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Fecal Microbiota Transplantation from Overweight or Obese Donors in Cachectic Patients with Advanced Gastroesophageal Cancer: A Randomized, Double-blind, Placebo-controlled, Phase II Study

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TRANSLATIONAL RELEVANCE

In the randomized phase II TRANSIT study we assessed the effect of allogenic fecal microbiota transplantation (FMT) from obese donors on cachexia in patients with HER2 negative advanced gastroesophageal cancer scheduled to receive first line chemotherapy. There was no difference between the autologous (control arm) and allogenic FMT on any cachexia parameter. However, in the allogenic group we observed better disease control rate and a numerical improvement in survival. Based on translational microbiome analyses engraftment of allogenic donor transplant was observed. We were not able to link the microbiome to cachexia as our intervention did not alter cachexia. Exploratory analyses linking the microbiome to response or survival did not reveal any difference in bacterial strains between responders and non-responders. Our trial provides a rationale for larger FMT trials to unravel the mechanistic biology behind chemotherapy response and microbiome modulation. Future translational studies may include more in depth analyses of the microbiome such as multi-kingdom profiling and evaluate the interaction between the immune-system and tumor biology.

ABSTRACT

Purpose: Cachexia is a multifactorial syndrome, associated with poor survival in cancer patients and is influenced by the gut microbiota. We investigated the effects of fecal microbiota transplantation (FMT) on cachexia and treatment response in patients with advanced gastroesophageal cancer (GEC).

Experimental design: In a double-blind randomized placebo-controlled trial performed in the Amsterdam University Medical Center, we assigned 24 cachectic patients with metastatic HER2-negative GEC to either allogenic FMT (healthy obese donor) or autologous FMT, prior to palliative chemotherapy (capecitabine and oxaliplatin). Primary objective was to assess the effect of allogenic FMT on satiety. Secondary outcomes were other features of cachexia, along with disease control rate (DCR), overall survival (OS), progression-free survival (PFS), and toxicity. Finally, exploratory analyses were performed on the effect of FMT on gut microbiota composition (metagenomic sequencing) and metabolites (untargeted metabolomics).

Results: Allogenic FMT did not improve any of the cachexia outcomes. Patients in the allogenic group (n=12) had a higher DCR at 12 weeks (p=0.035) compared to the autologous group (n=12), longer median OS of 365 vs 227 days, HR=0.38 (0.14-1.05; p=0.057) and PFS of 204 vs. 93 days, HR=0.50 (0.21-1.20; p=0.092). Patients in the allogenic group showed a significant shift in fecal microbiota composition after FMT (p=0.010) indicating proper engraftment of the donor microbiota.

Conclusions: FMT from a healthy obese donor prior to first-line chemotherapy did not affect cachexia, but may have improved response and survival in patients with metastatic GEC. These results provide a rational for larger FMT trials.

INTRODUCTION

Cachexia is associated with reduced tolerance to anticancer therapy and decreased survival.(1-4) The definition of this multifactorial syndrome includes the ongoing loss of (skeletal) muscle mass (with or without fat mass loss), which cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment.(5) The pathophysiology of cancer cachexia can be divided into four major domains: reduced dietary intake, elevated catabolism, a reduction in storage capacity (fat and muscle loss) and a deterioration in performance status.(6-8) Patients with gastroesophageal cancer (GEC) are particularly affected by the intake domain due to mechanical and digestive problems, leading to loss of appetite and early satiety.(9) Currently applied multimodal treatment interventions for cachexia are based on nutritional support and appetite stimulation.(9) However, these interventions often lack efficacy in counteracting cachexia and have no effect on survival in patients with GEC.

In recent years, it has become evident that the intestinal microbes, the so called gut microbiota, play a crucial role in regulating different aspects of cancer cachexia, including satiety and appetite regulation(10-12), host metabolism(13,14) and systemic inflammation.(15) This is mainly through circulating bacterial components and their metabolites interacting with different organ systems(12) (**Figure 1**). Both cancer and most anti-cancer treatments are able to directly or indirectly alter the gut barrier function. This can lead to an imbalance in the composition of the gut microbiota.(16) In turn this affects the pathways involved in the pathophysiology of cancer cachexia, including satiety(12), which alters eating behavior and host metabolism (**Figure 1**).(14) For example, in a cancer cachexia mice model, oral administration of specific *Lactobacillus spp.* partly restored the gut microbiota composition, reduced cachexia parameters and prolonged survival.(17)

Furthermore, the gut microbiota has also been implicated in modulating the response and toxicity to several classes of anti-cancer agents through immunomodulation and host metabolism(18) (**Figure 1**). Translational studies found a link between several different microbial species and the response to checkpoint-inhibitors.(19,20) Also, the effect of platinum agents seems to be partly driven by microbiome related attenuation of the tumor microenvironment.(21) In line with these findings, manipulation of the gut microbiota could potentially overcome the alterations in the metabolic

pathways and subsequently offset cancer related cachexia while at the same time serve as a means for improving clinical efficacy of currently used cancer therapy.

Several interventions are now being investigated to modulate gut microbiota composition in humans. One of these strategies is fecal microbiota transplantation (FMT), i.e. the administration of feces through a naso-duodenal tube from a healthy donor in the gut of a patient to treat disorders associated with gut microbiota aberrations. This concept has been proven to be safe and effective for patients with recurrent *Clostridioides difficile* (formerly *Clostridium difficile)* infections and has become the treatment of choice when resistance occurs to antibiotic treatment.(22) Also, human studies have revealed that metabolic traits are transmissible via FMT, including feeding behavior(23,24), glucose metabolism(25-27) and most notably body composition.(28)

To improve cachexia in patients with GEC, we conducted a randomized double-blind placebo-controlled pilot trial investigating the effect of allogenic FMT from healthy obese donors versus autologous FMT. We hypothesized that an allogenic FMT from an obese donor would reduce early satiety, improve metabolism and body composition. Primarily, to test this hypothesis we assessed cachexia-related parameters. Secondarily, we evaluated the efficacy of chemotherapy and survival in both groups. Exploratory mechanistic analyses were performed based on intestinal microbiota and plasma metabolite composition before and after FMT.

