Effect of C-Reactive Protein-Guided Antibiotic Treatment Duration, 7-Day Treatment, or 14-Day Treatment on 30-Day Clinical Failure Rate in Patients With Uncomplicated Gram-Negative Bacteremia: A Randomized Clinical Trial

Von Dach-Saouter, Elodie; Albrich, Werner C; Brunel, Anne-Sophie; Prendki, Virginie; Cuvelier, Clémence; Flury, Domenica; Gayet-Ageron, Angèle; Huttner, Benedikt; Kohler, Philippe; Lemmenmeier, Eva; McCallin, Shawna; Rossel, Anne; Harbarth, Stéphan Juergen; Kaiser, Laurent [**and 2 more**]

Supplementary Online Content

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Supplement 1. Trial protocol

This supplementary material has been provided by the authors to give readers additional information about their work.

The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic ThErapy in Gram-negative Bacteremia

Short title: Antibiotic durations for Gram-negative bacteremia

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Funding source

Swiss National Science Foundation, 74th National Research Program "Smarter Health Care" (no. 407440_167359)

THE PIRATE PROJECT: ANTIBIOTIC DURATIONS FOR GRAM-NEGATIVE BACTEREMIA

Study type	**Clinical trial** with products authorized in Switzerland, used according to the Swiss SmPC
Study categorization	**A**
Study registration	This trial is registered at www.clinicaltrials.gov (NCT03101072).
Sponsor	**University Hospitals of Geneva** Rue Gabrielle-Perret-Gentil 4 Geneva, Switzerland 1205
Funding source	**Swiss National Science Foundation**, 74th National Research Program "Smarter Health Care" (no. 407440_167359) PIRATE RESISTANCE nested study: Geneva University Hospitals & University of Geneva (Fondation Louis-Jeantet; no. S04-12)
Principal Investigator	**Dr. Angela Huttner, MD** Division of Infectious Diseases Rue Gabrielle-Perret-Gentil 4 Geneva, Switzerland 1205 Phone +41 79 553 3396 Email: angela.huttner@hcuge.ch
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External monitor	Clinical Trials Unit, HUG
Current protocol version	1.5 16 July 2018 Lazarevic, A. Huttner, B. Huttner

Version history

Date	Version	Changes	By
16.07.18	1.5	Addition of the nested study "PIRATE RESISTANCE" (section 6.4)	V. Lazarevic, A. Huttner, B. Huttner
8.06.18	1.4	Addition of the nested substudy "Endurance" (section 6.3)	W. Albrich, A. Huttner
8.06.17	1.3	Extension of informed consent to patient representatives of patients without decision-making capacity; clarification that recurrent bacteremia is an exclusion criterion; minor corrections & precisions	A. Huttner
27.03.17	1.2	Clarification that study sponsor will provide study insurance	A. Huttner
08.03.17	1.1	Clarification of informed consent procedures; addition of description of informed consent nested study (previously in Appendix 1) into protocol; addition of description of second substudy observing outcomes of non-included patients; clarification of AE recording procedures	A. Huttner

Table of Contents

SIGNATURE PAGE

PRINCIPAL INVESTIGATOR

I, the undersigned, have reviewed the protocol entitled "*The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic Therapy in Gram-negative bacteremia*," version 1.5, dated 16 July 2018. I will conduct the clinical study as described and I will adhere to Good Clinical Practices/International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (GCP/ICH) and all ethical and regulatory considerations stated under the Federal Law on Medicinal Products and Medical Devices (TPA/LPTh, 15 December 2000), Federal Law on Human Subjects Research (HRA/LRH, 30 September 2011) and the Ordinance on Clinical Trials in Human Research (ClinO/OClin, 20 September 2013).

Dr. Angela Huttner		16 July 2018
Principal Investigator	**Signature**	**Date**

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I, the undersigned, have reviewed the protocol entitled "*The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic Therapy in Gram-negative bacteremia*," version 1.5, dated 16 July 2018. I will conduct the clinical study as described and I will adhere to Good Clinical Practices/International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (GCP/ICH) and all ethical and regulatory considerations stated under the Federal Law on Medicinal Products and Medical Devices (TPA/LPTh, 15 December 2000), Federal Law on Human Subjects Research (HRA/LRH, 30 September 2011) and the Ordinance on Clinical Trials in Human Research (ClinO/OClin, 20 September 2013).

Dr. Pierre-Yves Bochud		16 July 2018
Principal Investigator, CHUV	**Signature**	**Date**

Dr. Werner Albrich		16 July 2018
Principal Investigator, Kantonsspital St. Gallen	**Signature**	**Date**

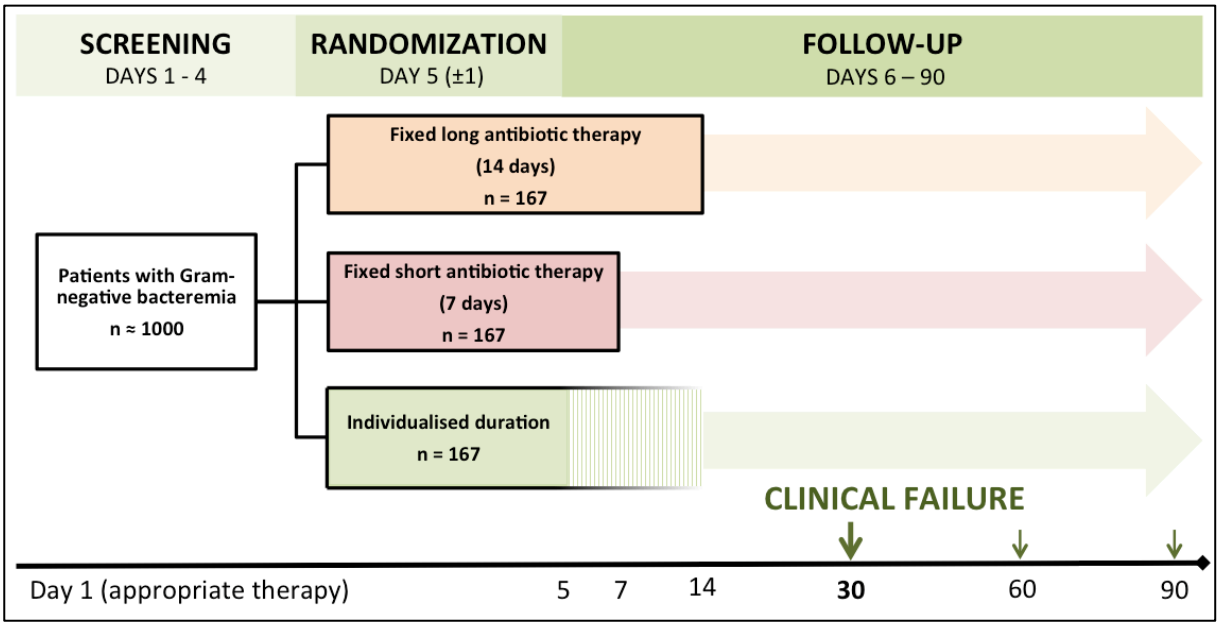
PROTOCOL SYNOPSIS

PROTOCOL SYNOPSIS – ANTIBIOTIC DURATIONS FOR GRAM-NEGATIVE BACTEREMIA	
PROTOCOL TITLE	**The PIRATE PROJECT: a Point-of-care, Informatics-based Randomized, controlled trial for decreasing over-utilization of Antibiotic ThErapy in Gram-negative Bacteremia**
PRINCIPAL INVESTIGATOR	Dr. Angela Huttner Division of Infectious Diseases University Hospitals of Geneva, Geneva, Switzerland
STUDY CENTERS	University Hospitals of Geneva, Geneva, Switzerland Centre hospitalier universitaire vaudois (CHUV): PI Pierre-Yves Bochud St. Gallen Kantonsspital (KSSG): PI Werner Albrich
SPONSORING INSTITUTION	University Hospitals of Geneva Rue Gabrielle-Perret-Gentil 4, Geneva, Switzerland 1205
FUNDER	Swiss National Science Foundation, 74th National Research Program "Smarter Health Care" (no. 407440_167359) University of Geneva, Fondation Louis-Jeantet (PIRATE RESISTANCE; S04-12)
CLINICAL STUDY COORDINATOR	Ms. Elodie von Dach Infection Control Program, HUG
EXTERNAL MONITOR	Clinical Trials Unit Rue Gabrielle-Perret-Gentil 4 Geneva, Switzerland 1205
TRIAL CATEGORY	A
TRIAL DESIGN	Phase IV, multicenter, assessor- and analyst-blinded, point-of-care randomized trial
STUDY POPULATION	Hospitalized adult patients diagnosed with bloodstream infections due to Gram-negative bacteria
SAMPLE SIZE	500 patients
FOLLOW-UP PERIOD	90 days after enrollment
FULL STUDY PERIOD PLANNED LAUNCH ACTIVE RECRUITMENT	January 2017 – March 2020 April 2017 April 2017 – March 2019
RATIONALE	Antibiotic resistance continues to grow and is now considered to be one of the most serious global threats of the 21st century. The key driver of resistance is antibiotic overuse; long antibiotic courses select for resistance among the trillions of bacteria hosted by the human body. No RCT evaluating the optimal duration of therapy for Gram-negative bacteremia (GNB), a frequent infection in hospitalized patients, has been published. Traditionally, guidelines have somewhat arbitrarily recommended long courses of two weeks, even though patients with no structural complications may recover after only five days of therapy. Evidence is mounting that longer courses leave patients with dangerous multi-resistant organisms. Indeed, given rising concerns over resistance, many physicians have reduced antibiotic durations for GNB to 7 days with no apparent untoward consequences. Durations could also be individualized, guided by objective markers, including inexpensive biomarkers such as C-reactive protein. We will conduct the first point-of-care (POC) randomized controlled trial (RCT) in Switzerland to test different antibiotic durations for GNB. The increasing equipoise toward different durations, the high incidence of this infection, the ease of its diagnosis, and the high stakes of antibiotic overconsumption combine to make antibiotic utilization for GNB an appropriate initial subject of study for the POC trial platform.

PROTOCOL SYNOPSIS – ANTIBIOTIC DURATIONS FOR GRAM-NEGATIVE BACTEREMIA	
MAIN OBJECTIVES	The primary objective is to determine whether shorter antibiotic courses (5-7 days) are non-inferior to a two-week antibiotic course in the treatment of GNB. Secondary objectives are to determine whether antibiotic durations can be safely determined via a simple algorithm employing clinical and laboratory (C-reactive protein) markers, whether shorter antibiotic courses for GNB will result in a decrease in antibiotic days, incidence of *Clostridium difficile* infection, the emergence of bacterial resistance, and length of hospital stay. Cost-benefit/health-economic analyses will also be performed.
INCLUSION CRITERIA	(1) Age ≥ 18 years (2) Presence of Gram-negative bacteria in at least one blood culture bottle (3) Treatment with a microbiologically efficacious antibiotic
EXCLUSION CRITERIA	(1) Immunosuppression (including HIV infection with CD4 cell count ≤500/µl, hematopoietic stem-cell transplantation in the first month after transplantation and at any time before engraftment, neutropenia in the 48 hours prior to randomization, receipt of high-dose steroids [>40 mg prednisone or its equivalent] daily for > 2 weeks) in the two weeks prior to randomization (2) GNB due to the following complicated infections: ○ Endocarditis or other endovascular infection without a removable focus ○ Necrotizing fasciitis ○ Osteomyelitis or septic arthritis ○ Confirmed prostatitis ○ Undrainable abscess or other unresolved sources requiring surgical intervention (e.g., cholecystitis) at the time of enrollment ○ Central nervous system infections ○ Empyema ○ Recurrent bacteremia (same bacterium [by resistance profile] causing bacteremia in the previous 60 days) (3) GNB due to non-fermenting bacilli (*Acinetobacter* spp., *Burkholderia* spp., *Pseudomonas* spp.), *Brucella* spp., *Fusobacterium* spp., or polymicrobial growth with Gram-positive organisms (4) Fever (≥38º C) or hemodynamic instability in the 24h prior to recruitment
INTERVENTIONS & CONTROL	With day 1 defined as the first day of appropriate (microbiologically efficacious) antibacterial therapy, patients will be randomized 1:1:1 on day 5 (±1) to one of the following three arms: ○ **"Fixed long" antibiotic course of 14 days (control arm)** ○ **"Fixed short" antibiotic course of 7 days (first intervention arm)** ○ **"Individualized" antibiotic course (second intervention arm):** • Starting on day 5, therapy will be discontinued after the patient has been *afebrile for 48 hours and the CRP level has decreased from its peak by at least 75%* In all arms, the choice and mode of administration (IV vs. PO) of antibiotic(s) will be left to the patient's attending physician and consulting infectious disease specialist and thus will follow usual standards of care.
PROCEDURE AND FOLLOW-UP	All patients will be assessed for the outcomes listed below on days 30, 60, and 90. While still hospitalized, they will be observed in their usual clinical setting by study investigators. After discharge, they will be contacted by telephone for further information. To avoid the introduction of bias in study arms, patients, hospital staff, and designated trial investigators will be blinded to patients' treatment duration assignment until that treatment is discontinued, thus either until day 7 (fixed short group), the clinical requirements for discontinuation have been met (individualized group), or day 14 (fixed long group). In the effort to avoid unblinding by the process of elimination after day 7, attending/treating physicians and nurses will not be made aware of the specific algorithm defining criteria for antibiotic discontinuation in the individualized group. They will thus be allowed to view all CRP results, but they will not be able to predict when an individualized duration will end.

