CLINICAL STUDY PROTOCOL

Exploratory Study of Fluoroquinolone Resistance for Patients Undergoing Autologous Hematopoietic Stem Cell Transplantation in the Treatment of Multiple Myeloma

Protocol Version

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Abbreviations

Abbreviation	Explanation
ALC	Absolute lymphocyte count
ANC	Absolute neutrophil count
CDC	Centers for Disease Control and Prevention
CDI	Center for Discovery and Innovation
CRP	C-reactive protein
ES	Engraftment syndrome
ESBL	Extended-spectrum beta lactamase
FN	Febrile neutropenia
FRE	Fluoroquinolone-resistant Enterobacterales
GvHD	Graft versus host disease
HAI	Healthcare-associated infections
HSC	Hematopoietic stem cell
HUMC	Hackensack University Medical Center
MDRO	Multi-drug resistant organism
MM	Multiple myeloma
MRSA	Methicillin-resistant Staphylococcus aureus
NCCN	National Comprehensive Cancer Network
NIF	Non-infectious fever
NIF	Non-infectious fever
PBSC	Peripheral blood stem cell
SDoH	Social determinants of health
SOC	Standard of Care
SOP	Standard Operating Procedures

TMP-SMZ	trimethoprim-sulfamethoxazole

1 Summary

This is an exploratory study to determine the prevalence of fluoroquinolone resistance in patients receiving dose-intense melphalan with autologous peripheral blood stem cell (PBSC) transplantation in the treatment of multiple myeloma (MM). Numerous previous studies showed a lower incidence of febrile neutropenia (FN) in neutropenic patients who received antibiotic prophylaxis during a period of neutropenia. However, a survival benefit has not been consistently demonstrated across the many studies performed. Further complicating the relevance of these observations is that few of these studies were limited to a more defined patient population, usually including a mixture of patients undergoing autologous or allogeneic transplantation, leukemia remission induction, and/or treatment of non-hematological malignancies.

Although a general benefit of antibiotic prophylaxis in the prevention of FN has been demonstrated, these studies were also conducted before the widespread development of colonization by fluoroquinolone-resistant Enterobacterales (FRE). The current relatively high proportion of patients harboring FRE challenges the assumption that prophylaxis with fluoroquinolones is still of benefit in preventing sepsis. This challenge applies especially to patients undergoing autologous PBSC transplantation in the treatment of MM. The period of neutropenia for these patients persists for only about 4-6 days. Yet, non-infectious fever (NIF), defined as fever without positive cultures or demonstration of a source of infection, is common in this patient population. NIF is increasingly attributed to a phenomenon termed “engraftment syndrome (ES)” when a source of infection is not identified, and patients with NIF are instead treated with a brief course of corticosteroids.

This study is to determine the current prevalence of FRE in a defined patient population treated with dose-intense melphalan with autologous PBSC transplantation. These data may be used in subsequent studies exploring the use of prophylaxis in this patient population. Secondary objectives are to explore the prevalence of FRE in minority patients or patients in stress as determined by scoring of social determinants of health, the benefit of fluoroquinolone prophylaxis in the subpopulation of patients with FRE colonization, if FRE colonization predicts FN, and if fluoroquinolone resistance is a factor in the development of ES. We will explore a correlation between the presence/absence of FRE with studies of stool microbiome for subjects willing to provide stool samples for analysis. A molecular and microbiological assessment of a perirectal swab prior to transplant admission (and other time points) will determine the presence of fluoroquinolone-resistant and multi-drug resistant gram-negative pathogens (MDRO, multi-drug resistant organisms). Subjects will be followed for development of fevers and blood stream infections, the development of ES requiring steroid use, and survival through the immediate period of treatment.

2 Introduction

Patients with hematologic malignancies who receive intensive chemotherapy frequently develop severe neutropenia. To prevent gram-negative bloodstream infections in patients with prolonged chemotherapy-induced neutropenia, fluoroquinolone prophylaxis became the recommended standard of care. A Cochran review of the use of antibiotic prophylaxis for

patients at risk of neutropenia identified 109 trials involving 13,579 patients conducted between 1973 and 2010, and concluded that antibiotic prophylaxis (compared to placebo):

- Reduced the incidence of FN,
- Reduced the occurrence of documented infection, microbiologically-documented infection, and other indicators of infection,
- Reduced the risk of death from all causes, and
- Reduced the risk of infection-related death.[1]

Included in this review were studies that compared various regimens such as use of a fluoroquinolone compared to prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMZ), or comparing one fluoroquinolone to another. Although TMP-SMZ and levofloxacin were found equivalent in preventing FN, the better tolerance of the latter led to fluoroquinolones being widely adopted as the primary agent used in the prophylaxis of FN.

However, the increasing prevalence in FRE pathogens and their association with multidrug resistant isolates raises clinical concern that prophylaxis may have negative effects, including observations that blood-stream infections found in patients harboring FRE are also frequently characterized as extended-spectrum beta-lactamase (ESBL) organisms resistant to first-line antibiotics commonly established at onset of FN as standard-of-care by transplant programs.[2] Thus, identification of patients harboring FRE and ESBL organisms before transplantation may improve transplant outcomes by avoiding use of a fluoroquinolone in prophylaxis and allowing a more judicial selection of antibiotics to be used in the setting of FN.

Furthermore, we do not know if fluoroquinolone prophylaxis contributes to the development of engraftment syndrome or may have other deleterious effects on treatment outcome for this select population of patients in which non-infectious fever is common. In a retrospective review of 100 consecutive patients undergoing dose-intense melphalan at Hackensack University Medical Center (HUMC) with autologous HSCT in 2020, we found that 27 patients developed fevers, but only one blood stream infection occurred and 28 patients were treated with a course of corticosteroids for NIF and/or diarrhea attributed to ES (data not published).

The intent of this study is to determine if fluoroquinolone prophylaxis protects against FN or possibly, increases the risk of NIF and ES in the defined population of patients undergoing autologous PBSC transplantation in the treatment of multiple myeloma. To determine a possible role of FRE colonization on these endpoints, patients will be tested for the presence of FRE prior to hospital admission, but colonization by FRE will not be a determining factor in whether a patient receives fluoroquinolone prophylaxis. We will explore secondary objectives including the incidence of FN in patients who harbor FRE. We will also explore the secondary objective that fluoroquinolone prophylaxis may influence the risk of ES. We

[1] Gafter-Gvili A, Fraser A, Paul M, et al. Antibiotic prophylaxis for bacterial infections in afebrile neutropenic patients following chemotherapy (Review). Cochrane Database of Systematic Review 2012, Issue 1.

[2] Satlin MJ, Chen L, Douglass C, et al. Colonization with fluoroquinolone-resistant Enterobacterales decreases the effectiveness of fluoroquinolone prophylaxis in hematopoietic cell transplant recipients. C lin Infect Dis. 2021 May 6:ciab404. doi: 10.1093/cid/ciab404. Epub ahead of print.

will explore if there are patterns of FRE resistance that differ by ethnicity or race or by differences in social determinants of health.