METHODS

Study Design and participants

We performed a double-blind randomized controlled trial with patients recruited at the Amsterdam UMC (Dutch Trial Register; NL5829). Eligible patients were men and women older than 18 years, with histologically-proven inoperable HER2-negative locally advanced or metastatic esophageal, gastric, or gastro-esophageal junction adenocarcinoma, who were scheduled to receive first-line chemotherapy in a three-weekly schedule: oral capecitabine 1000 mg/m^2 twice per day 1-14 and intravenous oxaliplatin 130 mg/m^2 day 1. Patients had to meet the criteria for cachexia: weight loss >5% over past 6 months (in absence of simple starvation); or BMI <20 and any degree of weight loss >2%; or (CT-scan based) appendicular skeletal muscle index consistent with sarcopenia (males <7.26 kg/m2; females <5.45 kg/m2) and any degree of weight loss >2%.[5] Additional eligibility criteria included a performance status score of 0, 1, or 2 according to the guidelines of the Eastern

Cooperative Oncology Group (ECOG).(29) Patients with an ECOG performance status higher than 2 were excluded as these patients are not eligible for treatment with chemotherapy. Finally, patients should be using a proton pump inhibitor (PPI), since the use of a PPI is common in patients with GEC, and can have a major influence on the gut microbiome composition.(30) Patients with non-cancer related gastrointestinal symptoms such as chronic nausea, altered taste sensation or swallowing difficulties were excluded due to their potential influence on the primary endpoint. Patients with a mechanical obstruction impairing the endoscopic placement of a naso-duodenal tube were also excluded.

In order to be eligible as a feces donor, subjects had to be older than 18 years of age with a BMI >25 kg/m^2 (overweight or obese), without any known underlying disease or use of medication and no signs of insulin resistance and/or metabolic syndrome(31), since FMT using metabolic syndrome donors adversely affects metabolism in humans(26), whereas healthy overweight or obese donor FMT improves bodyweight in human subjects with underweight.(28,31) A detailed description of patient and donor selection is available in the Data Supplement.

This trial was approved by the medical ethical review committee of the Amsterdam Medical Center (AMC) Amsterdam and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. An independent Data Safety Monitoring board (DSMB) was assigned to safeguard the interests of the participants, assess the safety and efficacy of the FMT during the trial, and monitor the overall conduct of the study. All patients provided written, informed voluntary consent. Every author had access to the study data and reviewed and approved the final manuscript.

Randomization and masking

In this double-blind randomized controlled trial patients were randomly assigned (1:1) to either receive allogenic (donor) (group A) or autologous FMT (group B) using computer-generated randomization. FMT donors and recipients were matched for sex.

Procedures

There were three study visits: the first visit (V1) was one week before start of chemotherapy (baseline, including FMT), week 4 (V2) and week 12 (V3) (**Supplemental Figure 1**). At every study visit, patients provided fresh morning fecal samples and completed the VAS questionnaires.

Furthermore, cachexia parameters were measured (BMI, REE, BIA) and fasting blood samples were drawn. Also, patients completed questionnaires regarding nutritional intake three days prior to each study visit. Response evaluation and level of sarcopenia was done by CT at baseline and after 3 cycles of chemotherapy. Adverse events (graded with Common Terminology Criteria for Adverse Events version 4.03) and performance score (graded with Eastern Cooperative Oncology Group) were monitored during each study visit.

Fecal microbiota transplantation was performed as previously described by de Groot and colleagues (**Figure 2**).(26) Briefly, on the day of fecal infusion, both donor and recipients delivered a fresh fecal sample (produced within 6 hours before use). After randomization, the feces were mixed until fully homogenized. This fecal solution was then filtered to remove food derived debris. The filtrate was transferred to a 1000-mL sterile bottle and stored at room temperature (17 ºC). Before and after fecal processing, samples were taken to study procedural effects on microbial composition.

To remove endogenous fecal contamination, patients first underwent bowel lavage with polyethylene glycol solution (Klean-Prep, Norgine BV, Amsterdam the Netherlands) through a naso-duodenal tube, followed by infusion of the gut microbiota solution in approximately 30 minutes (**Figure 2)**. Remaining study procedures are described in the Supplement methods section.

Outcomes

The primary outcome was to assess the effect of allogenic FMT on satiety after 4 weeks, determined by visual analogue scale (VAS) questionnaires (**Supplementary Methods**). A high VAS score (>5) indicates an increased feeling of satiety. To examine additional domains of cachexia, secondary outcomes included validated questionnaires to determine intake (mini nutritional assessment (MNA), dysphagia using Atkinsons-scale, VAS appetite). Also, in fasting plasma samples, gastrointestinal hormones involved in appetite regulation (ghrelin and leptin), low grade inflammation (C-reactive protein, IL-6, TGF-β activated and latent, adiponectin and MIC-1) were measured. In addition, resting energy expenditure (REE), BMI as well as muscle and fat mass measured using CT-scans and bioelectrical impedance analysis (for body composition) were determined at baseline and 12 weeks and finally, performance status (Eastern Cooperative Oncology Group, ECOG, performance score).

Secondary oncological outcomes included: disease control rate (DCR) within 3 months of enrollment by RECIST-criteria version 1.1, overall survival (OS), progression-free survival (PFS), and chemotherapy toxicity (graded with the Common Terminology Criteria for Adverse Events version 4.03). Responders (R) to chemotherapy were defined as patients with stable disease (SD) or partial response (PR); Non-Responders (NR) as patients with progression by the RECIST-criteria. OS was defined as time from randomization to death. PFS was defined as time from randomization until disease progression or death from any cause, whichever occurred first. The cut-off for follow-up was 1 year from randomization. The analysis for toxicity comprised all patients who received the intervention (FMT). To explore potential microbial-metabolite pathways, involved in cachectic and oncological outcomes, gut microbiota composition (as determined by shotgun sequencing performed by Clinical Microbiomics with Illumina Novaseq 6000) and fasting plasma metabolites (Metabolon, Durham, USA) were measured at all timepoints. Extensive description of methods is available in the Data Supplement.

