Therapeutic interventions targeting enteropathy in severe acute malnutrition modulate systemic and vascular inflammation and epithelial regeneration.

Therapeutic interventions targeting enteropathy in Severe Acute Malnutrition modulate systemic and vascular inflammation and epithelial regeneration

Short title: Interventions targeting enteropathy in SAM

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Abstract

Background

Severe acute malnutrition (SAM) is the most life-threatening form of undernutrition, and children hospitalized with complications have unacceptably high mortality. Complicated SAM is a multisystem disease characterised pathophysiologically by sarcopenia, systemic inflammation, metabolic dysfunction and malnutrition enteropathy including epithelial barrier dysfunction. There is a clear need for novel interventions to address the underlying pathogenic perturbations of complicated SAM.

Methods

In this analysis of tertiary outcomes from a phase II multi-centre trial in Zambia and Zimbabwe, multiplex biomarkers were measured in 122 children (57% male) with SAM randomized following stabilisation (the 'baseline') to one of four interventions for 14 days to treat malnutrition enteropathy: budesonide, N-acetylglucosamine, colostrum and teduglutide, compared with standard of care. Following measurement of 35 biomarker levels from day 15 plasma samples using Luminex and ELISA, the dimensionality of biomarker data was reduced using principal component analysis.

Findings

Both budesonide and colostrum reduced systemic inflammation (as measured by CD14, IL1-ra, CRP, and LBP), while children receiving colostrum had higher GLP2 and angiopoietin, and lower circulating lipopolysaccharide, suggesting better restoration of epithelial barrier function. N-acetylglucosamine, a precursor for epithelial glycosaminoglycan synthesis, increased biomarkers of epithelial regeneration (EGF, VEGF), and circulating growth factors (angiopoietin, IGFBP-3, and GCSF).

Interpretation

Interventions aimed at ameliorating malnutrition enteropathy showed plausible effects on biomarkers of inflammation and epithelial regeneration, reflecting the interdependence of the systemic inflammation and enteropathy markers seen in structural analysis. Given the interplay between inflammation and tissue restoration in malnutrition, this mechanism of action supports larger trials to determine the clinical benefits of interventions, either alone or in combination, in children with complicated SAM.

Funding

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Keywords: severe malnutrition; SAM; severe acute malnutrition; systemic inflammation; vascular inflammation; randomized trial

One Sentence Summary/Teaser: Therapeutic interventions targeting the gut dysfunction that characterises severe acute malnutrition reduced systemic inflammation and increased growth factors and biomarkers of tissue repair.

Research in context

Evidence before this study

Children admitted with severe acute malnutrition (SAM) mostly reside in lower- and middle- income countries. This, in part, explains that despite the high mortality attributed to this condition, the underlying pathophysiology is poorly understood. Few novel treatments have been trialled in this patient population, and plasma biomarkers have rarely been interrogated with multiplexed biomarker analyses. Children with SAM have higher plasma biomarkers of systemic and vascular inflammation compared with community control children without acute malnutrition, and elevated inflammation persists for at least 48 weeks after discharge. The higher concentrations of some of these markers were associated with poorer outcomes. The TAME trial has shown that several interventions aimed at treating the gut dysfunction seen in children with severe malnutrition are well tolerated, and one intervention teduglutide, positively impacts a composite biomarker representing intestinal inflammation.

Added value of this study

In this analysis of tertiary outcomes of the randomised controlled TAME trial, we show that two interventions aimed at treating gut dysfunction, budesonide and colostrum, improve systemic and vascular inflammation biomarkers. Administration of N-acetylglucosamine, a precursor for epithelial glycosaminoglycan synthesis, increased biomarkers of epithelial regeneration and circulating growth factors. This is potentially represents repair and tissue accretion in these children, and levels of some of these biomarkers have previously been associated with improved linear growth and outcomes following discharge. Finally, with structural modelling, we analysed the interplay between systemic inflammation and markers of growth and epithelial regeneration, which may suggest higher levels of inflammation can prevent the growth and regenerative processes in these children.

Implications of all available evidence

Our results suggest that interventions could reduce systemic and vascular inflammation in children with SAM, which we know from other studies exists up to at least 48 weeks post-discharge. It is also possible to increase the levels of some growth factors, which have previously been shown to be associated with better outcomes, and these growth factors are negatively related to the concentrations of systemic/vascular inflammation markers, highlighting the apparent relationship between the two. This data now needs to be taken forward with further trials to see if these changes in biomarkers persist for longer periods, or correspond with better outcomes in these high-risk children.

Introduction

Severe acute malnutrition (SAM) is the most life-threatening form of undernutrition, estimated to affect 13.7 million children under five years old in 2022[1]. The World Health Organization (WHO) defines SAM in children 6-59 months old as a weight-for-height z-score <-3, mid-upper arm circumference of <115mm, or bilateral pitting oedema[2]. Although most children with SAM receive community-based management, those with complications such as poor appetite, bilateral severe oedema, signs of metabolic disturbance or infections, or danger signs such as persistent vomiting or seizures are admitted to hospital[3]. The inpatient mortality of children with complicated SAM in a recent meta-analysis was 15.7% (range 3.7 %– 41.4%) across 19 studies largely from sub-Saharan Africa[4]. Mortality also remains high after discharge, with one Zimbabwe/Zambian study from the same centres as this trial showing the one-year post-discharge mortality almost matching the inpatient mortality[5,6], and being replicated in other studies across sub-Saharan Africa[7-10]. There is a clear need for transformative therapeutic approaches to improve outcomes.

Complicated SAM manifests as a multisystem disease, which includes sarcopenia and/or oedema, reduced intestinal villus height and crypt hyperplasia[11], metabolic disturbance, skin lesions likely due to extracellular matrix dysfunction in oedematous SAM[12], and immune changes consistent with a functional immunodeficiency[13]. A recent narrative review on this topic by us and others in this field has hypothesized that inflammation underlies many of these changes, with inflammation being a cause and/or consequence of these changes[14]. Children hospitalized with SAM have high inflammatory biomarkers from across several studies and settings[13], but as many children present with infection, the immune responses to acute illness may well be contributing to this. These changes do appear to persist over one-year post-discharge, suggesting infection is not the sole cause of changes seen in children with malnitrition[15]. These appears to be an interplay between inflammation and growth/regeneration, with these processes inter-competing and biomarkers reflecting these processes being negatively associated with each other[16], or independently predictive of mortality[15]. Higher systemic inflammatory biomarker concentrations are independently predictive of mortality in children with SAM[17-19], and associated with poorer linear growth in the 6 months following discharge[16]. Chronic inflammation has been shown to alter monocyte phenotypes[20], and likely to induce a state of functional immunosuppression[21], leaving children vulnerable to further infections, and to impaired tissue growth and repair. Currently, treatment of SAM does not adequately address this multi-organ dysfunction and inflammation.

