require medical treatment [33].

Common reported side effects of FMT include bloating, diarrhea, abdominal pain, constipation, and transient fever. A systematic review of FMT case reports documented a 0.6% incidence of worsening IBD symptoms following FMT, whereas a more recent study suggested worsening symptoms in 13% of adult patients with IBD post-FMT. Rates of flare in pediatric IBD patients may be lower than in adults, as was noted in the multicenter pediatric FMT cohort. Subsequent bacterial and viral infections have been reported as well, although causality is difficult to establish. Additional case reports describe medical conditions that developed post-FMT and include idiopathic thrombocytopenic purpura, Sjogren syndrome, peripheral neuropathy, and rheumatoid arthritis, but clear causation has not been established [14]. There is a minor possibility of the transfer of stool-borne multiresistant bacteria strain, however we have implemented testing for Vancomycin-resistant Enterococcus (VRE) in the FMT-Donor screening due to this risk. Any symptomatic infections will be immediately treated according to the guidelines. A Graft-versus-Host reaction reaction has never been described in the context of FMT and from a mechanistic point of view, it is highly unlikely, (if not excluded), since no human tissue is transplanted during FMT.

Considering all those data and particularly considering that we will perform FMT via colonoscopy and not via nasoduodenal tubes by an intragastral approach, we consider our FMT approach generally as very safe.

3.7 Study Population

Two distinct patient groups will be recruited for this trial: 1) FMT-Donors and 2) FMT-Recipients.

FMT-Donors are patients with any solid cancer, stages III or IV, who received any ICI therapy and have experienced a durable partial or complete response. These patients agree to donate stool as a source of fecal microbiota (bacteria, as part of their stool) that were potentially involved in a successful therapy response.

FMT-Recipients comprise of cancer patients with stage IV solid cancer of any origin who have not responded (stable disease or non-responder) after at least 1 full cycle of cancer immunotherapy, as decided by the treating physician. The patients agree to receive stool infusate from FMT-Donors via colonoscopy, as a source of bacteria that could potentially induce the response to next ongoing cancer immunotherapy.

4. STUDY OBJECTIVES AND OUTCOMES

4.1 Overall Objective

The purpose of this study is to evaluate the feasibility of FMT via colonoscopy to induce a change of the intestinal microbiome composition in cancer patient. The Donor stool will be derived from patient who responds to ICI therapy and applied to cancer patients who do not sufficiently respond to cancer immunotherapy in order to induce response in the recipients.

Feasibility will be assessed as measurable changes in the intestinal microbiome 24 weeks after FMT. It is important to understand whether FMT causes similar changes in the intestinal microbiome (and then in secondary test parameters, if applicable) in patients with different tumor entities in each case. This approach will tell us whether the FMT approach is effective in the same way in different tumor entities. This has not yet been investigated in any study and is, however, the fundamental requirement to be able to systematically initiate further FMT studies in cancer patients. From our point of view, an FMT approach only makes sense in patients with those cancer entities in which a change in the intestinal microbiome can be detected after FMT. Therefore, it is important here in the context of our study to investigate different tumor entities and also therapies (however, all belong to the same type of therapy, namely cancer immunotherapies). In summary, the microbiome changes will be systematically investigated in different tumor entities to provide meaningful results about microbiome changes and their impact on therapy response.

4.2 Specific Objectives and Outcomes

Primary Objective	**Primary Outcome**
To investigate whether one-time treatment with ICI responder-derived FMT is feasible **to induce a change of the intestinal microbiome after 24 weeks post FMT.** *Hypothesis: Utilization of FMT in patients with different solid cancer entities is a feasible approach to change the intestinal microbiome based on the data from patients with ICI-refractory melanoma.*	The Primary Outcome for this study is to assess the feasibility of the FMT approach to induce a change in the intestinal microbiome composition, which can bring a favorable cancer immunotherapy response in cancer patients previously not responding to cancer immunotherapy.
Secondary Objectives	**Secondary Outcomes**
1) To investigate the safety of FMT in cancer patients. *Hypothesis: FMT can be safely performed in cancer patients. The procedure does not lead to worsening of patient's health.*	1) Safety outcomes will be assessed based on: • Incidence of adverse events (AEs) • Incidence of serious adverse events (SAEs)

2) To study whether FMT induces response to cancer immunotherapy in patients without previous therapy success after 24 weeks. *Hypothesis: Failure to respond to immune checkpoint blockade in patients with advanced cancer is associated with intestinal dysbiosis and ICI responder-derived FMT can convert CI non-responders to responders.*	2) Efficacy of FMT approach as measured by objective response rate (ORR) by iRECIST, progression-free survival (PFS), and overall survival (OS)*.
3) To investigate whether FMT administration affects the composition and function of T-cells and innate/adaptive immune system subsets in recipients after 6, 12 and 24 weeks. *Hypothesis: ICI responder-derived FMT augments tumor antigen-specific CD8+ and CD4+ T-cell responses, dendritic cells maturation/function and innate immune responses to cancer.*	3) Evaluation of CD8+ and CD4+ T-cells, dendritic cells and innate cells composition and activity in peripheral blood of ICI responders (i.e. FMT-Donors), and additionally in intestinal and tumor tissue for FMT responders and FMT non-responders (i.e. FMT-Recipients).
4) To investigate whether FMT administration affects the composition of the intestinal microbiome in recipients after 6, 12 and 24 weeks. *Hypothesis: Endoscopic administration of stool from ICI responder induces changes in bacterial communities residing in the intestinal flora of FMT-Recipients.*	4) Analysis of bacterial taxa occurrence (within-sample and between-sample differences) in ICI responders, FMT responders and FMT non-responders.
5) To investigate whether FMT administration affects quality of life in FMT-Recipients. *Hypothesis: Enhanced response to cancer therapy after FMT improves the quality of life.*	5) Quality of life based on the questionnaire.
Exploratory Objectives	**Exploratory Outcomes**
1) To determine whether cancer immunotherapy response in patients is associated with a distinct gut, intestinal tissue and PBMC-derived microbiota profile. *Hypothesis: Cancer immunotherapy responders and non-responders have distinct gut commensal microbiota profiles; and that administration of responder-derived FMT can reconstitute responder-associated microbiota profile in CI non-responders.*	1) Stool samples will be collected pre-FMT (between screening visit and Baseline visit), after 6, 12 and 24 weeks using the OMNIgene GUT stool collection kits (1 kit per time point). Approximately 5g of stool per visit. Stool samples, intestinal tissue biopsies and PBMCs will be analyzed by 16s sequencing and shotgun metagenomics analyses.

2) To determine whether cancer immunotherapy response in patients is associated with common metabolomics profile. *Hypothesis: CI response can be induced by special microbiota profiles that exert distinct metabolic profiles.*	2) Serum and stool metabolome will be quantified by mass spectrometry at baseline and after 6, 12 and 24 weeks after FMT using 1 ml of serum per visit and at baseline and after 6 and 24 weeks after FMT using app. 5g stool per visit.
3) To determine whether cancer immunotherapy response in patients is associated with a distinct immune cell phenotype and T-cell subtypes (proteomics and single cell RNAseq). *Hypothesis: CI response can be induced by specific T-cell subsets.*	3) Proteomics analyses (from 1 ml of serum collected at the baseline and after 6, 12 and 24 week and peripheral blood mononuclear cells) and single cell RNAseq from PBMCs, intestinal biopsies and tumor tissue. PBMCs will be isolated from 20 ml whole blood collected at the baseline and after 6, 12 and 24 weeks. Intestinal tissue will be collected during colonoscopy as biopsies.
4) To determine whether cancer immunotherapy response in patients is associated with a distinct immune cell composition within the tumor tissue (imaging mass cytometry). *Hypothesis: CI response is dependent on specific spatial immune cell interactions.*	4) Imaging mass cytometry (IMC) will be performed on tumor tissue collected from the Pathology and Dermatopathology Departments
5) To determine whether the ICI treatment type received by FMT-Donor need to match the CI treatment received by FMT-Recipient with the refractory cancer (including single vs. combination and anti-CTLA-1 vs. anti-PD-1). *Hypothesis: Different microbiome composition is responsible for the response to different types of CI treatment.*	5) Metagenomic analyses will be performed on stool samples, PBMC and intestinal biopsy samples collected pre-FMT, after 6, 12 and 24 weeks.
6) To determine whether liquid biopsy (i.e. circulating tumor cells enumeration and composition) can be used as a predictive biomarker for the cancer immunotherapy response after receiving FMT. *Hypothesis: Response to CI can be linked to a particular composition and/or number of*	6) Enumeration and composition of circulating tumor cells (CTCs) in recipients at baseline, after 6, 12 and 24 weeks using 10 ml whole blood

CTCs and easily monitored with liquid biopsy. *7) To determine whether cancer immunotherapy response in patients is associated with serum cytokines.* *Hypothesis: Response to CI can be linked to a particular serum cytokines in FMT-Donors and FMT-Recipients.*	7) Serum cytokines will be quantified for FMT-Donors at Visit 1 and for FMT-Recipients at the baseline, and after 6, 12 and 24 weeks using 1ml serum per visit.