3 Background

3.1 Anti-microbial Prophylaxis for Neutropenic Patients with Cancer:

Patients with cancer are at increased risk of infection from a variety of opportunistic organisms including bacteria, viruses, fungi, and protozoa. Neutropenia and mucosal injury resulting from chemo/radiotherapy regimens places a patient, regardless of diagnosis, at significant risk of bacterial blood stream infections, with this risk rising proportionately with the depths and durations of neutropenia and mucosal damage. Recognition of this risk of opportunistic infections over five decades ago led to multiple studies exploring the role of prophylactic antibiotics to be given during the period of neutropenia. A Cochran review (published in 2012) of the use of antibiotic prophylaxis for patients at risk of neutropenia identified 109 trials involving 13,579 patients conducted between 1973 and 2010, and concluded that antibiotic prophylaxis (compared to placebo) decreased the risk of FN, and in some studies, the overall mortality risk.[1] These studies led to the recommendation by various groups including Centers for Disease Control and Prevention (CDC) in collaboration with the Infectious Diseases Society of America and the American Society of Blood and Marrow Transplantation, and by the National Comprehensive Cancer Network (NCCN) for use of prophylactic antibiotics for patients at risk of serious infections.[3,4]

3.2 Infectious Risk of Multiple Myeloma

Patients with MM are at higher risks of pneumonia and blood stream infections independent of neutropenia after dose-intense therapy. In a cohort study of 2557 newly diagnosed patients in Denmark, 1981 unique infections occurred during the first 6 months after diagnosis.[5] Pneumonia accounted for 27.7% of infections, and sepsis occurred in 17.7% of patients. Risk factors for pneumonia or sepsis in multivariate analysis were higher bone marrow plasma cell content, grade II or III ISS stage, and higher serum creatinine. (However, antibiotic prophylaxis is not widely used as a component of remission-induction therapy.)

3.3 Routine Prophylaxis for Autologous Transplantation of Multiple Myeloma

No prospective randomized studies of antibiotic prophylaxis during autologous HSC transplantation of patients with MM have been reported. Satlin et al described a retrospective review of the addition of routine prophylaxis with levofloxacin for cohorts of patients treated

[3] Centers for Disease Control and Prevention; Infectious Diseases Society of America; American Society of Blood and Marrow Transplantation. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2000;6(6a):7-83

[4] Prevention and Treatment of Cancer-Related Infections, Version: 2.2020. Published online (www.nccn.org).

[5] Sorrig R, lausen TW, Salomo M, Vangsted A, Gimsing P. Risk factors for infections in newly diagnosed multiple myeloma patients: A Danish retrospective nationwide cohort study. Eur J Haematol 2019;102:182-190.

before or after 6/2006.[6] After the adoption of routine levofloxacin prophylaxis, the rate of FN decreased from 91.6% to 60.9% and the rate of blood stream infections decreased from 41.2% (49 of 119) to 14.7% (23 of 156). These authors did note non-significant increases in blood stream infections with FRE (5% vs 1%) and Clostridium difficile infection (7% vs 3%). In contrast, these authors found that the addition of fluoroquinolone prophylaxis had no effect on the rate of fever in patients being treated for lymphoma.

We performed a retrospective review of autologous peripheral blood stem cell (PBSC) transplants for 100 sequential patients with a diagnosis of multiple myeloma treated at Hackensack University Medical Center (HUMC) commencing 1/1/2020 (last transplants 7/1/2020). Most patients were undergoing a first transplant (Table 1) after conditioning with melphalan 200 mg/m^2; 12 patients underwent a second transplant after conditioning with melphalan 200 mg/m^2 or melphalan 200 mg/m^2 in combination with two doses of bortezomib 1.6 mg/m^2. Antibiotic prophylaxis was administered to all patients with 97 patients receiving ciprofloxacin. Cefuroxime was the prophylaxis of choice for three patients unable to receive the fluoroquinolone. Febrile neutropenia was observed in 27 patients. Blood and urine cultures were obtained for all febrile patients at the onset of fever. Blood cultures for only one patient, who received cefuroxime prophylaxis, were positive for fluoroquinolone-sensitive Escherichia coli. No urine cultures returned positive. Two patients developed toxin-producing Clostridium difficile infection during their hospital course. A total of 45 patients were treated with corticosteroids, 17 as prophylaxis starting on day +8 of the transplant, the remainder (n=28) for onset of symptoms of engraftment syndrome including non-infectious fever, diarrhea, or rash.

Table 1: Febrile Neutropenia after Autologous PBSC Transplantation, Multiple Myeloma

Number Patients	100
Sex	
Male	63
Female	37
Age	
Median	62
Min	42
Max	82
Transplant	
First	88
Second	12
Conditioning	
Mel	93
MelVel	7
Ciproflox Prophylaxis	
Yes	97
No	3 (Cefuroxime)

[6] Satlin MJ, Vardhana S, Soabe R, et al. Impact of prophylactic levofloxacin on rates of bloodstream infection and fever in neutropenic patients with multiple myeloma undergoing autologous hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2015;21:1808-14.

Fever	
Yes	27
No	73
Blood Cultures	
Growth	1 (E coli)
No Growth	26
Steroid	
Yes	45 (17 planned prophylaxis)
No	55

The much lower observed rate of FN in our series compared to the 60.9% of patients receiving levofloxacin prophylaxis described by Satlin et al could be explained by numerous other factors including the choice and duration of initial remission-induction chemotherapy used during these different eras of treatment, and the time from diagnosis to transplantation. Most patients treated at HUMC undergo transplantation after only 2-3 cycles of remission-induction therapy. Savage et al noted a biphasic risk of infection in patients with multiple myeloma undergoing non-transplant therapy, initially with S. pneumoniae and H. influenzae, but later with S. aureus and gram-negative organisms.[7] It has been shown that patients with multiple myeloma undergoing remission-induction therapy are at risk of fever and death during the first three months of treatment (not including transplantation), with possible benefit from routine levofloxacin prophylaxis.[8] A Danish retrospective review of infections in patients with multiple myeloma found tumor burden and renal impairment were risk factors for pneumonia and sepsis in the early phases of treatment.[9,10] These factors suggest that differences in patient populations studied could result in differences in risk of infection.

3.4 Potential Toxicity of Fluoroquinolone Prophylaxis:

Moreover, antimicrobial resistance is a growing threat. Bacteria evolve and, in instances where antibiotics are used frequently, the possibility arises for a resistant bacterium to survive and flourish in the face of the medicine designed to kill it, making standard treatments ineffective. According to the CDC, more than 2 million people in the United States alone acquire an antibiotic-resistant bacterial infection, with over 23,000 deaths as a result each

[7] Savage DG, Lindenbaum J, Garrett TJ. Biphasic pattern of bacterial infection in multiple myeloma. Ann Intern Med. 1982;96(1):47-50.

[8] Drayson MT, Bowcock S, Planche T, et al. Levofloxacin prophylaxis in patients with newly diagnosed myeloma (TEAMM): a multicentre, double-blind, placebo-controlled, randomized, phase 3 trial. Lancet Oncol 2019;20:1760-72.

[9] Sørrig R, Klausen TW, Salomo M, Vangsted A, Gimsing P. Risk factors for blood stream infections in multiple myeloma: A population-based study of 1154 patients in Denmark. Eur J Haematol. 2018;101:21-27.