PROTOCOL SYNOPSIS – ANTIBIOTIC DURATIONS FOR GRAM-NEGATIVE BACTEREMIA	
PRIMARY OUTCOME	The primary outcome will be the *clinical failure rate in all arms at day 30*. Clinical failure is defined by the presence of at least one of the following: ○ Relapse: a recurrent bacteremia due to the same bacterium occurring from the day of treatment cessation and until day 30 ○ Local suppurative complication that was not present/apparent at infection onset (e.g., renal abscess in pyelonephritis, empyema in pneumonia) ○ Distant complications of the initial infection, defined by growth of the same bacterium causing the initial bacteremia (as determined by antibiotic susceptibility profiling) ○ The restarting of Gram-negative-directed antibiotic therapy after its initial discontinuation due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected ○ Death due to any cause through day 30
SECONDARY OUTCOMES	These include the incidence of clinical failure at days 60 and 90, all-cause mortality at days 30, 60 and 90, the total number of antibiotic days, the incidence of antibiotic-related adverse events (including *Clostridium difficile* infection), the emergence of bacterial resistance, length of hospital stay, and the number of patients in each arm whose assigned antibiotic duration was "overridden" by physicians in the absence of clinical failure (and the reasons for these deviations). Cost-effectiveness/health-economic analyses will also be performed. Various subgroup analyses will be performed for main causative organisms, resistance patterns, involved organ systems, antibiotic regimens including single vs. combination therapy and de-escalation. Risk factors for clinical failure will be determined. Four PIRATE substudies will be conducted: a nested observational prospective cohort comparing patients' recall and understanding after providing oral vs. written consent, an observational prospective cohort substudy examining the clinical outcomes of patients screened for but not included in the PIRATE trial, a prospective study evaluating the impact of antibiotic duration and antibiotic choice on the levels of urinary metabolites (3-indoxylsulfate, p-cresol sulphate, hippurate), as surrogate markers of microbiota diversity, and a prospective, matched cohort study for metagenomic analyses of PIRATE participants' intestinal flora for the presence of antibiotic resistance genes (ARG) and network analyses mapping interactions among different microbiota members, taxa, gene functions, ARGs and clinical parameters.
STATISTICAL ANALYSIS	We will perform the primary analysis on both the intention-to-treat (ITT) population (all patients randomized) and the per-protocol (PP) population (all patients adhering to the study protocol with no major deviations). Descriptive analyses with standard methods for randomized trials will be used to measure primary and secondary outcomes. Continuous variables will be compared between the three study arms with the use of Student's t-test or the Mann–Whitney U test, as appropriate; categorical variables will be compared with the chi-square test or Fisher's exact test. To test the hypothesis of non-inferiority for the pre-specified margin of 10 percentage points, a generalized linear regression model will be performed, with a log link and binomial distribution reporting risk differences. We will conclude non-inferiority of the "fixed short" arm then of the "individualized" arm compared to the "fixed long" if the 95% upper bound is less than the 10 percentage points' non-inferiority margin.

STUDY FLOWCHART



Day 1 is defined by the first day of microbiologically appropriate antibiotic therapy.

SCHEDULE OF ASSESSMENTS

Study visit/observation point	1	2	3	4	5	6	7
	Screening	Randomization	Follow-up				
Timeline (days)	**1-5**	**5**	**8**	**12**	**30**	**60**	**90**
Window period (days)	±1	±1	±2	±2	±7	±14	±21
Informed consent	X				(X)	(X)	(X)
Entry criteria	X						
CRP measurement* (2ml blood)			(X)	(X)			
AEs reviewed			X	X			
SAEs reviewed			X	X	X	X	X
Other outcomes data collected					X	X	X

* The CRP will not be requested in patients whose antibiotic therapy has already been discontinued.

ABBREVIATIONS AND DEFINITIONS

AE	Adverse event
ALT	Alanine transaminase
ARG	Antibiotic resistance gene
ABS	Antimicrobial stewardship
AST	Aspartate transaminase
CBC	Complete blood count
CCER	Geneva Cantonal Ethics Commission
CMU	Centre médical universitaire (University of Geneva)
CRF	Case report form
CRP	C-reactive protein
DPI	Dossier patient informatique
EC	Ethics committee
eCRF	Electronic case report form
EHR	Electronic health record
FDA	Food and Drug Administration
GNB	Gram-negative bacteremia
GRL	Genomic research laboratory
HIV	Human immunodeficiency virus
HUG	University Hospitals of Geneva (Hôpitaux universitaires de Genève)
ICF	Informed consent form
ITT	Intention to treat
LPTh	Loi sur les produits thérapeutiques (Therapeutic products law)
LRH	Loi relative à la recherche sur l'être humain (Law on Human Subjects Research)
NRP	National Research Program
OClin	Ordonnance sur les essais cliniques (Clinical trials ordinance)
PI	Principal investigator
PII	Personally identifiable information
PCS	p-cresol sulphate
POC	Point of care
PP	Per protocol
rANOVA	Repeated measure analysis of variance
RCT	Randomized controlled trial
SADR	Serious adverse drug reaction (also SAR)
SAE	Serious adverse event
SMB	Safety monitoring board
SUSAR	Sudden unexpected adverse reaction
3-IS	3-indoxyl sulfate

1 BACKGROUND AND RATIONALE

1.1 Background: Uncollected evidence and point-of-care randomization

Our best intentions for the patients of today may be harming the patients of tomorrow. Physicians in Switzerland are cognizant of the current level of resource waste, but are confronted daily by an unfortunate triad: sick individuals in need of immediate help, a relative wealth of diagnostic and therapeutic options, and only a meager evidence base proving that many of these options are simply unnecessary for the patient at hand. New methods to strengthen this evidence base—efficiently and at low cost—are long overdue. Physicians know that many of their clinical decisions are excessive, but lack the "cover" provided by high-quality clinical evidence to justify a drawdown in resource utilization.

Uncollected evidence

Clinical evidence is ubiquitous, present in every patient's outcome after any intervention. But our current model for collecting it is expensive and inefficient. Randomized controlled trials (RCTs), our most methodologically robust tool, remain the gold standard. Because of their superior internal validity, their results trump those of all other study designs in guiding individual clinical practice and health services delivery (Figure 1). But traditional RCTs are costly, time-consuming, and highly exclusive. They tend to select younger patients with few comorbidities, whereas the chronically ill, who represent the majority of hospitalized patients in Switzerland, are frequently excluded. Those included are then followed in relatively artificial study settings, calling the *external* validity of study results into question.[1,2] A paradigm shift in the way we conduct clinical and health services research is needed.

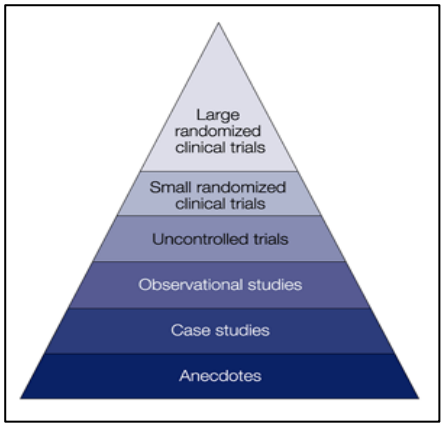


Figure 1. Hierarchy of clinical evidence. (From Engel LW, Straus SE. *Nat Rev Drug Discov* 2002; **1**(3): 229-37.)

Meanwhile, in the clinic, spontaneous "pseudo-randomizations" continue to occur every day, as healthcare providers are required to choose among several diagnostic and therapeutic strategies in their routine practice; their decisions are often based on arbitrary and individual preferences, local dogma, or anecdotal experiences. This abundance of clinical experience goes uncollected and unexamined.

Point-of-care randomization and learning healthcare systems

Novel point-of-care (POC) randomization trials exist to fill this gap. They use the electronic health record (EHR) already in place in the majority of healthcare systems to structure these spontaneous randomizations at the point of care, enabling the coherent study of patient outcomes. The use of the EHR facilitates more inclusive participant recruitment and data collection, minimizing study overhead and excessive follow-up visits for patients, and maximizing generalisability.[3] Uniquely positioned to compare approved treatments or diagnostic techniques toward which there is clinical equipoise, POC trials are embedded in the clinical setting and thus create "learning healthcare systems"[4] to benefit current and future patients. Clinical outcomes data from "real" patients, also retrievable from the EHR, can be analyzed efficiently and fed back to healthcare providers to guide later management. The gulf between research and the clinic is narrowed. And it must be: by definition, evidence-based medicine can come only from the clinic.

Even with the limitations of traditional RCTs described above, the RCT design remains the most reliable tool for determining the usefulness of an intervention and its cost-effectiveness.[5] Results from these trials outweigh those of all otherwise-designed studies in the preparation of far-reaching

guidelines and policies.[6] Yet the vast majority of RCTs are sponsored and conducted by industry.[7] Public health and academic milieus, so important for unbiased, independent scientific inquiry and patient representation, lag far behind because they cannot afford to routinely conduct traditional RCTs.

Point-of-care research is a novel approach to clinical study design that, at significantly lower cost, embeds randomized trials into regular medical care.[8] It is a direct offshoot of the learning healthcare system (LHS) movement,[4] which began in earnest in the last decade and is now championed by many groups, perhaps most notably the U.S.'s Institute of Medicine. The latter has called on healthcare leaders to transform their systems into LHS, in which patient care is integrated with medical research so that the healthcare practices offered in the system are continuously studied and improved. Importantly, bioethicists have become strong supporters of LHS, advocating a "Common Purpose Framework" in which healthcare providers and patients work side by side to effect these advances in medical care.[9] In recent years, several POC trials have been launched in the United States[3] and the United Kingdom. Two British trials, both still ongoing, have undergone a formal evaluation whose goal was to identify the challenges and weaknesses inherent in this novel study design.[10] The report concluded that the implementation of POC randomization was not particularly problematic, nor was data collection. The challenges, rather, were those that confront investigators of any RCT: long approvals processes, legalistic and convoluted informed consent procedures, and overly complex research governance procedures. To our knowledge, no POC RCT has been implemented in Switzerland, although the widespread use of EHR in the country's major hospitals makes it an appropriate setting for this innovative approach.

1.2 Rationale: a point-of-care trial to decrease excessive antibiotic consumption

First trial using the POC RCT platform
We will conduct the first POC RCT in Switzerland and thereby build a platform for further low-cost, methodologically rigorous POC trials. As a seminal prototype, and with a focus on conserving an endangered healthcare resource for an aging population, the POC randomized trial proposed here will assess **optimal durations of antibiotic therapy for Gram-negative bacteremia, a frequent—and frequently life-threatening—invasive infection in patients**.

Patients with chronic illnesses rely most on antibiotics and, through no fault of their own, misuse them the most.[11,12] Prolonged antibiotic courses result in unnecessarily long hospital stays[13] and, on a larger level, drive the increases we are witnessing globally in antibiotic resistance. While health services research clearly aims to optimize the prevention and treatment of illnesses in our aging population, its goal must also be to safeguard the precious, limited resource without which these individuals cannot safely undergo routine cardiovascular and joint-replacement surgeries, chemo- and other immunosuppressive therapy, and for whom common infections and minor injuries could once again become life-threatening.[14]

Antibiotic resistance is growing at an alarming rate and is now considered by many governments, including Switzerland's, to be one of the most serious global threats of the 21st century.[15-17] The key driver of antibiotic resistance is antibiotic overuse;[18] long antibiotic courses select for resistance among the trillions of bacteria hosted by the human body.[19] Patients who receive extended courses of broad-spectrum antibiotics are at significantly higher risk for later infections with difficult-to-treat, multidrug-resistant bacteria.[20,21] And the patients who develop and die from these highly resistant infections are overwhelmingly those with chronic conditions.[20]

No RCT evaluating the optimal duration of therapy for Gram-negative bacteremia (GNB), a frequent and severe infection in elderly and chronic-care patients, has been published. Traditionally,

guidelines have somewhat arbitrarily recommended long antibiotic courses of two weeks, even though patients with no structural complications may recover after only five days of therapy.[22] Direct evidence is mounting that longer antibiotic courses leave patients at risk of acquiring difficult-to-treat multi-resistant organisms. In one RCT comparing 8 to 15 days of antibiotic therapy for ventilator-associated pneumonia, multi-resistant pathogens emerged significantly less frequently in those who had received 8 days of antibiotics.[21] Indeed, given rising concerns over resistance, many physicians have reduced antibiotic durations for uncomplicated GNB to 7 days with no apparent untoward consequences.[23]

Although these shorter durations have not been directly studied, there is nonetheless mounting evidence that they do not increase patients' risk of relapse or other complications: several randomized studies evaluating the optimal duration of antibiotic therapy for pyelonephritis,[24] pneumonia,[25-27] peritonitis,[28,29] and surgical site infections[30] have included patients with concurrent bloodstream infections (both Gram-negative and positive). These have compared durations as short as 5 days with longer (7 or 14 days) durations, and none demonstrated differences in the subset of patients with bacteremia in clinical or microbiologic outcomes.[22] Nonetheless, none of these trials aimed specifically to assess the equivalence or the non-inferiority of shorter vs. longer durations of antibiotic therapy.