* Patients will undergo PET-CT/PET-MRI or CT scan before screening as well at around week 12 and week 24 of the study for clinical routine treatment outside of the frame of this study. By signing the ICF for this study, patient allows for the access to the previously stored samples and imaging data to be used for the purpose of this study, without any further contact.

5. STUDY DESIGN AND COURSE OF STUDY

5.1 General Study Design

This is a single-center, feasibility study of fecal microbiota transplantation (FMT) therapy, in patients with advanced malignant diseases who have not responded to cancer immunotherapy (CI). The impact of FMT, and not of the CI therapy, is the studied intervention, although CI therapy occurs also during the study duration as per clinical routine management of the patients.

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment details. No Comparator will be used for the study. A total of 30 patients will be enrolled, which includes 5 FMT-Donors and 25 FMT-Recipients as described in Chapter 6. Any discontinued participation will allow for the replacement with a new study subject until 30 subjects as specified above will reach follow-up.

Subjects will be screened between 0 and 14 days prior to baseline visit. At the baseline, subjects with cancer immunotherapy-refractory cancer will receive FMT via colonoscopy. All subjects will be followed up for 6 months after the FMT procedure (end of study for each patient) or early withdrawal. This study will include an additional research component involving collection of biological samples for exploratory immune, genomics, proteomics, metabolomics and microbiota analysis.

5.2 Study Duration and Study Schedule

Duration of the project in total: the project is expected to last 3 years.
Planned project start: 02.03.2022

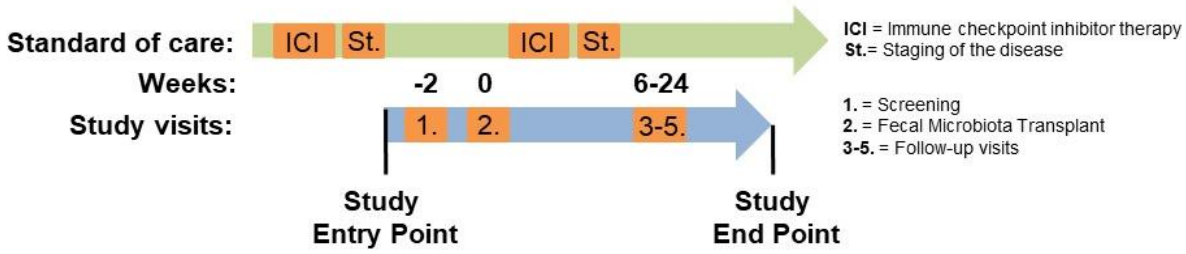
Planned project end: 31.02.2025

Study period for FMT recipients will start once the first patient has signed informed consent (planned in March 2022). Last patient's visit is planned for August 2024. The following molecular analyses will take place between March 2022 and December 2024. The final report to be

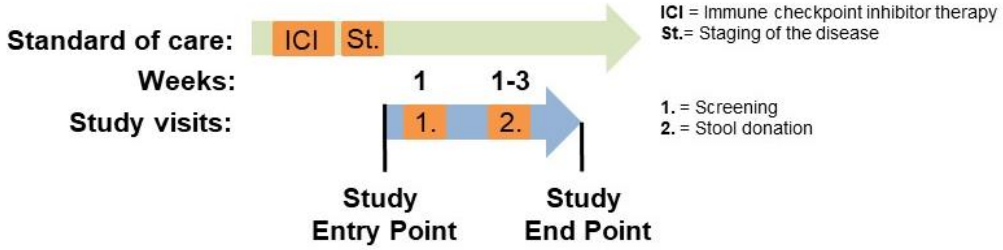
submitted by March 2025.

Study period for FMT donors will start once the first patient has signed informed consent (March 2022). Last patient's visit is planned for March 2024.

The trial starts with a screening (visit 1) at week -2, with signing the ICF for FMT-Recipients. Subsequently, visit 2 with the FMT procedure has to take place within 14 days (±7 days) after visit 1. After the 2nd visit, the patient returns back into standard of care treatment with the checkpoint inhibition therapy and staging necessary for clinical purposes. After 6 weeks, visit 3 will occur, where post-treatment samples will be collected. The same procedure will be repeated at 12 weeks and 24 weeks post intervention. The entire study can take up to 6 months.



For FMT-Donors the trials starts at screening upon ICF signature and finishes when a total of 170g of stool samples has been donated. The study will take a maximum of 3 weeks for this population.



5.3 Overview of study visits

Adverse events will be collected at every visit for every patient enrolled in the study, except Screening (Visit 1). In case of extreme difficulties to perform visits 3, 4 and 5 (e.g. due to the lockdown caused by pandemic), virtual assessments by Skype or Zoom will be allowed. In this case, no blood, serum or tissue samples will be collected, however stool samples will still be received (via mail).

Patient's eligibility for the study will be evaluated by the investigator based on medical history, physical examination, laboratory values and additional tests. To prepare for study participation, subjects will be instructed on the use of Concomitant Medications (Chapter 7.5). The study investigator or appropriate delegate at the site will discuss with each subject the nature of the study, its requirements, risks and benefits. Written informed consent must be obtained prior to performing any protocol-specific procedures. Detailed description of each visit can be found under Chapter 8.3.

5.4 Methods of Minimising Bias

The Tumor Board will suggest a study participant based on the response to current therapy and staging results as measured with iRECIST.

The therapy response after FMT will be confirmed by two physicians independently (1) leading physician (LP) or another physician from the same department as LP and 2) the PI of this study). The therapy response will be judged based on the most recent cancer staging results and will be documented on a dedicated Working Sheet as the source document (including therapy response, physicians name, date and signature) which will be then transferred to SecuTrial® (eCRF).

5.4.1 Randomisation

No randomization is necessary. The allocation of FMT-Donors to FMT-Recipients will be on “first come, first serve” bases. This means that FMT-Donors recruited at the beginning of the study will provide the stool samples for firstly enrolled FMT-Recipients.

5.4.2 Blinding Procedures

No blinding. Blinding of study participants or investigators is not foreseen due to the nature of the study intervention.

5.5 Unblinding Procedures (Code break)

No unblinding will be needed for the purpose of this study.

6. STUDY POPULATION

Adult subjects with advanced malignant disease can take part in the study depending on their previous standard of care treatment and staging status. Recipient eligibility is based upon prior exposure to cancer immunotherapy (CI). Patients must have received a minimum of 1 cycle of any CI therapy applied either as mono-therapy or combination therapy to be considered eligible. Patients who have received cancer immunotherapy in combination or in addition with other investigational agent(s) may be eligible at the discretion of the investigator. CI refractory disease is defined as stable or progressive disease (PD) at the first (or subsequent) radiographic while receiving cancer immunotherapy treatment as assessed by iRECIST on a restaging scan. Patients with stable disease as their best response are eligible; but patients with complete, mixed or partial response as their best response are ineligible. Other eligibility criteria include lack of contra-indications to FMT administration via colonoscopy.

6.1 Patient numbering

Patient will be numbered according to their entry date into the study. The first patient undergoing screening visit is patient number 1, the second is patient number 2, etc.

Total of 5 FMT-Donors and 25 FMT-Recipients will be included in the study. The enrollment goal is 1 FMT-Recipient per month. Importantly, at least 2 FMT-Donors should be recruited before the first FMT-Recipient. Each enrollment of FMT-Recipient will take place only when an infusate is already stored and can be assigned specifically to this patient.

6.2 Eligibility Criteria

Patients fulfilling all of the following inclusion criteria is eligible for the study. Written informed consent must be obtained after adequate information of the study patients before any assessment is performed. Patients must meet all of the following inclusion criteria to be eligible for the study.

6.2.1 Inclusion Criteria

General inclusion criteria for all participants:

- Patients, at minimum 18 years of age, male or female
- Signed informed consent obtained from subject according to local regulations
- ECOG[34] score at the time of study enrolment 0-1 according to the table:

Score	Definition
0	Fully active; no performance restrictions.
1	Strenuous physical activity restricted; fully ambulatory and able to carry out light work.
2	Capable of all self-care but unable to carry out any work activities. Up and about >50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair >50% of waking hours.
4	Completely disabled; cannot carry out any self-care; totally confined to bed or chair.

We will include patients/individuals fulfilling inclusion criteria for one of the following groups:

1. *Patients with refractory malignancy (FMT-Recipients)*. We will recruit 25 individuals with stable or progressing disease after minimum of 1 cycle of cancer immunotherapy.
2. *Patients with malignancy in remission after ICI therapy (FMT-Donors)*. We will recruit 5 patients willing to donate stool samples for the study.

Inclusion criteria FMT-Recipients:

1. Histologically or cytologically confirmed diagnosis of malignancy
2. Currently treated with cancer immunotherapy with at least 1 cycle completed. Multiple active malignancies are allowed.
3. Patient with stable or progressive disease as shown at the most recent staging method and decided by the treating investigator (based on the radiologic assessment).
4. Must be cancer immunotherapy refractory/resistant as judged by the treating physician based on a recent CT or PET-CT (PET-MRI) scan not older than 8 weeks before screening visit.
5. Willingness to receive FMT administered via colonoscopy and undergo necessary bowel preparation pre-procedure.
6. Demonstrate adequate organ function as defined below, all screening labs should be performed within 28 days of FMT intervention.