[10] Sørrig R, Klausen TW, Salomo M, Vangsted A, Gimsing P. Risk factors for infections in newly diagnosed Multiple Myeloma patients: A Danish retrospective nationwide cohort study. Eur J Haematol. 2019;102:182-190.

year.[11,12] In the healthcare setting, patients are increasingly susceptible to healthcare-associated infections (HAIs) often caused by antibiotic-resistant pathogens. This increase in anti-microbial resistance is also seen in the transplant setting, with increasing numbers of patients developing infections with multi-drug resistant organisms (MDRO).[13] Satlin et al described a prospective observation study of 234 patients undergoing allogeneic (n=119) or autologous (n=115) HSC transplantation, finding that 23% of patients harbored FRE, with recent antibiotic use being predictive for FRE colonization.[2] Seventeen (31%) FRE-colonized patients developed gram-negative sepsis despite prophylaxis with levofloxacin, compared to only two (1.1%) of 180 patients not colonized with FRE. These data suggest that prophylaxis with a fluoroquinolone antibiotic for patients harboring FRE could increase the risk of blood stream infection.

We also performed a retrospective review of 74 sequential patients who underwent allogeneic HSC transplantation at HUMC between 1/1/2020, and 8/31/2020, and identified 54 patients who developed neutropenic fevers, with 16 patients demonstrating bacteremia (n=15) or urinary tract (n=1) infection. FRE colonization was not tested during the transplant course, but six patients developed infection with gram-negative organisms, all of which were fluoroquinolone-resistant isolates, including (each in separate patients): Klebsiella pneumoniae (blood, fluoroquinolone-resistant; urine, ESBL), Escherichia coli (blood, fluoroquinolone-resistant (two patients); blood, ESBL (two patients)). Other organisms identified were Streptococcus mitis (five patients), Coagulase-negative Staphylococcus (three patients), Rophia mucilag (one patient), and Bacillus cereus (one patient). No patient in this series developed bacteremia with more than one organism. Although testing for colonization by FRE was not routinely performed for this patient population, that each of the gram-negative blood stream infections was with a fluoroquinolone-resistant organism would be consistent with the findings by Satlin et al that fluoroquinolone prophylaxis may increase the risk of life-threatening blood stream infections by MDRO.

3.5 Non-Infectious Fever and Engraftment Syndrome after Autologous HSC Transplantation for Multiple Myeloma:

Non-infectious fever (NIF) is increasingly recognized as a major characteristic of engraftment syndrome (ES). ES is a poorly defined constellation of symptoms including fever, diarrhea, skin rash, and pulmonary edema occurring proximal to neutrophil recovery after autologous or allogeneic HSC transplantation. The signs and symptoms of ES overlap with several other complications of HSC transplantation, such as acute graft-versus-host disease (GvHD, including hyperacute GvHD and autologous GvHD) and sepsis.

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[11] Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2013 (https://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf.).

[12] Jernigan JA, Hatfield KM, Wolford H, et al. Multidrug-Resistant Bacterial Infections in U.S. Hospitalized Patients, 2012-2017. N Engl J Med. 2020;382:1309-1319.

[13] Satlin MJ, Walsh TJ. Multidrug-resistant Enterobacteriaceae, Pseudomonas aeruginosa, and vancomycin-resistant Enterococcus: Three major threats to hematopoietic stem cell transplant recipients. Transpl Infect Dis. 2017;19:10.1111/tid.12762. doi:10.1111/tid.12762

*Table 2: Diagnostic Criteria of Engraftment Syndrome**

Spitzer[14]	Maolino[15]
Major Criteria	
Temperature ≥38.3° C with no identifiable infectious etiology	Noninfectious fever, **and**
Erythrodermatous rash >25% BSA not attributable to medication	Rash, or
Noncardiogenic pulmonary edema	Pulmonary infiltrates, or
	Diarrhea
Minor Criteria	
Hepatic dysfunction with either TBili ≥2 mg/dL or transaminase ≥2x normal	
Renal insufficiency (serum creatinine ≥2x baseline)	
Weight gain ≥2.5% of baseline weight	
Transient encephalopathy unexplainable by other causes	
Diagnosis: All three major criteria or two major criteria and one or more minor criterion, within 96 hr of engraftment	

* Adapted from Spitzer, TR.[16]

Criteria for the diagnosis of ES include fever not attributed to infection; features of systemic vascular leak such as hypotension, edema, ascites, non-cardiogenic pulmonary edema, and pleural effusions; and diarrhea without other cause such as chemotherapy toxicity (Table 2). The incidence of ES as reported by various transplant programs ranges 9-70% in the autologous HSC transplant setting (reviewed by Spitzer[16]).

Moreover, the pathophysiology of ES is not defined, complicating the diagnosis, but may be a result of proinflammatory cytokines. Elevated levels of C-reactive protein (CRP) are found in patients with ES, but also in patients with aGvHD or sepsis. The risk of developing ES has

[14] Spitzer TR. Engraftment syndrome following hematopoietic stem cell transplantation. Bone Marrow Transplant 2001;27:893-8.

[15] Maiolino B, Biasoli I, Lima J, et al. Engraftment syndrome following autologous hematopoietic stem cell transplantation: definition of diagnostic criteria. Bone Marrow Transplant 2003;31:393-7.

[16] Spitzer TR. Engraftment syndrome: double-edged sword of hematopoietic cell transplants. Bone Marrow Transplant 2015;50:469-75.

been correlated to the use of G-CSF after transplantation,[17] use of GM-CSF instead of G-CSF during the period of neutropenia,[18] higher numbers of CD34+ cells infused,[19] and more rapid recovery of neutrophils.[21] Tissue biopsies may also not help discriminate between ES and aGvHD. Dobryski et al in 2009 reported "severe autologous GvHD" in a series of patients treated for multiple myeloma,[20] but in a subsequent review of ES (2015) proposed that all forms of autologous GvHD be classified as ES and that the severity of ES be based on clinical symptoms and histological evidence of immune involvement.[21]

It must be considered that NIFs are also common in patients experiencing prolonged cytopenias such as patients undergoing leukemia remission-induction, or patients with lymphoma treated with more aggressive regimens such as Hyper-CVAD, settings in which NIF is not attributed to ES but may be related to mucositis. The risk of blood-stream infection can be correlated with duration of neutropenia and with mucosal barrier injury.

Steroids are used in the management of ES (more severe cases are treated with additional immunosuppressive agents such as a calcineurin inhibitor). Prophylactic use of corticosteroids has been reported in non-randomized trials as effective in preventing ES.[22,23] In these retrospective studies, all patients received antimicrobial prophylaxis with a fluoroquinolone.

The ES observed at this center fits more closely with the Maolino criteria (which were developed as a single-center experience for patients undergoing autologous HSC transplantation), usually presenting as fever and/or diarrhea. But cases with multi-organ failure even leading to death have been observed in our center. In our series of 100 patients undergoing autologous HSC transplantation in the treatment of MM, only 1 of 27 patients who developed fevers was found to have a blood stream infection. The other 26 can be classified as having NIF, and most were given a course of corticosteroids.

