Study title: **BALANCE+: A Platform Trial for Gram Negative Bloodstream Infections**

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Study Synopsis

Protocol Title	BALANCE+: A Platform Trial for Gram Negative Bloodstream Infections
Protocol Number	
Study Design and Phase	Perpetual multiple domain randomized controlled platform trial
Setting	International, multi-centre
Sample Size – BALANCE+ vanguard	The initial vanguard phase will target 72 patients for most domains
Sample size – BALANCE+ main platform	Successful domains will transition into the BALANCE + perpetual platform trial, which will have no fixed sample size
Platform Entry Criteria	*Inclusion criteria:* • admitted to a participating hospital • positive blood culture with Gram negative (GN) bacterium *Exclusion criteria:* • patient's goals of care are for palliation with no active treatment • moribund patient, not expected to survive > 72 hours There are additional domain-specific inclusion/exclusion criteria
Domains • **intervention arms**	De-escalation versus no de-escalation domain • No de-escalation ○ continue on the same empiric GN antibiotic(s) being used prior to blood culture finalization ○ companion antibiotics in a combination regimen (eg, vancomycin, azithromycin, aminoglycoside or metronidazole) can be discontinued at discretion of clinical team ○ if patient has a syndrome requiring prolonged duration (> 14 days) de-escalation is allowable after day 14 • De-escalation ○ empiric GN antibiotic(s) switched to narrower spectrum agent to which the blood culture isolate is susceptible (within 24 hours) ○ companion antibiotics in a combination regimen can be discontinued at discretion of clinical team (and will be encouraged to do so in this arm if not needed for another indication) Oral beta-lactam versus non beta-lactam domain • Non-beta-lactam arm: an oral fluoroquinolone (ciprofloxacin, moxifloxacin or levofloxacin) or trimethoprim-sulfamethoxazole

	• Beta-lactam arm: an oral beta-lactam agent including, but not limited to, amoxicillin, amoxicillin-clavulanate, cephalexin, cefadroxil, or cefixime Central vascular catheter retention versus replacement domain • Central vascular catheter retention arm: original line retained until non-functional, or no longer needed • Central vascular catheter replacement arm: replace the vascular catheter as soon as possible and within a maximum of 72 hours from blood culture finalization Low-risk AmpC domain • Cephalosporin arm: participants will be treated with ceftriaxone (at standard doses) during intravenous treatment, with oral step-down allowed to any susceptible agent • Carbapenem arm: participants will be treated with a carbapenem (at standard doses) during intravenous treatment, with oral step-down allowed to any susceptible agent Follow-up blood culture domain • Follow-up blood culture arm: participants will undergo routine repeat blood culture collection (at least one blood culture set) 4±1d from the calendar date of the index positive blood culture collection • No follow-up blood culture arm: participants will undergo no *routine* repeat blood culture collection between 4±1d from the calendar date of the index positive blood culture collection
Primary Feasibility Outcomes of BALANCE+ vanguard	• Domain-specific recruitment rate • Domain-specific protocol adherence
Primary Outcome of BALANCE+ domains	De-escalation versus no de-escalation domain • Patient-centered, ordinal Desirability of Outcome Ranking (DOOR) outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission) • tie-breaker within ordinal levels: new antimicrobial resistance (AMR) colonization or infection from routine cultures Oral beta-lactam versus non beta-lactam domain • ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)

	• tie-breaker within ordinal levels: new AMR colonization or infection from routine cultures Central vascular catheter retention versus replacement domain • ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission) • no tie-breaker Low-risk AmpC domain • ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission) • tie-breaker within ordinal levels: new AMR colonization or infection from routine cultures Follow-up blood culture domain • ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission) • no tie-breaker
Secondary Outcomes of BALANCE+	• 90-day mortality • 90-day reinfection • 90-day all cause readmission • 90-day AMR colonization/infection • 90-day Clostridioides difficile infection (CDI) • 30-day mortality • 60-day mortality • additional domain-specific secondary outcomes
Statistical Analysis BALANCE+ vanguard	• descriptive point estimates and 95% confidence intervals for domain-specific recruitment rates and protocol adherence • analyzed overall and by participating site
Statistical Analysis BALANCE+ Main Platform	• regular Bayesian interim analyses with uninformative priors • conducted at every 500th BALANCE+ platform enrolment • domains closed only if they meet pre-specified, stringent decision criteria for stopping based on superiority, non-inferiority or futility

1. THE NEED FOR A TRIAL

1.1 GENERAL BACKGROUND AND RATIONALE

1.1.1 Burden of Bloodstream Infections

Bloodstream infections (BSIs) are common and lethal, ranking among the top 7 causes of death, with 600,000 cases and 90,000 deaths per year in North America, and 1.2Million cases and 150,000 deaths per year in Europe.[1] Using population-based data, our team revealed an incidence of 150 BSIs per 100,000 population/year, a 17% mortality rate at 30 days, and an increased odds of mortality (2.62, 95%CI 2.52-2.73) compared to matched patients without BSI.[2]

1.1.2 Antimicrobial resistance (AMR) is a global public health threat

The World Health Organization, U.S. Centers for Disease Control, Association of Medical Microbiology and Infectious Diseases (AMMI) Canada, and Public Health Agency of Canada have all declared antimicrobial resistance (AMR) a global threat to health,[3-6] based on rapidly increasing resistance rates and declining new drug development.[7,8] Antibiotic overuse and misuse is rampant across all health care sectors, and poses a direct threat to patients in the form of avoidable allergy, adverse drug events, *C. difficile* infection and AMR. Globally, AMR is already associated with over 1 million attributable deaths per year,[9] and is particularly concerning among Gram negative (GN) pathogens,[10] with rapidly rising resistance to all antibacterial classes including last-line therapies.[11,12] We need efficient avenues to study new GN therapeutics, but also to conduct comparative effectiveness trials of how to best utilize existing agents to maximize benefits while minimizing harms and curtailing AMR.

1.1.3 Many fundamental aspects of BSI treatment remain untested

Our Canadian Institutes of Health Research (CIHR)-funded Bacteremia Antibiotic Length Actually Needed for Clinical Effectiveness (BALANCE) randomized controlled trial (RCT) is randomizing patients to 7 versus 14 days of antibiotic treatment across 73 sites in 7 countries,[13-15] and will establish the treatment duration paradigm for BSIs. BALANCE is already the largest BSI trial ever conducted, and will complete target recruitment of 3626 patients in June 2023. If 7 days is non-inferior for mortality as compared to the current most common standard of 14 days,[16,17] as we hypothesize, then that will translate to large reductions in antimicrobial use and pressure,[18] while still ensuring non-inferior cure rates. However, after BALANCE, additional crucial questions will remain regarding the optimal treatment of BSIs.

BALANCE**+** follows from BALANCE as a platform trial to enable efficient testing of multiple pressing questions in the management of GN BSIs, including cross-cutting questions that can be applied to the diverse population of patients with BSIs, as well as subgroup questions specific to particular pathogen(s) and underlying syndrome(s). This protocol describes a series of cross-cutting, nested research questions regarding antibiotic de-escalation and step-down (Figure 1). In addition, this protocol includes the first BALANCE+ questions regarding specific pathogen(s) and underlying syndrome(s). Beyond questions posed in this protocol, with the evolution of our BALANCE research program to a perpetual platform trial design, BALANCE**+**, we foresee *future* domains of study including, but not limited to, antibiotic selection, route of administration,

combination regimens, source control, novel therapeutics, diagnostics, personalized biomarkers/genomics, and secondary prevention.