Statistical Analysis

We based our sample size calculation on the satiety VAS-questionnaire results of the FATLOSE1 study (healthy lean donor fecal infusions in metabolic syndrome patients).(25) We calculated that with a mean 15mm (SD 10mm) decrease in VAS score upon an allogenic lean donor FMT versus a 5mm increase upon autologous FMT based on a two-sided alpha of 0.05 and 80% power, we needed 16 subjects in total. Subjects withdrawing for medical reasons (including antibiotic treatment) or death during the study period were replaced by new subjects. Our study was not powered to detect a difference in other cachexia or oncological outcomes.

All analyses regarding cachexia and oncological outcomes were performed in the intention-to-treat population. For the microbiome analyses, one patient was removed, due to a failed FMT. Comparisons between the two intervention groups (unpaired) were performed using the Mann–Whitney *U* or Chi-square test unless otherwise stated. Statistical comparisons between (paired) visits (V1, V2 and/or V3) were performed using the Wilcoxon Signed Rank test. The tests were performed two sided with a P value < 0.05 considered statistically significant.

OS and PFS were calculated using the Kaplan-Meier method, hazard ratios with the use of the Cox proportional-hazards model, and testing for statistical significance using the Breslow-Wilcoxon test.

To evaluate the effect of allogenic and autologous FMT on the overall composition of the gut microbiota, multilevel Principal Component Analysis (PCA) was performed on center log-ratio transformed species-level microbial composition using the mixOmics (v6.12.0) R package, removing between-individual variance and decomposing only within-individual variance. Significance was tested using MANOVA on the first 10 principal components and comparing the F statistic with 1000 permutations where time was shuffled within a subject and FMT allocation among subjects.

To calculate the difference in microbiome composition between a patient and its corresponding donor, binary Jaccard index was used (e.g method to evaluate the resembles between donor and recipient). Plots were constructed using the ggplot2 (v3.3.0) and ggpubr (v0.3.0) packages. Alpha (Shannon and species richness) and beta-diversity (Bray-Curtis) metrics were calculated in R (v4.0) using the vegan R package (v2.5.6). Non-parametric tests were used to assess correlations (Spearmans's rho). The Benjamini-Hochberg procedure was used to correct p-values for multiple comparisons.

The XGBoost (v. 0.90) implementation of gradient boosted trees was used in prediction models for response structured in a nested-cross validation system to prevent overfitting and ensure robustness of results (**Supplementary Methods**).

RESULTS

Patients Characteristics

Between August 2016 and January 2019, 24 patients were enrolled and randomly assigned to receive allogenic FMT (n=12) or autologous FMT (n=12) (**Figure 3**). One patient did not undergo an FMT due to severe constipation and seven subjects were replaced by new subjects because of antibiotic use (n=3), death (n=3), or withdrawal from chemotherapy after two doses (n=1) during the 12 weeks of study. All randomized patients, including the aforementioned, were included in the intention to treat analysis for response and survival (N=24). Patient demographics and baseline disease characteristics are listed in **Table 1**. The majority of patients were male (92% in the allogenic group and 67% in the autologous group) with a median age of 65 years (39-73) in the allogenic group and 62

years (51-78) in the autologous group, all patients had metastatic disease (**Table 1**). In the allogenic group there were two patients with gastric cancer (17%) vs. one in the autologous group (8%), three patients who received previous gastroesophageal cancer related surgery (25%) vs. five (42%), three patients with grade 2-3 dysphagia (25%) vs. eight (67%) and eight patients with two or more metastatic sites (67%) vs. five (42%) in the autologous group. Median time from randomization to FMT was 2 days (IQR 1-4) in the autologous group and 4 days (IQR 1-8) in the allogenic group.

We enrolled four healthy overweight (n=1) or obese donors (n=3) with a median BMI of 30 kg/m2 (26-33). Donor baseline characteristics are depicted in **Supplemental Table 1**.

Effect of FMT on Cachexia Outcomes

There was no significant difference in satiety levels (VAS questionnaire) at week 4 between the autologous group (mean=4.25, 95% CI= 1.63-5.96) and the allogenic group (mean=4.71, CI= 2.03-6.47) (p=0.663). In line with this finding there was also no apparent change in caloric intake between baseline and week 4 in both groups **(Supplemental Table 2)**. Moreover, there was no statistically significant difference in change in any other measure related to cachexia between both groups (**Supplemental Table 3**). Important to note, patients in the autologous group had a significantly higher level of dysphagia (p=0.018) but not of satiety (p=0.557) at baseline compared to the allogenic group **(Supplemental Table 3)**.

Effect of FMT on Adherence to Chemotherapy and Toxicity

Eighteen out of 24 patients completed the first three cycles of CAPOX without dose modifications (completion rate, 75%); there was no difference in completion rate between the autologous and allogenic group (p=0.336). One or more doses of capecitabine and/or oxaliplatin were omitted in six patients because of grade ≥2 neuropathy (n=5), grade ≥3 nausea and/or vomiting (n=1). Three patients in the autologous group died before end-of-study due to progression of disease. The incidence of common adverse events (AEs) associated with CAPOX (nausea/vomiting, anorexia, neuropathy) was similar between both groups. (**Supplemental Table 4**).