Antibiotic durations could also be individualized, guided by clinical response as measured by objective markers, including inexpensive biomarkers such as C-reactive protein (CRP).[31] This acute phase protein is a reliable and highly sensitive marker of inflammation across different patient populations and infections.[32-34] While procalcitonin has been studied in more than 20 RCT as a biomarker to guide the duration of antibiotic therapy in severe infections[35,36] and has indeed proved the concept of biomarker-guided therapy, observational and randomized studies[31,37] have demonstrated no substantial differences in the ability of these two markers to reflect improvement (or worsening) in the clinical course of severe infections. Indeed, a RCT comparing the two markers head-to-head for guiding antibiotic therapy duration in sepsis found that a procalcitonin-based protocol was not superior to a CRP-based protocol, while no difference in morbidity or mortality was observed.[31] As described below, we will ultimately employ a CRP-based protocol for the sake of pragmatism—and in the spirit of countering overuse to improve allocation of resources: in Switzerland, a procalcitonin assay costs roughly 8 times as much as a CRP assay (70-84 vs. 10-20 CHF).[37] In addition, CRP is substantially more accessible than procalcitonin across various clinical settings: many community hospitals do not offer or routinely perform the procalcitonin assay. Indeed, the significantly higher cost and relative overuse of procalcitonin recently led HUG decision makers to withdraw this assay from the laboratory formulary except for patients in the intensive-care unit and pediatric emergency room.

The increasing equipoise with regard to varying treatment durations, the high incidence of GNB, the ease of its diagnosis (via routine blood cultures), and the high stakes of antibiotic overconsumption in an aging population all combine to make antibiotic utilization for GNB an appropriate initial subject of study for the POC trial platform.

2 OBJECTIVES AND OUTCOME MEASURES

The study hypothesis is that shorter antibiotic courses for Gram-negative bacteremia reduce antibiotic treatment days without increasing relapse rate or mortality. The overall objective of this prototypical POC trial is to widen the evidence base on methods for safely drawing down the current over-utilization of antibiotics.

The primary objective is to determine whether shorter antibiotic courses (5-7 days) are non-inferior to a two-week antibiotic course in the treatment of GNB. Secondary objectives are to determine whether antibiotic durations can be safely determined via a simple algorithm employing clinical and laboratory (CRP) markers, whether shorter antibiotic courses for GNB will result in a decrease in antibiotic days, incidence of *Clostridium difficile* infection, the emergence of bacterial resistance, and length of hospital stay.

2.1 Primary outcome measure

The primary outcome will be the *clinical failure rate in all arms at day 30*. Clinical failure is defined by the presence of at least one of the following:

- Relapse: a recurrent bacteremia due to the same bacterium occurring from the day of treatment cessation and through day 30
- Local suppurative complication that was not present at infection onset (e.g., renal abscess in pyelonephritis, empyema in pneumonia)
- Distant complications of the initial infection, defined by growth of the same bacterium causing the initial bacteremia (as determined by antibiotic susceptibility profiling)
- The restarting of Gram-negative-directed antibiotic therapy after its initial discontinuation due to clinical worsening suspected to be due to the initial infecting organism and for which there is no alternate diagnosis/pathogen suspected
- Death due to any cause through day 30

2.2 Secondary outcome measures and other definitions

Secondary outcomes include the incidence of clinical failure at days 60 and 90; all-cause mortality at days 30, 60 and 90; the total number of antibiotic days; the incidence of antibiotic-related adverse events through day 90 (including *Clostridium difficile* infection, a common by-product of antibiotic overconsumption); the incidence of the emergence of bacterial resistance in those with recurrence; the number of patients in each arm whose assigned antibiotic duration was “overridden” by physicians in the absence of clinical failure (and the reasons for these deviations); and length of hospital stay. Cost-effectiveness and other health-economic analyses will also be performed.

Additional subgroup analyses will be performed for main causative organisms, resistance patterns, involved organ systems, antibiotic regimens including single vs. combination therapy, and de-escalation. Moreover, risk factors for clinical failure will be determined, such as age, antibiotic choice, anatomic focus of primary infection, comorbidity status, infection acquisition type (community vs. nosocomial), severity of illness at the time of diagnosis, etc.

Other definitions

Bacteremias will be categorized as nosocomial if the first positive sample is taken ≤48 h after hospital admission; otherwise they will be categorized as community-acquired. Additionally, if the patient has been admitted to hospital in the preceding 30 days, transferred from another healthcare facility (e.g., long-term care unit), is receiving chronic dialysis, or has metastatic cancer, their bacteremia will be considered healthcare-associated.[38]

Severity of illness at the time of bacteremia onset will be defined by the Quick SOFA Score (qSOFA), which can be determined for all patients (including non-ICU patients). The score consists of three

variables and has a maximum of 3 points (1 point each for systolic blood pressure ≤100 mmHg, respiratory rate ≥22 breaths/minute, and any altered mental state [Glasgow Coma Scale <15]); the presence of ≥2 points is associated with higher risks for mortality and extended ICU stay.[39]

3 INVESTIGATIONAL PLAN

3.1 Study design and setting

This analyst-blinded, point-of-care randomized controlled trial will enroll 500 hospitalized adult patients diagnosed with community- or hospital-acquired GNB. The trial will take place at the Geneva University Hospitals (principal site), the Centre hospitalier universitaire vaudois (CHUV), and the St. Gallen Cantonal Hospital (KSSG; Figure 2). The Geneva University Hospitals, CHUV and KSSG perform roughly 59,000, 43,000 and 35,000 admissions per year, respectively; each has both a microbiology laboratory and a team of consulting infectious disease physicians available at all times.

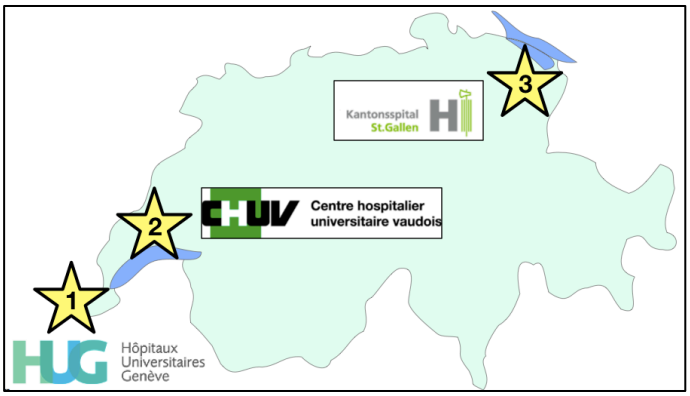


Figure 2: Trial sites. The HUG will be the principal site; CHUV and KSSG are participating peripheral sites.

3.2 Study population and entry criteria

Potential study patients will be identified via both the laboratory and the EHR: in all of these hospital systems, the microbiology laboratory is required to report daily all positive blood cultures to the infectious disease consult team.

3.2.1 Inclusion criteria

Inclusion criteria are as follows:

1) Age ≥ 18 years
2) The presence of Gram-negative bacteria in at least one blood culture bottle
3) Treatment with a microbiologically efficacious antibiotic

3.2.2 Exclusion criteria

Exclusion criteria are

1) Immunosuppression (including HIV infection with CD4 cell count ≤500/µl, hematopoietic stem-cell transplantation in the first month after transplantation and at any time before engraftment, neutropenia in the 48 hours prior to randomization, receipt of high-dose steroids [>40 mg prednisone or its equivalent] daily for > 2 weeks) in the two weeks prior to randomization
2) GNB due to the following complicated infections:
    a. Endocarditis or other endovascular infection without a removable focus
    b. Necrotizing fasciitis
    c. Osteomyelitis or septic arthritis
    d. Confirmed prostatitis
    e. Undrainable abscess or other unresolved sources requiring surgical intervention (e.g., cholecystitis) at the time of enrolment
    f. Central nervous system (CNS) infections
    g. Empyema
    h. Recurrent bacteremia (same bacterium [by resistance profile] causing bacteremia in the previous 60 days)
3) GNB due to non-fermenting bacilli (*Acinetobacter* spp., *Burkholderia* spp., *Pseudomonas* spp.,) *Brucella* spp., *Fusobacterium* spp., or polymicrobial growth with Gram-positive organisms
4) Fever (≥38º C) or hemodynamic instability in the 24h prior to recruitment

3.3 Intervention

With day 1 defined as the first day of appropriate (microbiologically efficacious per antibiogram results) antibacterial therapy, patients will be randomized 1:1:1 on day 5 (±1) to one of the following three arms (Figure 3):

1) "**Fixed long**" antibiotic course of **14 days** (control arm)

2) "**Fixed short**" antibiotic course of **7 days** (first intervention arm)

3) "**Individualized**" antibiotic course: starting on day 5, therapy will be discontinued after the patient has been *afebrile for 48 hours and the CRP level has decreased from its peak by at least 75%* (second intervention arm)

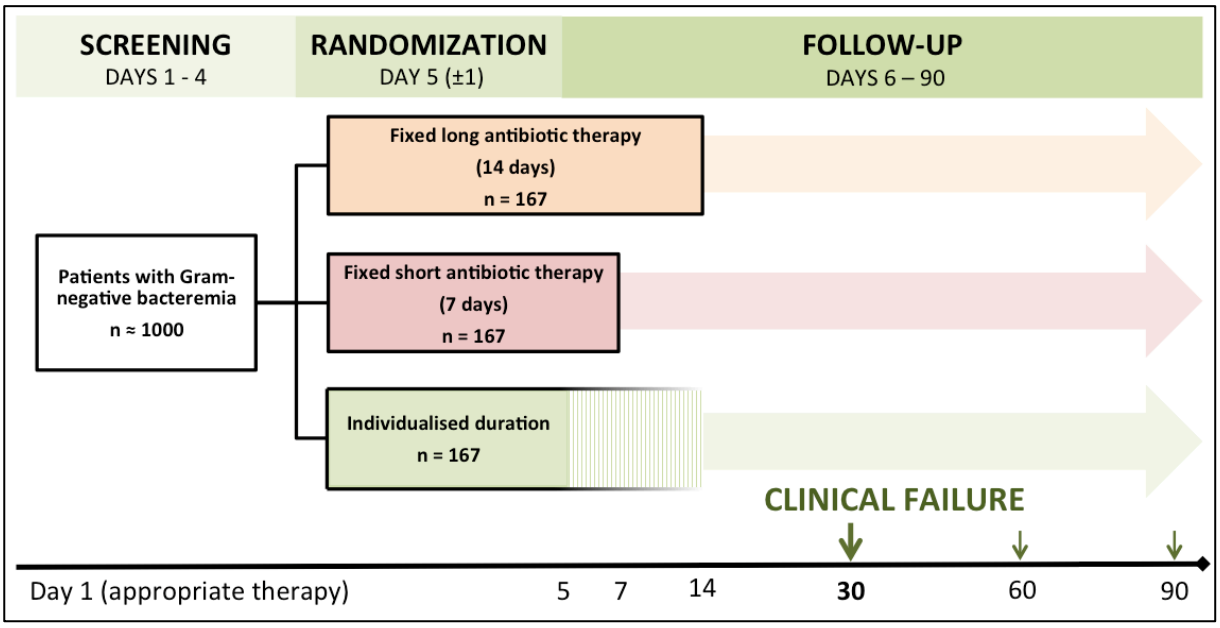


Figure 3. Trial flow. Day 1 is defined as the first day of microbiologically appropriate antibiotic therapy. Patients will be randomized on day 5 (±1) and followed until day 90.

The rationale for our "fixed short" treatment arm of 7 days derives from several observational studies suggesting the safety of shorter antibiotic durations (5-10 days) for patients with bacteraemia.[22,40,41]

CRP algorithm for the individualized-duration arm

The rationale for the individualized algorithm's specific use of a 75% reduction in peak CRP values is based on a RCT of patients in intensive-care units with severe sepsis or septic shock with or without bacteremia.[31] That study demonstrated that an even more restrictive algorithm (antibiotic stop once the CRP has decreased by ≥ 50% if peak CRP was ≥ 100 mg/l or once CRP is less than 25 mg/l if peak CRP was < 100 mg/l) was safe and effective to reduce antibiotic use. This study additionally used a 7-day maximum duration of antibiotic therapy for non-bacteremic patients while bacteremic patients received at least 7 day of antibiotics.[31] Our individualized algorithm is slightly adapted in analogy to procalcitonin-based algorithms, which have been successfully tested in several RCTs and used ≥ 80% decreases of procalcitonin to discontinue antibiotic therapy.[35,42-45] Our slight modifications take into account the slower decrease of CRP values compared to procalcitonin after resolution of an infection[44,46] and incorporate an additional safety margin compared to the study by Oliveira et al., which treated bacteremic patients differentially.[31] Of note, if the CRP value has not decreased by

75% by day 14, the marker will no longer be used to guide the duration of therapy. In these cases, the duration will be determined by clinical judgment per usual practice.