Figure 1: BALANCE+ Schematic Diagram

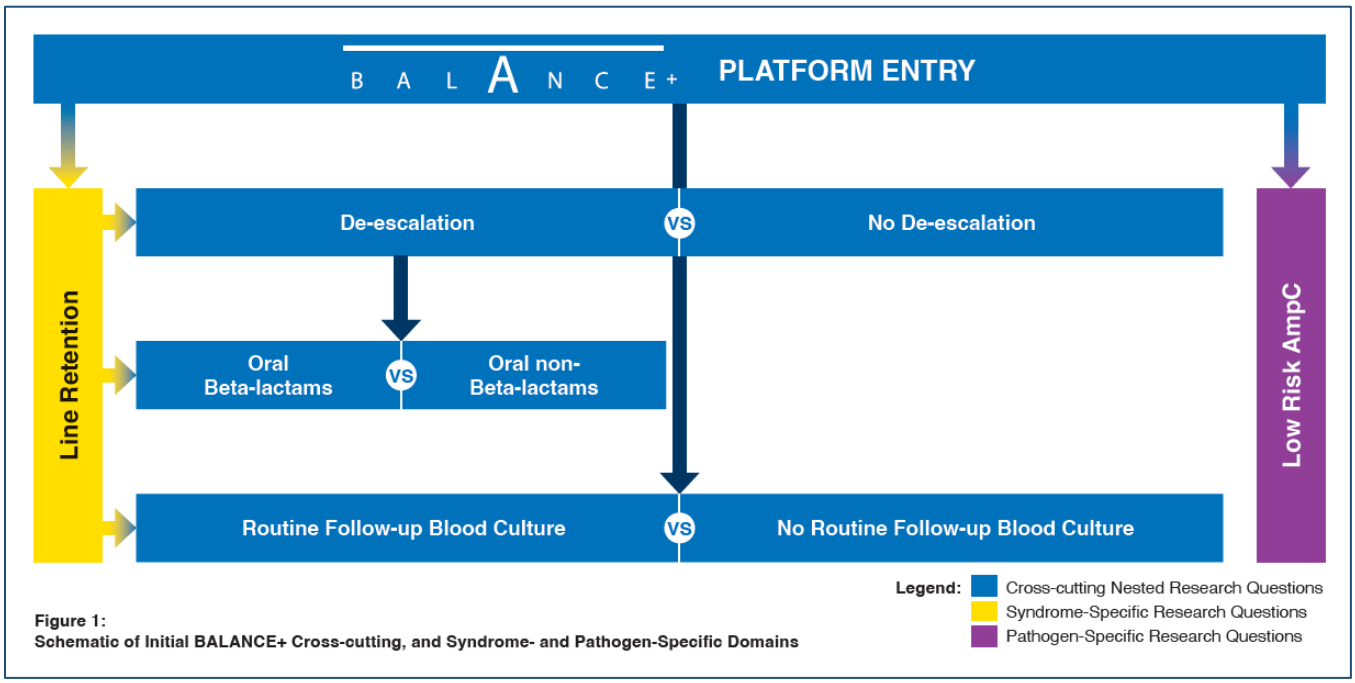

Figure 1:
Schematic of Initial BALANCE+ Cross-cutting, and Syndrome- and Pathogen-Specific Domains

1.2 SPECIFIC BACKGROUND AND RATIONALE FOR INDIVIDUAL BALANCE+ DOMAINS

1.2.1 Rationale for de-escalation vs no de-escalation domain

We need foundational evidence to support or refute the common clinical practice of antibiotic de-escalation. Antimicrobial stewardship guidelines recommend minimizing the use of broad-spectrum antibiotics by switching to narrower spectrum agents after microbiologic culture and susceptibility results become available.[19] The de-escalation maneuver consists of changing the empiric antimicrobial regimen to one which retains activity against the target pathogen, while minimizing ecologic impact on AMR.[20] *But is de-escalation truly safe and effective?* Two systematic reviews have evaluated existing research among patients undergoing de-escalation versus no-de-escalation and reached similar conclusions;[21,22] there have been 13 prior studies, which pooled together, indicate a substantial reduction in mortality associated with de-escalation (RR 0.68, 95%CI 0.52, 0.88).[22] However, 12 of these 13 studies are observational and have signs of selection bias, with de-escalation more likely among patients with lower baseline severity of illness, and lower organ failure scores at the time of de-escalation.[20] The single RCT has a mortality point estimate favoring the no de-escalation group (HR for de-escalation was 1.31, 95 % CI 0.64–2.67, P = 0.49).[23] This study was underpowered (N=120), but failed to establish non-inferior lengths of stay with de-escalation treatment, and also found higher total days of antibiotic use (14 vs 9.9, p=0.04) and super-infection (27% vs

11%, p=0.03) in the de-escalation group. Not only is the safety of this routine approach unclear, but benefits on AMR have yet to be established. Only 6 of the 13 studies measured AMR emergence; none documented a significant reduction in AMR, and AMR point estimates favoured no de-escalation in 3 of 6 studies.[24] Although narrowing antibiotic spectrum should theoretically lead to less selective pressure, there are also theoretical ecologic risks of de-escalation which by definition leads to greater risk of cross-exposure to multiple antibiotic agents.[24] Although some authors have recently lamented clinical under-utilization of de-escalation,[25] a large adequately powered trial is first needed to properly evaluate this practice and its effects on both clinical and microbiologic outcomes.

1.2.2. Oral beta-lactam versus non beta-lactam treatment domain

Many patients with BSI are transitioned to oral therapy during their treatment course, to facilitate shorter length of stay and reduced incidence of intravascular catheter complications.[19,26,27] Among patients transitioned to oral therapy there is a critical need to identify optimal treatment. Highly bioavailable non beta-lactam oral antibiotics (e.g., fluoroquinolones) achieve higher serum concentrations but have an expanded, and sometimes irreversible, side effect profile including connective tissue, neurologic and cardiac complications.[28,29] Other highly bioavailable non-beta-lactam drugs like trimethoprim-sulfamethoxazole are associated with drug-interactions, idiosyncratic severe toxicities, and renal effects such as elevation in serum creatinine and hyperkalemia.[30] By contrast, beta-lactams at standard doses have fewer potential side-effects but achieve lower serum concentrations which may impact effectiveness;[31] higher doses have been used to try to overcome lower bioavailability but these doses are not always well tolerated.

Prior observational studies of beta-lactam versus non-beta-lactam agents have not detected significant differences in outcomes, but have been limited by small sample sizes.[32,33] A recent systematic review pooling these small studies identified higher odds of re-infection (OR 2.06, 95% CI, 1.18 – 3.61; p = 0.01) with the use of beta-lactam antibiotics.[34] Therefore, we conducted a population-based observational study that included over 2000 patients and found those treated with highly bioavailable non-beta-lactam agents compared to matched patients receiving beta-lactam agents had a reduced risk of a composite outcome of mortality, re-infection, and hospital re-admission at 90 days [adjusted OR (aOR) 0.74 (95% CI, 0.60 – 0.92)].[35] Given the potential for residual confounding by indication, an RCT is crucial to answer this question.

1.2.3 Rationale for central line retention vs removal domain

In the subset of patients with GNB BSI in the context of an indwelling central vascular catheter, we need guidance addressing catheter retention. The rationale for replacement of central vascular catheters is that organisms may continue to colonize the catheter, persist in biofilm, and cause relapsing BSI after completion of antibiotic treatment. However, persistent central vascular catheter colonization may be less common with GN compared to Gram positive organisms, and there are challenges to replacing catheters including temporary compromise of vascular access and complications during new device insertion.[36] The Infectious Diseases Society of America (IDSA) guidelines only explicitly recommend removing long-term catheters

for patients with "suppurative thrombophlebitis; endocarditis; bloodstream infection that continues despite 72 hours of antimicrobial therapy to which the infecting microbes are susceptible; or infections due to *S. aureus, P. aeruginosa*, fungi, or mycobacteria."[36] There is no comment on how to respond more generally to GN BSI, due to lack of prior research. Indeed, a scoping review has been conducted to search for all RCTs evaluating the effectiveness of interventions to improve central venous access device outcomes.[37] The authors found 178 trials examining interventions related to catheter insertion, patency, infection prevention, education, and dressing and securement, but there were no trials evaluating line retention versus replacement.[37]