   Following laboratory parameters need to be met:

   – Platelet count ≥ 50 x 10^9 / L

- Hemoglobin ≥ 8.5 g/dL
- Prothrombin time (PT)-international normalized ration (INR) ≤ 1.5

7. Female subject of childbearing potential should have a negative urine pregnancy within minimum 8 hours prior to receiving the study intervention (FMT). If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
8. Female subjects of childbearing potential must be willing to use an clinically established method of contraception before the FMT procedure.

Inclusion criteria FMT-Donors:

1. Documented history of malignancy treated with ICI therapy.
2. Featuring partial or complete response of the malignancy as assessed by radiologic examination with a minimum duration of remission lasting ≥12 months measured since initiation of therapy.
3. Willingness to complete donor-specific questionnaire.
4. Willingness to complete donor-specific serologic and stool testing to evaluate infectious agents.
5. Patient tested negatively for all infectious agents specified.
6. Willingness to provide multiple stool samples, until total amount reaches 170g.
7. Absence of major gastrointestinal symptoms 3 months prior to stool donation (including frequent vomiting, diarrhea, bleeding, constipation).

6.2.2 Exclusion Criteria

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

Exclusion criteria FMT Recipients:

1. Presence of absolute contra-indications to colonoscopy and/or FMT administration:

- Toxic megacolon
- Inflammatory bowel disease
- Anatomic contra-indications to colonoscopy
- Colectomy

2. Patient is currently participating and receiving other study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of this study intervention.

3. Currently under any form of systemic antibiotics.

4. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy (> 10 mg prednisone daily or equivalent) or any other form of immunosuppressive therapy two weeks prior to trial treatment. Patients receiving systemic steroids at physiologic doses are permitted to enroll assuming steroid dose is not above the acceptable threshold (> 10 mg prednisone daily or equivalent).

5. Severe anaphylactic reaction to any food (food allergies).

6. Had a severe hypersensitivity reaction to propofol.

7. Has serious concomitant illnesses. The eligibility can be granted by the treating investigator on individual bases.

8. Has HIV infection or AIDS-related illness.

9. Has active infection of HAV, HBV or HCV. Patients with a history of Hepatitis B/C infection who have received anti-viral therapy and are disease free) may be considered for enrollment after discussion with Principal Investigator.

10. Patient has received a live vaccine within 4 weeks prior to the first dose of treatment. Seasonal influenza vaccines or COVID-19 vaccines for injection are generally inactivated virus vaccines and are allowed.
11. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
12. Females who are pregnant or breastfeeding.
13. Active central nervous system (CNS) metastases and/or leptomeningeal involvement.

Exclusion criteria FMT Donors:

1. History or current antibiotic treatment during the 2 month preceding donation.
2. History or current intrinsic gastrointestinal illnesses, including inflammatory bowel disease, irritable bowel syndrome, chronic diarrheal disorder (e.g. celiac disease or microscopic colitis) or major gastrointestinal surgical procedures.
3. History or current symptomatic autoimmune illness.
4. History or current documented neurologic or neurodevelopmental disorders.
5. History or current metabolic syndrome, obesity (BMI of >35), or moderate-to-severe malnutrition (as assessed clinically).
6. History or current infection with HIV (or AIDS-related illness).
7. Positive serological tests for Syphilis, HAV, HBV or HCV.
8. Positive stool test for Escherichia coli, Vancomycin-resistant Enterococcus, Norovirus, C. difficile, Yersinia, Campylobacter, Shigella or Salmonella.
9. Positive stool test for parasites.
10. Positive Sars-CoV-2 screening/testing (active infection).

Subjects whose pregnancy test on Screening visit shows a positive result, have to be excluded from the study. All pregnancies occurring during the treatment phase of the study and within 30 days after discontinuation of study medication have to be reported to the Investigator-Sponsor within one working day of the investigational sites knowledge of the pregnancy on the Initial Pregnancy Report Form. Female Subjects should be informed in this way before signing the informed consent form. Female partners of male participants may get pregnant without any consequence to the male participant.

6.3 Recruitment and Screening

The study population will consist of patients mentioned under the inclusion criteria. The subjects are patients of the investigators, who are being followed and treated at the University Hospital Zurich. After defining that a subject could take part at the present study project (e.g. during tumor board discussions), the possibility of participation will be explained by the treating physician. To this end, we will seek collaboration with investigators named in Section 1.2

The effective recruitment is the dialogue between an investigator and a potential participant prior to the initiation of the consent process. All subjects must sign and date the most current Competent Ethics Committee's (CEC) approved written informed consent before any project-specific assessments or procedures are performed. Participants may potentially benefit from this study. This information will clearly be stated in the patient information. Participation is voluntary and it will not affect medical treatment. Participants are allowed to retire at any point from the study.

Patients (both donors and recipients) will require only 1 screening visit. This visit will take place maximum 3 weeks before the FMT procedure for FMT-Recipients and at any time for FMT-Donors.

6.4 Assignment to Study Groups

The allocation of FMT-Donors to FMT-Recipients will be on “first come, first serve” bases. This means, that FMT-Donors recruited at the beginning of the study will provide the stool samples for firstly enrolled FMT-Recipients. This will eliminate the possibility of any impairment of frozen stool samples due to long-term storage. Each FMT-Recipient will receive stool from only 1 FMT-Donor. Stool infusates will not be pooled together.

6.5 Criteria for Withdrawal/ Discontinuation of Participants

A subject is free to withdraw from the study at any time at their own request and without providing a reason, or they may be withdrawn at any time at the discretion of sponsor or investigator for behavioral or administrative reasons. All documented data of the study will be analyzed and anonymized subsequently (see Art. 9 KlinV). If possible, the “Early Termination of Subject Visit” (see 6.6) should be performed.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative withdraws consent
- Investigator’s decision to withdraw the subject
- Repeated or serious noncompliance with trial procedure requirements
- The subject is lost to follow-up
- Administrative reasons
- Safety reasons

Study Coordinator will contact patient up to 3 times via phone or e-mail regarding the compliance with trial procedure requirements.

Any discontinued participation will allow for a replacement with a new study subject, until the total number of participants reaching follow-up (for FMT-Recipient) or donation of 170g stool (FMT-Donors) equals 25 and 5, respectively.

6.6 Early Termination of Study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient patient recruitment,
- when the safety or benefit of the subjects is doubtful or at risk
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise, or
- Early evidence of harm or benefit of the experimental intervention

7. STUDY PROCEDURES AND ASSESSMENTS

7.1 General Information

The responsible investigator from the Department of Gastroenterology and Hepatology, overall Sponsor-PI Prof. Dr. med. M. Scharl, will coordinate and oversee procedures with the collaborating departments and will be responsible for the performance and supervision of endoscopic procedures.

7.1.1 Study Intervention

FMT-Recipients will undergo the FMT procedure after the colon cleansing performed as per routine treatment protocols at the Department of Gastroenterology and Hepatology at the University Hospital Zürich.

The source of stool infusate for FMT in this study is derived from a patient that has a very good and longstanding response to ICI therapy.

FMT samples from suitable donors will be obtained and screened. FMT will undergo processing to produce FMT infusate. Generally 30 g of FMT-Donor stool will be used to generate 1 FMT infusate. Upon initial collection and processing, the FMT infusate will be passed through a filter to exclude particulate matter. Given the complexities of bacterial composition, the exact quantities of various bacteria may differ from batch to batch and an exact “FMT dose” cannot be calculated. FMT infusates will not be pooled at any time and single Recipient will receive stool only from a single Donor.

The infusate from individual Donor will be thawed and administered colonoscopically according to prepared guideline below. Using universal precautions, the infusate will be pre-loaded into standard 50cc syringes that have a tip compatible with the endoscope port for direct delivery of material through channel.

Infusate preparation

Reagents and Materials Required:

- Sterile physiological saline 0.9% NaCl
- Sterile 85% glycerol (final concentration 17%)
- BagMixer 400 Interscience™
- Sampling spatula (Bel-Art™ SP Scienceware™ Sterileware™ Sampling Spatulas)
- 250 ml screw cap container (Sarstedt 75.9922532)

Sample Procurement and Processing

Receiving sample:

- Samples can be received at any time from Monday-Friday, 9am-5pm. Samples should be processed within 3 hours of receipt.
- On receipt, samples should be stored in a biohazard bag labeled with time/date of receipt and de-identified FMT Donor code.

Sample processing:

- A sterile spatula is used to weigh 30 g of sample on a calibrated digital scale into a plastic bag compatible with BagMixer system.
- Addition of 40 mL of 85% glycerol (final concentration of 17%).
- Addition of 150 mL sterile, physiological saline (0.9% w/v of NaCl) .
- Suspension of fecal infusate with BagMixer 400.
- Passing of fecal suspension through a build-in 100 µm cell strainer to remove particulate matter and recover the fecal infusate in a 250 ml screw cap plastic container.

7.1.2 Storage Conditions

FMT samples are collected and handed over to the Study Coordinator at room temperature. Then, stool infusates are prepared within 3 hours of the receipt of the stool and stored in a -80°C freezer. The freezer is locked at all times with the code given only to selected USZ personnel. The room containing the freezer is not accessible without a key providing an additional method of limiting the access to samples. Stool infusates are kept secure from the influences of unwanted subjects.