Choice of antibiotic(s) and mode of administration
In all arms, the choice and mode of administration of antibiotic(s) will be left to the patient's attending physician and consulting infectious disease specialist and thus will follow usual standards of care, determined primarily by the three sites' local institutional antibiotic therapy guidelines and antimicrobial resistance patterns. (Resistance prevalences in these hospitals and communities are similar, and the sites' treatment guidelines contain no significant discrepancies with respect to acceptable antibiotic treatment standards of uncomplicated GNB.)

De-escalation (from a broad-spectrum to a more narrow-spectrum antibiotic), switches from intravenous to oral antibiotic therapy or from intermittent to continuous infusions or vice-versa, will be allowed, per current standard practice. Study investigators will not interfere with these clinical decisions, but will collect detailed data on all therapeutic management for later subanalyses. Although randomization and subsequent discontinuation of antibiotic therapy according to treatment arm will occur through the EHR, study investigators will also not interfere with attending physicians' decisions to prolong therapy, should a patient's clinical condition worsen. (See sections on statistical analysis and sample size calculation.)

3.4 Randomization
We determine day 5 as the appropriate randomization point because this is the usual timing of the study's, and clinicians', essential question: "*Now that my patient has been stabilized and appears to be improving, when can I safely discontinue antibiotic therapy?*"

3.4.1 General principles and peripheral site randomization procedures
Randomization will be based on investigator-blinded blocks of randomly varying size in order to protect against potential predictability of treatment assignments. Blocks will contain three, six, nine or twelve allocations. Randomization will be stratified by study site, given that the three sites do not share a common EHR system and that site launches will be staggered (HUG will be the first site to launch; CHUV and KSSG will follow). For randomization at HUG, see the next section. For randomization at CHUV and KSSG, a statistician not involved in the study analysis will produce the randomization list prior to the initiation of the study; the blocks' order will be generated by use of a computer-based randomized number system. The statistician and a designated non-member of the study team will keep copies of the randomization list.

3.4.2 Point-of-care randomization through the electronic health record at HUG
In POC trials, the extent of the EHR's involvement in randomization is variable: on one end of the spectrum, the EHR simply provides an automatic alert to a prescribing physician making her aware of the existence of the POC trial and the patient's likely eligibility for it, while simultaneously alerting a study investigator of the patient. At the other end of the spectrum, the EHR provides such alerts and, according to a physician-triggered EHR workflow, ultimately performs the actual randomization with treatment assignment.

In the present study, through the work of information technology (IT) specialists at HUG, this principal study site will implement a fully EHR-integrated process (Figure 4) using HUG's "Dossier patient informatique" (DPI), with an initial, early alert to the study team at the moment the positive blood culture is registered in the EHR, and automatic alerts for the treating physician at point of care and study personnel once the patient enters the eligibility window. Study personnel will be notified to (1) provide information to the patient, (2) verify study inclusion criteria, then (3) approve the EHR-based randomization.

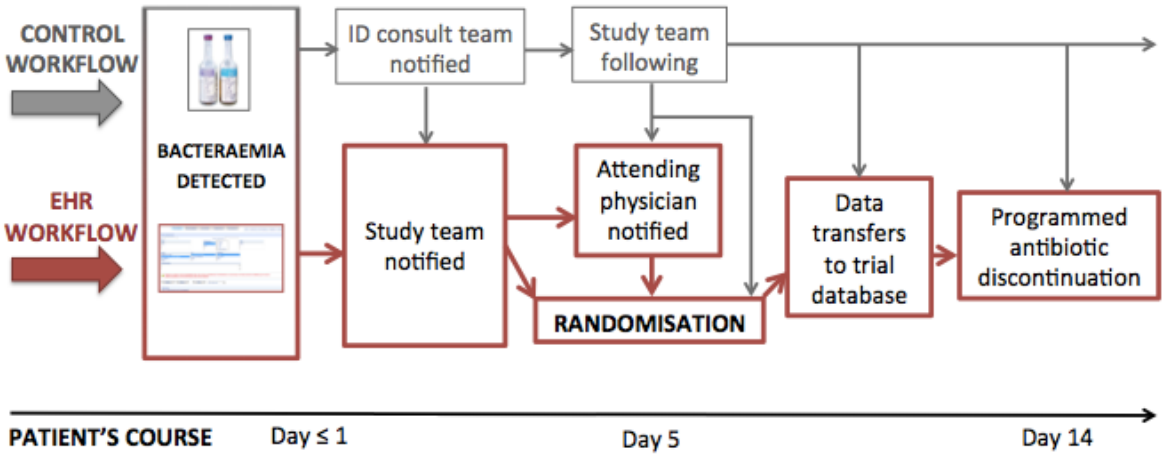


Figure 4: Electronic-healthcare record workflow for patient identification, randomization and follow-up. The EHR workflow is outlined in red, the control ("back-up") workflow in grey. Grey arrows indicate safety valves; these cover all points at which the EHR workflow could malfunction. In this hypothetical case, the patient has been randomized to the control arm (antibiotic therapy duration of 14 days).

At the moment of randomization, the EHR will (1) automatically place a note of participation in the medical record and (2) establish the duration of the patient's antibiotic prescription (7 or 14 days or the individualized duration, which will also be determined within the EHR, as it houses temperature and CRP data). On the predetermined day of antibiotic discontinuation, both physicians and nurses will receive alerts regarding that discontinuation. These alerts will be repeated until the physician electronically signs his acknowledgment of the discontinuation.

The control workflow
Importantly, the current study focus of GNB provides an ideal occasion for piloting and testing this process since, in reality, the study team will already be well aware of all patients hospital-wide with any Gram-negative bacteremia, given the established daily communication between microbiology laboratories and infectious disease teams described above. There will thus be a "control" workflow shadowing the automated EHR workflow at all times, with safety valves present at all workflow nodes (Figure 4).

The EHR-determined randomization procedure
The EHR will be programmed to randomize using the same principles described above: randomization will be based on randomly varying blocks with three, six, nine or twelve treatment duration assignments. The bio-informatics specialist (Dr. Rodolphe Meyer) leading the informatics component of this trial will keep a copy of the EHR's randomization list, as will a designated non-member of the study team (control workflow).

3.5 Blinding

3.5.1 "*Ad terminum*" blinding of patients, attending physicians, and investigators

The blinding of patients, hospital staff, and designated trial investigators to assigned treatment duration will be key to avoiding the introduction of bias in the follow-up/management of patients between study arms. It is conceivable that the knowledge that one's patient will receive a shorter course of antibiotics could result in defensive medicine, with that patient's receiving "special treatment" such as additional surveillance blood cultures and/or imaging tests just before antibiotic discontinuation. For this reason, we will carry blinding of patients, treating hospital staff, and designated investigators through the furthest study point possible. These parties will be blinded to

treatment assignment from randomization until antibiotic discontinuation ("*ad terminum*"). Thus, in the fixed-short arm, all parties will be blinded until day 7, and in the individualized arm, until the clinical requirements for discontinuation have been met. In the effort to avoid unblinding by the process of elimination after day 7, attending physicians and nurses will not be made aware of the specific algorithm defining criteria for antibiotic discontinuation in the individualized group. They will thus be allowed to view all CRP results, but they will not be able to predict when an individualized duration will end.

3.5.2 Blinded outcomes assessment and data analysis

Clinical data on all included patients will be collected regularly by study personnel and recorded in the electronic case report form (eCRF) and database (Secutrial® version 4.8). HUG data managers, experienced in generating blinded reports, will then provide data exports to a blinded outcomes assessor and a blinded data analyst; these exports will contain recoded ("scrambled") study identification numbers and no information on treatment assignments, allowing for both fully blinded outcomes assessment and data analysis.

3.5.3 Lifting the blind

Because (1) no experimental therapy will be given and (2) treating physicians will have the right to override the patient's treatment duration assignment in the case of clinical worsening, it is not anticipated that any early lifting of the blind will be necessary. Nonetheless, instructions and the means to access the randomization list overnight and on weekends will be available to the infectious disease physician on call, should early lifting of the blind be deemed necessary.

3.6 Study schedule

After randomization, patients will be followed for a total of 90 ±21 days. An important principle of POC trials is that patients be allowed to remain in their normal clinical setting; they are followed non-invasively for outcomes data. The only additional laboratory test that may be requested is later-phase CRP measurements if these have not already been ordered by treating physicians (on days 8±2 and 12±2 in patients whose antibiotic therapy was not already discontinued).

On days 30, 60, and 90, clinical data necessary for determining the primary and secondary outcomes listed above will be collected. If on these days the patient is no longer hospitalized, he will be contacted by study staff by telephone and interviewed according to a structured questionnaire including the clinical information in the following section. In the event that a patient reports clinical worsening at the time of follow-up, he will be asked to come for an in-person visit at the HUG's Policlinique des maladies infectieuses, or respective outpatient clinics at CHUV and KSSG, respectively. The study's schedule of assessments is shown in Table 1.

Of note, a fixed interim safety analysis will occur after randomization of the first 150 patients; this is described in more detail below.

Table 1. Schedule of assessments.

Study visit/observation point	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Screening	**Randomization**	**Follow-up**				
Timeline (days)	**0-5**	**5**	**8**	**12**	**30**	**60**	**90**
Window period (days)	±1	±1	±2	±2	±7	±14	±21
Informed consent	X				(X)	(X)	(X)
Entry criteria	X						
CRP measurement* (2ml blood)			X	X			
AEs reviewed			X	X			
SAEs reviewed			X	X	X	X	X
Other outcomes data collected					X	X	X

* The CRP will not be requested in patients whose antibiotic therapy has already been discontinued.

3.7 Data to be collected in the eCRF

The following clinical data will be collected from the patient's medical record and from structured telephone contacts and/or visits after hospital discharge.

Table 2. Data to be collected in the eCRF for the PIRATE trial.

Data to be collected in the eCRF
Demographic information • Age • Gender • Ethnicity • Employment status, education level, annual household income • Self-perception of health status
Study dates • Inclusion date • Start/end of treatment dates • Date of last follow-up
Enrollment • Inclusion criteria (checklist) • Exclusion criteria (checklist) • Study randomization number • Principal diagnosis
Clinical history & concomitant medications • Co-morbidities • Presence of invasive devices • Origin of bacteremia (community-acquired, healthcare-associated, nosocomial) • Results from diagnostic tests performed prior to inclusion • Concomitant medications (particularly antibiotic therapy)
Clinical course • Antibiotics used (class, dosage, route of administration) • Potential adverse events of study medications • Symptomatology related to the present illness • Additional medications • Discharge diagnosis
Laboratory (hematology and chemistry, both pre- and post-enrollment) • Complete blood counts including differential • Serum creatinine values • Liver function tests • CRP values • Procalcitonin values, if any
Laboratory (microbiology and immunology, both pre- and post-enrollment) • Results of stains, cultures, and molecular diagnostic testing (e.g., PCR) • Antibody titers if related to the GNB under study
Imaging • Results of X-rays, CT, MRI, or PET scans, ultrasounds
Compliance • Reported compliance with antibiotic treatment (e.g., number of applications missed)
Follow up • Regular end of study, subject/patient withdrew consent, withdrawal by investigator, protocol violation, loss to follow-up, death

3.8 Interim analyses

An interim analysis for safety will be performed after roughly 150 patients have reached 30 days of follow-up (described in more detail below). At this time, and if determined necessary, a non-blinded investigator will also assess the performance of the CRP as a marker for guiding durations. If the marker is proving either impractical (e.g., logistically difficult to obtain) or its algorithm difficult to follow (e.g., CRP initially decreases but not quite by 75%, then rises again due to another inflammatory process), an alteration in the algorithm, or in the arm itself, will be explored. Any recommended changes will undergo review by all study investigators and methodologists, and any proposed amendment to the protocol will be submitted to the central ethics committee per usual routine.

4 SAFETY CHECKPOINT ANALYSES AND OVERALL SAFETY ASSESSMENT

4.1 Safety "checkpoint" analyses

An initial safety "checkpoint" analysis will be performed after 150 patients (roughly one third of the target) have reached the 30-day follow-up mark in order to assess whether arms with shorter therapy durations (fixed-short duration of 7 days and individualized arm) could potentially result in worse clinical outcomes, specifically with increased clinical failure. The outcomes assessment and data analysis for this assessment will be done in blinded fashion. If, however, results show a significant difference in outcomes in any arm, the blind will be lifted for the data analyst. (Blinding and unblinding of data will occur with the aid of the data manager, as described above.) Given the relatively small number of patients to be included in the first safety analysis (necessarily reducing power), statistical support in the interpretation of the data will be provided by Dr. Gayet-Ageron and Prof. Perneger.