1.2.4 Rationale for low-risk AmpC domain

For the first pathogen-specific question, we will test whether ceftriaxone is non-inferior to carbapenems for ampC producing organisms at lower risk of inducible beta-lactamase production. AmpC beta-lactamases are class C serine beta-lactamases that are produced by a number of different GN organisms that have traditionally been labelled by the "SPICE" acronym.[38] These beta-lactamases are inducible such that initial *in vitro* sensitivity results could be unreliable, as the beta-lactamase becomes induced leading to potential treatment failure with penicillin and cephalosporin antibiotics. However, observational and *in vitro* data suggests that this phenomenon may only be clinically important in a subset of these organisms: *Enterobacter cloacae*, *Klebsiella* (formerly *Enterobacter*) *aerogenes* and *Citrobacter freundii*.[39-41] In contrast, other so-called "SPICE" organisms are now felt to be uncommon producers of AmpC, including *Serratia spp, Morganella spp, Providencia spp,* and *Citrobacter koseri.* Therefore, recently released IDSA guidelines suggest that cephalosporins or penicillins can be used for sensitive strains of these organisms – to reduce overall use of carbapenems.[38] However, it is challenging to undo decades of recommendation against non-carbapenem beta-lactams in these patients. The proposed carbapenem-sparing approaches have never been tested for these organisms in an RCT, and the BALANCE**+** platform offers a rare opportunity to embed such a trial.

1.2.5 Rationale for follow up blood culture domain

Follow up blood cultures (FUBCs) are blood cultures that are collected in the setting of a known BSI (i.e., after a known index positive blood culture) to test for the presence of persistent bacteremia or document the clearance of known bacteremia. FUBCs are suggested in the management of *Staphylococcus aureus* bacteremia,[42] endocarditis,[43] and candidemia[44] but there is controversy as to whether they are required for patients with GN BSI. In prior work by our team, we found that FUBCs are common (collected in 27% of 901 episodes of GN BSI) but only rarely yielded a pathogen (10.9%), and were not associated with detectable differences in mortality.[45] This study suggested that the practice of FUBC testing may result in excessive resource use among patients with GN BSI, providing no discernable clinical benefit, while potentially leading to unnecessary antibiotic use as a sequelae of contaminated cultures. Indeed, a separate study by Mitaka et al found that FUBC collection in GN BSI was associated with longer duration of antibiotic treatment and hospital length of stay, without a significant difference in mortality.[46]

However, subsequent studies reported a mortality benefit associated with FUBC testing, and generated controversy in this field, which has been heightened by the publication of two recent meta-analyses.[47,48] Thaden et al detected 15 observational studies, of which 5 were assessed to be at low risk of bias. Among this subset of 5 studies, FUBCs were associated with substantial decreased mortality (hazard ratio 0.56, 95%CI 0.45-0.71).[47] Shinohara et al. identified 9 eligible observational studies, in which use of FUBC was highly variable (18-89% of patients with GN BSI). Random-effects meta-analysis estimated a similar mortality benefit to that seen in the other meta-analysis (hazard ratio 0.54, 95%CI 0.42-0.69).[48]

It is difficult to understand how the practice of FUBC testing could lead to a halving of mortality in patients with GN BSI given that only a minority have positive FUBC results. Although selection bias could potentially be weighted against those undergoing FUBC (sicker patients more likely to be tested) there is a greater methodologic problem in observational studies on this research question: immortal time bias. Patients must survive long enough to undergo FUBC testing, and existing research has not properly accounted for this concern, especially given a wide time interval of 1-7 days used as the definition for FUBC in most previous studies. Therefore, the ideal method to resolve this controversy is a randomized controlled trial.

1.3 RESEARCH QUESTIONS, HYPOTHESES AND OBJECTIVES

1.3.1 Overarching Objective

The overarching objective of the BALANCE+ program is to transform random care to randomized care for patients with Gram negative BSI to inform best treatment approaches and optimize outcomes.

1.3.2. Research Questions for the Initial BALANCE+ Domains

(i) De-escalation versus no de-escalation domain

Is antibiotic de-escalation (narrowing of antibiotic spectrum based on blood culture susceptibility results) associated with superior clinical outcomes and reduced microbiome disruption / resistance burden compared to continuation of an initial empiric treatment regimen?
We hypothesize that de-escalation will be superior.

(ii) Oral beta-lactam versus non-beta-lactam treatment domain

Among those who receive oral antibiotics, are non-beta-lactam agents (fluoroquinolones, trimethoprim-sulfamethoxazole [TMP-SMX]) superior to oral beta-lactams?
We hypothesize that non-beta-lactams will be superior to beta-lactams.

(iii) Central vascular catheter retention versus replacement domain

Is mandated vascular catheter replacement superior to retention among those with GN BSI in the context of an indwelling central vascular catheter?
We hypothesize that vascular catheter replacement will be superior to catheter retention.

(iv) Low-risk AmpC domain

Is ceftriaxone non-inferior to the carbapenems for AmpC producing GN organisms which are now considered to have a low risk of inducible beta-lactamase production?
We hypothesize that the ceftriaxone strategy will be non-inferior.

(v) Follow up blood culture domain

Are routine follow up blood cultures (collected 4±1 days after initial positive blood culture collection) associated with superior clinical outcomes?
We hypothesize that a strategy of no routine follow up blood culture collection will be associated with non-inferior mortality compared to a strategy of routine follow up blood culture collection.

1.3.3 Specific Objectives for the BALANCE+ Feasibility vanguard

The specific objectives of this BALANCE + vanguard are to establish feasibility *of each individual trial domain* by confirming sufficient:
(1) domain-specific recruitment rates
(2) domain-specific protocol adherence

2. THE TRIAL

2.1 General study design

BALANCE**+** is an adaptive platform trial, as defined by the goal of determining the best treatment strategies for a disease by simultaneously investigating multiple treatments, using specialized statistical tools and recognizing rather than avoiding heterogeneity in the study population.[49] Adaptive platform trials offer efficiencies over traditional trials by focusing on a disease rather than a single experimental question and by using pre-specified statistical plans to respond to accumulating evidence in a timely manner.[50,51] BALANCE**+** focuses on GN BSI, and tests both cross-cutting and subgroup focused questions. The domains are open-label given the pragmatic design embedded in routine care, and the assessment of treatment strategies rather than individual agents.

This protocol describes the initial vanguard phase of BALANCE+. We anticipate amendment submission at the end of the vanguard phase.

2.2 Trial interventions

(i) De-escalation versus no de-escalation domain

Clinicians and researchers have used variable definitions of de-escalation, but this study will use a thoughtful consensus definition proposed by a task force of the European Society of Intensive Care Medicine and the European Society of Clinical Microbiology and Infectious Diseases: which involves stopping components of an antimicrobial combination or replacing broad-spectrum antimicrobials with ones with a narrower spectrum or lower ecological impact.[20]
We will further operationalize the de-escalation and no de-escalation intervention arms in a manner similar to the only prior (small) RCT on this topic.[23]

No de-escalation arm

- continue on the same empiric GN antibiotic(s) with which patient was being treated prior to blood culture finalization as long as that treatment is active against the identified pathogen(s)
- companion antibiotics in a combination regimen (eg, vancomycin, azithromycin, aminoglycoside or metronidazole) can be de-escalated at the discretion of the prescribing team
- if the patient has a syndrome requiring prolonged duration (eg, endocarditis, osteomyelitis, undrained abscess) de-escalation is allowable after day 14

De-escalation arm

- the empiric GN antibiotic(s) will be switched to a narrower* spectrum agent to which the blood culture isolate is susceptible (within 24 hours)
- companion antibiotics in a combination regimen (eg, vancomycin, azithromycin, aminoglycoside or metronidazole) can be de-escalated at the discretion of the prescribing team, but de-escalation will be encouraged if not needed for another indication (within 24h)

***** We will provide the clinical team with an antibiotic spectrum score-informed recommendation of narrowest effective agent.[52,53] There is no gold standard spectrum score. We have used the most detailed available spectrum score which was developed through a multi-stage Delphi panel process (Appendix Table 1, column 1).[52] However, we have modified this scoring system to rank carbapenems as broader spectrum than beta-lactam beta-lactamase inhibitors, as supported by another consensus ranking specific to beta-lactam agents (Appendix Table 1, column 2).[53] We have made other modifications to move other drugs higher in the ranking (eg, colistin) due to breadth of Gram negative activity – since the Delphi panel process was based on both Gram positive and negative activity. Lastly, we have added newly licensed agents into the ranking based on literature surveillance studies of their Gram negative spectrum of activity. Our study ranking is provided in Appendix Table 1, column 3.