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[17] Gutierrez-Garcia G, Rovira M, Magnano L, et al. Innovative strategies minimize engraftment syndrome in multiple myeloma patients with novel induction therapy following autologous hematopoietic stem cell transplantation. Bone Marrow Transplant 2018;53:1541-7.

[18] Chen, J, Pan K, Zhan T, et al. Autologous stem cell transplantation for multiple myeloma: growth factor matters. Biiol Blood Marrow Transplant 2019;25:e293-7.

[19] Ravoet C, Feremans W, Husson B, et al. Clinical evidence for an engraftment syndrome associated with early and steep neutrophil recovery after autologous blood stem cell transplantation. Bone Marrow Transplant 1996;18:943-7.

[20] Drobyski WR, Hari P, Keever-Taylor C, et al. Severe autologous GVHD after hematopoietic progenitor cell transplantation for multiple myeloma. Bone Marrow Transplant 2009;43:169-77.

[21] Cornell RF, Hari P, Drobyski WR. Engraftment syndrome after autologous stem cell transplantation: an update unifying the definition and management approach. Biol Blood Marrow Transplant 2015;21:2061-8.

[22] Rodriguez-Lobato L-F, Martinez-Roca A, Castano-Diez S, et al. The avoidance of G-CSF and the addition of prophylactic corticosteroids after autologous stem cell transplantation for multiple myeloma patients appeal for the at-home setting to reduce readmission for neutropenic fever. PLoS One 2020;15:e0241778.

[23] Betticher C, Bacher U, Legros M, et al. Prophylactic corticosteroid use prevents engraftment syndrome in patients after autologous stem cell transplantation. Hematol Oncol 2021;39:97-104.

3.6 Social Determinants of FRE Prevalence

This study will be open in two metropolitan areas (Bergen County, New Jersey, a suburb of New York City, and Washington, DC) with differing ethnic/racial and socioeconomic populations. It is possible that FRE prevalence will differ in these two metropolitan regions, and that FRE may also differ by various social determinants of health (SDoH) as described, for example by differences in methicillin-resistant Staphylococcus aureus (MRSA) infections or SARS-CoV-2 (Covid) hospitalizations.[24,25]

3.7 Microbiome

The gut microbiome is a key player in shaping the innate and adaptive immune responses to anti-cancer therapies. In patients with hematological malignancies undergoing conditioning and allogeneic HSC transplantation, loss of gut microbiota diversity during peri-engraftment is associated with blood-stream infections, GVHD-induced mortality and overall survival.[26] Conditioning regimens and use of broad-spectrum antibiotics to treat FN lead to a loss of intestinal homeostasis, depletion of beneficial obligate anaerobes and a shift towards a microbiome dominated by antibiotic-resistant microbiota.[27] Furthermore, obligate anaerobes such as *Eubacterium limosum* and other *Clostridia* strains were associated with reduced risk of progression in allogeneic HSC transplant patients, including MM patients.[28] Pretransplant gut microbiota diversity and composition has been shown to be predictive of multidrug-resistant blood-stream infections and unfavorable outcomes,[29] and fecal microbiota transplant from healthy donors to patients prior to allogeneic HSC transplant ameliorated adverse outcomes in patients colonized with multi-drug resistant organisms.[30] A recent study reported that intestinal domination of *Enterococcus* or *Escherichia* at abundances as low as 1% and 0.1% respectively increased the risk of BSI and other clinical complications in allogeneic HSC transplant patients by these bacteria significantly.[31]

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[24] See I, Wesson P, Gualandi N, et al. Socioeconomic Factors Explain Racial Disparities in Invasive Community-Associated Methicillin-Resistant Staphylococcus aureus Disease Rates. Clin Infect Dis. 2017;64:597-604.

[25] Wortham JM, Meador SA, Hadler JL, et al. Census tract socioeconomic indicators and COVID-19-associated hospitalization rates-COVID-NET surveillance areas in 14 states, March 1-April 30, 2020. PLoS One. 2021;16:e0257622.

[26] Hono Y, van den Brink MRM. Gut microbiota injury in allogeneic HSC. Nat Rev Cancer. 2018;18:283-95

[27] Van Lier YF, Van den Brink MRM, Hazenberg MD, Markey KA. The post-hematopoietic cell transplantation microbiome: relationships with transplant outcome and potential therapeutic targets. Haematologica 2021; 106:2042-53

[28] Peled JU, Devlin SM, Staffas A, et al Intestinal microbiota and relapse after hematopoietic-cell transplantation. J Clin Oncol 2017;35:1650-9.

[29] Samet A, Sledzińska A, Krawczyk B, et al. Leukemia and risk of recurrent Escherichia coli bacteremia: genotyping implicates E. coli translocation from the colon to the bloodstream. Eur J Clin Microbiol Infect Dis. 2013;32:1393-400

[30] Innes AJ, Mullish BH, Ghani R, et al. Fecal Microbiota Transplant Mitigates Adverse Outcomes Seen in Patients Colonized With Multidrug-Resistant Organisms Undergoing Allogeneic Hematopoietic Cell Transplantation. Front Cell Infect Microbiol. 2021;11:684659.

[31] Liao C, Taylor BP, Ceccarani C, et al. Compilation of longitudinal microbiota data and hospitalome from hematopoietic cell transplantation patients. Sci Data. 2021;8:71

Recent studies have begun to correlate gut microbiota dysbiosis with outcomes in the autologous HSC transplant settings. Pre-transplant composition of oral and fecal bacteria as well as fungi are linked with post-transplant associated gastrointestinal toxicities and rate of neutrophil engraftment in MM patients.[32] In a multi-center observational study of 534 patients (MM, amyloidosis and lymphoma), greater microbiota diversity during peri-engraftment was associated with longer progression-free survival in recipients of high-dose chemotherapy and autologous HSC transplantation.[33] While this cohort of autologous HSC transplant patients had fewer blood-stream infections compared to allogeneic HSC transplant patients, similar domination of genera such as *Enterococcus* and *Streptococcus* occurred in the first two weeks post-transplant and pre-transplant gut microbiota diversity was lower than healthy volunteers.[31] Consistent with these findings, our interim 16S taxonomic analysis of stool samples serially collected from 25 MM patients who received high-dose melphalan conditioning and autologous HSC transplant at the JTCC observed a significant loss of microbiota diversity during peri-engraftment. Specific genera were associated with increased risk of engraftment syndrome (*Bacteroides*) and relapse within 2 years post-transplant (*Enterococcus, Streptococcus*).

This sampling study will allow us to associated specific taxa with increased risk of BSI, fluoroquinolone resistance, engraftment syndrome and progression of disease.

4 Rationale, Objectives and Hypothesis

4.1 Problem Statement/Research question

Infections with antibiotic-resistant coliform bacteria are an increasing threat to the survival of patients undergoing hematopoietic stem cell transplantation.

4.2 Hypothesis

Avoidance of routine prophylaxis with a fluoroquinolone in patients harboring FRE will reduce the risk of blood stream infection by FRE or MDRO, without increasing the risk of FN.

4.3 Primary Objective

What is the prevalence of FRE in patients undergoing autologous PBSC transplantation with dose-intense melphalan?