Unblinded results will be forwarded to an independent Safety Monitoring Board (SMB) for review. Should patients in either of the intervention arms demonstrate significantly worse clinical outcomes, recommendations made by the SMB will be followed. Other safety checkpoints may be scheduled depending on the outcome of this first fixed analysis.

4.2 Safety Monitoring Board

Members of the SMB will be selected before the start of the project and will include at least three experts in the field, including one biostatistician or epidemiologist, one infectious disease physician and one general internist or surgeon. The names of SMB members will be forwarded to the ethics committee when determined.

4.3 Adverse events and reactions

4.3.1 Adverse events

An AE is any untoward medical occurrence in a subject that may occur during or after administration of a pharmaceutical product and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

For the purposes of this study, whose focus is not on the choice of antibiotic but on its duration, and within which only post-market, non-experimental antibiotics with well-established safety profiles will be used, only AE considered possibly, probably, or certainly related to the antibiotic(s) being administered for the Gram-negative bacteremia will be recorded into the eCRF. The AE will thus be recorded during and in the two days following discontinuation of antibiotic therapy targeting the Gram-negative bacteremia. After that point and for the remainder of the study period, only symptomatic *Clostridium difficile* infection and SAE (as defined in Section 4.5) will be recorded in the eCRF. AE will be graded according to the tables for AE severity below (Section 4.9).

4.3.2 Adverse reactions

An adverse reaction (AR) is any untoward or unintended response to a pharmaceutical product. This means that a causal relationship between the product and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting Investigator as having a reasonable suspected causal relationship to a pharmaceutical product (i.e. possibly, probably or definitely related to the product) will qualify as AR.

4.4 Serious adverse events

A SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention:

- Death
- Life-threatening event (i.e., the subject was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e., substantial disruption of one's ability to carry out normal life functions)
- Hospitalization, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalization (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a SAE.
- An important medical event (that may not cause death, be life-threatening, or require hospitalization) that may, based upon appropriate medical judgment, jeopardize the subject and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive care in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.
- Congenital anomaly or birth defect

4.5 Serious adverse drug reaction (SADR)

An event that is expected or unexpected and is both serious and, in the opinion of the reporting investigator or Sponsors, believed to be possibly, probably or definitely due to a pharmaceutical product or any other study treatments, based on the information provided, is considered a serious adverse drug reaction (SADR or SAR).

4.6 Suspected unexpected serious adverse reaction (SUSAR)

A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to a pharmaceutical product. No category of SAE has been defined as 'expected.'

4.7 Causality assessment

For every AE, an assessment of the relationship of the event to the administration of the study antibiotic will be undertaken. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a pharmaceutical product. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of antibiotic administration; and the known biology of the antibiotic therapy (Table 3).

Table 3: Guidelines for assessing the relationship of medication administration to an AE.

Causality grading		**Explanation**
0	**No Relationship**	No temporal relationship to study drug ***and*** Alternate etiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study drug

Causality grading		Explanation
1	**Unlikely**	Unlikely temporal relationship to study drug ***and*** Alternate etiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study drug
2	**Possible**	Reasonable temporal relationship to study drug; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other medications
3	**Probable**	Reasonable temporal relationship to study drug; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other medications
4	**Definite**	Reasonable temporal relationship to study drug; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other medications ***or*** Event reproducible upon re-challenge

4.8 Reporting procedures for adverse events

Adverse events will be recorded in the study database as described above. In this phase 4 study, the principal investigator (PI) will report all SAE to the CCER and SMB by means of the annual safety report. In addition, any SAE considered possibly, probably or definitely related to the study antibiotic (SUSAR) and resulting in death will be reported by the PI to the CCER and SMB within seven calendar days and to co-investigators within 24 hours.

4.9 Assessment of severity

The severity of AE will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0, using the following scales (Table 4).

Table 4: Toxicity grading scale for AEs.

Severity grade	Description
Grade 1	**Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; no intervention indicated
Grade 2	**Moderate**; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL
Grade 3	**Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL
Grade 4	**Life-threatening** consequences; urgent intervention indicated
Grade 5	**Death related to AE**

ADL: activities of daily living
From CTCAE v4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

5 STATISTICAL ANALYSIS

5.1 Sample size calculation

Previous observational studies and randomized controlled trials including patients with bacteremia[25,31,38,47] have demonstrated clinical failure rates between 10 and 30% in settings with access to broad-spectrum antibiotics and resistance prevalences similar to those in Switzerland. The primary reason for failure in these and other studies is the lack of appropriate antibiotic therapy (either due to antimicrobial resistance or a delay in therapy initiation). Because this trial's inclusion criteria require five days of microbiologically adequate antibiotic therapy at the time of randomization, we assume the upper limit of "success" or, inversely, the clinical failure will be 10% in both the control and intervention arms. To establish non-inferiority, we will allow a difference up to 10% in the primary outcome. The chosen margin is wide because the expected gain from reducing the use of antibiotics is significant (decreased odds for antibiotic resistance, reduction in treatment adverse events, reduced medical costs by shorter length of hospital stay).[24] Furthermore, as we will have excluded immunosuppressed patients and those with complicated infections, there will be decreased risk for life-threatening events and serious deterioration.

Further assuming a one-sided type I error (α) of 0.025, a power (ß) of 0.80, an attrition (loss to follow-up) of ~5%, and potential treatment switching of ~12%, 167 patients will be needed in each of the three arms to prove non-inferiority, making the total sample size 500.

5.2 Statistical analysis

We will perform the primary analysis on both the intention-to-treat (ITT) population (all patients randomized) and the per-protocol (PP) population (all patients adhering to the study protocol with no major deviations). While ITT analyses are critical in all studies, in non-inferiority trials PP analyses take on a particularly important role in the effort to avoid commission of a type II error.[48] (One can imagine, for example, that non-adherence to the full 14-day antibiotic regimen could lead to this arm's appearing generally less effective, thus lowering the bar for the two intervention arms.) In this particular study, however, the PP analysis will have another key role. As stated above, attending physicians will have the freedom to override their patients' treatment duration assignments in the event of perceived clinical worsening. There may thus be some switching from intervention towards control arms (non-adherence to treatment assignment) with the risk of diluting the difference in clinical failure rates between the intervention groups and the control group, and thus increasing the risk of incorrectly concluding non-inferiority of shorter antibiotic duration. The sample size has thus been increased to accommodate treatment switching of ~12% study-wide, or some 20 patients per group (see above).

Descriptive analyses with standard methods for randomized trials will be used to measure primary and secondary outcomes. Continuous variables will be compared between the three study arms with the use of Student's t-test or the Mann–Whitney U test, as appropriate; categorical variables will be compared with the chi-square test or Fisher's exact test, as appropriate. To test the hypothesis of non-inferiority for the pre-specified margin of 10 percentage points (see below), we will perform a generalized linear regression model with a log link and binomial distribution reporting risk differences of clinical failure between intervention arms compared to the control arm. The treatment assignment will be the main predictor with the control arm ("fixed long") as the reference and the model will be adjusted for the study centre.[49] If some differences in the use of antibiotics are described between the three intervention arms, we will also adjust for it in the regression model. We will conclude non-inferiority of the "fixed short" arm then of the "individualized" arm compared to the "fixed long" if the 95% upper bound is less than the 10 percentage points' non-inferiority margin. With three centers, a mixed regression model is less appropriate. We will also present risk ratios or odds ratios with 95% confidence intervals.

Missing data

Missing data will be taken into consideration using several methods, among them responder analysis, complete case analysis (modified ITT) and potentially multiple imputation. These sensitivity analyses will be used to validate study findings.

6 OBSERVATIONAL SUBSTUDIES

6.1 Nested prospective observational cohort study on recall and understanding after oral vs. written informed consent

6.1.1 Background and rationale: oral consent with witness testimony

In line with the recent advice of the Geneva Ethics Commission and with OClin Art.8 al.1b, patients deemed to have decision-making capacity and consenting to participate in this study may provide oral consent when an independent witness can sign testimony to that consent. The background and justification for this informed consent (IC) model are detailed below in Section 8.3. The decision to obtain oral or written consent will be left to the discretion of the including investigator and will depend on the clinical and cognitive state of the patient.

Very little is known about patient recall and understanding after oral versus written consent, whether that consent was granted for a research study or a clinical procedure; we therefore propose a nested study to compare these outcomes among the patients providing oral consent to those of patients providing written consent for participation in this study, as well as to outcomes of historical controls (patients providing written consent for participation in other trials, such as those followed by Chenaud et al.[50] and described further in Section 8.3). We know that recall and understanding in the weeks after written consent are not optimal; our hypothesis is that they will not differ much (will not be significantly worse) after oral consent.

6.1.2 Nested oral consent study design, setting & population

All patients approached for inclusion in the PIRATE study throughout its three trial sites will be eligible for participation in this nested, prospective observational cohort study. This substudy will begin and end in step with the larger PIRATE trial, thus patients are expected to be included from the spring of 2017 through the spring of 2019. (Addendum, 8 June 2017: For patients who are not deemed to have decision-making capacity and whose participation in the trial is allowed by their representative [see Section 8.3 below and Art. 24 of the LRH], that representative will be contacted for follow-up of clinical outcomes on days 30, 60 and 90; during these interviews, the representative will be asked the same questions on recall provided in the next section. This group of non-patients will serve as a control arm [with the assumption being that their recall should be superior to that of hospitalized, acutely ill patients granting either oral or written consent].)

6.1.3 Nested oral consent study outcomes

The primary outcome is the percentage of patients in both groups (patient oral vs. written consent) who recall granting informed consent to participate in the PIRATE trial on day 30 (±7 days) of the PIRATE trial. Secondary outcomes will include the same endpoint on days 60 (±14 days) and 90 (±21 days), as well as the ability to recall the purpose and risks of the trial as stated in the information brochure at all named time points. For these, the simple questionnaire in the accompanying box will be used. Another outcome will simply be the number of patients included in each arm, and the investigator's cited reason for pursuing an oral vs. written consent. Finally, we will perform correlation analyses for recall and understanding, looking at baseline demographic factors, other factors such as whether the patient asked a question during the initial information encounter,[50] whether family members were present, and whether an attending physician or family member signed the witness testimony.

INFORMED CONSENT - RECALL AND UNDERSTANDING POST CONSENT

1. **Do you recall agreeing to participate in this study?**
   (For patient representatives: do you recall allowing the patient to participate in this study?)
2. **Do you recall the purpose of this study?** *(per information brochure: "The purpose of this research study is to compare 14 days of antibiotic therapy to either 7 days or an "individualized" number of days…for their efficacy and safety."*
3. **Do you recall the risks of the study?** *(per information brochure: "Rarely an additional blood draw may be needed to continue to measure the response to therapy. Blood draws can lead to bruising and pain at the point of puncture. The total loss of blood (2 ml, or half a teaspoon) is not higher than for a blood donation and thus not enough to cause medical problems in people with no underlying illness. An unknown risk cannot be excluded.")*

6.1.4 Substudy statistical considerations

Given the hypothesis of non-inferiority in recall after oral vs. written consent, a presumed recall after consent in the control (written) arm of roughly 80%,[50] and assuming a significance (alpha) level and power of 5% and 80%, respectively, roughly 198 patients would be needed in each arm to demonstrate non-inferiority with a margin of 10%, a sample size achievable given the context of the larger PIRATE trial. Nonetheless, we appreciate that we will ultimately be relying on a convenience sample. This is because we cannot confirm that PIRATE trial inclusions by oral and written consent will occur at a 1:1 rate; patients will not be randomized to either mode of consent.

Descriptive statistics will be used to describe patient characteristics and measure recall outcomes in each arm. Continuous variables will be compared with the use of Student's t-test or the Mann–Whitney U test, as appropriate; categorical variables will be compared with the chi-square test or Fisher's exact test, as appropriate. Logistic or log binomial regression models, where appropriate, will be used for the correlation analyses described above.

6.2 Observational study on excluded patients' clinical outcomes (EPCO)

6.2.1 Background and rationale

Traditional randomized controlled trials have historically excluded patients who are "too old," "too sick," and "too comorbid"; these exclusions reduce their external validity and thus the relevance of their results for clinicians dealing with real patients. Paul et al. recently provided a striking example of this problem: they observed the clinical outcomes of the 220 patients who were not included in a randomized controlled trial comparing vancomycin to trimethoprim-sulfamethoxazole for invasive methicillin-resistant *Staphylococcus aureus* infections in comparison with those of the 252 patients who were included. Most patients were excluded because of an inability or unwillingness to provide written informed consent. The clinical failure rate in this group was 80%, while only 33% of included patients experienced clinical failure. Within the trial, mortality in the vancomycin group was non-significantly lower (mortality odds ratio 0.76, 95%CI 0.36-1.62), but among excluded patients, mortality was significantly higher with vancomycin treatment (OR 2.63, 95%CI 1.04-6.64).[1]

As described in the first pages of this protocol, point-of-care randomized trials seek a greater inclusiveness and should thus theoretically provide stronger external validity. One particular aspect of the PIRATE trial is its option to allow witnessed, oral consent from patients who are too sick and/or tired to be able or willing to hand-sign a consent form. We hope that this will allow for a more inclusive trial and thus more methodologically robust and applicable outcomes data. We therefore propose to follow excluded patients' clinical outcomes ("EPCO"), as Paul et al. did, in an observational cohort study, but with the hypothesis that in this case, outcomes will be less divergent among included vs. non-included patients.