Effect of FMT on Response and Survival

There were no complete responders in either group after three cycles of CAPOX. In the allogenic group seven patients had a PR (58%), three SD (25%), and two had disease progression (17%). In the autologous group four patients had a PR (33%), one SD (8%), and seven had disease progression (58%). Exploratory analysis of response revealed in the allogenic group a higher DCR 83% compared to the autologous group 42%, p=0.035 **(Figure 4A)**. Median OS was 365 days and 227 days in the allogenic and autologous group, respectively (HR=0.38, 95%CI=0.14-1.05; p=0.057; **Figure 4B**). Median PFS was 204 days in the allogenic group and 93 days in the autologous arm (HR=0.50, 95% CI=0.21-1.20; p=0.092; **Figure 4C**). Per protocol analyses (without failed FMT; N=23) showed comparative results (**Supplemental Figure 2**).

In the autologous group, three out of 12 patients (25%) needed treatment with antibiotics during the first cycle of chemotherapy, while none of the patients in the allogenic group received antibiotics. To explore the effect of antibiotics on oncological outcomes, we performed a sensitivity analysis by omitting patients who received antibiotics; patients in the allogenic group (n=12) had a DCR of 83% vs. 56% (p=0.16) in the autologous FMT group (n=9), median OS was 365 days vs 158 days (HR=0.29, 95%CI=0.09-0.92; p=0.035) and median PFS was 204 vs 89 days (HR=0.52, 95% CI=0.20-1.37; p=0.124) for the allogenic and autologous group, respectively.

Effect of FMT on gut microbiota composition

Next, we analyzed the effect of FMT on the gut microbiota composition in the two groups (n=23), only excluding one participant who did not receive autologous FMT due to constipation. Reassuringly, there was a significant decrease in the binary Jaccard index between baseline (V1) and 4 weeks (V2) in the allogenic group (p=0.01), which was not present in the autologous FMT group (**Figure 5A)**. Secondly, there was a clear engraftment of donor species after FMT, defined as species being present in the donor, absent in recipient prior to FMT and present after FMT (**Supplemental Figure 3**). Thus, the microbiome composition from the allogenic recipients resembled the donor microbiome more closely after the FMT compared to baseline. To further explore the impact of FMT on the overall community composition of the gut microbiota, we performed a multilevel PCA, examining within-individual variation in microbiota composition (i.e. pre-FMT baseline microbial composition compared to microbiota composition observed at 4 weeks and 12 weeks post-FMT). There was a clear shift after FMT in the allogenic group, while no such shift could be detected in the autologous group

(**Figure 5B**). To extend our understanding of the physiological mechanism of the improved DCR in the allogenic FMT group, we aimed to identify specific bacterial species and/or bacterial communities that where enriched or deprived in the allogenic group compared to the autologous FMT group. However, no significant differences were found in any of the alpha-diversity measures (Shannon index and species richness, **Supplemental Figure 4**) between patients who received allogenic or autologous FMT at any of the three visits. Moreover, no significant relation between gut microbiota diversity (both alpha and beta diversity) and DCR, OS or PFS were observed (**Supplemental Figure 5)**. Also, no individual species or groups of functionally related species were found to be associated with DCR, OS or PFS at 4 or 12 weeks following allogenic FMT (**Supplemental Figure 6-7**). A machine learning model for DCR based on the feces sample obtained in week 4 also showed no predictive value (AUC: 0.52; top 15 microbes **Supplemental Figure 8**).

Effect of FMT on Plasma Metabolites

To further elucidate potential metabolic mechanisms explaining the beneficial effect of allogenic FMT, we explored the change of plasma metabolites after FMT. We found a significant effect of chemotherapy on the plasma metabolome, visible as a marked shift in the multilevel PCA plot between baseline, week 4 and week 12 (**Supplemental Figure 9**). However, there was no clear difference in change between the two intervention groups. In line, we observed no specific plasma metabolite that was significantly different between both intervention groups and no association with DCR following allogenic donor FMT. The model was a poor predictor of DCR based on the plasma sample drawn in week 4 (AUC: 0.49) or week 12 (AUC: 0.60; top 15 metabolites of both models **Supplemental Figure 10 and 11**)

DISCUSSION

To our knowledge, this is the first randomized controlled trial of donor FMT derived from healthy obese donors prior to first-line palliative chemotherapy in patients with advanced GEC. Several conclusions can be drawn from this pilot study. First, allogenic FMT did not improve satiety or cachexia related parameters. However, based on exploratory efficacy analyses we observed better DCR in the allogenic group and higher median survival (OS and PFS). Second, we observed a significant and prolonged shift in gut microbiota composition up to 12 weeks in the allogenic group

after FMT (confirming that allogenic transplantation was sustainable, despite treatment with chemotherapy). We could not identify specific intestinal bacterial species that were associated with oncological outcomes in the allogenic donor group. This may have been due to the limited sample size combined with the multidimensional effects of transferring an entire microbial ecosystem on the gut microbiota composition and functionality.

For advanced GEC, response to first-line palliative chemotherapy is heterogeneous and survival rates are still poor, with a 5-year survival of less than 20%.(32-35) The initial hypothesis was to modulate the microbiome through FMT from a healthy (non-insulin resistant) obese donor in an attempt to counteract cancer cachexia and consequently improve therapeutic response. In contrast to the hypothesis, we did not observe any statistically significant change in any of the cachexia related parameters in the allogenic donor FMT group. This could be due to several factors including: 1. The patients suffered from refractory cachexia and therefore no robust intervention could have altered their metabolic state; or 2. other factors apart from low grade systemic inflammation and therapy-related side effects had a larger impact on cachexia than the donor FMT. Moreover, several baseline characteristics might have affected the primary endpoint including the distribution between both groups of dysphagia and previous cancer related surgery. It is important to note that we based our hypothesis on previous studies, indicating that cachexia was associated with decreased PFS, OS and increased toxicity in various cancer types.(1,3) However, results from a recently published study addressing the relationship between survival and cachexia in patients with advanced GEC suggested that response to chemotherapy and survival in advanced GEC depends on factors beyond cachexia.(36)

Despite the fact that we did not find any effect of the intervention on cachectic features, the results from our exploratory analyses for response and survival favored the allogenic group. Survival in the allogenic group was also higher compared to historical data in advanced GEC patients treated with CAPOX or doublet chemotherapy.(37,38) In this regard, the observed improvement in both DCR and PFS suggests that (repetitive) treatment with obese donor FMT could benefit patients with advanced GEC. Importantly, the incidence of common adverse events (AEs) associated with CAPOX (nausea/vomiting, anorexia, neuropathy) was similar between both groups.