4.4 Secondary Objectives

4.4.1 Does the risk of febrile neutropenia differ in FRE carriers compared to non-carriers?

4.4.2 Do FRE carriers have an increased the risk of GNR sepsis compared to non-carriers?

4.4.3 Do FRE carriers experience different grades of GI toxicity after dose-intense melphalan compared to non-carriers?

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[32] El Jurdi N, Filali-Mouhim A, Salem I, et al. Gastrointestinal Microbiome and Mycobiome Changes during Autologous Transplantation for Multiple Myeloma: Results of a Prospective Pilot Study. Biol Blood Marrow Transplant. 2019;25:1511-9.

[33] Khan N, Lindner S, Gomes ALC, et al. Fecal microbiota diversity disruption and clinical outcomes after auto-HCT: a multicenter observational study. Blood 202;137:1527-37

4.4.4 Do FRE carriers experience a longer length of hospital stay compared to non-carriers?

4.4.5 Do FRE carriers experience different engraftment kinetics compared to non-carriers?

4.5 Exploratory Objectives

4.5.1 Are there differences in FRE carriage in different racial/ethnic groups?

4.5.2 How does the use of fluoroquinolone prophylaxis in FRE carriers compared to non-carriers affect the microbiome and immune reconstitution?

4.5.3 Do FRE carriers have a different risk of engraftment syndrome compared to non-carriers?

4.5.4 Do FRE carriers have an increased risk of progression of disease compared to non-carriers?

4.5.5 Do FRE carriers have higher incidence of domination of genera such as *Enterococcus* or *Streptococcus* within the first 2 weeks of transplantation compared to non-carriers?

4.6 Variables/Endpoints

4.6.1 Primary Outcome Variables

4.6.1.1 Detection of FRE prior to initiation of transplant procedures

4.6.2 Secondary Outcome Variables

4.6.2.1 Occurrence of neutropenic fever during the first 28 days (4 weeks) after autologous PBSC transplantation in the treatment of multiple myeloma.

4.6.2.2 Occurrence of blood stream infections during the first 28 days (4 weeks) after autologous PBSC transplantation in the treatment of multiple myeloma.

4.6.3 Exploratory Outcome Variables

4.6.3.1 Self-reported racial/ethnic classification

4.6.3.2 Self-reported social determinants of health

4.6.3.3 Severity (frequency, consistency and volume) of diarrhea for patients undergoing autologous HSC transplantation

4.6.3.4 Use of corticosteroids in the management of non-infectious fever and diarrhea.

4.6.3.5 Use of empiric broad-spectrum antibiotics in the management of FN in patients in the first two weeks of transplant

4.6.3.6 Peripheral blood immune cell counts of patients during conditioning before transplant, peri-engraftment and immune reconstitution

5 Study Design

5.1 General Design

This is a prospective, exploratory study to determine the incidence of FRE colonization for patients with a diagnosis of multiple myeloma undergoing autologous PBSC transplantation. Patients will be tested for the presence of FRE before receiving fluoroquinolone prophylaxis at multiple points during the transplant course, including before chemotherapy mobilization (if used) using fluoroquinolone prophylaxis, at

initial transplant hospitalization, at time of hospital discharge, and at or after day 84 after transplantation (day 0 is defined as day of HSC infusion). FRE colonization will not be a determining factor in the use of fluoroquinolone prophylaxis during the treatment course. Selection of antibiotics for treatment of FN will be by the ID service in consideration of the results of FRE testing (if known at time of transplant admission) and patient history of previous infections.

5.2 Study Duration (if applicable)

We anticipate a 12-month enrollment period with monitoring of subjects for 84 days (twelve weeks) after transplantation. Data will be analyzed over a three-month period (total study period of 18 months).

5.3 Number of Study Sites

This study will be open at two transplant units: Hackensack University Medical Center and MedStar Georgetown University Hospital.

5.4 Study Population

5.4.1 Subjects eligible for this study will meet the defined eligibility criteria to undergo autologous transplantation established at the transplant centers as per standard of care (SOC).

5.4.2 All adult patients with a diagnosis of multiple myeloma undergoing autologous transplantation after dose-intense melphalan conditioning are candidates for enrollment into this study. These subjects will be approached for enrollment into this study by the transplant attending physician caring for the patient.

5.5 Number of Participants:

5.5.1 We anticipate enrollment of subjects over a 12-month time frame. Based on the numbers of patients treated at these two institutions in CY2020, we estimate the numbers of subjects to be enrolled to be:

5.5.1.1 HUMC: **124**

5.5.1.2 MGUH: **20**

5.6 Eligibility Criteria

5.6.1 Inclusion Criteria:

5.6.1.1 Subjects >18 yrs of age.

5.6.1.2 Diagnosis of multiple myeloma undergoing (first or subsequent) autologous PBSC transplantation.

5.6.1.3 Transplant conditioning with melphalan 200 mg/m^2.

5.6.1.4 PBSC cell dose of >2x10e6 CD34+ cells/kg.

5.6.1.5 Able to receive fluoroquinolone prophylaxis.

5.6.1.6 Subjects must give consent for enrollment into this study.

5.6.2 Exclusion Criteria:

5.6.2.1 Unwillingness to provide informed consent.

5.6.2.2 Enrollment into a treatment protocol prescribing antibiotic prophylaxis.

5.6.2.3 Undergoing transplantation for a diagnosis other than multiple myeloma.

5.6.2.4 Receiving a conditioning regimen other than melphalan 200 mg/m^2.

5.6.2.5 Known light-chain amyloid deposition in any organ.

5.7 Withdrawal Criteria

5.7.1 Subjects will be removed from study if they withdraw consent for participation in the study.

5.7.2 Unless consent for analysis is also withdrawn by the subject, subjects who voluntarily withdraw from the study will continue to be monitored until day 84 after transplantation for primary and secondary endpoints.

5.7.3 Subjects who withdraw consent for analysis will have study-related documents destroyed.

5.7.4 Subjects who withdraw from the study will be replaced.

6 Study Procedures

6.1 Subjects will be recruited from among the patient populations undergoing transplantation at Hackensack University Medical Center or MedStar Georgetown University Hospital.

6.2 Subjects will be approached by the transplant physician caring for the patient or designee.

6.3 Informed consent for study enrollment and all associated procedures will be obtained by the transplant physician caring for the subject or designee.

6.4 One perirectal swab for microbiological and molecular analysis, including the determination of fluoroquinolone resistance, will be obtained at the following time points:

6.4.1 At the “apheresis consultation” (or up to day of mobilization administration) if undergoing chemotherapy mobilization with use of fluoroquinolone prophylaxis, and/or

6.4.2 At the “data review consultation” (or up to day of admission prior to initiation of fluoroquinolone prophylaxis) held before transplant admission, all subjects.

6.4.3 On the day of discharge from the transplant unit.

6.4.4 At day 84-120 after transplantation.

6.5 One optional stool sample for microbiome analysis will be obtained at the same time points listed in 6.4 (for patients opting to provide this sample)

6.6 Fresh perirectal swabs and stool samples will be couriered to the laboratories of Barry Kreiswirth, PhD, and Rena Feinman, PhD at CDI, where they will be processed. Samples can be stored under refrigeration for 24hrs or frozen at -80 C for later delivery, if necessary.