Each FMT unit (infusate for 1 FMT-Recipient) is marked with:

- FMT-Donor ID (FMT-D_001 to FMT-D_005 assuming 5 donors)
- Date of sample preparation
- Container ID (e.g. 1/5 to 5/5 assuming 5 containers containing 1 portion of stool infusate were prepared from the given stool sample)

An expiration date for FMT infusates is 12 months after collection, assuming storage in a -80°C freezer during this time. Normal temperature fluctuations in freezer temperature of up to 5°C are acceptable and monitored through the installed system.

One container will contain the infusate amount required for one recipient. Upon opening, standard protocols for handling biohazardous material will be followed at all times. Sterile microbiological technique will be followed when handling material to avoid contamination. If treatments need to be destroyed, internal protocols for disposal of human stool should be followed.

Transport of human specimens will occur according to USZ regulation, i.e. with triple-packaging method and adequate labeling of specimen, personnel and sample destination place.

7.2 Administration of Study Intervention

7.2.1 Study Intervention

The FMT will be performed via colonoscopy. For this purpose the patient will receive a peripheral intravenous catheter to receive saline and propofol as sedation medication during the colonoscopy as per routine treatment protocol.

The FMT infusate (stool dissolved in 0.9% NaCl) will be administered via colonoscopy. This means that we will perform a colonoscopy and inject the FMT infusate by a catheter via the colonoscopy into the terminal ileum and the right-sided colon.

FMT infusate will be thawed prior to administration according to prepared guidelines. Using universal precautions, FMT infusate will be pre-loaded into standard 50cc syringes that have a tip compatible with the endoscope port for direct delivery of material through channel. Before applying the FMT infusate, 6 intestinal biopsies will be taken for study purposes using a biopsy

forceps (as per routine procedure), 3 biopsies for proteomic analyses and 3 for immune cell composition analyses.

FMT via colonoscopy is the standard procedure for performing FMT at the University Hospital Zürich.

7.2.2 Modification of Interventions

Upon patient request, the colonoscopy can also be performed without sedation without any harm to the patient. The FMT procedure itself cannot be altered.

7.3 Compliance with Study Intervention

The FMT will be done at the Department of Gastroenterology and Hepatology at the University Hospital Zürich. This will ensure compliance of the patient.

7.4 Data Collection and Follow-up for Withdrawn Participants

A subject is free to withdraw from the study at any time at their own request and without providing a reason, or they may be withdrawn at any time at the discretion of sponsor or investigator for behavioral or administrative reasons.

If a subject has any clinically significant, study-related abnormalities at the conclusion of the study, the clinical monitor should be notified and every effort should be made to arrange follow-up evaluations at appropriate intervals to document the course of the abnormalities.

The reason for a subject discontinuing from the study will be recorded in the eCRF. A discontinuation occurs when a randomized subject ceases participation in the study, regardless of the circumstances, prior to completion of the protocol. The investigator must determine the primary reason for discontinuation.

Withdrawal due to adverse event should be distinguished from withdrawal due to insufficient response according to the definition of adverse events. The final evaluation required by the protocol will be performed at the time of the study discontinuation. The investigator will record the reason for study discontinuation, provide or arrange for appropriate follow-up (if required) for each such subject, and document the course of the subject's condition.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal and request the subject to return all unused investigational product(s) and to return for a final visit, if applicable, as well as follow-up with the subject regarding any unresolved adverse events. If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations will be performed, and no additional data will be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

7.5 Concomitant Interventions (Treatments)

Patients enrolled in the study cannot be under any form of systemic antibiotics or receive systemic steroid therapy (> 10 mg prednisone daily or equivalent) or any other form of immunosuppressive therapy two weeks prior to trial treatment.

All concomitant and/or rescue interventions or treatment(s) have to be recorded in the CRF/eCRF. Other treatment should be continued without alteration.

8. STUDY ASSESSMENTS

8.1 Assessments of Outcomes

8.1.1 Assessment of Primary Outcome

The primary outcome of this study is feasibility of endoscopically administered FMT in cancer patients to induce a change of intestinal microbiome composition measured with a Metagenomic approach. Microbial species are expected to facilitate the response to CI-therapy in cancer patients previously refractory to CI.

8.1.2 Assessment of Secondary Outcomes

The secondary outcome of the study are:

- Safety
- Efficacy
- Evaluation of immune cell composition
- Analyses of bacteria taxa occurrence
- Determination of FMT impact on patient's quality of life

8.1.2.1 8.2.2.1. Safety

Safety testing will be performed at any study visit for FMT-Recipients and during a screening visit for FMT-Donors. In specific, the following safety parameters will be determined:

- Vital signs
- Body temperature
- Respiratory rate
- Blood pressure, pulse rate
- Physical examination

Additional measures will be taken for FMT-Recipients during screening:

- Pregnancy test (at screening, in women only)
- Hematology (differential blood cell count, hemoglobin, protrombin), blood chemistry (sodium, potassium, serum creatinine, bilirubin, ASAT, ALAT, CRP)
- Adverse events: from time of consent to Study Completion
- Serious adverse events: from time of consent until 4 weeks after Study Completion
- Serum testing for HIV, HAV, HBV or HCV
- PCR testing for Sars-CoV-2

Extensive safety labs from stool and blood will be performed during the screening visit for FMT-Donors and will include:

- Stool testing for bacteria (Escherichia coli, Vancomycin-resistant Enterococcus, Norovirus, C. difficile, Yersinia, Campylobacter, Shigella or Salmonella)
- Stool testing for parasites
- Serum testing for HIV, Syphilis, HAV, HBV or HCV
- PCR testing for Sars-CoV-2

Positive results are directly reported to BAG (Bundesamt für Gesundheit) as per current federal requirements.

8.1.2.2 Efficacy

Efficacy of FMT approach will be measured by objective response rate (ORR) by iRECIST, progression-free survival (PFS), and overall survival (OS).

8.1.2.3 Evaluation of immune cell composition

Immune cell composition (CD4+ and CD8+ T cells and dendritic cells and innate immune cells) will be evaluates in the tissues (biopsy from colon and tumor tissue) with immunohistochemistry as well as in the circulation with flow cytometry. Both methods will be performed at the USZ. Pre-selection of targeted cells will be guided based on murine models treated with FMT-Recipient baseline stool saved in 17% glycerol.

8.1.2.4 Analyses of bacteria taxa occurrence

To investigate whether FMT administration affects composition of the intestinal microbiome in recipients we will perform the analysis of bacterial taxa occurrence (within-sample and between-sample differences) in ICI responders, FMT responders and FMT non-responders. Samples will be analyzed at the baseline and after 6, 12 and 24 weeks.

8.1.3 Assessment of Exploratory Outcomes

- Stool samples will be collected pre-FMT (between screening visit and Baseline visit), after 6, 12 and 24 weeks using the OMNIgene GUT stool collection kits (1 kit per time point). Approximately 5g of stool per visit. Stool sampels will be analyzed by 16s sequencing and shotgun metagenomics analyses.
- Serum and stool metabolome will be quantified by mass spectrometry at baseline and after 6, 12 and 24 weeks after FMT using 1 ml of serum per visit.
- Proteomics analyses (from 1 ml of serum collected at the baseline and after 6, 12 and 24 week and peripheral blood mononuclear cells) and single cell RNAseq from PBMCs, intestinal biopsies and tumor tissue. PBMCs will be isolated from 20 ml whole blood collected at the baseline and after 6, 12 and 24 weeks. Intestinal tissue will be collected during colonoscopy as biopsies.

- Imaging mass cytometry (IMC) will be performed on tumor tissue collected from the Pathology and Dermatopathology Departments).
- Metagenomic analyses will be performed on stool samples, intestinal tissue biopsies and PBMCs collected pre-FMT, after 6, 12 and 24 weeks.
- Enumeration and composition of circulating tumor cells (CTCs) in recipients at baseline, after 6, 12 and 24 weeks using 10 ml whole blood
- Serum cytokines will be quantified for FMT-Donors at Visit 1 and for FMT-Recipients at the baseline, and after 6, 12 and 24 weeks using 1ml serum per visit.

All samples will be analyzed together (all time points collected from all patients).

8.1.4 Assessment of Safety Outcomes

8.1.4.1 Serious Adverse Events

Clinical investigators and ultimately the protocol Principal Investigator (PI) have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention.

Clinical study subjects will be routinely questioned about AEs at study visits. Documentation of AEs will start at the time of first protocol-specific measure taken (i.e. ICF signature). The well-being of the subjects will be ascertained by neutral questioning ("How are you?"). The investigator is responsible for reporting all AEs occurring during the course of the study.

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the patient file and subsequently in the eCRF.

AEs or abnormal test findings felt to be associated with the study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.
An abnormal test finding will be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy

  Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.
- The test finding leads to a change in study dosing or discontinuation of subject participation in the clinical study.

All AEs, serious and non-serious, will be fully documented in the patient source and in the appropriate eCRF. For each AE, the investigator will provide the onset, duration, intensity, treatment required, outcome and action taken with the investigational product.