The gut microbiota can directly and indirectly influence the pharmacological effects of chemotherapy through several mechanisms, including immunomodulation and metabolism.(18,21,39)

To extend our understanding of the role of the gut microbiome and its association with the favorable oncological outcomes in the allogenic group, we investigated a potential link between the intervention, inflammation and plasma metabolites. However, no difference in pro-inflammatory cytokines known to be related to tumor progression, nor specific metabolites that could potentially explain the difference in response between the two groups, were found.

Some limitations need to be acknowledged. First, our primary endpoint was satiety, which is usually altered in patients with GEC, leading to inadequate intake and in some cases cachexia. However, it is a subjective outcome measure and not always related to cachexia. Therefore, we assessed other cachexia related parameters including: body composition, cytokines and intake which did not show any difference between the intervention and placebo group.

Second, our study was not powered to detect a difference in response rate or survival. However, despite potentially being underpowered, a numerically higher median survival in the allogenic group was observed, which warrants further investigation in a larger phase II trial.

Third, even though microbiome analyses revealed a significant shift in microbiome composition after allogenic FMT, we did not identify a specific microbe or group of microbes mediating the beneficial oncological outcomes of the allogenic group. In this regard, it is important to stress that bacteria are not the only microorganisms present in the gut, but rather coexist alongside with fungi, unicellular parasites, and phages, which were not investigated in this study. Therefore, multi-kingdom profiling (e.g. viruses, phages, parasites etc) is essential to exclude that other components of the gut microbiota, comprising > 60% of the feces, might explain the beneficial effects of obese donor FMT on response and survival.(40) Moreover, the type and abundance of proteins and metabolites produced by the gut microbiota will not only depend on its composition, but also on the ecological networks formed between members of the microbial community as well as on host-microbe interactions (for example, co-metabolites).(41)

Fourth, in this study patients in the autologous group received an FMT from their own feces, though studies have shown that autologous FMT can also change host metabolism.(26) Future studies could alternatively subdivide the subjects in chemotherapy treatment with or without FMT.

Finally, the beneficial oncological outcomes in the allogenic group might be caused by modulation of the host innate and adaptive immune-system.(42) In our study we did not perform

extensive analyses on the tumor immune microenvironment or different immune-cell subtypes in the systemic circulation.

In conclusion, this hypothesis generating study suggests that healthy obese donor FMT was not able to alter cachexia in patients with advanced GEC through the manipulation of the gut microbiota. Based on secondary efficacy analyses, chemotherapy response and survival seemed to favor the allogenic intervention group. However, larger studies in humans are essential to replicate these findings and address the link between the gut microbiota composition and innate/adaptive immunity in relation to chemotherapy response. Ultimately, this could lead to the development of personalized treatment modalities, such as subject specific microbiome based pre- and probiotics enhancing the efficacy of anti-cancer agents.

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TABLES

Table 1. Baseline characteristics (n=24)

Characteristic	Allogenic (N = 12)	Autologous (N = 12)
Age - years		
Median	65	62
Range	39-73	51-78
Sex		
Male	11 (92)	8 (67)
Female	1 (8)	4 (33)
Subsite of tumor		
Esophagus	9 (75)	10 (83)
Gastroesophageal junction	1 (8)	1 (8)
Stomach	2 (17)	1 (8)
Histology		
Adenocarcinoma	11 (92)	10 (83)
Squamous-cell carcinoma	1 (8)	2 (17)
Extent of disease		
Metastatic	12 (100)	12 (100)
No. of metastatic sites		
1	4 (33)	7 (58)
≥2	8 (67)	5 (42)
Previous surgery		
Yes	3 (25)	5 (42)
No	9 (75)	7 (58)
Previous cytostatic therapy		
Yes	7 (58)	5 (42)
No	5 (42)	7 (58)
ECOG performance-status score		
0 or 1	10 (83)	9 (75)
2	2 (17)	3 (25)
Dysphagia (grade)		
0 or 1	9 (75)	4 (33)
2 or 3	3 (25)	8 (67)
Enteral feeding		

Yes	1 (8)	1 (8)
No	11 (92)	11 (92)

Data shown are for the intention-to-treat population. Parenthesis indicate the percentage of patients. Eastern Cooperative Oncology Group (ECOG) performance-status score ranges from 0 to 4, with 0 indicating fully active and higher scores indicating greater restrictions in physical activities.

FIGURE LEGENDS

Figure 1. Role of the gut microbiota in cancer cachexia and anti-tumor response.

The gut microbiota influences important physiological functions, including host metabolism and immunity through microbiota-derived metabolites. Both cancer and anti-cancer therapy disturb the gut microbiota composition, resulting in intestinal dysbiosis and gut barrier dysfunction. In turn, microbiota-derived metabolites are affected, leading to dysregulation in metabolic and immunological pathways, including appetite and satiety (gastrointestinal hormones) and systemic inflammation (such as CRP, IL-6, TGF-β and adiponectin). Systemic inflammation is the main driver leading to four domains associated with cancer cachexia: reduced intake, elevated catabolism, reduced storage and decreased performance. As for anti-tumor response, the gut microbiota affects the tumor micro-environment through several mechanisms, including host metabolism and immunomodulation (IL-1, IL-6, TGF-β and T cell response). CRP, C-related protein; IL, Interleukin; MIC1, Macrophage inhibitory cytokine-1; TGF-β, Transforming growth factor β.

Figure 2. Fecal microbiota transplantation procedure

Figure 3. Enrollment flow chart

Figure 4. Disease Control Rate (A) Overall survival (B) and Progression-free survival (C), Intention-to-treat analysis

(A) Diseases Control Rate (DCR): allogenic versus autologous FMT (p=0.035).