6.7 Samples collected at MGUH will be frozen and batched for delivery by a courier service such as FedEx.

6.8 The analysis of the perirectal swab, which will include both microbiological and molecular testing, will determine whether the patient is carrying a fluoroquinolone resistant Enterobacteriaceae and it will identify the presence of carbapenemase and ESBL producing pathogens.

6.9 The results of the fluoroquinolone resistance testing will not be a factor in whether prophylaxis is given or not given, nor in the choice of prophylaxis if FRE is detected.

6.10 Subjects known to carry FRE will be seen in consultation by the infectious disease service at transplant for recommendations regarding antibiotic(s) to be used in the treatment of febrile neutropenia, if it occurs during the inpatient transplant course.

6.11 The stool sample will be banked in the laboratory of Rena Feinman, Ph.D. and used to profile the gut microbiome by 16S rRNA sequencing.

6.12 Isolates of bacteria will be obtained from the microbiology laboratory for patients who develop urinary or blood stream infections during the peri-transplant period (defined as day -1 to day of hospital discharge).

6.13 Standard Transplant Procedures (Table 1)

NOTE: Participation in the study will not alter patients' standard of care (SOC) treatment.

6.13.1 Determination of patient eligibility for transplantation will be by the transplant physician caring for the subject.

6.13.2 Choice of PBSC cell dose will be by the transplant physician caring for the subject.

6.13.3 The mobilization and collection of PBSC will be per the decision of the transplant physician caring for the subject and will be per the SOPs of the transplant program at which the subject is being treated.

6.13.4 Subjects will undergo pre-transplant evaluation in accordance with the SOPs of the transplant program at which the subject is being treated.

6.13.5 Fluoroquinolone prophylaxis will be in given in accordance with the standard practice of the transplant program treating the subject.

6.13.6 Vital signs and temperature will be monitored per the SOPs of the transplant unit in which the subject is located.

6.13.7 For subjects developing fever during the transplant course anti-bacterial prophylaxis will be discontinued and appropriated antibiotics in the treatment of FN will be initiated, in accordance with transplant programs' SOP(s).

6.13.8 The ID service will be consulted for recommendations regarding antibiotics in the treatment of FN for subjects with FRE, if this information is available. The perirectal swab resistance data should be evaluated in making this recommendation.

6.13.9 Blood and urine cultures will be obtained at the onset of neutropenic fever, in accordance with transplant programs' SOP(s).

6.13.10 Other prophylaxis guidelines will be in accordance with transplant program SOPs and may be modified for the needs of the individual subject.

6.13.11 All subjects will receive HSV prophylaxis with valacyclovir or acyclovir in accordance with the transplant program SOPs.

6.13.12 All subjects will receive fungal prophylaxis with fluconazole 400 mg daily until hematological recovery and discharge, or completion of corticosteroid treatment, or as otherwise recommended by the infectious disease consultant.

6.13.13 PCP prophylaxis may be given after hematological recovery as decided by the transplant physician caring for the subject.

Table 1:

Procedure	Apheresis Consultation	Data Review Consultation	Day -1	Day +0	Hospital Discharge	Day +84-120
Study consenting	X					
SDoH questionnaire	X					
Perirectal swab	X[1]	X[2]			X	X
Stool sample (optional)	X[1]	X[2]			X	X
Fluoroquinolone prophylaxis	X[1]		X[3]			
Anti-viral prophylaxis	X[1]		X[3]			
Anti-fungal prophylaxis			X[3]			
Melphalan conditioning			X			
PBSC infusion				X		
Study completion						X

1. Only for subjects undergoing chemotherapy mobilization with use of fluoroquinolone prophylaxis.
2. For all subjects including subjects not undergoing chemotherapy mobilization of PBSC and not receiving pre-transplant fluroquinolone prophylaxis.
3. Fluoroquinolone, anti-viral, anti-fungal prophylaxis regimens will be per the SOP of the transplant center at which the subject is being treated.

6.14 Subject Follow-Up.

6.14.1 Subjects will be followed for survival, transplant-related non-hematological adverse events, and incidence of ES requiring initiation of corticosteroids or other immunosuppressive medications.

6.14.2 Subjects will be followed (for study purposes) for 84 (up to120) days after transplantation.

7 Risks and Benefits

7.1 Benefits:

7.1.1 There is unlikely to be a direct benefit to the subject enrolled into this study.

7.1.2 The information obtained through participation in this study may benefit future patients undergoing autologous PBSC transplantation.

7.2 Risks:

7.2.1 All subjects will be treated in accordance with the SOPs of the transplant program at which they are being treated.

7.2.2 Participation in this study is not likely to increase the medical risks of the transplant regimen.

7.2.3 There is a risk of breach of confidentiality for subjects enrolled into this study.

7.2.4 There is a risk of embarrassment with the collection procedures. Subjects will be allowed to collect their own samples in privacy to minimize this risk.

8 Methods

8.1 One perirectal swab will be obtained at each study endpoint will be provided to the laboratory of Barry Kreiswirth, Ph.D. A stool sample (optional) will be provided to the laboratory of Rena Feinman, Ph.D.

8.2 Identification of FRE will be performed on fresh or frozen samples in the laboratory of Barry Kreiswirth, Ph.D,

8.3 Identification of microbiome will be performed on fresh or frozen samples in the laboratory of Rena Feinman, Ph.D,

8.4 Perirectal swab specimens will be labeled with a subject ID number and specimen number and frozen at -70°C to -80°C at each study site within 24 hours of collection. The swabs will then be shipped on dry ice to the laboratory of Barry Kreiswirth, Ph.D. monthly in batch. Upon receipt, the specimens will be thawed and 300 µL will be thawed and plated for growth as described in 8.7 below. The remaining 700 µL will be transferred into seven 100 µL aliquots prior to re-freezing and stored at -80°C.

8.5 Gram-negative bloodstream or urinary tract isolates (patients who develop infection during the peri-transplant period) will be shipped fresh to the laborataory of Barry Kreiswirth, Ph.D., or stored at -70°C to -80°C at each study site in 2 Microbank cryovials that are labeled with a subject ID number and specimen number.

8.6 Every six months or more frequently one “copy” of the blood stream isolate will be shipped on dry ice to the Kreiswirth laboratory in batch. The other “copy” will be kept by the primary site in case there are any issues with the first “copy” of the isolate.

8.7 Specimen Shipment

8.7.1 As above, perirectal swabs and stool samples will be shipped monthly or more frequently from study sites to the Kreiswirth and Feinman laboratories fresh or frozen on dry ice. Gram-negative bloodstream or urinary tract isolates from study participants will be shipped fresh or frozen on dry ice.

8.8 Laboratory Evaluations/Assays

8.8.1 No samples other than perirectal swabs and stool samples (optional) will be collected from study participants.

8.8.2 Laboratory testing of these swabs will only occur in the Kreiswirth laboratory after shipment of fresh or frozen swabs. Swabs will be tested fresh or thawed and broth enrichment cultures will be performed with selective media to detect FRE and ESBL-E colonization.