The intensity of AEs will be assessed as being

- mild (hardly noticeable, negligible impairment of well-being),
- moderate (marked discomfort, but tolerable without immediate relief), or
- severe (overwhelming discomfort, calling for immediate relief).

The investigator will determine the relationship of the investigational drug to all AEs as defined on the AE page of the eCRF.

8.1.4.2 Laboratory Parameters

Vital signs and body measurements:
• Body height
• Body weight
• Body temperature
• Respiratory rate
• Blood pressure, pulse rate
• Physical examination
• Pregnancy test
• Hematology; Blood chemistry;
• Adverse events: from time of consent to Study Completion.
• Serious adverse events: from time of consent until 4 weeks after Study Completion
• Concomitant medications/significant non-drug therapies
• Blood collection: maximum 10 ml blood per sample (taken in two 3ml tube and two 10 ml tubes), total 26ml of blood.

Adverse events, vital signs, and safety laboratory tests data will be explored through the use of standard presentations of descriptive statistics.

Serum creatinine and calculated creatinine clearance

Serum creatinine will be reported within the standard presentations of chemistry data. The Cockcroft-Gault GFR calculation will be used as an estimate of creatinine clearance. GFR data will be listed and summarized by time. Mean changes from baseline for serum creatinine and GFR will be plotted and summarized across time. Week 8 changes from baseline will be plotted and summarized by dose to visually assess dose-related changes.

Significant infections

Significant infections, defined as any infection (viral, bacterial, and fungal) requiring hospitalization or parenteral antimicrobials, will be summarized as part of the study report narratives.

8.1.4.3 Vital Signs

Vital signs will be taken at screening and will include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only. Vital signs will be collected by a Study Coordinator (with a nurse-background) or an investigator, while patient is in supine position and after minimum 5 minutes of resting.

8.1.5 Assessments in Participants Who Prematurely Stop the Study

We will perform the following steps for patients terminating the study before the last follow-up visit:

- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Safety Lab, stool samples for Microbiota Analysis
- Blood and serum samples for molecular analyses

- Collection of Subject Diaries
- Assessment of quality of life (based on the questionnaire)
- Rektosigmoidoscopy

A rektosigmoidoscopy will be performed to take 6 intestinal biopsies to assess changes in the intestinal immune cell composition after FMT.

8.2 Procedures at Each Visit

8.2.1 FMT-Recipients

8.2.1.1 Screening visit – day -28 to -0

- Information of patient and Informed Consent
- Check for Inclusion and Exclusion Criteria
- Assessment of most recent CT or PET-CT/MRI scan with respect to treatment response/tumor size
- Medical History, Demographics, Concomitant Therapy and Physical Examination with Vital Signs
- Blood sampling, stool sampling (Microbiota Analysis), Urine analysis (only for females), nasal swab (Sars-COV-2 test)
- Distribution of questionnaires

During the screening visit patient will:

- Sign the ICF (the treating physician will explain the study and the ICF at least 24 h before that day)
- Answer any questionnaires
- Receive Physical examination and vital signs measurements
- Provide blood sample
- Receive stool collection tubes to be taken home and sent via post in the next 7 days
- Receive MoviPrep for bowel preparation on day before FMT

The ICF will be provided to all patient a minimum of 24h before the visit, either in person or via e-mail. Next, at the beginning of the screening visit patient will be given time to ask and resolve any remaining questions. Then, patient will sign the ICF together with the PI and. the patient will complete the questionnaires regarding quality of life and diet. Further, the patient will be asked by the investigator about demographics and medical history with an emphasis on the subject's family history, eventually surgery procedures, current medications and past therapies 2 years prior to Screening. All data must be documented in the patient source (KISIM) and eCRF.

Then, the investigator will perform a physical exam during the screening period which will include the examination of the heart, lung and abdomen. Clinically significant abnormal findings should be recorded as medical history.

Vital signs will be taken at screening and will include temperature, pulse, respiratory rate, weight and blood pressure. Height will be measured at screening only.

Patient will donate blood for laboratory testing and will receive stool collection kit including instructions to be taken and filled at home. The kits can be returned either via post or in person within 7 days after screening visit. Additionally, pregnancy test (from urine) will be performed for female participants with child-bearing potential.

A request can be sent to the Pathology Department at USZ in order to obtain already harvested tumor tissue that is no longer necessary for patients diagnosis and treatment decisions.

8.2.1.2 Visit 2: Baseline visit (Baseline = FMT procedure) – day 0

- Check for inclusion and exclusion criteria
- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Stool samples for Microbiota Analysis (metagenomics and metabolomics)
- Blood and serum sampling for molecular analyses
- Colonoscopy for FMT and obtaining intestinal biopsies

On the day before FMT a patient is required to perform bowel cleansing using MoviPrep provided during the screening visit. MoviPrep is an osmotic laxative indicated for cleansing of the colon as a preparation for colonoscopy in adults 18 years of age or older. MoviPrep instructions can be found in Appendix II. Not food intake will be possible between the colon cleansing and FMT procedure on the next day.

The FMT will be applied via colonoscopy. During the colonoscopy and before the application of the FMT material, 6 intestinal biopsies will be taken from the colon with a regular biopsy forceps (3 biopsies for proteomic analyses and 3 for immune cell composition analyses).

8.2.1.3 Visit 3: Study visit - day 42 ± 14

- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Stool samples for Microbiota Analysis
- Blood and serum sampling for molecular analyses
- Quality of life questionnaire

The safety lab values will be taken from the internal patient clinical information system (KISIM). The patient will undergo regular cancer immunotherapy as part of the regular medical treatment. CI therapy will be applied every 3 weeks, a safety lab will be taken before the administration of the CI therapy. Visit 3 corresponds to the 2nd administration of ICI therapy after FMT procedure. This will reduce the burden of study procedures for the patient.

8.2.1.4 Visit 4: Study visit - day 84 ± 21

- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Stool samples for Microbiota Analysis
- Blood and serum sampling for molecular analyses
- Quality of life questionnaire

The safety lab values will be taken from the internal patient clinical information system (KISIM). The patient will undergo regular cancer immunotherapy as part of the regular medical treatment. CI therapy will be applied every 3 weeks, a safety lab will be taken before the administration of the CI therapy. Visit 4 corresponds to the 3rd administration of CI therapy after FMT procedure. This will reduce the burden of study procedures for the patient.

After around 12 weeks after FMT, a regular CT or PET-CT/MRI scan will be performed to judge clinical course of cancer immunotherapy. This imaging analyses is part of the regular medical treatment of the patient. Routinely performed imaging will be assessed with respect to changes compared to the last imaging before FMT procedure.

8.2.1.5 Visit 5: FUP-Visit (Follow up = End of Study) – day 168 ± 21d

- Quality of life questionnaire
- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Stool samples for Microbiota Analysis
- Blood and serum sampling for molecular analyses
- Rektosigmoidoscopy for intestinal biopsies

The safety lab values will be taken from the internal patient clinical information system (KISIM). The patient will undergo regular cancer immunotherapy as part of the regular medical treatment. CI therapy will be applied every 3 weeks, a safety lab will be taken before the administration of the CI therapy. Visit 5 corresponds to the 4th administration of CI therapy after FMT procedure. This will reduce the burden of study procedures for the patient.

After around 24 weeks after FMT, a standard-of-care CT or PET-CT/MRI scan will be performed to judge clinical course of cancer immunotherapy. This imaging analyses is part of the regular medical treatment of the patient. Routinely performed imaging will be assessed with respect to changes compared to the last imaging before FMT procedure.

A rektosigmoidoscopy will be performed to take 6 intestinal biopsies to assess changes in the intestinal immune cell composition after FMT. This procedure will take place after 6 and 24 weeks after FMT. Patient needs to come to the examination on an empty stomach. This means no food 6 hours before, drinking clear liquid is allowed until 2 hours before.

8.2.2 FMT-Donors

Donor's eligibility for the study will be evaluated based on medical history, physical examination, laboratory values and additional tests. To prepare for study participation, subjects will be instructed on the use of Concomitant Medications (Chapter 7.5). Subjects will be screened between 0 and 7 days prior to stool collection to confirm that they meet the entrance criteria for the study (except stool safety labs). Note, safety testing will be performed on stool sample collected during Visit 2 and only then meeting of the Inclusion and Exclusion criteria will be finally confirmed. The study investigator or appropriate delegate at the site will discuss with each subject the nature of the study, its requirements, risks and benefits. Written informed consent must be obtained prior to performing any protocol-specific procedures.

- Information of patient and Informed Consent (Visit 1)
- Medical History (including the cancer stage and type), Demographics, Concomitant Therapy and Physical Examination with Vital Signs (Visit 1)
- Distribution of questionnaires and stool collection container (Visit 1)
- Blood sampling and blood safety testing (Visit 1)
- Check for Inclusion and Exclusion Criteria (Visit 1 and Visit 2)
- Stool donation, microbiome sequencing and stool safety testing (Visit 2)

Visit 1.

Procedures defined for Visit 1 will be performed upon obtaining Informed Consent signature. Patient will be then also given a stool collection kit together with instructions, which will allow stool donation for: 1) microbiome sequencing, 2) safety labs and 3) FMT. Donation takes place during Visit 2.