(B) Kaplan-Meier estimates of overall 1-year survival and (C) progression-free survival (PFS) in patients randomized for allogenic (blue) or autologous (red) FMT.

R= Responder (stable disease or partial response), NR= Non-responder (progression).

Figure 5. Effect of FMT on gut microbiota composition

5A. Jaccard distance in Allogenic group. The Jaccard distance measures *dis*similarity between sample sets (in a binary manner: presence or absence). The larger the Jaccard distance the more the gut microbiota composition of recipients differ from the donor. The smaller the distance, the more similar. There is a significant decrease in Jaccard distance (e.g. increase in similarity) at week 4 and week 12 versus baseline. Thus, the gut microbiota of patients receiving allogenic FMT becomes more similar to the donor gut microbiota composition.

5B. 'Multilevel' PCA: Only within-individual variance is depicted. Allogenic subjects (left) show a shift in microbiome composition after FMT; autologous subjects show no visible shift.

Figure 1.

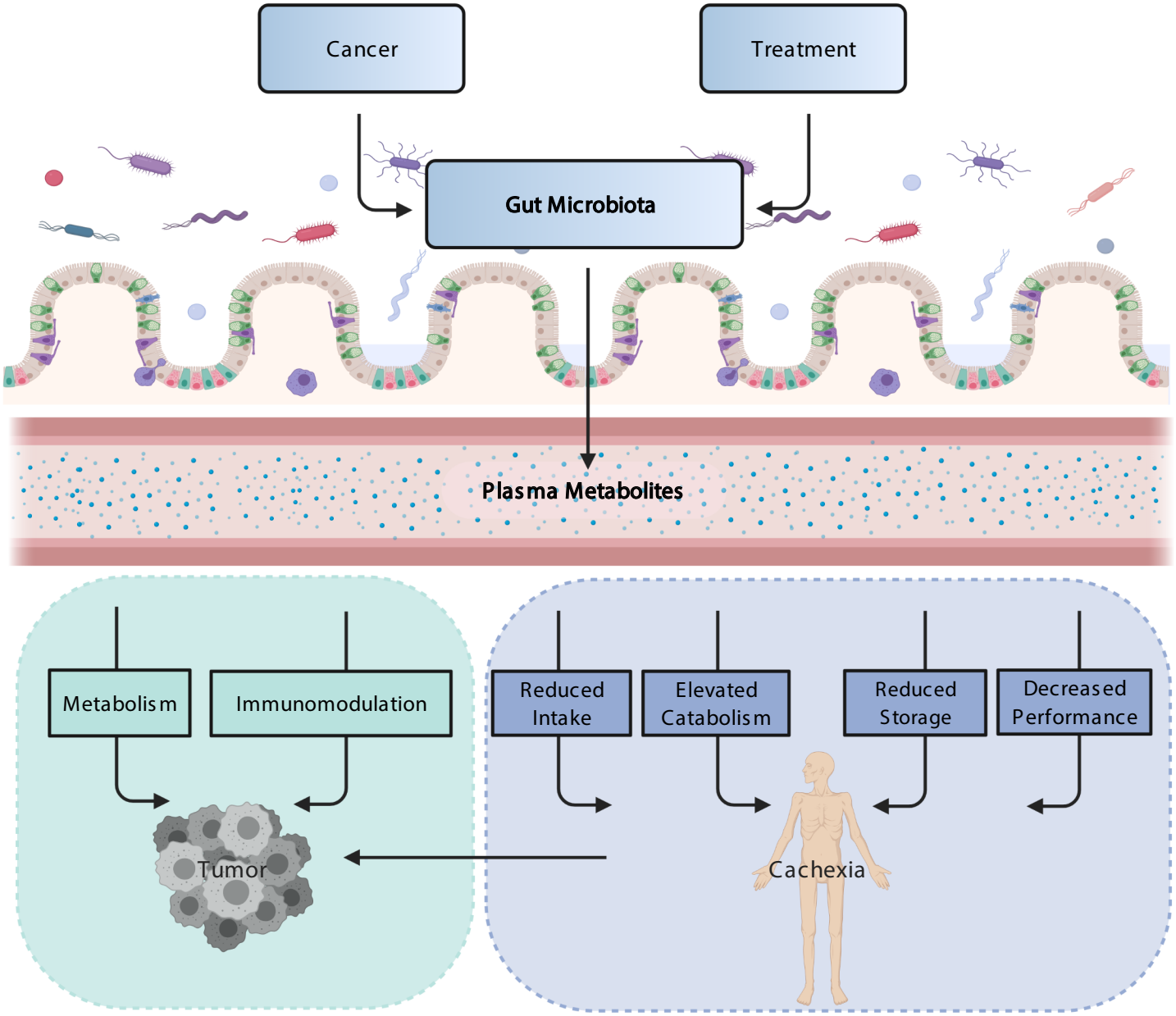
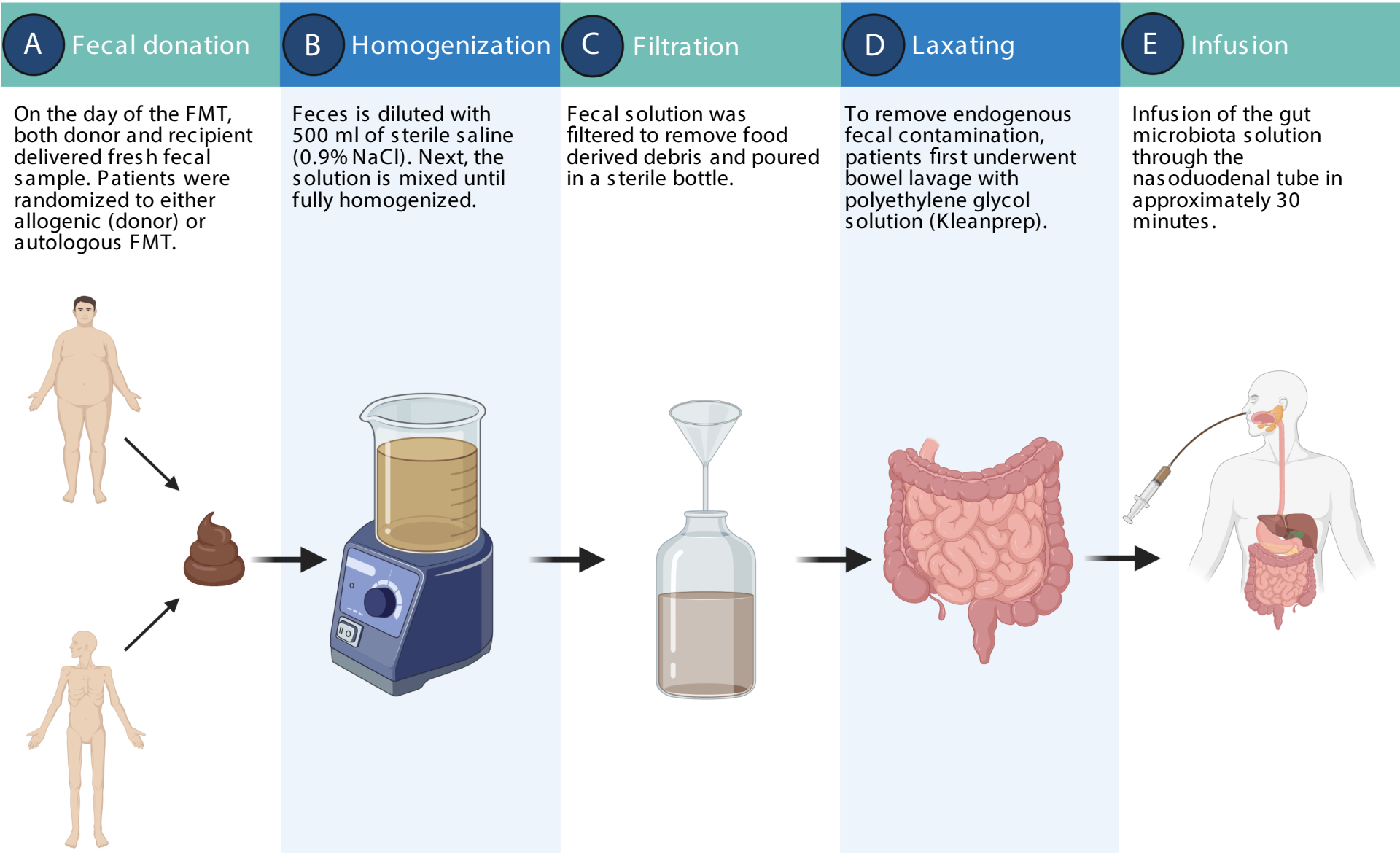