Even though we will inform the clinical team as to what we have determined to be the narrowest spectrum agent active against the blood culture pathogen, the final de-escalation decision will be at their discretion. As long as the selected agent is narrow*er* than the empiric agent, the switch will be considered adherent to protocol (even if it is not the narrow*est* recommended agent) (see definition of adherence below).

(ii) Beta-lactam versus non-beta-lactam oral treatment domain

- **Non-beta-lactam arm:** clinicians can select an oral fluoroquinolone (ciprofloxacin, moxifloxacin or levofloxacin) or trimethoprim-sulfamethoxazole; all of these agents offer bioavailability exceeding 90%
- **Beta-lactam arm:** clinicians can select an oral beta-lactam agent including, but not limited to, amoxicillin, amoxicillin-clavulanate, cephalexin, cefadroxil, or cefixime. The list of available agents is provided in Appendix Table 2.

Our prior observational study, suggested that the subgroup receiving higher doses of beta-lactams achieved similar outcomes as matched patients on non-beta-lactam agents.[35]

However, this subgroup was very small, and routine beta-lactam doses were used in most (78%) patients. We will provide dosing recommendations to maximize beta-lactam outcomes (Appendix Table 3), but to maintain the pragmatic nature of the trial, the ultimate dosing decision will be at the discretion of the treating physician. Doses and intervals will be tracked during the vanguard phase to assess variability in prescribing practices. Treatment duration will also be at the discretion of the treating physician, but they will be required to declare their intended treatment duration prior to randomization, to ensure that they are agreeable to the patient receiving the same duration regardless of randomization arm.

(iii) Central vascular catheter retention versus replacement domain

- **Central vascular catheter replacement arm:** for participants randomized to the catheter replacement arm, the clinical team will replace the vascular catheter as soon as possible and within a maximum of 72 hours from blood culture finalization
- **Central vascular catheter retention arm:** For participants randomized to the line retention arm, the line will be retained until non-functional, or no longer needed

(iv) Low-risk AmpC domain

- **Cephalosporin arm:** participants will be treated with ceftriaxone (at standard doses) with oral step-down allowed to any susceptible agent
- **Carbapenem arm:** participants will be treated with a carbapenem (at standard doses) with oral step-down allowed to any susceptible agent

(v) Follow up blood culture domain

- **Follow-up blood culture arm:** participants will undergo routine repeat blood culture collection (at least one blood culture set) 4±1d from the calendar date of the index positive blood culture collection.
- **No follow-up blood culture arm:** participants will undergo no *routine* repeat blood culture collection between 4±1d from the calendar date of the index positive blood culture collection.

Although most prior observational studies of follow-up blood culture collection used a definition spanning 1-7d from culture collection,[47,48,54-56] we believe that the definition should start later because cultures collected in the first 1-2d will usually be sent before the original culture is detected to be positive. We also believe that the time window should end earlier such that the results of repeat cultures are available prior to the end of routine treatment durations.

2.3 Consent and Randomization

After engagement with the treating team, the research coordinator/ site primary investigator will follow usual good research practice at each site to approach eligible patients (or substitute decision-makers) with GN BSI for possible inclusion in the study.

BALANCE+ uses a patient-centered, layered, integrated consent process, approved by the local/provincial research ethics board (REB), to ensure patients can understand the concepts

and have agency to obtain as much detail as they would like regarding the multiple platform domains.[57] Critically ill patients are frequently unable to provide initial consent due to altered level of consciousness or understanding. Hence, BALANCE+ uses standard operating procedures to seek assistance from substitute-decision makers on behalf of patients.
Written informed consent, with an option for telephone informed consent where appropriate, and approved by the local REB, will be obtained from all participants prior to inclusion in this study. Discussion will take place in locations which protect confidentiality. All the study related information will be explained in simple terms and a study information and consent form document will be given to the patient or substitute decision-maker (in-person or via email). They will be given sufficient time to read, understand the study related information and ask any questions prior to participation in this study. All questions will be answered before obtaining informed consent. The participant will receive a copy of the signed consent form.

Once consent is obtained, patients will then be immediately centrally randomized (www.randomize.net) to all domains to which they consent, and then allocation will be revealed if/when they are eligible for each particular domain. All initial interventions will use 1:1 randomization with variable block sizes stratified by site and severity (PITT bacteremia score);[58] BALANCE+ will not involve response adaptive randomization in these initial domains.[59] Prior to transition to main trial we may consider adding additional domain-specific stratifications.

2.4 Protecting against bias

Randomization will occur centrally, with allocation concealment enhanced by random, large block sizes. Blinding is not feasible for the main cross-cutting questions because the arms include multiple drugs for diverse underlying pathogens and syndromes causing GN BSI. However, potential information bias will be mitigated by using objective outcome measures and blinding outcome adjudicators and analysts to group assignment.

2.5 Inclusion and exclusion criteria

To maximize diversity of participants and generalizability of trial findings we aim for broad BALANCE**+** platform criteria. Within the BALANCE+ platform, there will also be domain specific inclusion/exclusion criteria.

2.5.1 BALANCE+ Platform inclusion and exclusion criteria

Inclusion criteria:

- admitted to a participating hospital
- positive blood culture with GN bacterium

Exclusion criteria:

- patient's goals of care are for palliation with no active treatment
- moribund patient, not expected to survive > 72 hours

2.5.2 Domain specific inclusion and exclusion criteria

(i) De-escalation versus no de-escalation domain

Inclusion Criteria

- included in BALANCE+ platform

Exclusion Criteria

- receiving an empiric antibiotic regimen at the time of blood culture finalization to which the GN pathogen(s) are not sensitive
- carbapenem-resistance (so that patients will not need to remain on reserve-use agents)
- no de-escalation option due to any or all of:
    - resistance
    - allergies
    - medical contraindications
    - drug-interaction risk
    - other relevant reason
- patients with a suspected or proven polymicrobial source of infection

Note: Patients with foci requiring prolonged treatment durations (such as undrained abscesses) will not be excluded, but de-escalation will be allowed after day 14 in these patients.

(ii) Beta-lactam versus non-beta-lactam oral/enteral treatment domain

Inclusion Criteria

- included in BALANCE+ platform
- initially treated with intravenous antibiotics, but clinical team transitioning patient to oral/enteral antibiotic within 7 days of starting treatment

Exclusion Criteria

- enrolled in an arm of another BALANCE+ platform domain which limits the use of oral/enteral therapy:
    - no-de-escalation arm
- no non-beta-lactam options due to any or all of:
    - resistance
    - allergies
    - medical contraindications
    - drug-interaction risk
    - other relevant reason
- no beta-lactam options due to any or all of:
    - resistance
    - allergies
    - medical contraindications
    - drug-drug interaction risk
    - other relevant reason

(iii) Central vascular catheter replacement domain

Inclusion Criteria

- included in BALANCE+ platform

- has an indwelling central vascular catheter that was already in place within the 48-hour period before the onset of bloodstream infection (i.e. is not a new catheter placed within 48 hours of the onset of infection)
    - can be either a tunneled (Port-a-Cath, Hickman line, dialysis catheter), temporary centrally inserted (internal jugular, subclavian, femoral), peripherally inserted central (PICC) catheter, or central (femoral) arterial catheter

Exclusion Criteria

- patient has no ongoing need for a central vascular catheter
- patient has definite indication for central vascular catheter removal
    - ongoing septic shock with definite/probable line source
    - concomitant *S. aureus* bacteremia
    - concomitant candidemia
    - local suppurative signs (severe redness, warmth, pain, swelling or fluctuance/collection) necessitating catheter removal, or other clinical evidence of infected line (e.g. imaging/echocardiographic findings)
    - definite alternative source of GN BSI