Therapeutic **A**pproaches to **M**alnutrition **E**nteropathy (TAME) was a Phase II trial conducted among children admitted with complicated SAM in Zimbabwe and Zambia. Four interventions were trialled at stabilization to improve the malnutrition enteropathy: budesonide, colostrum, N-acetylglucosamine, and teduglutide. These have 4 distinct modes of actions: 1) oral budesonide to improve local inflammation, 2) colostrum aimed at reducing epithelial permeability 3) N-acetylglucosamine, a sugar needed for epithelial regeneration, and 4) teduglutide, a glucagon-like preptide-2 (GLP2) analogue promoting growth and repair in the intestine. Teduglutide showed an improvement in the trial primary outcome – an enteropathy score comprising faecal biomarkers of intestinal inflammation[22]. Here, we progress to examine the tertiary endpoints of the TAME trial, looking at multiple plasma biomarkers using a multiplex platform. Building on our findings, we now explore whether the randomized interventions improve the interplay between systemic/vascular inflammation and markers of intestinal repair and growth, which are also known pathophysiological pathways that burden children with complicated SAM[15].

Materials and Methods

The TAME trial investigated the impact of four interventions on enteropathy biomarkers in a multi-arm, phase II, randomised controlled trial in southern Africa between 4th May 2020 to 27th April 2021. The protocol has been published[23]. Briefly, 125 children hospitalised with SAM were randomized to standard of care, or one of four investigational medicinal products (IMPs) for 14 days: oral budesonide, oral colostrum, oral N-acetylglucosamine, and subcutaneous teduglutide [Supplementary Table 1]. Randomization and interventions were given following stabilisation of the child admitted with SAM, which we have called the 'baseline' of the trial. TAME was conducted at two tertiary referral hospitals: Harare, Zimbabwe (Sally Mugabe Hospital), and Lusaka, Zambia (Children's Hospital - University Teaching Hospital).

Endpoints of the trial

The primary endpoint of the TAME trial was the change in a composite enteropathy biomarker score derived from Kosek et al, incorporating faecal alpha-1-antitrypsin, neopterin and myeloperoxidase[24] between baseline and day 15. Only teduglutide improved the composite primary outcome[25]. The current analysis evaluated tertiary endpoints included in the trial protocol, which were designed to explore changes between baseline and day 15 in circulating biomarkers of systemic inflammation (n=16), endothelial activation (n=6), and growth factors (n=7). A full list of the soluble plasma biomarkers analysed is shown in Supplementary Table 2.

Recruitment and randomization

Inclusion criteria were children aged ≥6 and <60 months hospitalised with SAM according to WHO criteria[2]. Children were recruited after initiation of transition (from F75 milk to either F100 milk, or ready-to-use therapeutic food), provided they were clinically stable as judged by the treating physician, and had written informed consent provided by the caregiver. Exclusion criteria included those who were clinically unstable; less than 5kg bodyweight; with neurological or oro-facial abnormalities that would explain poor feeding; where the caregiver was unwilling to learn the HIV status of the child; severe anaemia (haemoglobin <6g/dL at enrolment); if they had an underlying condition which would put the child at undue risk of failing study completion or would interfere with analysis of study results; or if they had a contraindication to any of the trial treatments. Around initial transition, caregivers were asked to provide written informed consent and participants were randomly allocated to an IMP or continuation of standard of care. Randomisation codes were pre-prepared by the trial statistician following permuted blocks to better ensure equal split of

participants across groups. A sealed envelope was opened for each participant at the time of recruitment to ensure allocation concealment. TAME was non-blinded for caregivers and ward staff, but laboratory technicians were blinded to intervention arm. Prior to randomization, all children received standard of care according to current WHO guidelines, which included universal use of antibiotics[2]. Demographic information including the caregiver-reported biological sex of the child was collected at baseline. No information on gender was collected.

Investigatory Medical Products (IMPs)

These IMPs chosen are therapies are directed at restoration of the mucosal barrier and tight junction damage in malnutrition enteropathy, which is histologically similar to environmental enteropathy[26,27], and would aim to cause the reversal of the cascade of downstream inflammatory derangements seen in SAM. The duration of 14 days was chosen as a pragmatic length as the maximum time likely to be acceptable to carers for keeping their child in hospital during the trial. The IMPs chosen for this study were oral budesonide, oral colostrum, oral N-acetylglucosamine, and subcutaneous teduglutide.

1) **Budesonide** is a corticosteroid that reduces inflammation through direct local action on inflammatory cells in the gut, and has extensive first-pass elimination. This reduces the systemic side-effects following oral administration. It is standard therapy for Crohn's disease.
2) **Bovine colostrum** is a nutraceutical, which is generally regarded as safe and freely available as food supplements in health food shops. Colostrum is the foremilk secreted by the cow and contains nutrients and immunoglobulins, and is higher in protein and nutritional and growth-promoting bioactive compounds compared with milk[28]. It has been shown to reduce the epithelial permeability seen in heat shock in adults[29], and have cross-species effect on human health. Benefit for children in SAM is hypothesised through the protective and anti-inflammatory properties of the immunoglobulins which are the largest fractional protein component, as well as growth factors and hormones which encourage intestinal differentiation and proliferation[30]. Bovine colostrum also contains large amounts of exomes and extracellular vesicles enriched with many miRNAs and proteins involved in the immune response and growth[31].
3) **N-acetylglucosamine** is an amide derivative of glucose and is present on every cell surface. It is a substrate for synthesising glycosaminoglycans – polysaccharides that protect the bowel mucosa from toxic damage. N-acetylglucosamine is a significant component of the mucous produced by the goblet cells which protects the intestinal lining, and synthesis is impaired in

patients with inflammatory bowel disease (IBD)[32], and its administration has been shown to induce disease remission in a pilot study in paediatric IBD cases[33].

4) **Teduglutide** is a long-acting analogue of glucagon-like peptide-2 (GLP-2), a protein secreted by the distal small intestine and colon which preserves mucosal integrity by promoting growth and repair of the intestinal epithelium. It has proven efficacy in intestinal failure associated with short bowel syndrome, including improving absorption and reducing the need for parenteral support[34].