Figure 2.



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Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

Figure 3.

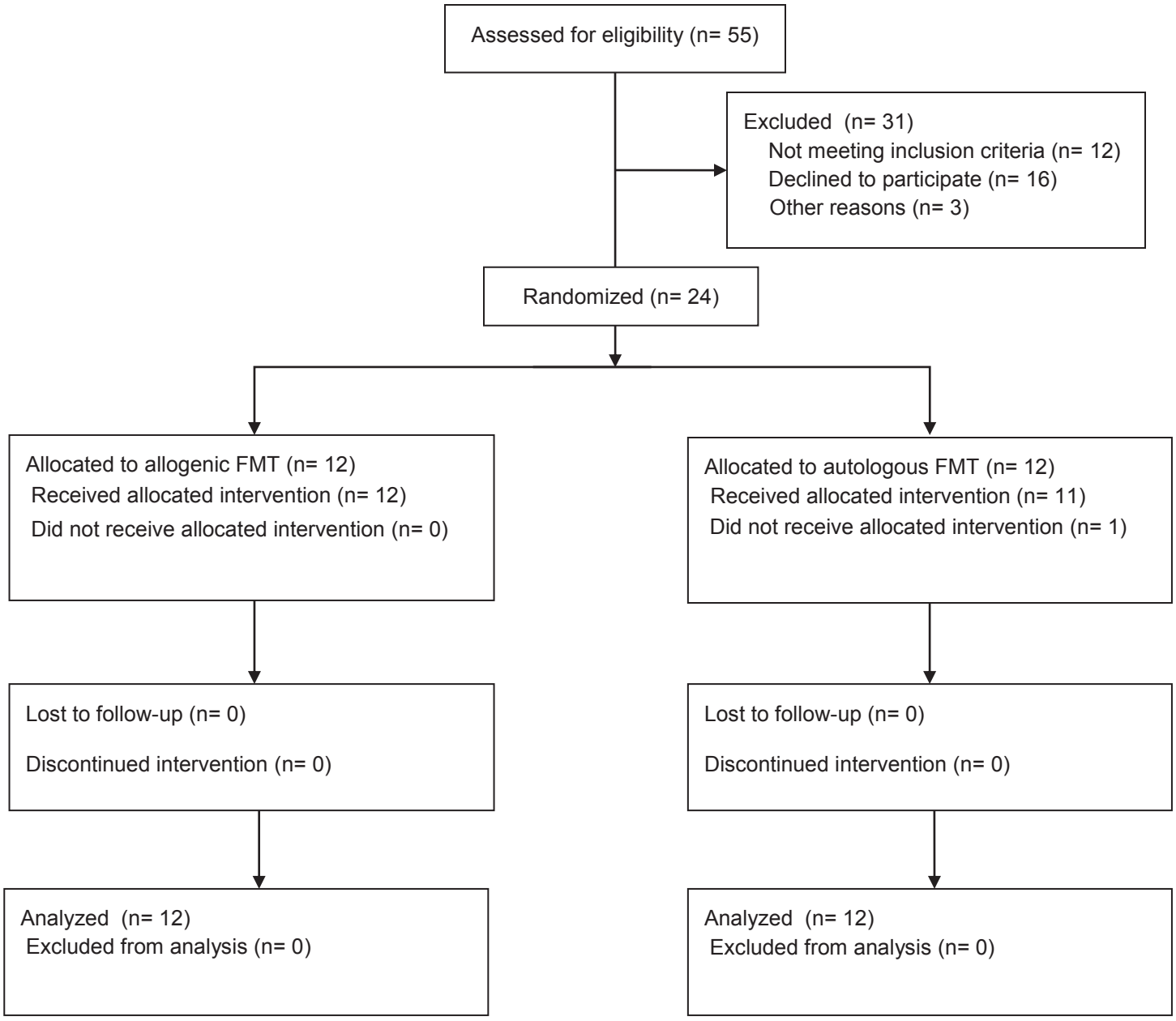

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Figure 4.