6.2.2 EPCO study design, setting & population

All patients approached for but not included in the PIRATE trial will be eligible for participation in this prospective, multicenter observational cohort study. This study will begin and end in step with the larger PIRATE trial, thus patients are expected to be included from the spring of 2017 through the spring of 2019.

6.2.3 EPCO study outcomes

The primary outcome will be the same as that of the PIRATE trial: the rate of clinical failure rate, as defined in Section 2.1, at day 30 ± 7 days among excluded patients receiving 7 vs. 14 days (vs. other durations) of antibiotic therapy. Secondary outcomes will include the median duration of antibiotic therapy, length of hospital stay, and the reasons for exclusion (consent, patient characteristics, and infection characteristics). We will further assess associations, if any, between baseline patient and infection characteristics and willingness or ability to provide informed consent, whether oral or written.[51-55] Given the difficulties of obtaining follow-up information in this type of population, patients' clinical outcomes will be followed only through day 30 ±7 (with day 1 being the first day of microbiologically appropriate antibiotic therapy).

6.2.4 EPCO study statistical considerations

As described in Section 5.1, we estimate that included patients will experience a clinical success rate between approximately 80 and 90%. Assuming a significance (alpha) level of 5%, power of 80%, and attrition (inability to glean follow-up information) of roughly 20%, approximately 90 patients will be needed to enable the detection of at least a 20% difference in the primary outcome rate between the observation cohort and the PIRATE randomized controlled trial.

Descriptive statistics will be used to describe patient and infection characteristics. Continuous variables will be compared with the use of Student's t-test or the Mann–Whitney U test, as appropriate; categorical variables will be compared with the chi-square test or Fisher's exact test, as appropriate. Unadjusted odds ratios for the comparison between the different antibiotic durations in each cohort (observational and PIRATE trial) will be computed with 95% confidence intervals, and compared using the Breslow-Day test.

6.2.5 Request for waiver of informed consent for the EPCO observational study

Given this substudy's purely observational nature and the disproportionate difficulty that obtaining informed consent would pose (given that a sizable number of patients will have just declined participation in the PIRATE trial), we will ask, in accordance with Article 34 of the Swiss Human Research Act (HRA; 810.30, 2011), for a waiver of informed consent for participation.

6.2.6 Data handling for the EPCO observational study

No data from excluded patients will be entered into the PIRATE eCRF and database. EPCO patients will be identified by means of an EPCO study number, and no personally identifying information will be transcribed into the EPCO CRF, which will be stored under lock and key, independently and apart from PIRATE data. EPCO data will be entered into a separate, password-protected electronic database that will serve the EPCO study only.

6.3 The PIRATE ENDURANCE Project: the Effect of aNtibiotic DURation oN bacterial Ecology: a single-center nested prospective matched cohort study

6.3.1 Background and rationale

Systemic broad-spectrum antibiotics are indispensable for successful treatment of infectious diseases. However, their use is accompanied not only by the development of antimicrobial resistance but in addition by collateral effects with reduction of the human microbiota diversity, that are still poorly understood. The loss of a diverse composition of the microbiota after antibiotic therapy has been associated with a variety of conditions including metabolic syndrome, autoimmune diseases, inflammatory bowel disease, susceptibility to infections and mortality after stem cell transplantation, improved overall survival in cancer patients and treatment response to checkpoint inhibitors in patients with cancer, amongst others.[56-60] The role that commensal bacteria play in shaping and maintaining the integrity and functionality of the immune system and the epithelia as well as their anti-inflammatory effects in the intestinal tissues is increasingly recognised.[56] The biodiversity of bacteria composing the gut microbiota has been shown to be an indicator of health in older populations and associated with frailty levels as wells as other co-morbidities.[61]

Recently a theory has been proposed to explain why the current microbiome of humans and animals is not accidental, but the result of millennia of selective evolution. The microorganisms populating the human gut have been selected over generations to optimize host interaction.[62] A major factor for the loss of certain bacterial taxa and microbial richness in intestinal microbiota of humans in developed countries is antibiotic exposure which disturbs host physiology[63] and can last for many months after antibiotic exposure.[64] It has been suggested that microbial composition and diversity should be assessed and monitored.[65] This underscores the need to evaluate easily measurable markers for their clinical value to assess the collateral effects of antimicrobial treatment on human microbiota diversity.

Many organic waste soluble compounds are produced by colonic bacteria, of which 3-indoxyl sulphate (3-IS) and p-cresol sulphate (PCS) have been studied the best.[66] Similarly, hippurate, which is formed through gut bacterial metabolism of dietary components, is associated with greater microbiota diversity.[60] It has been recently described that immediate changes in gut microbiota diversity after antibiotic use can be indirectly captured by detection of 3-indoxyl sulphate (3-IS) in the urine. 3-IS is a metabolite which originates from the processing of tryptophan into indole by intestinal microorganisms, which is subsequently oxidised and sulphonated in the liver. 3-IS is easily accessible and measurable in urine. 3- IS levels in blood were higher in conventional than germ-free mice.[67] It has been demonstrated that low urinary 3-IS is related to gut flora disruption in patients receiving broad-spectrum antibiotic therapy.[68-70] Another recent pilot study revealed a distinct change in the abundance of several microbial taxa after the administration of oral vancomycin; this correlated with measured concentrations of 3-IS and PCS in patients with kidney failure, confirming a link between decreased gut microbiome diversity and plasma levels of these solutes also in these patients.[70] Yet evidence of the effect on microbiota biodiversity of shorter antibiotic courses or de-escalation of antibiotic therapy from a broad-spectrum to a more narrow-spectrum antibiotic therapy is still lacking; the implications of this possible effect are still unknown.

The ENDURANCE Project, a prospective matched cohort substudy, will explore whether antibiotic duration differentially affects the gut microbiota diversity and in particular, whether shorter courses have less negative impact on it than longer durations; whether different antibiotics have differential effects; whether de-escalation can ameliorate these effects and modify the risk of adverse events (including *C. difficile* infection, a common side effect of antibiotic therapy) and the extent of disruption to the gut ecology. Even though microbiota diversity will be assessed only indirectly by measuring urinary 3-IS, changes of this metabolite have been correlated previously with microbiota diversity in other clinical settings. To our knowledge these correlations have not been performed yet

in human clinical studies. If shorter antibiotic durations or certain antibiotics are associated with a lower risk of decreased microbiota diversity, this might support antibiotic stewardship efforts. It may generate further hypotheses and provide a basis for future microbiota studies within the area of antibiotic stewardship.

The PIRATE study provides a good opportunity to study the changes of urine metabolite levels (such as 3-IS, hippurate and PCS) after antibiotic exposure in an exploratory manner. Choices of effective antibiotics are left to the treating physicians and therefore vary. Some patients will undergo de-escalation or escalation during therapy according to microbial sensitivity, physician preferences and possibly other factors (*e.g.*, allergies). We will collect urine samples for the determination of urinary metabolite levels from consenting patients who are enrolled and randomised in the PIRATE study to variable antibiotic durations. This study may help to support the use of urinary metabolites as easily measurable markers of gut microbial diversity and a predictor of worse outcome or adverse events before the start of antibiotic therapy. This nested prospective cohort study attempts to address the following questions:

- *Is microbial diversity negatively affected by longer courses of antibiotic therapy in comparison to shorter ones?*
- *Is the change of diversity different for different antibiotics or antibiotic combinations?*
- *Is there a correlation between initial microbiota diversity or its relative change and clinical outcome (e.g. complication rate, relapse, length of stay or mortality)?*

6.3.2 ENDURANCE study design, setting & population

For this substudy, urine samples of patients included in the PIRATE trial at the St. Gallen site only and providing informed consent for ENDURANCE will be recovered from their initial presentation, at day 5±1 (randomization), at the end of therapy (EOT), at day 30±4, and at day 60±7 to measure metabolites in the urine. Exclusion criteria are (1) the receipt of antibiotic therapy up to seven days prior to enrolment and (2) Hartnup disease (as these patients might have different 3-IS levels at baseline). See the table below for full inclusion and exclusion criteria.

The ENDURANCE substudy receives no funding and will be conducted at the St. Gallen site as a dissertation project.

ENDURANCE

(schedule of sample collection)

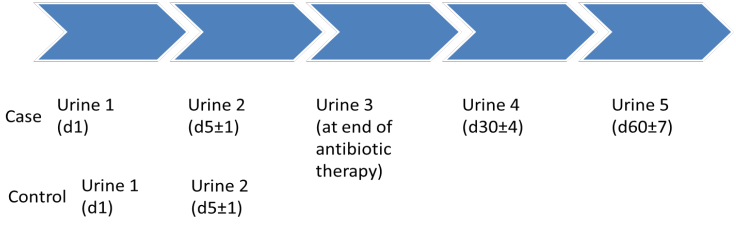


Figure 5. ENDURANCE's study schedule.

Table 5. ENDURANCE's entry criteria.

Endurance case patients		Endurance control patients	
Inclusion criteria	**Exclusion criteria**	**Inclusion criteria**	**Exclusion criteria**
• Enrollment in PIRATE • Written informed consent	• Hartnup disease • Antibiotic therapy up to 7 days prior to enrolment • Otherwise identical to PIRATE study	• Age ≥ 18 years • Hospitalization • Written informed consent	• Hartnup disease • Immunosuppression: o HIV infection with CD4 cell count ≤500/µl o hematopoietic stem-cell transplantation in the first month after transplantation and at any time before engraftment o neutropenia in the 48 hours prior to randomization o receipt of high-dose steroids [>40 mg prednisone or its equivalent] daily for > 2 weeks) in the 2 weeks prior to randomization • Antibiotic therapy up to 7 days prior to enrolment • Fever (≥38° C) in the 24h prior to recruitment or any other signs/symptoms suggesting possible infection potentially requiring antibiotic therapy

In order to control for effects of the hospital environment and for temporal fluctuations in urinary metabolite levels, we aim to enroll controls expected to be hospitalized for at least 5 days without receipt of antibiotics. From these patients, we will collect urine on admission and on day 5 ±1. Urinary metabolites will be tested on samples from both admission and day 5±1. will be tested on both urines from admission and day 5±1. If control patients receive antibiotics before day 5 of hospitalization, urinary metabolites will not be examined. For each enrolled case patient, we will aim to enroll one sex- and age group- (by age categories 18-45, 46-65 and >65 years of age) matched control hospitalized around the same time as cases. Further matching will likely be too difficult for practical reasons. This study will begin in spring/summer 2018 and continue until the end of the PIRATE trial, thus patients are expected to be included through to the spring of 2019.

6.3.3 ENDURANCE study outcomes

The primary outcome of the ENDURANCE study is the change in urinary metabolite concentrations detected through reversed-phase liquid chromatography-electrospray ionization-tandem mass spectrometry from admission to day 30 (±4), comparing patients with short durations of antibiotic therapy (5-9 days) and longer durations of antibiotic therapy (10-14 days).
Secondary, exploratory outcomes are:

- longitudinal change in urinary metabolite concentrations from admission to end of therapy, effects of different antibiotic choices, both empirical and definitive as well as of treatment de-escalations
- correlation between initial and subsequent urinary metabolite levels and risk of *Clostridium difficile* associated diarrhea, secondary infections, other adverse events, overall complication rate, length of hospital stay and mortality.

6.3.4 ENDURANCE statistical considerations

For this exploratory nested study there will be no formal sample size calculation; instead we will enroll a convenience sample of as many patients as possible until the end of recruitment of PIRATE. The change of urinary metabolite levels over time within participants will be compared between participants according to treatment duration and antibiotic choices. For these analyses we will use parametric t-tests for 2 group comparisons or ANOVA for multiple group comparisons, or non-parametric Mann-Whitney U-test, as appropriate. For categorical values, comparison will be done by X^2 or Fisher's exact test, as appropriate. The change in urinary metabolite levels in controls from admission to day 5 ±1. If there is no relevant change over time in urinary metabolite levels in controls, the analysis of metabolite level dynamics will not be adjusted for cases. If there are relevant changes in metabolite levels in controls during hospitalisation, the analysis of metabolite level dynamics will be adjusted for cases. For this purpose, matching controls will be chosen using a risk-set sampling strategy. The continuous longitudinal variables will be analysed using linear mixed models. All analyses will be done using the R-statistical software, including the extension package 'lme4'.