[note: Study does not exclude *Pseudomonas* spp. bacteremia; this organism is identified as a rationale for line removal in IDSA guidelines but based only on expert opinion not high grade evidence]

(iv) Low-risk AmpC domain

Inclusion Criteria

- included in BALANCE+ platform
- positive blood culture with GN bacterium, of the following species:
    - *Serratia spp.*
    - *Morganella spp.*
    - *Providencia spp.*
    - *Proteus spp. other than P.mirabilis*
- organism is sensitive to ceftriaxone

Exclusion Criteria

- severe allergy to beta-lactams (eg, type 4 hypersensitivity reaction or DRESS)
- baseline phenotypic resistance to ceftriaxone

(v) Follow up blood culture domain

Inclusion Criteria

- included in BALANCE+ platform

Exclusion Criteria

- Patient already discharged home prior to day 4
- Definite indication for repeat blood culture testing
    - Concomitant Staph. aureus bacteremia
    - Concomitant Candidemia

- Clinical suspicion for infective endocarditis (e.g., presence of prosthetic valve, implantable cardiac device)

2.6 Antibiotic treatment duration

Antibiotic treatment durations in this trial will be guided by the pending findings from the BALANCE trial. If 7 is non-inferior to 14 days for patients in BALANCE then 7 days will be the typical *recommended* treatment duration by the study team for uncomplicated infections. However treatment duration will be at the discretion of the clinicians caring for the patient. The care team will need to specify the intended duration prior to randomization.

There will be future opportunity to implement additional duration randomization domains in BALANCE**+**; for example, 7 versus 14 days treatment for patients with organ transplantation (patients excluded from BALANCE); ultra-short treatment versus standard treatment for syndromes with early source control (e.g., hepatobiliary bacteremia after successful release of obstruction); different treatment approaches for complicated GN BSI related to syndromes excluded from BALANCE (endocarditis, osteomyelitis, deep abscesses).

2.7 Frequency and duration of follow up and study withdrawal

Participants will be assessed daily during their hospital stay, and again for outcome ascertainment at days 30, 60 and 90. The schedule of visits is displayed in Appendix Table 5.

If a patient is withdrawn from the study prematurely due to one of the following reasons, a withdrawal form that is part of the CRF will be completed. If permitted by the patient or substitute-decision maker, complete data collection will continue for withdrawn patients, as would be done for non-withdrawn patients. Anticipated reasons for withdrawal include:

- Consent withdrawn by patient or substitute-decision maker
- Patient's physician believes patient should be withdrawn from the study (please specify reason: _____)
- Patient's final blood culture report showed no GN organism or non-GN organism
- Inadvertent duplicate randomization (please specify first actual patient ID: _____)
- Other reason (please specify: _____)

2.8 Primary and secondary outcome measures

2.8.1. Primary Outcomes for BALANCE+ Platform Domains

The primary outcome in most of the BALANCE+ domains will use the patient-centered Desirability Of Outcome Ranking (DOOR) approach.[60] As a first step, patients will be assessed according to an ordinal DOOR outcome which prioritizes patient-important clinical outcomes: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection or readmission). DOOR outcomes have been used extensively in antimicrobial RCTs.[61-64] The DOOR prioritizes clinical outcomes as most important, but in some of the domains acquisition of new antimicrobial resistant (AMR) organisms detected on routine clinical and surveillance testing as part of usual

care will be used as a tie-breaker within ordinal levels – given that antimicrobial treatment strategies should aim to maximize clinical cure and survival for infected patients, while minimizing selection of AMR (see organisms included on AMR list below).

De-escalation versus no de-escalation domain

- ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)
- new AMR as tie-breaker

Oral beta-lactam versus non beta-lactam domain

- ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)
- new AMR as tie-breaker

Central vascular catheter retention versus replacement domain

- ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)
- no tie-breaker

Low-risk AmpC domain

- ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)
- new AMR as tie-breaker

Follow-up blood culture domain

- ordinal DOOR outcome: (dead at 90 days) < (alive at 90 days with reinfection and readmission) < (alive at 90 days with reinfection or readmission) < (alive at 90 days with neither reinfection nor readmission)
- no tie-breaker

Note: the primary outcomes will be revisited at the end of the vanguard phase, to account for feasibility based on observed recruitment rates and outcome event rates in the vanguard, and also to provide opportunity to harmonize with other trials in the field (including emerging platforms).

2.8.2 Secondary outcomes

The secondary outcomes for all BALANCE+ domains include the individual components of the DOOR outcome:

- 90-day mortality
- 90-day re-infection
- 90-day all cause readmission
- 90-day AMR colonization/infection

Other secondary outcomes include:

- 90-day Clostridioides difficile infection (CDI)
- 30-day mortality
- 60-day mortality

2.8.3 Additional Secondary Outcomes for Individual Domains

We will collect additional secondary outcomes for individual BALANCE+ domains:

(i) De-escalation versus no de-escalation

- change in total microbiome diversity (Shannon diversity index) between the day of randomization and day of discharge home from hospital (or day 30 if earlier)
- net change in resistome AMR burden between the day of randomization and day of discharge home from hospital (or day 30 if earlier).

(ii) Beta-lactam versus non-beta-lactam oral treatment

- antibiotic-related allergic reaction
- antibiotic-related (non-allergic) adverse event (grade 4+ or 3+ with treatment change)

(iii) Central vascular catheter replacement versus retention

- pneumothorax or thoracotomy tube insertion related to vascular catheter
- clinically important bleeding related to vascular catheter insertion as measured by clinically apparent hematoma or transfusion of ≥1 unit of packed red blood cells within 48h of line insertion, with no other recognized cause
- line associated thrombus
- persistent bacteremia >5d from initial index culture
- secondary bloodstream infection with new bacterial or fungal organism

(iv) Low-risk AmpC

- isolation of extended-spectrum beta-lactamase (ESBL) producing organism in any routine clinical or surveillance culture
- isolation of a carbapenem-resistant organism in any routine clinical or surveillance culture

(v) Follow up blood culture domain

- total duration of antibiotic therapy
- hospital length of stay

2.8.4 Primary Feasibility Outcomes for the BALANCE+ Vanguard Phase

The BALANCE+ vanguard phase will be focused on feasibility, with two domain-specific co-primary feasibility outcomes:

(i) recruitment rate

(ii) protocol adherence

(i) **Recruitment rate**

Recruitment rate will be measured as the number of patients randomized to each study domain, overall, and by individual participating site. We will target a minimum overall recruitment rate of 1 patient/site/month in the de-escalation domain, beta-lactam versus non-

beta-lactam stepdown domain, and FUBC domain; we will target recruitment of 0.25 patients/site/month in the line replacement domain. We anticipate these recruitment rates will be achievable based on recruitment rates of patient subgroups in the BALANCE RCT, which has very comparable screening processes.[13-15] However, we anticipate low numbers of organisms eligible for the low risk ampC domain and so will not have a specific recruitment target. Importantly, during the vanguard phase of the RCT, we will monitor recruitment closely, and work with sites to optimize recruitment, and identify barriers to recruitment with potential adjustment of inclusion/exclusion criteria if needed.

(ii) **Protocol adherence**

Protocol adherence will be calculated differently depending on the domain, but in each case will require adherence to the specific intervention arm and complete follow-up for the primary outcome. We will target ≥90% adherence in each arm of each domain. As with recruitment rate, we will monitor adherence rates closely, and liaise with individual sites around any protocol deviations, and communicate learnings to other participating sites.

De-escalation arm

Adherence will require that the patient's empiric GN antibiotic be stopped and switched to another narrower spectrum agent within 24h of blood culture susceptibility results or randomization, whichever happens later; this can be any agent with narrower spectrum in the study ranking (Appendix Table 1, column 3) and does not have to be the narrowest effective agent that was recommended by the study team. Decision-making for companion antibiotics will not impact the adherence measure.