8.8.3 FQRE broth enrichment cultures: After vortexing, 100 µL of Amies liquid will be added to 5 mL tryptic soy broth with a 5-µg levofloxacin disk and incubated at 37° overnight. The next day 100 µL of broth will be inoculated onto a MacConkey agar plate that contains 0.5 µg/mL of levofloxacin and incubated overnight.

8.8.4 Colonies present after 24 hours of incubation at 37°C on the MacConkey/levofloxacin agar plate will be subcultured onto a blood agar plate and incubated overnight. These colonies will then be identified by matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Organisms that are identified as a member of the Enterobacterales will then undergoing antimicrobial susceptibility testing by reference broth microdilution.12 All FQRE isolates will be stored at -80°C.

8.8.5 ESBL broth enrichment cultures: After vortexing, 100 µL of Amies liquid will be added to 5 mL tryptic soy broth with a 30-µg cefpodoxime disk and incubated overnight. The next day 100 µL of broth will be inoculated onto a chromogenic ESBL agar plate (Hardy Diagnostics). Colonies present after 24 hours of incubation at 37°C on the Mac Cipro plate will be subcultured onto a blood agar plate and incubated overnight. These colonies will then be identified by MALDI-TOF. Organisms that are identified as a member of the Enterobacterales will then undergoing antimicrobial susceptibility testing by reference broth microdilution using frozen panels.12 Any ceftriaxone-resistant Enterobacterales (CRO-R-E) will undergo phenotypic testing for ESBL production13 and all CRO-R-E isolates will be stored at -80°C.

8.8.6 Positive control culture: After vortexing, 100 µL of Amies liquid will be inoculated directly onto a blood agar plate and incubated overnight at 37°C. Growth of any organism on this plate will be required for the sample to be eligible. No growth on the blood agar plate will render the swab sample invalid.

8.8.7 Microbial DNA will be extracted as specified by the commercially available for fecal samples.

8.9 Special Assays or Procedures

8.9.1 All gram-negative bloodstream or urinary tract isolates from participants will also be shipped (fresh or frozen) to the Kreiswirth laboratory. For participants who develop a BSI or urinary tract infection due to FRE, their colonizing and bloodstream FRE isolates will undergo whole-genome sequencing. This sequencing will be performed using the Illumina NextSeq platform and the number of single nucleotide variant (SNP) differences between colonizing and bloodstream FRE isolates for each patient will be assessed and compared to SNP differences among FRE from different patients. Antimicrobial resistance determinants will also be compared between colonizing and bloodstream strains.

8.9.2 For microbiome profiling studies, batches of serially collected DNA samples from patients will be shipped to a commercial vendor for 16S rRNA sequencing of the V4 amplicon on the Illumina MiSeq platform.

8.9.3 Social Determinants of Health questionnaire will be requested of all subjects at time of study entry. Also collated will be self-reported race/ethnicity and home address.

8.10 Patient Care

8.10.1 Subjects who experience neutropenic fevers will have blood and urine cultures obtained that will be assessed for the presence, identification, and antibiotic sensitivity of bacterial organisms using standard of care techniques established in the microbiology laboratory of the institution at which the transplant is being performed.

8.10.2 Subjects will be monitored from initiation of PBSC mobilization until 84 days after transplantation for:

8.10.2.1 Development of neutropenic fevers.

8.10.2.2 Development of bacteremia/sepsis.

8.10.2.3 Development of urinary tract infection.

8.10.2.4 Development of Clostridium difficile enteritis.

8.10.2.5 Development of viral reactivation or infection.

8.10.2.6 Development of fungal infection.

8.10.2.7 Development and severity of mucositis including:

8.10.2.7.1 Oral mucositis

8.10.2.7.2 Odynophagia

8.10.2.8 Opioid equivalents in the treatment of mucositis will be calculated.

8.10.2.9 Diarrhea (daily quantity).

8.10.2.10 Duration of neutropenia.

8.10.2.11 Onset and severity of ES, and the duration and quantity of steroids if prescribed.

8.10.2.12 Relapse of disease.

8.10.2.13 Survival after transplantation until day +84.

8.11 Statistical Analysis:

8.11.1 The primary objective of this study is to estimate the prevalence of FRE in patients with MM undergoing autologous transplantation, and secondary goals will include an examination of whether having FRE colonization is associated with risk of febrile neutropenia and blood stream infection during the initial transplant course.

8.11.2 Sample Size Consideration

The study plans to enroll 144 subjects with MM who have been treated with fluoroquinolone prophylaxis per SOC. We expect testing to detect FRE will fail for about 10% of subjects and adjusting for this dropout will leave 129 evaluable patients. The sample size calculation for proportion of autologous transplantation recipient with FRE was based on 90% confidence interval. Assuming that ~30% of the subjects are expected to be colonized by FRE, enrolling 144 subjects with 10% dropout rate will yield 7.0% margin of error on the proportion of FRE in

this population. The table below provides 90% CI's using exact probability method (Clopper-Pearson) for a range of possible prevalence rate out of 129 subjects.

Table 2. Observed rate of FRE and 90% Confidence interval out of 129 subjects given FQ prophylaxis

Number of responses (%)	33 (25%)	34 (26%)	35 (27%)	37 (28%)	38 (29%)	39 (30%)
Lower Limit of 90% CI	0.1981	0.2051	0.2121	0.2262	0.2333	0.2404
Upper Limit of 90% CI	0.3236	0.3318	0.3399	0.3562	0.3643	0.3723

8.12 Analysis Plan

8.12.1 Primary Endpoint Analysis

The observed proportion with 90% exact method confidence interval will be used to estimate the prevalence of FRE in MM who have undergone HSCT.

Characteristics of C will be summarized by mean (standard deviation) or median (interquartile range) depending on whether normality assumption of the continuous variables holds, with the validity of this assumption tested by the Shapiro-Wilk test. Categorical variables among the FRE carriers and non-carriers will summarized using frequency (percentages). Comparisons of continuous variables between the FRE carriers and FRE non-carriers will be performed using two-sided t tests or two-sided Wilcoxon rank sum tests, as appropriate. Comparisons of categorical variables between the two groups, FRE carriers an FRE non-carriers, will be conducted using Fisher's exact test or Pearson's Chi-square test, as appropriate. Time to event such as onset of fever, blood stream infection, or engraftment, will be evaluated using Kaplan-Meier method and 95% confidence interval of the median time will be computed and reported. Occurrence of time dependent events will be estimated with cumulative incidence functions and the corresponding 95% confidence interval.

8.12.2 Secondary Endpoint Analysis

8.12.2.1 Occurrence/Risk of Febrile Neutropenia (FN)

The occurrence of FN among the FRE carriers and FRE non-carriers will be estimated by proportion with 95% CI of MM patients undergoing autologous SCT who develop FN within 28 days of the transplantation.

Comparison of FN risk between the FRE carriers and FRE non-carriers will be examined using logistic regression analysis and reporting odds ratio (OR) of FN along with 95% confidence interval. With FRE non-carriers as reference group, OR>1 and 95% CI that exclude 1 would indicate FRE carriers are at a higher risk for FN.

8.12.2.2 Occurrence/Risk of Blood Stream infection (BSI)

The occurrence of Gram-negative BSI and/or urinary tract infections among the FRE carriers and FRE non-carriers will be estimated by proportion with 95% CI of MM patients undergoing autologous SCT who develop FN within 28 days of the transplantation.