Visit 2.

Within 3 weeks of Visit 1 (exact date defined by the patient) stool donation will take place. Stool collection container will be filled by the patient with fresh stool and handed over to the Clinic of Gastroenterology on the same day. A small proportion (swab) of donated stool will be taken for safety labs as well as microbiome sequencing and the remaining stool in large container will be weighted (while patient is still at the clinic). In case when stool sample in is not sufficient to prepare at least 5 infusates (i.e. less than 170g stool in total), patient will be requested to provide an additional stool sample, until total of 170g of fresh stool is collected. Distribution of the collection kit and safety screening will be repeated for each donation. Positive testing at any time point for any of the infectious agents will automatically exclude the FMT-Donor from the Study.

8.3 Early Termination of Subject (Anytime)

- Update of Medical History, Concomitant Therapy, AE, SAE
- Physical Examination, Vital signs
- Stool samples for Microbiota Analysis

- Blood and serum samples for molecular analyses
- Collection of Subject Diaries
- Assessment of quality of life (based on the questionnaire)
- Rektosigmoidoscopy

A rektosigmoidoscopy will be performed to take 6 intestinal biopsies to assess changes in the intestinal immune cell composition after FMT. Patient needs to come to the examination on an empty stomach. This means no food 6 hours before, drinking clear liquid is allowed until 2 hours before.

Early termination (i.e. not reaching follow-up) will result in enrolment of new study participant.

9. REGULATORY ASPECTS AND SAFETY

9.1 Local regulations/Declaration of Helsinki

This study is conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH, the HRA as well as other locally relevant legal and regulatory requirements.

9.2 (Serious) Adverse Events and notification of safety and protective measures

An Adverse Event (AE) is any untoward medical occurrence in a patient or a clinical investigation subject which does not necessarily have a causal relationship with the trial procedure. An AE can therefore be any unfavorable or unintended finding, symptom, or disease temporally associated with a trial procedure, whether or not related to it.

A Serious Adverse Event (SAE) (ClinO, Art. 63) is any untoward medical occurrence that

- Results in death or is life-threatening,
- Requires in-patient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability or incapacity, or
- Causes a congenital anomaly or birth defect

Sponsor-Investigator make a causality assessment of the event to the trial intervention, (see table below based on the terms given in ICH E2A guidelines). Any event is assessed as related or unrelated to the trial intervention.

Unrelated	• The event started in no temporal relationship to the medical intervention applied and • The event can be definitely explained by underlying diseases or other situations.
Related	• The event started in a plausible temporal relationship to the medical intervention applied and

	• The event cannot be definitely explained by underlying diseases or other situations.

Sponsor-Investigator make a severity assessment of the event as mild, moderate or severe. Mild means the complication is tolerable, moderate means it interferes with daily activities and severe means it renders daily activities impossible.

The Sponsor Investigator is responsible for ensuring that all adverse events (see Section 9.1 for definition) are recorded on the Adverse Event eCRF accordance with instructions provided in this section. For each adverse event recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section 9.1.2 for seriousness criteria), severity (see Section 9.2), and causality (see Section 9.3).During the entire duration of the study, all serious adverse events (SAEs) that may be causally related to the study intervention are collected and documented in source documents. Reportable events are recorded in the case report form (CRF). Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the FMT is also an adverse event.

Adverse events may occur any time during the course of the clinical trial. Progression of the cancer under study is not considered an adverse event.

Reporting of SAEs (see ClinO, Art. 63)

All SAEs are documented and reported immediately (within a maximum of 24 hours) to the Sponsor-Investigator of the study.

If it cannot be excluded that the SAE occurring in Switzerland is attributable to the intervention under investigation, the Investigator reports it to the Ethics Committee via BASEC within 15 days.

Follow up of (Serious) Adverse Events

Participants terminating the study (either regularly or prematurely) with

- reported ongoing SAE, or
- any ongoing AEs of laboratory values or of vital signs being beyond the alert limit

will return for a follow-up investigation. This visit will take place up to 30 days after terminating the treatment period. Follow-up information on the outcome will be recorded on the respective SAE page in the CRF/eCRF.

Follow-up investigations may also be necessary according to the investigator's medical judgment even if the participant has no SAE at the end of the study. However, information related to these investigations does not have to be documented in the CRF/eCRF but must be noted in the source documents.

Notification of safety and protective measures (see ClinO, Art 62, b)

If immediate safety and protective measures have to be taken during the conduct of the study, the investigator notifies the Ethics committee of these measures, and of the circumstances necessitating them, within 7 days.

9.3 (Periodic) safety reporting

An annual safety report (ASR/DSUR) is submitted once a year to the local Ethics Committee by the Investigator (ClinO, Art. 43 Abs).

9.4 Amendments

Substantial changes to the study setup and study organization, the protocol and relevant study documents are submitted to the Ethics Committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Ethics Committee. Such deviations shall be documented and reported to the Ethics Committee as soon as possible. A list of all non-substantial amendments will be submitted once a year to the competent EC together with the ASR.

9.5 (Premature) termination of study

The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, e.g.

- Ethical concerns,
- Insufficient participant recruitment,
- When the safety of the participants is doubtful or at risk (e.g. when the benefit-risk assessment is no longer positive),
- Alterations in accepted clinical practice that make the continuation of the study unwise, or
- Early evidence of harm or benefit of the experimental intervention

Upon regular study termination, the Ethics Committee is notified via BASEC within 90 days (ClinO, Art. 38).

Upon premature study termination or study interruption, the Ethics Committee is notified via BASEC within 15 days (ClinO, Art. 38).

9.6 Insurance

Insurance is covered by “Versicherung für klinische Versuche und nichtklinische Versuche“ by Zürich Versicherungs-Gesellschaft AG (Policy no.: 14.970.888).

Any damage developed in relation to study participation is covered by this insurance. So as not to forfeit their insurance cover, the participants themselves must strictly follow the instructions of the study personnel. Participants must not be involved in any other medical treatment without permission of the principal investigator (emergency excluded). Medical emergency treatment must be reported immediately to the investigator. The investigator must also be informed instantly, in the event of health problems or other damages during or after the course of study treatment.

The investigator will allow delegates of the insurance company to have access to the source data/documents as necessary to clarify a case of damage related to study participation. All involved parties will keep the patient data strictly confidential.

A copy of the insurance certificate will be placed in the Investigator’s Site File.

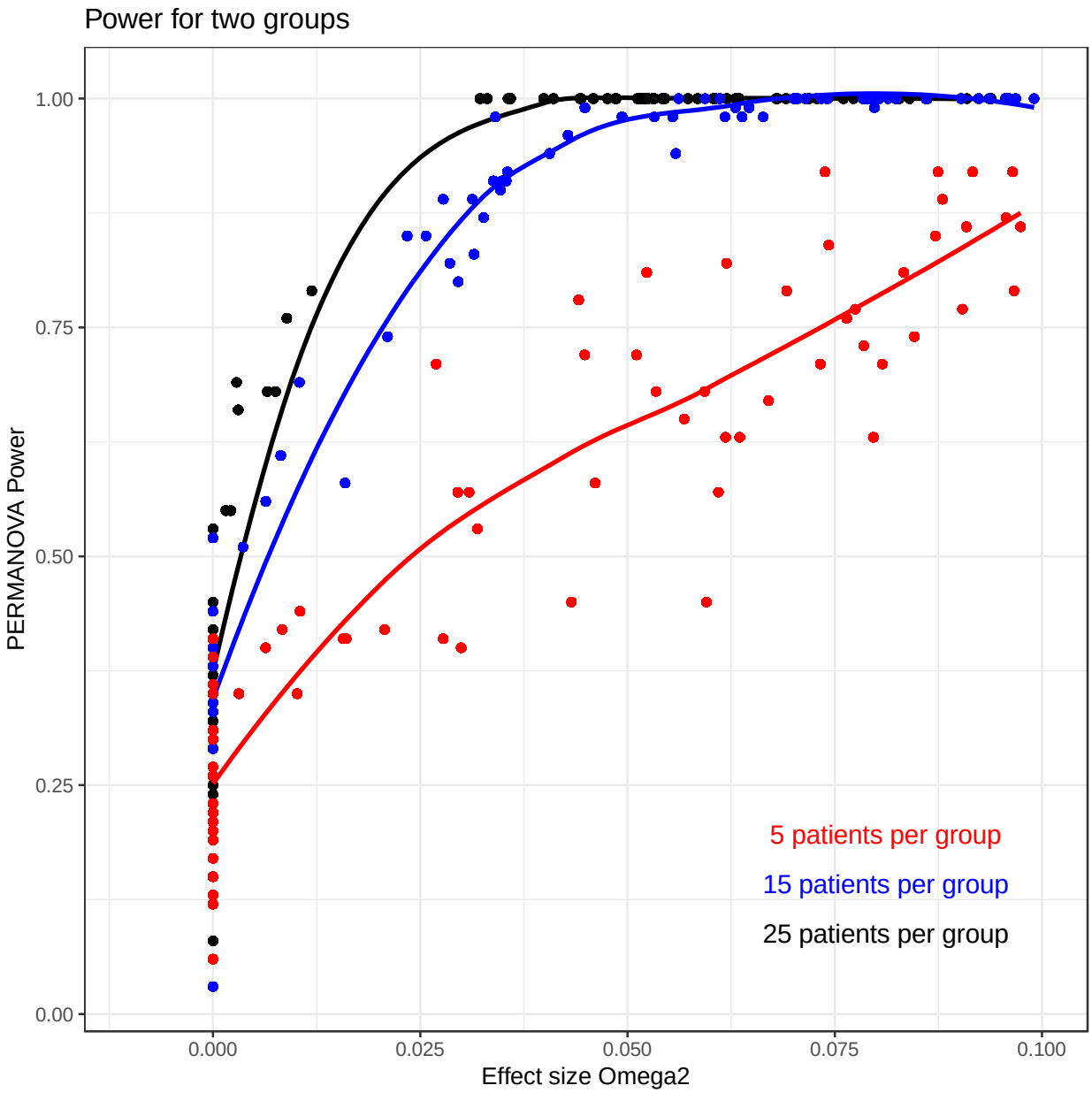
10. STATISTICAL METHODS

10.1 Hypothesis

The null hypothesis is that there is no difference before and after the fecal microbiome transplantation.
The alternative that the FMT administration affects microbial composition after 12 weeks.