No de-escalation arm

Adherence will require that the patient remain on the same empiric GN antibiotic they were receiving when the index blood culture susceptibility results became available. This agent must continue for the entire intravenous portion of the antibiotic course, unless the course is longer than 14 days (such as for complicated infections such as undrained abscesses) in which case a change is allowable thereafter.

Beta-lactam versus non-beta-lactam oral stepdown domain

Adherence will be calculated as the proportion of randomized patients receiving an oral antibiotic agent from the correct treatment group only, with no contamination by treatment with oral antibiotic agents from the other group. We will also examine proportion adherence to the declared intended treatment duration within ±15% of planned duration in days, with a target of ≥90% adherence.

Line replacement arm

Adherence will require that the patient's line be replaced within 72h of blood culture susceptibility results or randomization, whichever occurs later.

Line retention arm

Adherence will require that the line be retained until it is deemed to be no longer needed (so that it can be removed without replacement) or stops working.

Low risk ampC domain

Protocol adherence will be calculated as the proportion of randomized patients receiving an antibiotic agent from the correct intravenous treatment group only, with no contamination by treatment with antibiotic agents from the other group after the day of randomization.

Follow up blood culture arm

Adherence will require that the patient undergo at least one blood culture test collection 4±1 calendar days after the calendar date of index blood culture collection (unless already discharged from hospital by day 4).

No follow up blood culture arm

Adherence will require that the patient undergo no *routine* blood culture test collection 4±1 calendar days after the calendar date of index blood culture collection. If blood cultures are drawn at 4±1 days, the clinical team will be asked if these were conducted as routine surveillance or for clinical concerns (such as patient instability or concerns of new infection). If the clinical team reports that the indication was routine surveillance then these will be counted as protocol non-adherence. This differentiation is subjective, though, and so the FUBC domain will be analyzed only with intention to treat approach. No per protocol analysis will be undertaken, because this will be biased in favour of the no follow-up culture arm by removing some of the sicker patients.

2.9 Outcome Ascertainment and Data Linkage

Mortality, reinfection and readmission will be measured by contacting patients, or their substitute decision makers, at 30, 60 and 90 days from index blood culture. We will include consent for linkage to administrative datasets to supplement detection of these clinical outcomes (partnering with Canadian Health Data Research Network) and for consent to communicate with their treating doctors even if transferred to another institution.

Data will be linked to other local and provincial administrative databases to determine the primary and secondary outcomes at day 90.
The data linkage will be done only for the participants that provide informed consent for this linkage. The linkage will be done using the following identifiers:

- Hospital Medical Record Number
- First, last name
- Date of birth
- Sex
- Admission date
- Health card number (optional)

These identifiers may differ in some other jurisdictions and corresponding protocol amendments will be undertaken as appropriate.
Appropriate security measures will be implemented to safeguard information. Linking of data will be done in a way that participant's identity is protected and is unlikely to be known by anyone other than those directly part of the BALANCE+ research team.

2.10 Outcome definitions

30-, 60- and 90-day mortality

Participant status 30, 60 and 90 days post index blood culture collection date will be recorded as dead or alive.
Note: Participants that are lost to follow-up at day 90 will be tracked via the provincial administrative database, family doctors office or death registry.

Reinfection

Reinfection will be defined as a repeat positive blood culture with the same pathogen, or recurrence of the same underlying source of infection (as diagnosed by the clinical team) with or without pathogen isolation.

AMR organisms

In this pragmatic trial, new AMR will be detected based on positive surveillance and clinical cultures collected during routine care in their index hospitalization to 90d.

AMR-qualifying organisms will be based on a modification of the Dutch nosocomial infection surveillance guidelines – as per the BALANCE RCT:[13,65] methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* spp, extended spectrum beta-lactamase producing *Enterobacteriaceae* (defined by third generation cephalosporin-resistance), carbapenem-resistant *Enterobacteriaceae*, carbapenem-resistant *Pseudomonas*, carbapenem-resistant *Acinetobacter spp*; or *Enterobacteriaceae* resistant to at least two of fluoroquinolones, aminoglycosides or trimethoprim-sulfamethoxazole, *Acinetobacter* spp resistant to at least two of fluoroquinolones, aminoglycosides or ceftazidime; or non-*Enterobacteriaceae* resistant to at least three of fluoroquinolones, aminoglycosides, carbapenems, ceftazidime or piperacillin. The BALANCE+ modification of this definition will also include *C.difficile*. AMR outcomes will be adjudicated by pairs of independent reviewers blinded to treatment assignment.

All cause re-admission

All cause re-admission will be defined as admission to the same or different hospital within 90 days of index blood culture collection. Reason for re-admission will also be recorded.

Microbiome Diversity and Resistome Measurement

As a secondary analysis in the de-escalation domain, we will also collect stool specimens at randomization day and day of discharge (or day 30 if that is earlier) to measure the change in stool microbiome diversity and change in total antimicrobial resistance burden via resistome testing.

Stool will be collected using an innovative and simple toilet-paper like collection system which can then be preserved safely in room temperature buffer (https://www.zymoresearch.com/pages/stool-collector-device). This can be self-collected by patients who are physically able to wipe for self-care, or by nursing staff for those who are unable.

Shotgun metagenomic sequencing will be conducted on the stool specimen, which will facilitate both microbiome and resistome analysis.

The primary microbiome related outcome will be Shannon Diversity Index which is a quantitative overall measure of diversity that incorporates the number of different bacteria that are present in a stool sample and the uniformity in distribution of these bacteria.

There is no universally accepted overall measure of resistance burden in metagenomic resistome analysis. We will count presence/absence of clinically relevant resistance genes and resistance-conferring mutations for a defined set of clinically-relevant antimicrobial resistance genes based on published lists.[66] The list will include all 'high risk' genes based on the World Health Organization framework, as well as additional genes.[67] Resistance genes for gene presence/absence are listed in Appendix Table 4.

2.11 Sample size

2.11.1. *Sample Size for the BALANCE+ Vanguard RCT:*

For this vanguard phase, we require 72 patients (for each domain) to estimate protocol adherence within ± 5% margin of error and 95% confidence if the adherence is 95%, or ± 7% margin of error and 95% confidence if the adherence is 90%. One exception will be the low risk AmpC domain, where given the low expected numbers of eligible patients, we will aim for 36 patients to estimate protocol adherence within ± 7% margin of error and 95% confidence if the adherence is 95%, or ± 10% margin of error and 95% confidence if the adherence is 90%.

2.11.2. *Sample Size for domains in BALANCE+ full RCTs*:

As a perpetual platform, BALANCE**+** will use Bayesian methods with no fixed sample size. However, we will generate frequentist sample size calculations individually for each domain at the end of the vanguard phase and prior to registration of the main platform trial. We will also incorporate a maximum feasibility sample size based on a minimally clinically important difference for each domain.

2.12 Cost-Effectiveness

Each BALANCE**+** domain will be complemented by separately funded cost-effectiveness sub-studies, beyond the scope of this protocol.

2.13 Anticipated recruitment rate

BALANCE sites have achieved average recruitment rates of 1/month in ICU, 1/month in non-ICU wards. Therefore, it should be possible to accrue sufficient patients for each domain after the first 6-12 sites have been enrolling for 12 months.

In BALANCE we have achieved recruitment of 80 patients/month. BALANCE**+** will be limited to GN BSIs, which represented 1980/2400 (83%) of BALANCE patients at the last interim analysis. Therefore, we can expect to recruit 66 patients/month (or 2376 BALANCE**+** platform patients over the first 3 years). The actual recruitment rate should exceed that because BALANCE**+** will have less restrictive exclusion criteria and additional international sites. Examining the BALANCE GN BSI population characteristics indicates that we can expect the following

conservative monthly (and 3 year) recruitment rates for each domain: de-escalation domain 59/month (2,124 total), beta-lactam versus non-beta-lactam stepdown 20/mo (720), line retention/removal 18/mo (648), AmpC pathogen-specific domain 4/mo (144), follow up blood culture domain 70/month (2,520).