Full details of dosing and timing of the IMPs are shown in Supplementary Table 1.

Laboratory methods

Luminex multiplex analysis was carried out using a 25-plex custom Luminex panel [Bio-techne Corporation (R&D); Minneapolis, MN, USA] as detailed in Supplementary Table 2 as per manufacturer's instructions. Plasma samples were run in singlicate at 1:2 dilution using a MAGPIX reader [Luminex Corp; Austin, TX, USA].

ELISAs for stool α1-AT [Immunochrom GmbH; Germany], neopterin [Arigo Biolaboratories; Taiwan], and myeloperoxidase [Immundiagnostik; Germany] were carried out following dilution of 100mg stool in wash buffer; following centrifugation, the ELISA was carried out on the supernatant. CRP, sCD14, sCD163 were quantified in plasma with ELISA [R&D Quantikine; Minneapolis, MN, USA], and LBP [R&D Duoset; Minneapolis, MN, USA] according to the manufacturers' instructions.

Lipopolysaccharide (LPS, also known as endotoxin) was measured using the Limulus amoebocyte lysate (LAL) assay [Associates of Cape Cod Inc.; MA, USA], which uses clotting components harvested from the blood of horseshoe crabs[35]. Plasma was diluted 1:10 with endotoxin-free water, heated to 70C for 30 minutes, and then run according to the manufacturer's specification with readings taken using a kinetic plate reader.

Sample collection

Amongst other samples, blood and stool were collected in-hospital at baseline and on follow up day 15-19. Following universal precautions, blood (maximum 4 mL on any occasion and total not exceeding 2 mL/kg over two weeks) was collected in endotoxin-free EDTA tubes spun to recover plasma which was stored at -80°C until further analysis. Stool was collected by study nurses or caregivers into plain tubes without fixative and placed in a cool box. Samples were sent to the

laboratory within four hours and stored at -80°C until analysis. HIV testing was conducted at baseline.

Statistical methods

This manuscript presents tertiary outcomes of the main TAME study. The analysis of our tertiary endpoints followed the same approach as used for our primary and secondary endpoints, as detailed in our pre-published protocol[23]. Day 15 biomarkers were analysed by trial arm using an ANCOVA model, adjusted for baseline values and the pre-specified variables: HIV status (positive/negative), sex (male/female), oedema (yes/no), WHZ (continuous), diarrhoea (yes/no), site (Zimbabwe/Zambia). Following assessment with Tukey's ladder of powers, soluble biomarkers were normalised by $\log_{10}$ transformation. Where measurements were below the limit of quantification of the assay, a value was assigned according to the following formula[36]: assigned value = lowest level of detection / sqrt(2). Correlations between normalised biomarkers were calculated using Pearson's correlation coefficient. Differential correlation was conducted using the Fisher's Z transformation of the Pearson correlation coefficient in each group, with significance determined by Fisher's Z test.

Dimensional reduction of the biomarker data was subsequently undertaken using two principal component analyses (PCA): the first included systemic inflammatory, endothelial inflammatory/activation, and growth factor biomarkers; the second included all enteropathy biomarkers (α1-AT, neopterin, myeloperoxidase, glucagon-like peptide 2, and intestinal fatty acid-binding protein). Biomarkers with a missingness of >10% were not included in the PCA, due to high missingness. Following normalisation as described above, biomarkers were zero-centred (i.e., each biomarker's values were universally adjusted so that the mean was zero) and standardised (universally adjusted so that the standard deviation was 1) prior to PCA. The PCA was carried out on the day 15 (ie post-intervention) data, and scores for each component were generated for the day 15 and baseline results from this analysis. The PCA was carried out using a complete-case approach. For the larger PCA of systemic biomarkers, components with an eigenvalue of >2 were retained. For the smaller PCA of enteropathy biomarkers, components with an eigenvalue of >1 were retained. When considering the impact of individual biomarkers on a component, a loading of >0.20 was used as a cutoff to select which biomarkers were used to describe the component. Analysis of day 15 PCA biomarkers by trial arm used an ANCOVA model, adjusted for the same pre-specified variables: HIV status (positive/negative), sex (male/female), oedema (yes/no), WHZ (continuous), diarrhoea (yes/no), site (Zimbabwe/Zambia), along with the baseline PCA value.

Partial least squares (PLS) path modelling was used to further explore the relationship between PCA components by assessing the relationship between the gut components and the systemic components, along with a set of covariates as set out in Supplementary Figure 1. Any impact of the randomisation was assessed by comparing one randomisation group with the standard of care arm.

As pre-specified in the protocol, the α was set at 0.1 for all analyses, consistent with an exploratory phase II trial. All results were therefore estimated with a 90% confidence interval. Although no correction was made for type 1 error, consistent with the view this is not required for exploratory multi-arm multi-stage trials in Phase II[37], the adjusted P values were shown for reference to give an indication of the effect of multiple analysis, having been adjusted using Benjamini-Hochberg correction. Statistical analysis was carried out using Stata v18 (StataCorp., College Station, TX), except for the differential correlation analysis, which was carried out using R Statistical Software (v4.3.1; R Core Team 2023).

Sample size

The sample size was pre-specified as n=225 (45 in each arm), based on the primary outcome of the composite biomarker score[23]. Given low mortality and little loss to follow-up, plus slow enrolment due to COVID-19 which closed one recruitment site, the trial steering committee and data monitoring and ethics committee reviewed the sample size in January 2021, and allowed reduction in enrolment to 125 (25 in each arm). This was based on a Cohen's d effect size of 0.3, with 80% power and 90% confidence, and conservative correlation between baseline and follow-up estimates of 0.5, requiring 23 per group across 5 groups to analyse the primary outcome by ANCOVA. Allowing for 5% losses, the sample size of 115 was rounded up to 125 participants in total (25 per group).

Trial Registration and Ethics

Informed consent was gained from all trial participants. Ethical approval was obtained from the University of Zambia Biomedical Research Ethics Committee (006-09-17), the National Health Research Committee of Zambia, the Zambia Medicines Regulatory Authority (CT 082/18), the Joint Research Ethics Committee of Harare Central Hospital (JREC/66/19), the Medicines Control Authority of Zimbabwe (CT/176/2019), and the Medical Research Council of Zimbabwe (MRCZ/A/2458). The trial sponsor was Queen Mary University of London. The trial was registered at www.clinicaltrials.gov (NCT03716115) and the protocol was published[23].