10.2 Determination of Sample Size

The primary endpoint of this study is to evaluate the differences in community composition before and after the FMT. While the within-group variation in microbiome composition, as well as the effect size between the groups is uncertain and hard to predict, we have performed a power analysis. We used R package micropower to simulate a range of different effect sizes for subject groups comprising 5, 15 and 25 subjects, and show that having 25 subjects allows 90% power to detect effect size (ω^2) of 0.0025, which is less than the effect observed in other microbiome studies.



10.3 Planned Analyses

Microbial taxa changes and abundances will be tested for statistical significance by using Wilcoxon rank-sum test (adjustments for multiple comparisons are done with FDR method at an alpha level of 0.05).

10.3.1 Primary Analysis

For microbiome analysis (16S rRNA sequencing), the difference in alpha diversity (diversity of species within each sample) before and after the FMT, will be assessed using Shannon index (significance will be determined using Mann-Whitney test). The differences in microbiome composition before and after the FMT will be estimated by calculating several distance metrics, such as weighted UniFrac distance and Bray-curtis distance. The between-group differences will then be analyzed using multivariate analysis of variance with permutation

(PERMANOVA), and analysis of group similarities (ANOSIM). Qiime2 pipeline will be used to detect ASV.

For microbiome shotgun metagenomics, after removal of host and contaminating reads, metagenomic sequencing data will aligned using DIAMOND, whereas species-level taxonomic relative abundances estimation. Functional binning will be performed using MEGAN. Additionally, relative abundances of KEGG orthologous groups and pathways will be estimated by summing up the relative abundances of genes annotated to belong to the same group/pathway. The differences in microbial taxa composition, gene/pathway composition will be assessed before and after the FMT, as well as between responder patients and non-responder using non-parametric tests (Mann-Whitney or Kruskal Wallis tests, and FDR method for multiple-testing correction).

10.3.2 Secondary Analyses

Our secondary objective it to detect if the FMT could alter the response to immune therapy and therefore the subgroups would be responders vs non responders and the microbiome would be analyzed between these two groups aiming to specify species/strains responsible to alter the response of immune therapy in cancer patients.

More specifically:

1) Safety outcomes:

The exploratory approach will be used to identify unanticipated and rare adverse effects of the intervention, and mean cumulative function (MCF) test will be used to analyze multiple/repeated adverse effects during the whole time of the study based on their percentage (rate).

2) Efficacy of FMT approach as measured by objective response rate (ORR) by iRECIST, progression-free survival (PFS), and overall survival (OS):

- Objective response rate (ORR), consisting of complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD),; will be measured according to RECIST .
- Progression-free survival (PFS) rate at , 6, 12 and 24 weeks according to RECIST.
- Overall survival (OS) rate at , 6, 12 and 24 weeks.

A Kaplan-Meier plot for PFS follow-up duration will be generated to assess the follow-up time in the treatment group (recipients).

3) Evaluation of CD8+ and CD4+ T-cells, dendritic cells and innate cells composition and activity in peripheral blood, intestinal and tumor tissue from ICI responders (FMT-Donors), FMT responders and FMT non-responders.

One way ANOVA test will be used to assess the differences in cells population followed by Tukey HSD post hoc test.

4) Analysis of bacterial taxa occurrence (within-sample and between-sample differences) in ICI responders, FMT responders and FMT non-responders.

One way ANOVA test will be used to assess the differences in cells population followed by Tukey HSD post hoc test.

5) Analysis of changes in quality of life after FMT utilizing a Quality of life questionnaire.

A provided Scoring Manual designed specifically for EORTC QLQ-C30 questionnaire will be utilized.

10.3.3 Interim Analyses

No interim analyses are planned.

10.3.4 Safety Analysis

Safety assessments will consist of monitoring and recording adverse events, including serious adverse events and adverse events of special interest, performing protocol-specified safety laboratory assessments, measuring protocol-specified vital signs, and conducting other protocol-specified tests that are deemed to the safety evaluation of the study.

Safety data will be evaluated using descriptive statistics: that means: numbers, frequency and severity of Adverse events. For details see Chapter: 10.3.2.

10.3.5 Deviation(s) from the Original Statistical Plan

All deviations from the original statistical plan will be amended if necesssary.

10.4 Handling of Missing Data and Drop-Outs

Any discontinued participation will allow for a replacement with a new study subject, until the total number of participants reaching follow-up equals 25 for FMT-Recipients and 5 for FMT-Donors.

11. ELIGIBILITY OF THE PROJECT SITE(S)

The Department of Gastroenterology and Hepatology is the largest Gastroenterology department in Switzerland. Additionally, it has a broad clinical trial unit that is regularly inspected by Swissmedic and the Clinical Trials Center at the University Hospital Zürich. All needed infrastructure and expertise for performing FMT as well as clinical trials are available. FMT for refractory C. difficile infection is routinely done at our department ensuring sufficient expertise and knowledge how to perform FMT.

12. DATA QUALITY ASSURANCE AND CONTROL

The Sponsor-Investigator is implementing and maintaining quality assurance and quality control systems with written SOPs and Working Instructions to ensure that trials are conducted and data are generated, documented (record), and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s).

The trial manager and study coordinators will receive training by the CTU Zurich with regards to maintaining quality assurance and study documentation and conducting basic source data verification (on site and / or online). Furthermore, the trial manager and study coordinators will attend documented and standardized elementary training on the use and application of the EDC software system SecuTrial® conducted by the Data Management Department of the CTU Zurich in order to ensure reliable database management and contemporaneous and accurate eCRF completion and oversight.

System-supported automatic notifications on eCRF completion status will be implemented to ensure data completeness (where possible). In addition, study coordinators will check the completeness of paper-reported outcome measures and follow-up with participants should missing values be identified. Data will be entered into the SecuTrial® database in a contemporaneous manner and in line with the study monitoring plan, a minimum of 10% of this data will be verified against source data. Further data quality assurance measures are provided by implementing study-specific reports, programming of system-operated automatic alerts and by implementing data validity and plausibility checks wherever possible within the eCRF. In addition, customizable data quality rules will allow spot checks on rates of case report form completion, identification of missing data and detection of protocol deviations or out-of-range data values.

A data monitoring plan specific to the study assessment schedule has been prepared by the CTU Zurich. Data monitoring will be initiated after inclusion of 4 study participants (with at least 2 FMT-Recipient), with a focus on the participants' eligibility, documentation of the intervention at the corresponding study centres (engaging, supporting and communicating) and completion of questionnaires (ePRO – if applicable) by the participants. Observations and findings will be documented by the responsible Clinical Research Associates (CRAs) of the CTU Zurich and made available to the trial manager for feedback and support. Patient eligibility will then be reviewed for 50% of additional study participants. Furthermore, random clinical data monitoring will be undertaken by the CRAs of the CTU Zurich, where source data for up to 10% of the enrolled patients will be verified.

12.1 Data handling and record keeping

The study will strictly follow the protocol. If any changes become necessary, they must be laid down in an amendment to the protocol. All amendments of the protocol must be signed by the Sponsor-Investigator and if essential submitted to CEC.

12.1.1 Case Report Forms

The investigators will use electronic case report forms (eCRF described in Section 12), one for each enrolled study participant, to be filled in with all relevant data pertaining to the participant during the study. All participants who either entered the study or were considered not-eligible or were eligible but not enrolled into the study additionally have to be documented on a screening log. The investigator will document the participation of each study participant on the Enrolment Log.

eCRFs must be kept current to reflect participant status at each phase during the course of study. Participants must not to be identified in the eCRF by name. Appropriate coded identification (e.g. Participant Number) must be used.

It must be assured that any authorized person, who may perform data entries and changes in the eCRF, can be identified. A list with signatures and initials of all authorized persons will be filed in the study site file and the trial master file, respectively.