Comparison of BSI risk between the FRE carriers and FRE non-carriers will be examined using logistic regression analysis and reporting odds ratio of BSI along with 95% confidence interval.

Alternatively, if this event is anticipated to be of rare occurrence, comparison of risk of BSI will be compared between the FRE carriers and FRE non-carriers using Poisson regression and relative risk ratios with 95% confidence will be reported.

In either case, OR>1 or RR>1, and corresponding 95% CI that exclude 1 would indicate FRE carriers are at higher risk for BSI than FRE non-carriers.

8.12.2.3 Comparison of severity of GI toxicity between FRE carriers and FRE non-carriers will be performed using ordinal logistic regression analysis with outcomes as the ordered categories of the grade of GI toxicity. For this analysis, the proportional odds assumption will be examined before reporting the OR and 95% CI of observing more severe GI vs less severe odds between the FRE carriers and FRE non-carriers.

8.12.2.4 Comparison of Length of Stay (LOS) between FRE carriers and FRE non-carriers will be performed by comparing the KM curves estimating time to discharge using a two-sided log-rank test and the hazard risk of longer LOS and 95% CI will be examined using Cox proportional hazard regression model.

8.12.2.5 Comparison of Engraftment Kinetics (absolute neutrophil count (ANC), absolute lymphocyte count (ALC), platelet) between FRE carriers and FRE non-carriers will be performed by using two-sided two-sample t-tests or Wilcoxon rank sum tests of the Land myeloid cell populations. Longitudinal analysis of engraftment kinetics will be performed using mixed regression analysis with factors FRE carrier status and time.

8.13 Exploratory Endpoint Analysis

8.13.1 Comparison of effect of FRE colonization and fluoroquinolone prophylaxis on the microbiome between FRE carriers and FRE non-carriers will be performed using two-sided t-tests and Wilcoxon rank sum tests with false discovery rate correction of p values.

8.13.1.1 Comparison of time to ES will be estimated by the KM method and risk of ES will be compared between FRE carriers and FRE non-carriers using Cox proportional hazards regression model and report HR and 95% confidence interval.

8.13.1.2 The USEARCH global alignment algorithm will be used to assign operational taxonomic units (OTUs) of ≥ 97% distance-based similarity and annotate OTUs using reference-based methods such as GreenGenes and StrainSelect. Once all OTUs and Kellog Orthologs are assigned, α-diversity, β-diversity, ordination, clustering, PERMANOVA and differential abundance testing will be performed in R software. These analyses will provide information regarding phylogenetic differences in community composition among standard-risk and high-risk patients and taxa distribution per patient are correlated with FRE, blood-stream infections, engraftment syndrome, immune reconstitution and depth of response post-apheresis and post-ASCT (≤1 yr and ≤2 yr median PFS rates). Analyses will also be done to identify shifts in gut microbiota profiles associated with beta lactam exposures in patients that develop FN. Linear Discriminant Analysis Effect Size (LEfSe) analysis, using a logarithmic LDA cutoff of 2.0, and generalized linear mixed-effects modelling will be performed in order to identify taxa associated with engraftment syndrome, outcome and prophylactic and treatment-associated antibiotic exposures prior to transplant and peri-engraftment period. Spearman's rank correlation test and cause-specific Cox proportional hazards multivariable regression models will be performed on the modules produced by the Linear Discriminant Analysis to correlate distinct microbiota with outcome variables. Risk factors such as systemic steroids, prior treatments, obesity, age, gender, race and ethnic disparity will be incorporated as part of our analyses.

8.13.2 Multivariate Analysis.

8.13.2.1 In all the analyses above, in which FRE carriers are being compared with FRE non-carriers, we will perform both univariate and multivariate analyses for the corresponding endpoints. In the patients that tested negative for FRE pre-transplant, changes in the FRE status overtime will be examined to characterize the FRE profiles using generalized estimating equations (GEE) method.

8.13.2.2 General Data analysis

All study data will be imported into SAS or R software. Statistical analysis will be conducted using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA) or R version 4.1.1 or higher (R Foundation for Statistical Computing, Vienna, Austria). Unless otherwise specified, any $P < 0.05$ will be considered statistically significant. Any results yielding $0.05 \leq P < 0.10$ will be considered trending towards statistical significance.

8.14 Stopping Rules

8.14.1 Study accrual will cease if subject enrollment fails to meet expected numbers.

9 Trial Administration

9.1 Ethical Considerations - Institutional Review Board (IRB) Review

9.1.1 The study will be conducted according to the International Conference on Harmonization (ICH), Good Clinical Practice (GCP), the Declaration of Helsinki, Institutional Review Boards (IRB) and in accordance with the U.S. Code of Federal Regulations on Protection of Human Rights (21 CFR 50).

9.2 Data management (storage etc.)

9.2.1 Routine transplant outcome data will be stored in the EMR of the institution at which the subject is being treated.

9.2.2 A research database that includes transplant outcome data and the results of the FRE testing will be established by the research team at HUMC.

9.3 Confidentiality

9.3.1 The subject charts, collected data, and all analysis of the data will adhere to HIPAA and institutional subject confidentiality requirements.

More specifically, a coding system will be used for which a unique identifier (study ID number) will be assigned to each subject name and contact details. Only the study number will be included in the data collection tool, data analysis software and potential publications. The list with the direct identifiers (for the purposes of linking data with the samples and keeping track of subjects) will be stored separately in a secure server at each site.

Analytical datasets will be stored on secure servers that also limit access to the investigator team. Should results of the study be published or reported, individual names or other identifying information will not be used.

9.4 Informed consent

9.4.1 Informed consent will be obtained from each participant prior to entering the study. The informed consent form provides information and explanations of the aims, methods, anticipated benefits and potential risks of the study.

9.4.2 The acquisition of informed consent will be documented in the participant's record and the informed consent form will be signed and personally dated by the participant and by the person who conducted the informed consent discussion. The original signed form will be retained in the Investigator Site File and a copy of the original will be provided to the subject prior to participation in the study.

9.4.3 The participant will be informed that they may withdraw from the study at any time without prejudice and compilation of that person's data will cease as of the date of his/her written request for withdrawal.

9.5 Data Quality Assurance

9.5.1 The database is subject to validation for quality purposes by the research department of the institutions at which this study is conducted.

9.6 Study Records (retention etc.)

Records will be retained in accordance with regulatory, organizational and sponsor requirements, but for no less than six (6) years following the completion of the research. Disposal of records will be done in such a manner that no identifying information can be linked to research data.

9.7 Credentials, Training

The principal investigator, sub-investigator and all study team member have completed their CITI training as per institutional policies. The principal investigator, sub-investigators, and all study team members are experienced in the care of the neutropenic patient undergoing HSC transplantation or leukemia remission induction.

9.8 Financing and Insurance (if applicable)

9.9 Publication Plan (if applicable)

9.9.1 Results from this study will be presented internally and will also be published in peer-reviewed journals such as Blood or Biology of Blood and Marrow Transplantation.

9.9.2 No individual names or other identifying information will be used in potential reports or publications.

10 Appendices

Appendix #	Name	Title	Section	Topic

11 References