Role of funders

Funders had no role in study design, data collection, data analyses, interpretation, or writing of this manuscript.

Results

Of 143 children who were screened for the TAME trial, 133 were eligible; 8 caregivers declined consent, leading to 125 children being enrolled and randomised after a median of 5.5 days (range 1-21) since admission to hospital with complicated SAM, as shown in Figure 1. The baseline characteristics of enrolled children are shown in Table 1. Baseline blood and stool samples were collected from all children prior to randomisation. Randomised IMPs were provided in hospital for 14 days by study nurses. Adherence and completion were very high, with 122/124 (98%) children who survived to day 15 receiving all planned doses of IMPs, as measured by the nurses in hospital. Of 125 children with baseline samples, 2 withdrew and 1 died before collection of a follow-up blood and stool sample at day 15, therefore 122 children (98%) contributed to the post-intervention biomarker analysis.

Biomarker results

Levels of biomarkers at baseline and at day 15 and number of samples at each timepoint are summarised in Supplementary Table 3. The level of correlation between the individual biomarkers is shown in Supplementary Figure 2, with the differential correlations among the day 15 biomarkers by treatment group being shown in Supplementary Figure 3. The results for the day 15 biomarker concentrations, adjusted for sex, oedema, HIV, diarrhoea, WHZ, site, and the baseline biomarker value, are shown in Figure 2, with the full dataset shown in Supplementary Table 4. Children who received the corticosteroid budesonide had significantly lower concentrations of plasma CRP [-0.387 $\log_{10}$ mg/L (90%CI -0.710,-0.065)], soluble CD163 [-0.108 $\log_{10}$ ng/L (90%CI -0.197,-0.019)], ICAM-1 [-0.062 $\log_{10}$ pg/mL (90%CI -0.123,-0.002)], and GM-CSF [-0.239 $\log_{10}$ pg/mL (90%CI -0.382,-0.096)] at day 15. Children who received N-acetylglucosamine had higher day 15 concentrations of G-CSF [0.099 $\log_{10}$ pg/mL (90%CI 0.025,0.173)], IGFBP-3 [0.200 $\log_{10}$ pg/mL(90%CI 0.010,0.389)], angiopoietin [0.348 $\log_{10}$ pg/mL (90%CI 0.103,0.593)], and L-selectin [0.099 $\log_{10}$ pg/mL(90%CI 0.015,0.183)]. Children who received colostrum had higher post-intervention GLP-2 (0.118 $\log_{10}$ ng/mL (90%CI 0.008,0.229)] and angiopoietin [0.262 $\log_{10}$ pg/mL (90% CI 0.012,0.512)], and lower LPS concentrations [-0.492 $\log_{10}$ EU/mL (90%CI -0.965,-0.018)]. Children who received teduglutide had higher plasma neopterin [0.263 $\log_{10}$ nmol/L (90%CI 0.017,0.509)] and IL-6 [0.118 $\log_{10}$ pg/mL (90%CI 0.011,0.225)].

Differential correlation network analysis showed that there were some significant changes in correlations between groups, as shown visually in Supplementary Figure 3. In the teduglutide group,

there was stronger relationships between IL-6 and several other biomarkers. In the NAG group D-dimer was more weakly associated with several other pro-inflammatory markers.

Principal component analysis

A PCA of plasma biomarkers at day 15 was used to reduce data dimensionality. LPS was not included in the PCA analysis due to missingness > 10% (Supplementary Table 3). The PCA identified three components with an eigenvalue > 2, with the results shown in Figure 3A. The full PCA plots are shown in Supplementary Figure 5.The loadings of each factor were used to biologically interpret the principal components. The first systemic biomarker component was pro-inflammatory, with top positive loadings of TNF-α, IL-6, IL-33, IL8, IL-2, IL-1β, and interferon-γ, as well as the macrophage activation proteins CCL3 and CCL4. The second systemic biomarker component was also pro-inflammatory, and captured pathways associated with host-endotoxin responses, containing positive loadings of soluble CD14, lipopolysaccharide binding protein, CRP, soluble CD163, IL-1ra, and VCAM-1. The third systemic biomarker component represented tissue restoration, containing the growth factors epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and angiopoietin, as well as d-dimer, P-selectin, and negative loading of L-selectin, IL-10, GM-CSF and PlGF.

A PCA was similarly carried out on the gut biomarkers, resulting in two principal components with an eigenvalue of > 1, and their loadings are shown in Figure 3B, and detailed in Supplementary Figure 6. Gut component 1 had strong positive loadings of plasma GLP2 and IFABP, and a negative loading of faecal myeloperoxidase, neopterin, and alpha-1-antitrypsin (α1-AT), thus, this GLP2 dominant PC likely to represent increased signaling for intestinal epithelial regeneration. Gut component 2 had strong positive loadings of MPO, α1-AT and IFABP, with negative contribution from neopterin. This likely represents intestinal inflammation and damage, although given the small negative input from neopterin, this may suggest this damage/inflammation is neutrophil rather than macrophage mediated.

Analysis of covariance (ANCOVA) analysis was used to assess the effect of the randomised interventions on the day 15 PCA scores (Table 2), adjusting for baseline PCA score, HIV, site, oedema, WHZ, sex, and presence of diarrhoea. Budesonide and colostrum were associated with reduced values of the pro-inflammatory systemic component 2, suggesting a modulation of host endotoxin responses, and N-acetylglucosamine was associated with increased values of systemic component 3, suggesting recovery of growth factors and tissue repair. Teduglutide had no significant effect on any of the principal components.

PLS path modelling

PLS path modelling was used to explore the relationships between the principal components in each of the intervention arms compared with standard of care, with results shown graphically in Figure 4, and fully breakdown shown in Supplementary Table 5. In all cases the baseline biomarker concentrations had the strongest associations with day 15 biomarker concentrations. There were significant interactions in all randomised groups between the enteropathy components and the systemic components, showing the interdependence of the gut and systemic inflammatory processes in SAM. This mirrored the correlations seen between biomarkers from different body systems in Supplementary Figure 3. The effect of the randomised group on the individual components closely mirrored the results from ANCOVA modelling in Table 2, with an additional effect of budesonide on gut component 2.