Documented medical histories and narrative statements relative to the participants' progress during the study will be maintained. These records will also include the following: originals or

copies of laboratory and other medical test results which must be kept on file with the individual participants eCRF.

The investigators assure to perform a complete and accurate documentation of the participant data in the eCRF. All data entered into the eCRF must also be available in the individual participant file either as print-outs or as notes taken by either the investigator or another responsible person assigned by the investigator.

Essential documents must be retained for at least 10 years after the regular end or a premature termination of the respective study (KlinV Art. 45).

Any patient files and source data must be archived for the longest possible period of time according to the feasibility of the investigational site, e.g. hospital, institution or private practice.

12.1.2 Specification of Source Documents

Study monitors (i.e. CRAs) will perform ongoing source data verification and review to confirm that critical protocol data (i.e., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial. Before study initiation, the types of source documents that are to be generated will be clearly defined in the Trial Monitoring Plan and Source Data location list. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data. Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained. To facilitate source data verification and review, the sponsor investigator provide the Monitor (i.e. CRA) direct access to applicable source documents and reports for trial-related monitoring.

The following documents are considered source data (clearly documented on the Source Data location list), including but not limited to:

• SAE worksheets

• Nurse records, records of clinical coordinators, and

• Medical records from other department(s), or other hospital(s), or discharge letters and correspondence with other departments/hospitals, if participant visited any during the study period and the post study period.

Source data must be available at the site to document the existence of the study participants and substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

The following information (at least but not limited to) should be included in the source documents:

• Demographic data (age, sex)
• Inclusion and Exclusion Criteria details
• Participation in study and signed and dated Informed Consent Forms
• Visit dates
• Medical history and physical examination details
• Key efficacy and safety data (as specified in the protocol)
• AEs and concomitant medication
• Results of relevant examinations
• Laboratory printouts

• Dispensing and return of study drug details
• Reason for premature discontinuation

12.1.3 Record Keeping / Archiving

All study data must be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. The Department of Gastroenterology at USZ will be responsible for archiving the records.

Data will be stored according instructions of the University Hospital Zurich at IronMountain® (providing secured facilities and environmental control systems).

12.2 Data Management

For the present clinical study, the electronic data capture (EDC) software secuTrial® (interActive systems; iAs, Berlin) will be used for data processing and management.

secuTrial® is a commercially available browser-based EDC system, fulfilling FDA-stipulated 21 CFR part 11 requirements, is GCP compliant and meets the Swiss regulatory requirements regarding the collection of patient data in clinical or non-interventional studies and patient registries.

secuTrial® allows the generation of electronic Case Report Forms (eCRFs) to collect patient data using a web-based interface. Operating requirements include a web-based Front End (e.g. MS Internet Explorer 9.0 or greater, Mozilla Firefox 20 or greater, Safari, Opera), WebObjects applications server, Enterprise Linux Back End and Oracle database server. The application is hosted by the Clinical Trials Center of the University Hospital Zurich, which holds a commercial License for this EDC system.
Data collection occurs via eCRFs, which are generated by the CTC Data Management department in close collaboration with the study team and undergo a thorough data validation process.

eCRFs consists of:
- forms and data entry fields to enter data (parameters as defined in the study protocol)
- visits, that specify the events for which data collection is scheduled

Ongoing maintenance and use of this software is ensured by the Data Management Department of the Clinical Trials Center, University Hospital Zurich, Switzerland (CTU Zurich).

12.2.1 Data Security, Access and Back-up

Appropriate coded identification (e.g. pseudonymisation) is used in order to enter subject data into the database. All data entered into eCRFs is transferred to an Oracle database using 128 bit Secure Sockets Layer (SSL) encryption.

The server hosting the EDC system and the database is kept in an off-site locked server-room. Only system administrators have direct access to the server and back-up tapes.

A role-based user concept with personal login and passwords (e.g. for site investigator, statistician, monitor (i.e. CRA), administrator etc.) regulates permission for each user to access the system and database when required.

Role- and user-based settings will be implemented to control access to various functionality and modules, such as being able to export data, to enter data, export reports and view the logging records. Study Centres will be implemented to help segregate users so that the data they enter is only accessible by someone in their group, especially useful for multi-centric studies where the data entered by one institution should not be accessible or viewable by others within the same project. A current list with signatures and names of all authorized study personnel with access to the study records will be filed in the study site file and the trial master file, respectively.

A built-in data logging tool (audit trail) ensures that any changes to the project or user activity (date and time stamp and user log), including contextual information (e.g. the project record being accessed), are continuously tracked in real-time and accessible online or via downloadable audit table.

A multi-level back-up system is in place. Whole system internal back-ups including the database are run several times per day and an additional external back-up onto tape once a day. The back-up tapes are stored in a secure place in a separate building.

12.2.2 Analysis and Archiving

eCRFs are kept current to reflect subject status at each phase during the course of the study. For ad interim (if applicable) and final analyses, data files are extracted from the database in CSV (case-delimited) format, typically supported by Microsoft Excel, SAS, Stata, R, or SPSS software systems. Direct import into these statistical packages is advised for best data analyses. This study foresees the use of R package for statistical analysis of study outcome.

The study database will be securely stored by the sponsor for at least 15 years (after the regular end or a premature termination of the respective study). The sponsor further maintains essential documents and source data in the Trial Master File and archives interim and final reports in electronic and hard copy format for at least 10 years.

12.2.3 Electronic and Central Data Validation

The EDC system supports data checks completeness and plausibility. Furthermore, selected data points are cross-checked for plausibility with previously entered data for that participant. Additional central data validity checks against pre-determined parameters are run either automatically or *ad hoc*, to detect inconsistencies and identify missing data for source data verification.

12.3 Routine Monitoring

Monitoring visits at the investigator’s site prior to the start and during the course of the study will help to follow up the progress of the clinical study, to assure utmost accuracy of the data and to detect possible errors at an early time point. The Sponsor-Investigator organises professional independent monitoring for the study.

All original data including all patient files, progress notes and copies of laboratory and medical test results must be available for monitoring. The monitor will review all or a part of the CRF/eCRFs and written informed consents. The accuracy of the data will be verified by reviewing the above referenced documents. The investigator's site will collaborate with the Clinical Trials Center (CTC) of the University Hospital Zurich to ensure monitoring. According to the CTC's

Monitoring SOP the extent and nature of monitoring activities based on the objective and design of the study will be defined in a study specific Monitoring Plan.

12.4 Audits and Inspections

A quality assurance audit/inspection of this study may be conducted by the competent authority or CEC, respectively. The quality assurance auditor/inspector will have access to all medical records, the investigator's study related files and correspondence, and the informed consent documentation that is relevant to this clinical study.

The investigator will allow the persons being responsible for the audit or the inspection to have access to the source data/documents and to answer any questions arising. All involved parties will keep the patient data strictly confidential.

12.5 Confidentiality, Data Protection

Direct access to source documents will be permitted for purposes of monitoring, audits and inspections.

12.6 Storage of Biological Material and Related Health Data

The investigators will use electronic case report forms (eCRF), one for each enrolled study participant, to be filled in with all relevant data pertaining to the subject during the study. All subjects who either entered the study or were considered not eligible or were eligible but not enrolled into the study additionally have to be documented on a screening log. The investigator will document the participation of each study subject on the Enrollment Log.

It is the responsibility of the investigator to assure that all data in the course of the study will be entered completely and correctly in the respective data base. Corrections in the eCRF may only be done by the investigator or by other authorized persons. In case of corrections the original data entries will be archived in the system and can be made visible. For all data entries and corrections date, time of day and person who is performing the entries will be generated automatically.

eCRFs must be kept current to reflect subject status at each phase during the course of study. Subjects must not to be identified in the eCRF by name. Appropriate coded identification (e.g. Subject Number) must be used.

It must be assured that any authorized person, who may perform data entries and changes in the eCRF, can be identified. A list with signatures and initials of all authorized persons will be filed in the study site file and the trial master file, respectively.

Documented medical histories and narrative statements relative to the subject's progress during the study will be maintained. These records will also include the following: originals or copies of laboratory and other medical test results which must be kept on file with the individual subject's eCRF.

The investigators assure to perform a complete and accurate documentation of the subject data in the eCRF. All data entered into the eCRF with exception of (for which data the eCRF will be source data to be specified for each study) must also be available in the individual subject file either as print-outs or as notes taken by either the investigator or another responsible person assigned by the investigator.

Essential documents related to the trial must be retained for at least 10 years after the regular end or a premature termination of the respective study (Art. 45 KlinV). Essential documents must be retained according to local law in case of international multicenter studies.

Any patient files and source data must be archived for the longest possible period of time according to the feasibility of the investigational site, e.g. hospital, institution or private practice.

13. PUBLICATION AND DISSEMINATION POLICY

After the statistical analysis of this trial the sponsor will make every endeavour to publish the data in a medical journal.

14. FUNDING AND SUPPORT

Financial support specified for the development and establishment of the above-mentioned therapy within this clinical trial is given by institutional funds (i.e. Innovationsgrant from USZ and Comprehensive Cancer Center Zurich at USZ) of Prof. Dr. Michael Scharl.

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16. APPENDICES

I. BRISQ Guideline
II. MoviPrep instructions