6.3.5 Ethical considerations for the ENDURANCE study

Due to the nature of this substudy, we will need to additionally collect urine samples. Both case and control patients approached for the ENDURANCE substudy will need to provide written informed consent through addenda to the current PIRATE informed consent form (ICF addendum PIRATE+ENDURANCE (case) v1.0, dated 28.05.2018 and ICF addendum PIRATE+ENDURANCE (control) v1.0, dated 28.05.2018). At the time of enrollment and randomization of the PIRATE study, all patients will have received between 4 and 6 days of efficacious antibiotics. However, we anticipate rapid declines in 3-IS levels after initiation of antibiotic therapy already prior to PIRATE enrolment. Therefore the initial urinary metabolite levels (not yet affected by the antibiotic therapy) and initial decline will be of great importance for this study. Most patients who present with Gram-negative bacteremia will have a urine specimen collected on admission for routine clinical purposes. At this point patients will not have been identified by the study investigators yet and therefore will not have been approached to provide informed consent for the study. We therefore plan to delay the regular disposal of urine specimens, which are collected from patients with simultaneous blood culture collection, and freeze these urine samples (typically obtained at the Emergency Department). Only those samples from patients who eventually qualify and are enrolled in the PIRATE study will be kept and tested retrospectively. All other urine samples will be discarded if there is no accompanying bacteremia, if patients do not qualify for or consent to the ENDURANCE study. These aspects can be found in the ENDURANCE informed consent form addendum. Control patients will be approached and urine samples collected on admission and on day 5±1.

6.3.6 Data handling for the ENDURANCE study

For each enrolled study participant a paper CRF is maintained and functions as source data. CRFs will be kept current to reflect subject status at each phase during the course of study. Data from ENDURANCE patients and controls will be entered by a study team member into a separate password-protected database (Excel). There will be plausibility checks but no double data entry. ENDURANCE patients and controls will be identified by means of an ENDURANCE study number. No personally identifying information will be entered into the ENDURANCE CRF, which will be stored under lock and key. Only study team members have access to the CRF and the password-protected database. All study data will be archived for 10 years after study termination or premature termination of the clinical trial at the archives of the Division of Infectious Diseases / Hospital Epidemiology of the Cantonal Hospital of St. Gallen.

Biological samples are collected, coded and then shipped to a collaborating center in Innsbruck, Austria for urinary metabolite analysis. There they will be analyzed and stored for 2 years.

Afterwards samples will be destroyed or returned to St. Gallen for further analyses.

6.4 The PIRATE RESISTANCE Project: Network analysis of the microbiota and host intestinal response with mapping of the antibiotic Resistome after antibiotic therapy

6.4.1 Background and rationale

Metagenomic data are beginning to capture the immediate changes in gut flora antibiotic resistance genes (ARG) after antibiotic use. Yet we still lack direct evidence that shorter antibiotic courses or "de-escalation" from a broad- to narrow-spectrum antibiotic actually reduces selection for ARG in "bystander" microorganisms. Metagenomic data are beginning to capture the immediate changes in gut flora composition and resistance genes (the "resistome") after administration of commonly used antibiotics such as ciprofloxacin[71] and imipenem.[72] Yet we still lack hard evidence that shorter antibiotic courses, or even de-escalation from a broad-spectrum antibiotic to a more narrow-spectrum agent, do indeed reduce emergence of resistance in "bystander" microorganisms. To our knowledge, there is no metagenomic evidence from patients randomized to different antibiotic durations proving what should be obvious: that more days of antibiotics lead to increased antibiotic resistance genes (ARG) in patients' microbiomes. Additionally, there is no prospectively collected evidence to determine whether de-escalation has a significant impact on a patient's antibiotic resistome. The answer to this question has important implications, since antimicrobial stewardship programs (ABS) throughout the world dedicate significant resources to encouraging and managing de-escalation. If de-escalation does not meaningfully reduce the number of ARGs a patient carries during and after antibiotic therapy, then other strategies such as shorter durations ("go hard and go home") should be favored.

Also lacking is an understanding of the host intestinal response to antibiotic therapy and its duration. Metagenomic studies typically report the *relative* abundance of bacterial taxa or gene functions. Since intestinal bacterial loads vary between and within individuals (*e.g.*, during antibiotic treatment), an assessment of absolute abundance may reveal associations yet unseen in classical microbiota analysis. We hypothesize that antibiotic-driven changes in intestinal microbiota composition and mass may alter intestinal epithelial turnover via immune control, since gut microbiota produce both immunostimulatory and immune tolerance signals.

We propose to use the unique opportunity provided by the PIRATE trial to collect fecal samples from randomized PIRATE patients for metagenomic examination of the intestinal microbiota. This nested prospective cohort study, the PIRATE RESISTANCE project, will answer two fundamental, clinically oriented questions: *Does halving the duration of antibiotic therapy for a common hospital- and community-acquired infection lead to a commensurate decrease in the emergence of ARGs in the human intestinal microbiome? Does de-escalation from a broad-spectrum to a narrow-spectrum antibiotic reduce the emergence of ARG?* Further, the proposed network analysis, which will include metagenomic and metadata from different time points and different treatment groups, will allow a mapping not only of interactions among different microbiota members but also of associations between microbial taxa, gene functions, ARGs and clinical parameters (*e.g.*, CRP levels). Identification of key organisms involved in positive (co-occurrence) and negative (exclusion) correlations will pave the way for interventional studies (*e.g.*, controlled probiotics use) aimed at reducing collateral damage of antibiotic therapy and improved recovery after such treatments.

6.4.2 RESISTANCE study design, setting & population

Study population and entry criteria

All patients included in the PIRATE trial at the Geneva site will be eligible for enrollment in the PIRATE RESISTANCE nested study. Within this population, PIRATE RESISTANCE will specifically target patients receiving ceftriaxone, cefepime, cefuroxime, imipenem, meropenem, ertapenem, piperacillin/tazobactam, ciprofloxacin, levofloxacin, or cotrimoxazole for genomic analysis.

Additionally, 10 hospitalized adult patients not receiving any antibiotic therapy will be approached for consent to participate as matched controls (see below).

Study design and schedule

The PIRATE RESISTANCE study will be conducted entirely within the framework of the PIRATE randomized controlled trial, whose flow is depicted in Figure 6. The RESISTANCE study's main components are found on the right side of the figure and are depicted by the orange circles ( ).

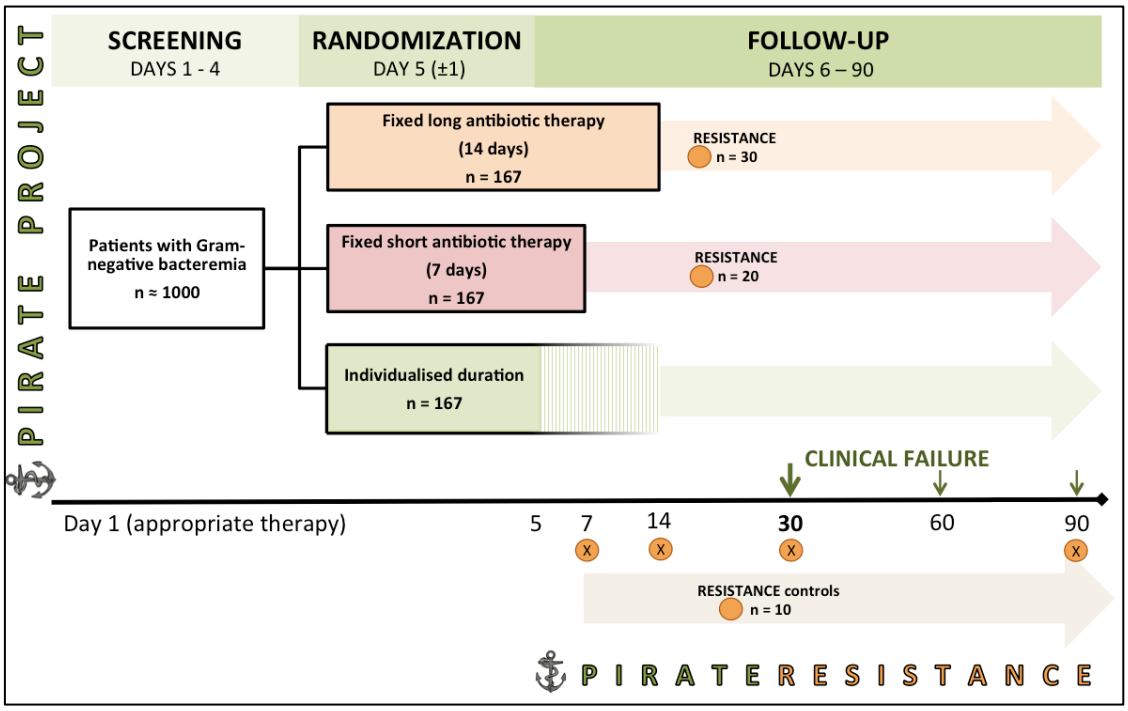


Figure 6. Study flow for the PIRATE point-of-care randomized controlled trial and the PIRATE RESISTANCE nested genomic cohort study ( ).

In this nested, prospective, observational matched cohort study, we will perform metagenomic analysis on stool samples collected from:

(1) 30 patients randomized to 14 days of antibiotic therapy; among these, 15 will have undergone de-escalation, and the remaining 15 will have remained on broad-spectrum antibiotics for at least 10 days
(2) 20 patients randomized to 7 days of antibiotic therapy
(3) 10 adult hospitalized patients not receiving any antibiotic therapy in the previous three months but matched for demographic characteristics and Charlson comorbidity scores (controls)

Stool samples will be collected from case patients on days 7 (±2), 14 (±3), 30 (±7) and 90 (±14) after the start of antibiotic therapy. Samples will be collected from control patients 7 (±2) and 14 (±3) days after hospitalization.

6.4.3 RESISTANCE study outcomes

The primary outcome of the RESISTANCE study is the abundance of ARGs detected using the ResFinder database (the number of read matches to each resistance gene normalized to the total number of reads assigned to bacteria or specific taxa; see below) at day 30 in the antibiotic arms (as compared to days 7 and 14 in the control arm). Secondary outcomes include the prevalences of the species of origin for each identified resistance gene, the relative and absolute levels of bacterial community clustering and diversity, and changes in overall species- and higher-level prevalence within each study group over time.

For the purposes of this study we will define de-escalation as any switch from a broad-spectrum to a narrow-spectrum antibiotic (whether occurring in the setting of pathogen identification or not); prospective spectrum classifications for the antibiotics commonly used in Gram-negative bacteremia are listed in Table 6. It should be noted that there is no universal definition of de-escalation and no universal consensus on which antibiotics actually have a "broad" or "narrow" spectrum. For example, while ciprofloxacin has a somewhat wide spectrum of microbiologic activity, we include it in the "narrow-spectrum" category since, in practice, it is frequently used for targeted therapy after the empiric phase of treatment.

Table 6. Broad and narrow-spectrum antibiotics typically used for the treatment of Gram-negative bacteremia at HUG.

"Broad" spectrum	**"Narrow" spectrum**
• ceftriaxone • cefepime • carbapenems (imipenem / ertapenem / meropenem) • piperacillin-tazobactam	• cotrimoxazole • ciprofloxacin • cefuroxime

6.4.4 RESISTANCE laboratory methods

Stool sampling for gut microbiota assessment. Stools will be collected from a Commode Specimen Container (Covidien) placed over the toilet seat opening. At least three pea-sized (or bigger) sections of fecal material will be added to Sarstedt Feces Tube 76x20mm and frozen at -80°C until processing.

DNA extraction. DNA will be extracted from a 100 mg aliquot of each stool sample on a MagCore HF16 Automated Nucleic Acid Extractor (RBC Bioscience) as described previously[73].

qPCR assays. Human and bacterial DNA concentrations will be determined by qPCR experiments targeting beta-actin and 16S rRNA reference genes, respectively, as described previously[74].

Sequencing. Indexed paired-end metagenomic libraries will be prepared from 200 ng DNA using the TruSeq Nano DNA Library Preparation Kit (Illumina) and size selected at about 350 bp. The pooled libraries will be sequenced in Rapid Run mode for 2x250+8 cycles on an Illumina HiSeq 2500 instrument at Fasteris (Plan-les-Ouates, Switzerland) The Trimmomatic package[75] will be used to remove bases that correspond to the standard Illumina adapters. Paired reads will be quality filtered and joined using PEAR[76]. About 10 million paired-end reads will be obtained per sample.

Sequence processing. Deconseq[77] with ≥90% coverage and ≥94% identity will be used to remove sequences matching human genome data (EMBL em_rel_std_hum database). To filter out putative artificial replicate reads, we will use a home-made script which retains only reads with the longest sequence among those with identical first 100 bases. Reads will be classified using Kraken with default parameters,[78] than Bracken[79] will be used to estimate the abundance of taxa at each level (phylum to species).

Functional assignments of merged reads assigned to domain Bacteria will be made using the SEED subsystem[80] database filtered to only contain prokaryotic sequences. Merged reads will be mapped (translated search) against the filtered database by means of USEARCH[81]. For each read, all top USEARCH matches will be selected and the hit corresponding to the reference entry most frequently assigned in the entire dataset will be retained.