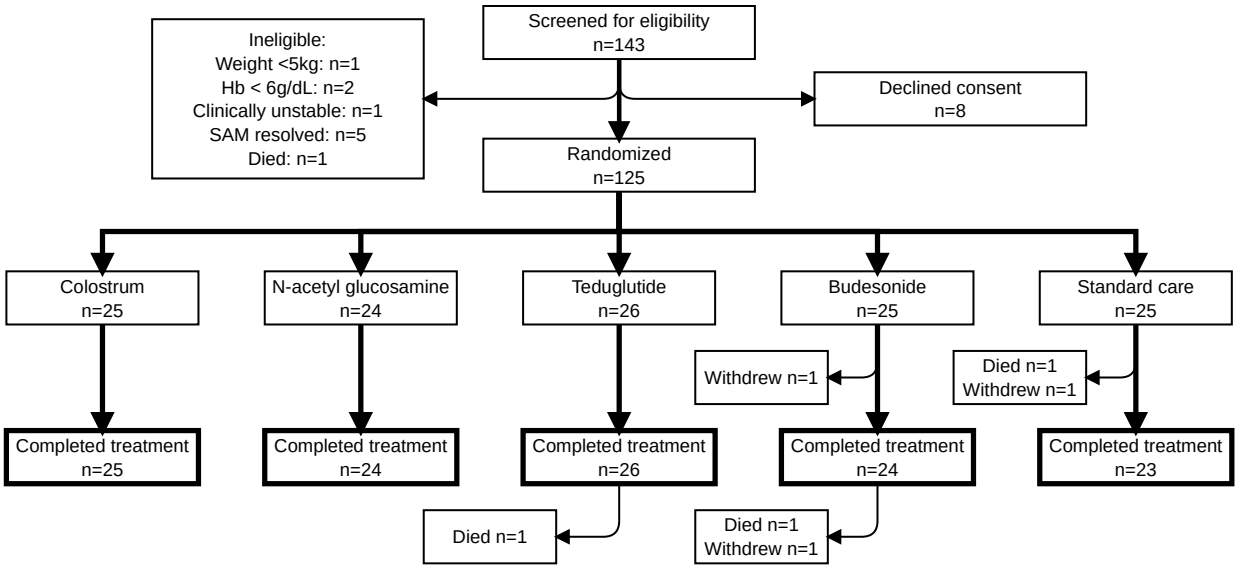

Figure 1: CONSORT diagram for children in the TAME trial

Children were randomised to one of five treatment arms (4 IMPs, and standard of care). Three children died, and three children withdrew. Three children exited the study prior to the day 15 endpoint, so their data were not included in the final endpoint analysis. Three children exited between day 15 and day 28, so their data were included in the current analysis.

	Randomisation					
	Colostrum	**NAG**	**Teduglutide**	**Budesonide**	**Standard Care**	**Total**
	N=25	N=24	N=26	N=25	N=25	N=125
Age (months)	20 (15,23)	19 (14,23)	18 (12,20)	17 (13,22)	16 (13,27)	18 (13,22)
Male	13 (52%)	16 (67%)	16 (62%)	11 (44%)	15 (60%)	71 (57%)
Site						
Zambia	12 (48%)	12 (50%)	13 (50%)	12 (48%)	13 (52%)	62 (50%)
Zimbabwe	13 (52%)	12 (50%)	13 (50%)	13 (52%)	12 (48%)	63 (50%)
HIV positive	4 (16%)	4 (17%)	6 (23%)	2 (8%)	8 (32%)	24 (19%)
Days to recruitment	6 (5,8)	5 (3,6)	5 (4,7)	4 (3,5)	5 (3,7)	5 (3,7)
MUAC (cm)	11.4 (10.8,11.9)	11.5 (11.2,12.9)	11.6 (11.0,12.3)	12.0 (11.4,12.6)	11.7 (11.0,12.3)	11.6 (11.0,12.5)
WAZ	-3.65 (-5.45,-3.25)	-4.05 (-4.71,-2.86)	-3.39 (-4.32,-2.75)	-2.99 (-3.86,-2.36)	-3.39 (-4.50,-2.70)	-3.59 (-4.50,-2.74)
WHZ	-2.72 (-4.19,-2.35)	-2.59 (-3.74,-1.24)	-2.22 (-3.34,-1.44)	-2.21 (-2.69,-1.59)	-2.62 (-3.60,-1.89)	-2.38 (-3.47,-1.59)
WHZ (oedematous)	*-2.57 (-3.47,-2.15)*	*-2.26 (-3.34,-1.22)*	*-1.78 (-2.35,-1.37)*	*-2.01 (-2.34,-1.58)*	*-2.10 (-3.44,-1.33)*	*-2.21 (-3.15,-1.51)*
HAZ	-3.07 (-3.82,-2.72)	-4.11 (-4.63,-2.73)	-3.29 (-4.38,-2.66)	-3.04 (-3.44,-2.21)	-3.17 (-4.41,-1.98)	-3.19 (-4.39,-2.39)
Oedema						
None	8 (32%)	7 (29%)	5 (19%)	7 (28%)	5 (20%)	32 (26%)
+	7 (28%)	6 (25%)	15 (58%)	8 (32%)	10 (40%)	46 (37%)
++	10 (40%)	10 (42%)	5 (19%)	10 (40%)	9 (36%)	44 (35%)
+++	0 (0%)	1 (4%)	1 (4%)	0 (0%)	1 (4%)	3 (2%)
Birthweight (kg)	3.00 (2.70,3.20)	2.80 (2.58,3.05)	2.92 (2.70,3.44)	3.20 (2.80,3.40)	3.10 (2.60,3.40)	3.00 (2.70,3.40)
Premature (<37 weeks)	6 (24%)	4 (17%)	6 (24%)	4 (16%)	2 (8%)	22 (18%)
Acute diarrhoea	14 (56%)	14 (61%)	16 (67%)	13 (54%)	13 (57%)	70 (59%)
Persistent diarrhoea	5 (20%)	5 (22%)	7 (29%)	6 (25%)	5 (22%)	28 (24%)
Pneumonia	5 (20%)	4 (17%)	4 (17%)	6 (25%)	3 (13%)	22 (18%)
TB	4 (16%)	4 (17%)	2 (8%)	2 (8%)	3 (13%)	15 (13%)
UTI	0 (0%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (1%)
Cerebral palsy	0 (0%)	2 (9%)	2 (8%)	2 (8%)	0 (0%)	6 (5%)

Table 1: Baseline characteristics of children in the TAME trial by randomization group