2.14 Protocol adherence

We expect high compliance with the de-escalation domain given that both arms are within current standard of care. In a prior systematic review of de-escalation studies the rate of de-escalation varied from 1/3 to 2/3 of patients with sepsis.[22] However, it is possible that there will be some deviations from protocol. For example, in sicker ICU patients the clinical team may change their mind post-randomization and opt not to de-escalate, and in less sick non-ICU ward patients the team may change their mind post-randomization and opt to de-escalate. In the line replacement domain there is potential for nonadherence among unstable ICU patients; the clinical team may change their mind post-randomization and opt not to replace a line. The same could occur among non-ICU patients with tunneled catheters (Hickman lines, Port-a-caths). Therefore, we will track domain-specific protocol adherence closely, and if rates are unacceptable (adherence <90%) the steering committee will determine reasons and trouble-shoot solutions. If adherence is very low then a domain could also be removed from the platform after piloting during the vanguard phase.

2.15 Rate of loss to follow up

We anticipate >90% follow-up for the primary outcome. We achieved >99% follow-up in the BALANCE ICU pilot trial,[15] the BALANCE non-ICU pilot trial,[14] and the main BALANCE RCT, but the BALANCE+ primary outcome is more complex than the primary outcome of 90 day vital status in these prior trials.[13]

2.16 Participating sites

The BALANCE RCT is enrolling at 73 hospitals in 7 countries (Canada, Australia, New Zealand, Israel, Saudi Arabia, Switzerland, United States), and most sites have confirmed ongoing participation in BALANCE**+**, with the potential to add further sites and countries (including Singapore). This offers an efficient start-up for BALANCE**+**, compared to the 3-year period over which BALANCE sites were initially scaled up and launched.

Given that this is a vanguard phase, we will aim for seamless transition to a main trial. We will initiate sites as soon as ethics and contracts are in place, and aim to launch in concert with the end of the BALANCE study. We anticipate that the target of 72 patients for each domain during the vanguard phase of the RCT will be achieved by the first 6-12 active centers within 12 months, or a shorter duration with more sites.

2.17 Statistical analysis plan

BALANCE**+** will be conducted, analyzed and reported according to CONSORT guidelines, including analyzing patients in the groups to which they were assigned (intention-to-treat).[69] As a perpetual platform BALANCE**+** will involve regular Bayesian interim analyses with uninformative priors,[68,69] designed and monitored by a statistical working group with experience in platform trials. These analyses will be conducted (by a statistical working group

and reviewed by the data monitoring committee) at every 500th BALANCE+ platform patient enrolment. Domains will be closed only if they meet pre-specified, stringent decision criteria for stopping based on superiority, non-inferiority or futility. Superiority will be called if an intervention exhibits a >99% posterior probability of a proportional odds ratio < 1 for the primary outcome. Non-inferiority will be called if the posterior probability of the proportional odds ratio >0.7 is >99%. Futility will be called (for non-inferiority or superiority) if the posterior probability is <1%.

The feasibility outcomes for each domain involve descriptive point estimates and 95% confidence intervals for recruitment rate and protocol adherence. We will analyze these overall and stratified by participating site. In addition to these quantitative outcomes, we will communicate with sites on a monthly basis to assess facilitators and barriers to recruitment and adherence, and work to optimize trial processes.

In secondary analyses in the de-escalation domain we will examine the differences in change in microbiome diversity and AMR resistome burden between randomization day and acute care hospital discharge (or day 30 if earlier) across the treatment arms. The primary microbiome related outcome will be Shannon Diversity Index which is a quantitative overall measure of diversity that incorporates the number of different bacteria that are present in a stool sample and the uniformity in distribution of these bacteria. For the resistome outcome will analyse as the net change in clinically relevant mutations from baseline to follow-up stool specimen.

2.18 Subgroup analyses

Across all BALANCE**+** platform domains, we will examine subgroups by sex, gender, underlying syndrome (urinary tract, lung, skin and soft tissue, vascular catheter, abdominal, hepatobiliary, other/unknown), pathogen group (Enterobacterales, Non-Enterobacterales), baseline colonization with antimicrobial resistant organism, and by ICU versus non-ICU location at time of GN BSI diagnosis. Additional subgroup analyses will be conducted for some BALANCE**+** platform domains. In the de-escalation domain, we will examine patients de-escalated to the narrowest effective agent recommended by the research team versus those de-escalated to different options by the treating team. In the beta-lactam versus non-beta-lactam oral stepdown domain, we will also examine subgroups with stated intended duration <8 versus ≥8 days, and protocol recommended versus lower beta-lactam doses (see Appendix table 3). In the line replacement domain, we will also examine subgroups of patients labelled as definite, probable or possible line source of BSI.

3. TRIAL MANAGEMENT

3.1 Data Centre

The BALANCE**+** platform will have a structured approach to day-day trial management through Sunnybrook Research Institute (SRI) Centre for Clinical Trials Services (CCTS) methods center. CCTS has successfully overseen large platform trials including the Canadian Treatments for COVID-19 (CATCO) trial. Asgar Rishu, who has 15 years of experience in trial coordination including BALANCE, will be the international BALANCE**+** coordinator at SRI, with support from a regional coordinator in each participating country, and a site coordinator at each institution.

3.2 Steering Committee

The BALANCE**+** steering committee includes

- Nick Daneman, MD (Principal investigator)
- Rob Fowler, MD (co-Principal investigator)
- Jennie Johnstone, MD (Canadian co-PI)
- Derek MacFadden, MD (Canadian co-PI)
- Emily McDonald, MD (Canadian co-PI)
- Todd Lee, MD (Canadian co-PI)
- Ben Rogers, MD (national lead Australia)
- David Paterson, MD (national lead Singapore)
- Dafna Yahav, MD (national lead Israel)
- Asgar Rishu, MBBS (Project Manager)
- Ruxandra Pinto, PhD (Biostatistician)
- Sean Ong, MBBS (Trainee)
- Priyanka Chaubey (patient with lived experience)

The steering committee will add additional national leads as other countries join BALANCE+. The BALANCE**+** team, is purposefully diverse across sex, gender, race, geography, career stage, clinical specialty, and areas of methodologic expertise.

3.3 Data Safety and Monitoring Committee

The BALANCE**+ Data Safety and Monitoring Committee** will provide independent review of study reports, procedures, indicators of trial management, efficacy and safety reports, and interim and final analyses; the BALANCE**+** DSMC charter will be modified from the BALANCE charter, which in turn derived from the Data Monitoring Committees: Lessons, Ethics, Statistics (DAMOCLES) Study Group charter.[70]

3.4 Protocol Deviations and Violations

Protocol Deviation: A protocol deviation is an incident involving non-compliance with the REB approved protocol that may or may not have a significant effect on patient's rights, safety or welfare, or on the integrity of the data.

In this taxonomy, protocol deviations would involve non-adherence to the randomization arm in a domain to which the patient is enrolled. The definitions of non-adherence are specific to each domain and randomization-arm (see section 2.8.4 Primary Feasibility Outcomes).

Protocol Violation: A protocol violation is an accidental or unintentional change to, or non-compliance with the REB approved protocol that generally increases risk or decreases benefit, affects the subject's rights, safety, or welfare, or the integrity of the data and could have been prevented by the investigator. All protocol violations should be reported to the REB and sponsor/coordinating centre.

Examples include:

- enrolling a non-eligible patient
- randomizing a patient before obtaining consent
- assigning the wrong study intervention within an enrolment domain
- study participants failing to comply with the trial protocol regarding a study intervention (e.g. not completing antibiotic course after discharge from hospital)

3.5 Adverse Event Reporting

Some of the common known antibiotic side effects are: rash, diarrhea, nausea/vomiting, headache, abdominal pain, hypersensitivity (allergic) reactions, renal (kidney) toxicity, ototoxicity (hearing loss), dizziness, *Clostridioides difficile* infection, antibiotic related kidney injury, antimicrobial related hepatitis, and other antimicrobial related organ toxicity. However, these risks already exist outside the research study participation because all patients with bloodstream infection will be prescribed antibiotics, and all randomization arms in all BALANCE+ domains are within the current standard of care in participating hospitals.