To identify antibiotic-resistance genes, merged reads will be mapped to the ResFinder database[82] using USEARCH with minimum identity of 90% and minimum alignment length of 100 bases. The regions flanking the antibiotic-resistance genes will be compared to bacterial genomic sequences using BLAST[83] to determine the species of origin for each resistance gene. To calculate the relative abundance of gene functions and antibiotic-resistance genes, the number of read matches to each

function or resistance gene will be normalized to the total number of reads assigned to bacteria, and alternatively, to bacterial 16S rRNA gene count. In addition, the number of some antibiotic-resistance genes will be normalized to specific taxa (e.g. Enterobacteriaceae) expected to be the major reservoir of these genes. The regions flanking the antibiotic-resistance genes will be compared to bacterial genomic sequences using BLAST[83] to determine the species of origin for each resistance gene.

To obtain an approximate estimation of the 'absolute' abundance (expressed in arbitrary units) of bacterial taxa, as well as of gene functions and antibiotic resistance genes, we will multiply the corresponding relative abundance (determined by sequencing) by DNA concentration of the purified extract (determined by qPCR).

Clustering of bacterial communities. Bacterial community comparisons will be carried out using Bray-Curtis similarity.[84] The similarity matrix, based on the square-root-transformed relative abundance of taxa (species, genera, families, orders, classes, phyla) or gene functions (subsystem levels 1–4) will be constructed in PRIMER (Primer-E Ltd., Plymouth, UK). Principal coordinates analysis (PCoA) of Bray-Curtis similarity matrices will be performed in PRIMER.

Patterns of microbial communities. Co-presence and mutual exclusion of bacterial taxa will be assessed by CoNet[85] using Cytoscape plugin[86].

Ecological indices. Ecological indices (diversity, richness) will be calculated from the relative abundance of species or genera in PRIMER, using a dataset normalized to the same number of sequences across all samples (i.e., the lowest number of sequences found in any sample).

6.4.5 RESISTANCE statistical analysis

Sample size. An effect size is difficult to estimate given the lack of pre-existing data in this understudied domain. For this reason, and because metagenomics analyses are costly, we will use a convenience sample whose size is guided chiefly by feasibility.

Statistical analysis. To assess differences in overall microbiota taxonomic or functional composition between pre-defined groups of samples (categorical variables such as "treated" and "untreated"), we will use permutational multivariate analysis of variance (PERMANOVA, PRIMER) of the Bray-Curtis similarity matrix. Analysis of similarity percentage (SIMPER, PRIMER) will be used to identify taxa and gene functions that contribute to the distinction between pre-defined groups of samples. Distance-based linear model (DISTLM, PRIMER) will be used for analyzing the relationship between bacterial community structures and quantitative continuous variables (e.g. duration of antibiotic treatment). In addition, Wilcoxon rank-sum and Wilcoxon signed-rank tests will be used to assess statistical significance of differences in the relative abundance of taxa, gene functions and resistance genes. Statistical significance will be set at the 95% confidence level ($p<0.05$). The Benjamini and Hochberg correction will be applied for multiple testing. The correlation between individual microbial taxa or gene functions with continuous variables will be measured by Spearman's rank correlation coefficient. The values >0.5 and <–0.5 will be considered to reflect significant positive and negative correlations.

6.4.6 RESISTANCE ethical considerations

Both case and control patients approached for the RESISTANCE study will need to provide written informed consent (or oral and witnessed, or consent by proxy, as per the main PIRATE trial's procedures) through addenda to the current PIRATE informed consent form.

6.4.7 Data handling for the RESISTANCE nested study

Included case patients will continue to be identified by their PIRATE study randomization number, and control patients will be given a RESISTANCE study number. This code will be used to label the stool samples that will be transferred to the Genomic Research Laboratory (GRL), where DNA extraction and metagenomic analysis will be performed.

Stool samples will be stored at GRL at -80°C in the secured (locked) deep-freezers (24/7 monitoring system) in the CMU (or HUG) to maintain pre-set temperatures and respond quickly to emergencies such as temperature out of range. Biological materials will be destroyed 10 years after termination of metagenomic analysis following HUG's routine destruction procedure for biological samples.

Coded raw metagenomic data and result files (obtained by data analysis) will be kept for at least 10 years on a Genomic Research Laboratory server (separate from the Secutrial database) with an appropriate system for backup generation. If a central (HUG) data storage system (appropriate for metagenomic data) becomes available, the raw data will also be directly submitted to this facility. Access to metagenomic data on these servers will be restricted by entering a user name and password of GRL investigators. GRL investigators involved in the project will not have any access to the electronic patient's files on the HUG intranet - they will not be able to associate metagenomic data with patient's identity.

In line with SNSF policies and general principles of fair data sharing, publication of metagenomic results will require depositing the raw sequence data into a public sequence database. Before deposition to a database, any sequence read matching the human genomic sequence will be removed. Sequence file names and metadata that will be deposited will not contain any direct identifier or strong indirect identifier (e.g., name, initials, address, e-mail, phone number) and will contain only a minimum of indirect identifiers (sex, age [years], diagnosis, symptoms, treatment details).

7 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

7.1 Investigator procedures

Approved SOPs (blinding procedures, AE recording, etc.) will be used at all clinical sites.

7.2 Monitoring

External monitoring will be performed according to ICH Good Clinical Practice (GCP) by the Unité d'investigation Clinique of the HUG. Following a Monitoring Plan and written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigating team will provide direct access to all trial-related source data, documents and reports for the purpose of monitoring and auditing by the Sponsor and inspection by local and regulatory authorities.

7.3 Modification to protocol

The PI is responsible for ensuring that changes to an approved trial, during the period for which EC approval has already been given, are not initiated without further EC review and approval except to eliminate apparent immediate hazards to the subject, or when the changes involve only logistical or administrative aspects of the study (e.g. change in monitor(s), change of telephone number(s)).

7.4 Protocol and GCP deviation

Any deviations from the protocol and GCP will be documented in a protocol deviation form and filed in the trial master file.

7.5 Trial progress

The progress of the trial will be overseen by the Principal Investigator.

8 ETHICS

8.1 Declaration of Helsinki

Study investigators will ensure that this study is conducted according to the principles of the latest revision of the Declaration of Helsinki (Fortaleza, Brazil, October 2013).

8.2 ICH Guidelines for Good Clinical Practice (GCP)

Study investigators will ensure that this study is conducted in full conformity with the ICH Good Clinical Practice (GCP), the requirements of the Swiss Human Research Act (HRA; 810.30, 2011) and the Swiss ordinance on clinical trials (ClinO; 810.305, 2013), and local regulatory requirements.

8.3 Informed consent

All patients will be informed of the study by means of a written information brochure, per usual ICH standards, with full details of the study including its risks and benefits (see information brochure, v1.3, dated 8.06.2017) before enrollment. In accordance with the recent advice of the Geneva Ethics Commission, and in line with ClinO 810.305, we propose to allow (1) consenting patients with decision-making capacity to grant oral consent when an independent witness can provide signed testimony to that oral consent, and (2) patients deemed not to have decision-making capacity to be included in the study if their representative (legal proxy or next of kin) provides informed, written consent. The rationale for this proposal is described below, and the substudy detailed in Section 6 will compare patients' post-consent recall and understanding of the study after oral vs. written consent.

As we have written elsewhere,[87] the right of today's patient to be informed of an experiment and to refuse participation in it is the direct result of a series of historical, criminal episodes in which vulnerable human beings were harmed by physicians with too much power and ambition. The now traditional, written informed consent (IC) process is an attempt to level the field and restore basic rights of self-determination to the individual patient. Yet a growing body of literature indicates that sick patients are unlikely to be "protected" by their signed IC forms.

By definition, all patients are weakened and vulnerable; the patients who will be approached for inclusion in this study are no exception. There is increasing evidence—some collected in a landmark study conducted at HUG—that even those who appear to be ideal candidates for understanding and granting IC rarely are. Among 44 patients whose illness was improving and who granted "informed," written consent for participation in a clinical trial, only 32% could remember the purpose and potential risks of the trial ten days later.[50] This is not surprising; a recent review of 85 IC forms for clinical trials including adults found their median length to be 27 pages. The process has become complex, legalistic, and often intimidating to sick patients. Importantly, and not surprisingly, *patients often do not recognize written consent as serving their interest, but rather the interest of researchers and hospitals*.[88]

A mounting number of bioethicists are in agreement that, *where a framework of ethically robust oversight and policies exists*, the traditional, written IC process may not be ethically necessary for comparative effectiveness research (CER).[9,89] By definition, CER does not include experimental interventions on patients; rather, this type of research compares outcomes following already licensed therapies. Its focus is thus quality improvement. Where any randomization occurs, as in point-of-care trials, informed consent remains essential, but *the complexity of the process should match the actual risks of the study*.[8] The general public appears to be in agreement. In a population-based survey conducted in the US, when faced with the trade-off of requiring documentation of consent and allowing comparative effectiveness research to go forward, a majority of survey respondents who were asked to imagine participation in a hypothetical trial preferred to forego

documentation rather than see valuable research halted.[90] And we note the irony of the fact that the scores of "pseudo-randomizations" occurring throughout a patient's hospitalization—those moments when a physician does not know the best management option because good-quality evidence on outcomes is lacking, and thus chooses somewhat haphazardly among options—require no informed consent at all. Those pseudo-randomizations are potentially harmful and, left haphazard, will never provide the evidence or education they could.

Point-of-care randomized trials are an arm of quality improvement. They examine already licensed, non-experimental therapies and are thus low-risk. They are not intended for regulatory submission, nor do they require it. Their need for elaborate governance procedures such as those required of experimental phase I, II, III trials should thus be questioned.[10]

8.4 Benefits and risks to the participant

The only material benefit to the individual participant is the additional follow-up medical attention he or she will receive on days 30, 60 and 90. Because this study does not deviate from clinical practice, and because physicians will have the right to override their patients' treatment duration assignments at any moment, clinical risks beyond those inherent to established clinical practice are difficult to identify.

8.5 Ethics committee review

The protocol, ICF, and all other study documents will be submitted to the Geneva Cantonal Ethics Commission (CCER) as the lead ethics committee for approval. The Principal Investigator will submit and, where necessary, obtain approval from for all subsequent substantial amendments to the protocol and ICF.

8.6 Subject confidentiality

All data will be coded: subject data will be identified by a unique study identification number containing no personally identifiable information (PII) in the CRF and database. A separate confidential file containing PII will be stored in a secured (locked) location in accordance with data protection requirements. Only the sponsor representative, study investigators, the study monitor, and the CCER will have access to the records.

9 DATA HANDLING AND RECORD KEEPING

9.1 Data handling and management

Data management will be contracted to the Unité d'Investigation Clinique (UIC). Baseline and outcomes clinical data from primary source records will be entered into an electronic case report form (eCRF) for integration into the electronic database (SecuTrial™ platform). At the HUG site, in an effort to modernize data collection in this POC trial, the UIC data management team will work together with the DPI informatics team to pilot algorithms for the regular transfer of coded, post-randomization clinical data (see Table 2) directly from DPI into the SecuTrial database, reducing both staffing/resource use and the risk of manual transcription errors. At the peripheral sites (neither of which uses DPI), study investigators will enter data into SecuTrial™.

The Principal Investigator will be responsible for overseeing the receipt, entering, cleaning, querying, analysis and storage of all data that accrue from the study by designated persons.

For each set of data, quality control and triggers to computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. After integration of all corrections in the complete set of data, the database will be locked and saved before being released for statistical analysis. Each step of this process will be monitored through the implementation of individual passwords and/or regular backups in order to maintain appropriate database access and to guarantee database integrity.

9.2 Record keeping

The Investigator will maintain appropriate medical and research records for this trial, in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of patients. The Principal Investigator, co-investigators and clinical research nurses will have access to records. Investigators will permit authorized representatives of the Sponsor, monitors, as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of monitoring, quality assurance reviews, audits and evaluation of the study safety and progress.

9.3 Source data and case report form (CRF)

All protocol-required information will be collected from source documents and entered into the eCRF either by study investigators and algorithm (HUG site) or simply by study investigators. Source documents are original documents, data, and records from which the subject's eCRF data are obtained. For this study, these will include, but are not limited to, clinical notes, laboratory records, radiologic imaging, and correspondence. All source data and CRFs will be stored securely. The information to be recorded in the CRF is listed in detail in Table 2. The full CRF accompanies this study protocol.

9.4 Data protection, storage and ownership

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party. The data generated throughout the course of the study will be owned by the study Sponsor. All data will be stored for 10 years from the end of the trial. Electronic data will be password protected, and paper documents will be stored in a locked cabinet.

10 FINANCING AND INSURANCE

10.1 Financing

The PIRATE trial is funded by a grant to A. Huttner and the aforementioned investigators from the Swiss National Science Foundation within the framework of the 74th National Research Program "Smarter Health Care" (grant no. 407440_167359). The PIRATE RESISTANCE nested study is funded by a grant to A. Huttner and the RESISTANCE investigators from the Geneva University Hospitals & University of Geneva (Fondation Louis-Jeantet; no. S04-12).

10.2 Insurance

Study insurance will be provided by the Sponsor. The finalized insurance policy has been forwarded to the Ethics Commission.

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