This table shows the baseline demographics of children with SAM (n=125) by randomized group. Continuous data are displayed as the median value, with lower and upper interquartile range. Categorical data show the number (n) in that category, and the percentage of the total randomization group. Persistent diarrhoea is diarrhoea present for ≥14 days. NAG: N-acetylglucosamine; MUAC: mid-upper arm circumference; WHZ: weight-for-height z-score; HAZ: height-for-age z-score; TB: tuberculosis; UTI: urinary tract infection

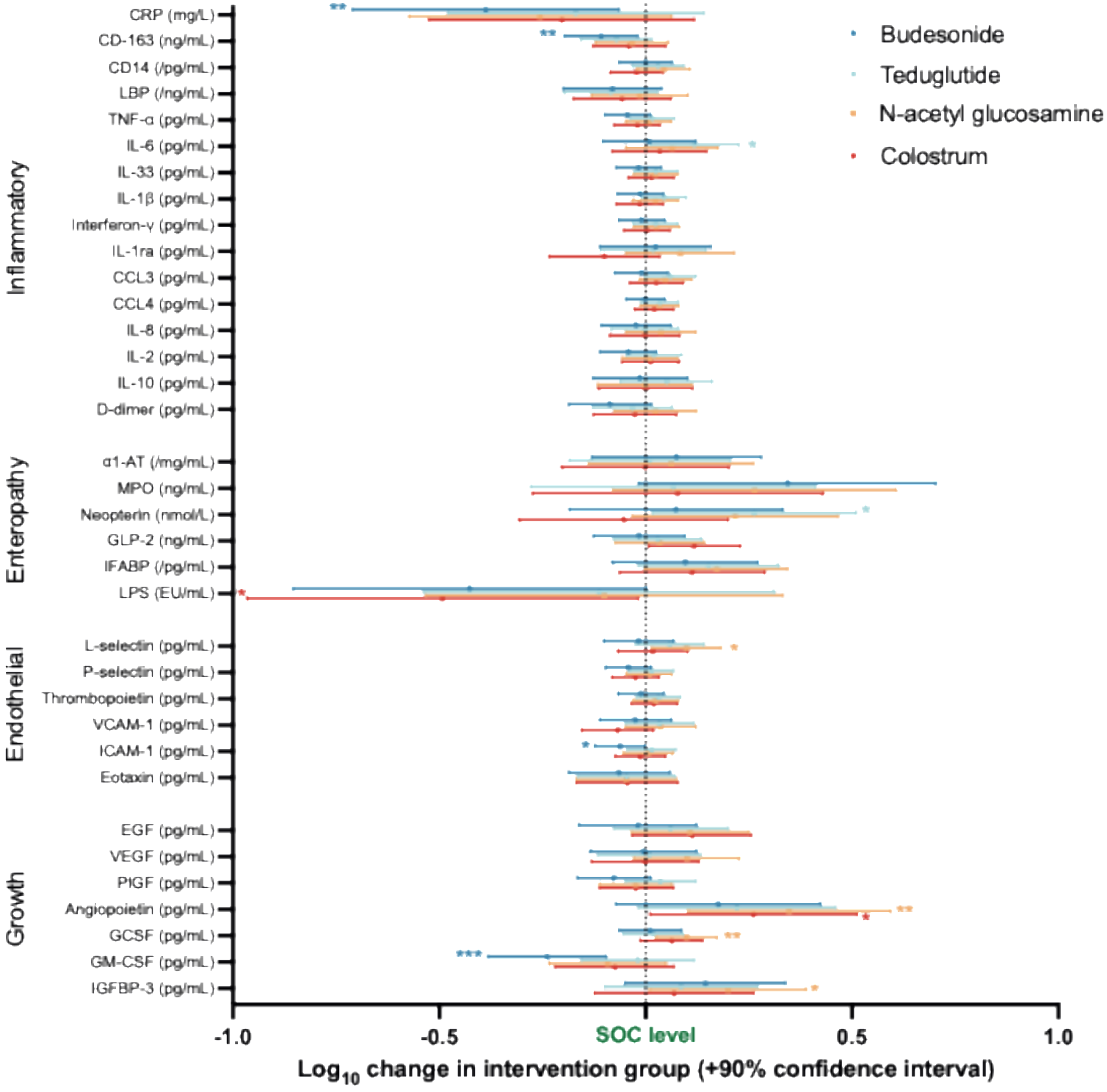

Figure 2: Changes of the adjusted log_{10} D15 biomarker value attributable to randomized intervention, over the standard of care (SOC) group

Differences are shown in the log10 levels of budesonide, teduglutide, N-acetylglucosamine (NAG), and colostrum groups. Results were adjusted for sex, oedema, HIV, diarrhoea, WHZ, site, and the baseline biomarker value. Reslts are shown as the change in log10 value compared with SOC group. A p value threshold <0.10 was pre-specified as statistically significant since this is a phase II trial and marked with *; P<0.05 **; P<0.01 ***.The full numerical results are shown in Supplementary Table 4.