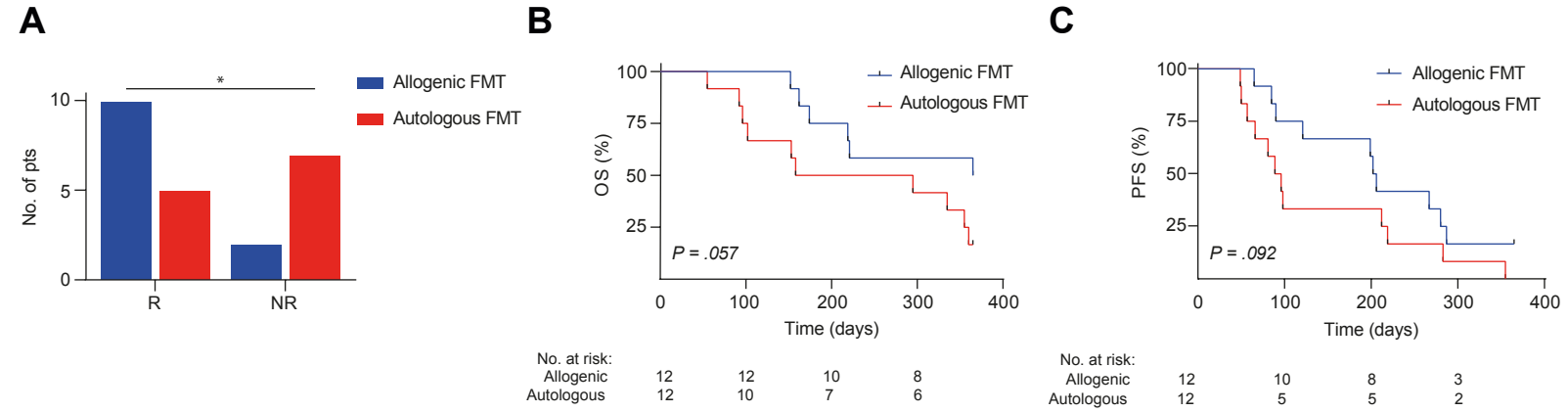
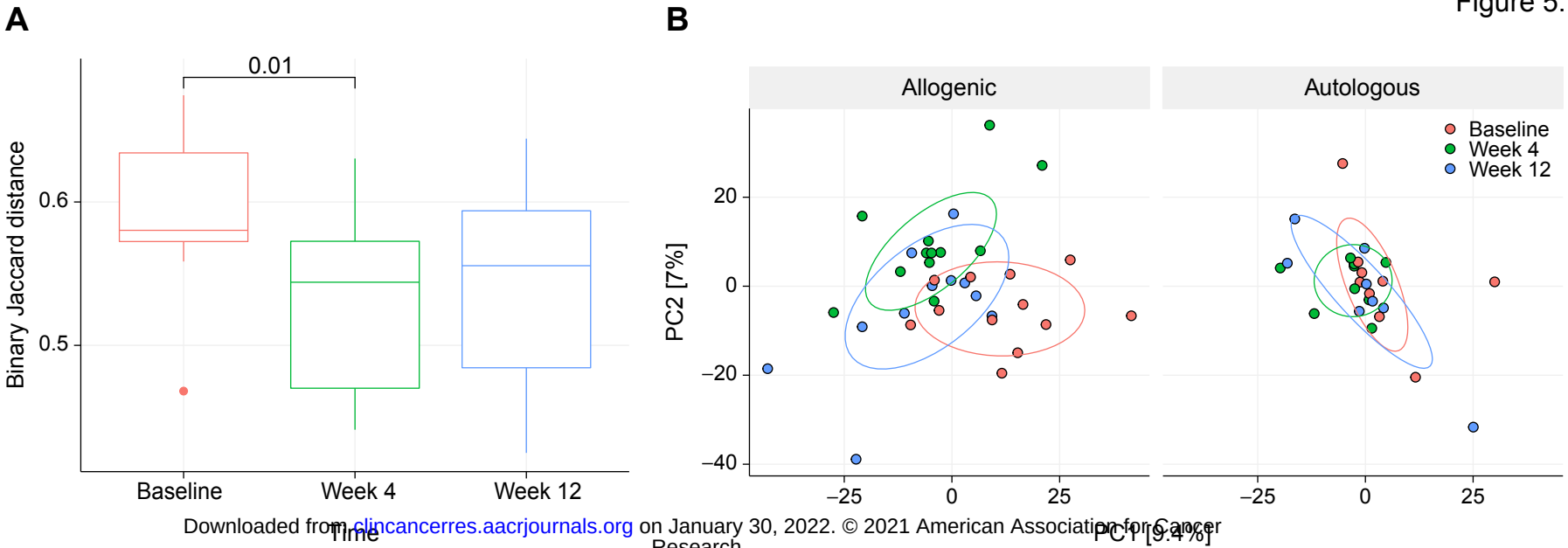


Figure 5.



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Clinical Cancer Research

Fecal Microbiota Transplantation from Overweight or Obese Donors in Cachectic Patients with Advanced Gastroesophageal Cancer: A Randomized, Double-blind, Placebo-controlled, Phase II Study

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