Morbidity and mortality are expected among critically ill patient populations with BSI. Accordingly, mortality at 30, 60 and 90 days are outcomes. Outcomes will be reported as such, not as Serious Adverse Events (SAEs), Serious Unexpected Adverse Reactions (UARs), or Suspected Unexpected Serious Adverse Reactions (SUSARs). These outcomes will be reported to the DSMC at all interim analyses. Nevertheless, we will closely monitor patient safety in the trial by recording the antimicrobial-related adverse events and serious unexpected adverse drug reactions.

3.6 Trial registration

BALANCE+ will be registered on clinicaltrials.gov first as the BALANCE+ vanguard phase (prior to enrolment of first patient), and then this will either be updated or a new registration will be made when the trial transitions to the main platform.

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APPENDIX TABLE 1: SPECTRUM RANKINGS		
Madaras-Kelly et al. Spectrum Scores Across All Antibiotics (exact scores†, sorted by class)	Weiss et al. Spectrum Ranking Within Beta-Lactams (ascending spectrum)	BALANCE+ Rankings (descending spectrum)
Aminoglycosides • Amikacin 35.50 • Gentamicin, tobramycin 35.50 b-lactamase inhibitors • Ampicillin/sulbactam, amoxicillin/clavulanate 29.50 • Piperacillin/tazobactam 42.25 • Ticarcillin/clavulanate 40.50 Carbapenems • Ertapenem 30.25 • Imipenem, meropenem 41.50 Cephalosporins • Cefazolin, cephalexin 19.25 • Cefuroxime 23.50 • Ceftriaxone, cefotaxime 25.25 • Ceftazidime/cefepime 33.25 • Ceftaroline 26.00 Fluoroquinolones • Ciprofloxacin, levofloxacin 39.75 • Moxifloxacin 36.25 Glycopeptides/lipopeptides • Vancomycin 13.00 • Daptomycin 14.25 Macrolides/lincosamides • Azithromycin, clarithromycin 12.25 • Clindamycin 10.75 Penicillins • Ampicillin, amoxicillin 13.50 • Nafcillin, oxacillin 4.25 Tetracyclines • Tetracycline, doxycycline 38.75 • Tigecycline 49.75 Miscellaneous • Aztreonam 21.50 • Colistin, polymyxin B 34.00 • Linezolid 18.00 • Metronidazole 4.00 • Trimethoprim/sulfamethoxazole 33.50	1. Amoxicillin 2. Amoxicillin + clavulanic acid 3. Third generation cephalosporins, ureido/carboxy-penicillins 4. Piperacillin-tazobactam, Ticarcillin-clavulanate, 4th generation cephalosporins, anti-pseudomonal third generation cephalosporins 5. Ertapenem 6. Meropenem, Doripenem, Imipenem	• Cefiderocol • Colistin • Polymyxin B • Meropenem-vaborbactam • Imipenem-relebactam • Ceftazidime-avibactam • Ceftolozane-tazobactam • Tigecycline • Meropenem, Doripenem • Ertapenem • Piperacillin-tazobactam • Ticarcillin-clavulanate • Fosfomycin • Moxifloxacin • Levofloxacin • Ciprofloxacin • Doxycycline • Amikacin • Tobramycin • Gentamicin • Cefipime • Ceftazidime • Trimethoprim-sulfamethoxazole • Ampicillin-sulbactam • Amoxicillin-clavulanate • Ceftaroline • Ceftriaxone • Cefuroxime • Cefazolin • Ampicillin • Amoxicillin

†higher score represents broader spectrum

APPENDIX TABLE 2: NON-BETA-LACTAM AND BETA-LACTAM ORAL TREATMENT OPTIONS	
Non-beta-lactam options	Beta-lactam options
Fluoroquinolones • ciprofloxacin • levofloxacin • moxifloxacin†	Penicillins • amoxicillin • amoxicillin-clavulanate
Sulfonamides • trimethoprim-sulfamethoxazole	Cephalosporins • cephalexin • cefadroxil • cefixime

†this agent is not acceptable for BSI from urinary tract source due to poor urinary penetration

NB: dose and interval will be left to discretion of the prescribing physician but will be monitored during the vanguard phase of the RCT to assess for variability

NB: Treatment duration will also be at the discretion of the treating physician, but they will be required to declare their intended treatment duration prior to randomization, to ensure that they are agreeable to the patient receiving the same duration regardless of randomization arm

APPENDIX TABLE 3: STANDARD VERSUS HIGHER BETA-LACTAM DOSES		
Beta-lactam	Higher (recommended) doses	Lower (but acceptable) doses
Amoxicillin	1g PO TID	500mg PO TID
Amoxicillin-clavulanate	875/125mg PO TID	875/125mg PO BID or 500/125 mg PO TID
Cephalexin	1g PO QID	500 mg PO QID
Cefadroxil	1g PO BID	500 mg PO BID
Cefixime	400mg PO BID	400mg PO BID

APPENDIX TABLE 4: CLINICALLY RELEVANT RESISTANCE GENES INCLUDED IN PRIMARY RESISTOME MEASUREMENT	
Antibiotic Class	Relevant Resistance Genes
Aminoglycosides	• aphA6 • aadA1 • aacC4 • aacC2 • aacC1
Beta-lactams	• mecA • BES-1 • BIC-1 • CTX-M1/8/9 • GES • IMI/NMC-A • KPC • SHVs • ccrA • IMP-1/2/5/12 • NDM • VIM-1/7/13 • ACC-1/3 • ACT-1/5/7 • CFE-1 • CMY-10 • DHA • FOX • LAT • MIR • OXA-2/10/18/23/24/45/48/50/51/54/55/58/60/60/62
Fluoroquinolones	• QnrA • B-4/B-5/B-8/B-31/C/D/S • QepA • AAC • oprM • oprj
Other	• msrA • mefA • ermC • ermB • ermA • vanB/C • tetA/B

*The list of clinically relevant resistance genes will be updated based on any evolution of the World Health Organization framework during the study, with the list finalized immediately prior to sequencing/analysis

APPENDIX TABLE 5: SCHEDULE OF EVENTS						
Procedure	**Day 1**	**Day 4 ± 1**	**Discharge day**	**Day 30 (+2d)**	**Day 60 (+3d)**	**Day 90 (+5d)**
Informed consent	X					
Randomization[#]	X					
Demographics & baseline data	X					
Reveal randomization						
De-escalation vs No de-escalation domain[%]	X					
Oral beta-lactam versus non beta-lactam domain	X					
Central vascular catheter replacement domain[@]	X					
Routine versus no routine follow-up blood cultures[$]	X					
Low-risk AmpC domain	X					
In hospital collection						
Follow-up blood cultures[$]		X				
Assigned treatment for required duration*	X	X				
Daily data collection		X				
Concomitant medication review	X	X				
Microbiome/resistome specimen[¥]	X			X		
Protocol adherence review	X					
Adverse event review						
De-escalation vs No de-escalation domain	X	X	X			
Oral beta-lactam versus non beta-lactam domain	X	X	X			
Central vascular catheter replacement domain	X	X	X			

Routine versus no routine follow-up blood cultures	X	X	X			
Low-risk AmpC domain	X	X	X			
Follow-up for outcomes						
Vital status				X	X	X
Any new positive blood culture				X	X	X
Any OTHER positive culture				X	X	X
Readmission to any hospital				X	X	X
Any new antibiotic(s) since discharge				X	X	X

max randomization window of 72 hrs from culture collection date

% within 24 hours of blood culture finalization. Finalization means when antibiogram (antimicrobial susceptibility) is available

@ maximum of 24 hours from blood culture finalization

$ for patients who consented for "follow-up" domain and are randomized to follow-up blood culture arm

*3-5 days or hospital discharge whichever comes first

¥ day of randomization +2, day of discharge or day 30 (±2) whichever comes first