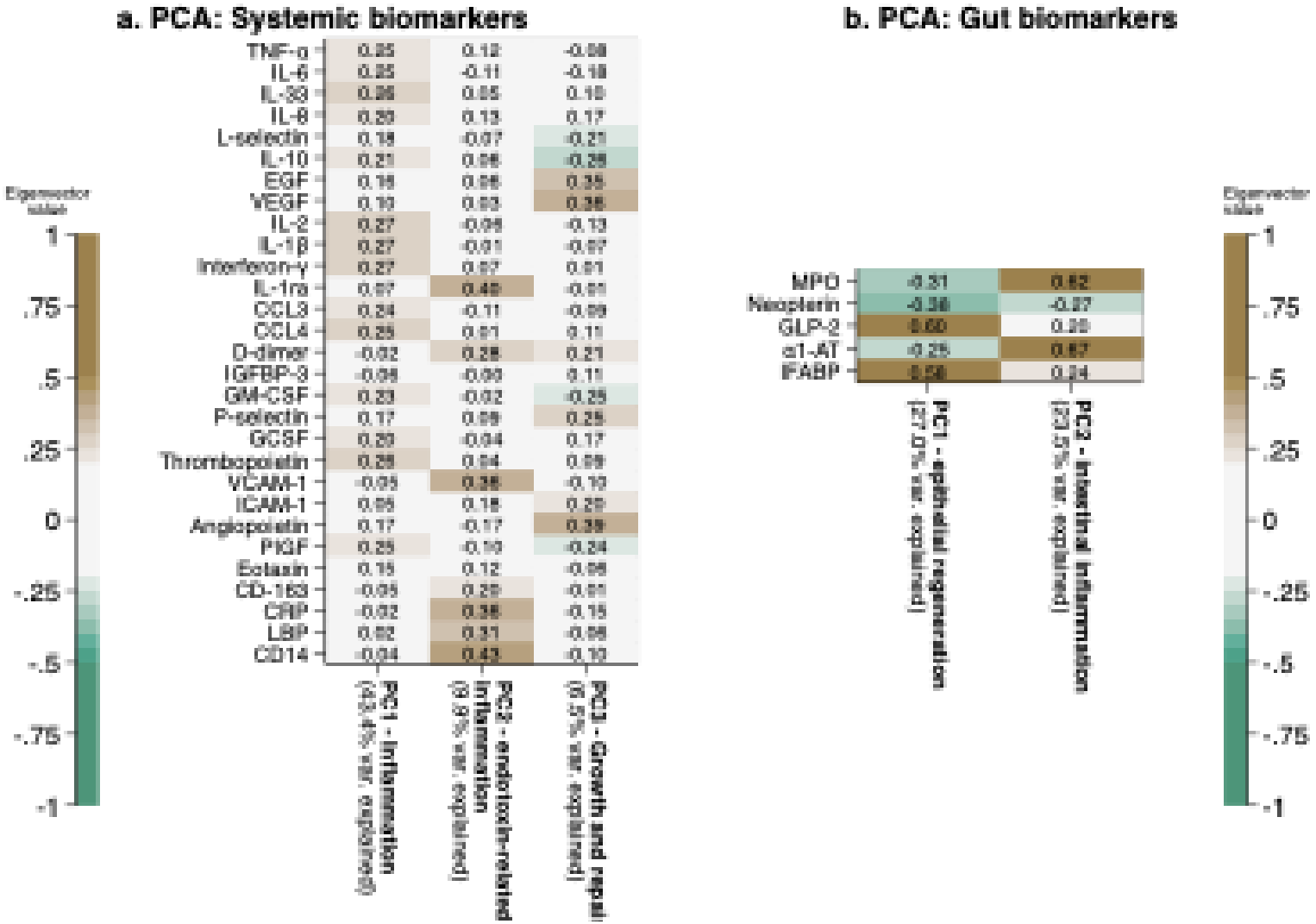

Figure 3: PCA analysis of A) day 15 systemic markers and B) day 15 enteropathy markers

Biomarkers were $\log_{10}$ transformed, standardized, and normalized before undergoing PCA. Eigenvector values, which correspond to a coefficient of orthogonal projection attributable to each biomarker following transformation for that component, are shown.

	Colostrum		NAG		Teduglutide		Budesonide	
	change over SOC	**90% CI**	**change over SOC**	**90% CI**	**change over SOC**	**90% CI**	**change over SOC**	**90% CI**
Systemic component 1 – *Inflammation*	0.20	(-0.92, 1.31)	0.61	(-0.49, 1.71)	0.62	(-0.45, 1.69)	-0.41	(-1.51, 0.70)
Systemic component 2 – *endotoxin-related*	-0.71*	(-1.36,-0.07)	-0.04	(-0.68, 0.59)	-0.26	(-0.88, 0.36)	-0.74*	(-1.39,-0.09)
Systemic component 3 – *growth and repair*	0.39	(-0.22, 1.00)	0.73**	(0.12, 1.34)	0.25	(-0.34, 0.84)	0.20	(-0.41, 0.81)
Gut component 1 – *epithelial regeneration*	0.35	(-0.18, 0.88)	-0.03	(-0.55, 0.49)	-0.00	(-0.52, 0.52)	-0.18	(-0.71, 0.36)
Gut component 2 – *intestinal inflammation*	0.30	(-0.20, 0.80)	0.42	(-0.08, 0.91)	0.07	(-0.43, 0.57)	0.45	(-0.06, 0.97)

*** p-value<0.01, ** p-value<0.05, * p-value<0.10

Table 2: Differences in component scores derived from biomarkers measured at Day15 between treatment group and the standard of care (SOC)

Results obtained using ANCOVA with adjustment for baseline PCA score, HIV, site, oedema, WHZ, sex, and presence of diarrhoea. Pre-specified significance threshold, p value <0.10 equivalent to 90% confidence intervals (CI).

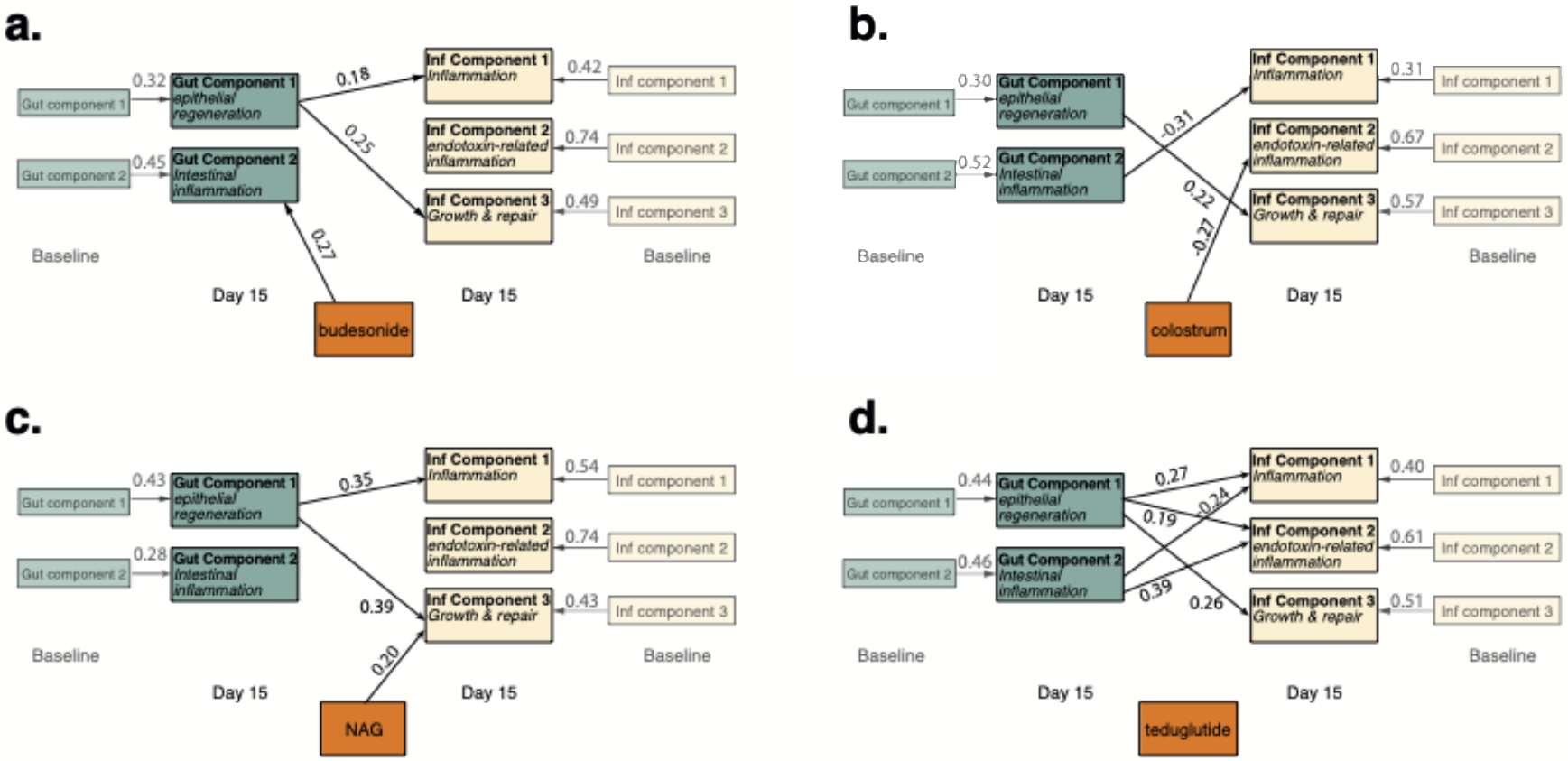

Figure 4: PLS path modelling of the relationship between the components and the effect of the trial intervention by a) budesonide b)colostrum c) N-acetylglucosamine (NAG), and d) teduglutide

The relationship of the PCA components (detailed in Figure 3) are assessed for each of the trial arms, compared with the standard of care. Results displayed are the standardized path coefficients, which are shown in full in Supplementary Table 5. Paths are displayed here is the P<0.10 for the path coefficient. In addition to the above, HIV, site, oedema, diarrhoea, and WHZ was added and connected to each of the D15 components (covariates not shown). The full model tested is shown in Supplementary Figure 1,

Discussion

This study explored the impact of gut-targeted interventions on systemic and faecal biomarkers of inflammation and tissue repair, in children admitted to hospital with complicated SAM in a phase II randomised multi-centre trial in sub-Saharan Africa. All four interventions aimed at improving enteropathy appeared to show evidence of benefits beyond the gut. Budesonide led to reductions in a suite of systemic pro-inflammatory biomarkers, while colostrum and N-acetylglucosamine led to restoration of growth factors and tissue repair. Given the deleterious effects of inflammation in children with malnutrition, which drives tissue damage and prevents epithelial restoration, these interventions showed intriguing evidence of ameliorating pathological processes that hinder recovery from SAM. Together with teduglutide, which showed effects on the primary enteropathy outcome previously reported [22], all four interventions show laboratory evidence of distinct mechanisms of action on an inflammatory-growth factor interplay that might lead to clinical benefits in children with SAM.

Increased inflammation is likely to be central to the damaging multisystem processes that characterise SAM[14]. Children with SAM demonstrate altered innate and adaptive immune responses with elevated systemic inflammatory markers[13]. Higher inflammatory markers are associated with mortality and readmission to hospital[15,17,38], and lower growth velocity[16]. Higher systemic and vascular inflammation continues for at least 48 weeks post-discharge, despite nutritional recovery, and the presence of increased growth factors in the face of this pro-inflammatory milieu is predictive of better outcomes [15]. It is therefore becoming apparent that children with SAM could benefit from interventions which reduce inflammation and restore tissues, both during hospitalisation, and also in the year following discharge. Although several trials have examined the effect of medications[39] or feeds[40] aimed at improving the gut in SAM, little has changed in the management of children with SAM since WHO guidelines were introduced in 1999. The current trial aimed to specifically combat inflammation using several therapies targeting the gut.

Budesonide, an orally administered glucocorticoid with high first-pass metabolism with minimal systemic absorption. It is used to limit local intestinal inflammation in inflammatory bowel disease, with the aim of minimal systemic effects. In the current study, 2 weeks of budesonide led to lower concentrations of CRP, CD163, and ICAM compared with the standard of care (SOC) arm, with a corresponding reduction in the pro-inflammatory systemic component 2 in this group. The exact mechanism for these changes in systemic markers is unclear, since oral budesonide theoretically acts

locally on the intestine, and should have minimal extra-intestinal effects. In selecting oral budesonide, we hypothesised that the beneficial systemic effects are principally indirect, by improving the local inflammation that characterises malnutrition enteropathy. Therefore this would reduce the translocation of antigenic substances across the damaged gut barrier. However, there is also some evidence for systemic effects of local steroids in other diseases. For example, inhaled budesonide in COPD has a systemic effect similar to methylprednisolone[41], and cases of oral budesonide causing iatrogenic Addison's disease have been reported[42]. PLS path modelling suggested that budesonide directly affects gut component 2, which contains inflammatory biomarkers such as myeloperoxidase and alpha-1 antitrypsin (Figure 4). In the PCA, there was no difference in Gut components in the budesonide group, but there was in one of the Systemic components (Table 2). It is therefore plausible that improvements in circulating pro-inflammatory biomarkers occur through a more direct systemic effect, despite the high first-pass metabolism of budesonide.

N-acetylglucosamine (NAG), a naturally occurring amino sugar which can be used as a precursor for epithelial glycosaminoglycan synthesis, was associated with an increase in the growth factor-containing principal component by day 15. As a sugar which is depleted in patients with IBD[43], NAG can improve IBD symptoms in children[33] and inhibits inflammation and neurogeneration in adults with multiple sclerosis, through controlling the N-linked glycans in cells which subsequently help to suppress T-helper-1 cells[44]. This nutraceutical product, along with budesonide, is one of the cheapest and easiest to administer, as the product can be kept at room temperature and is given orally. Bovine colostrum, the foremilk rich in immunoglobulins, growth factors and other bioactive components with anti-inflammatory properties, decreased values of the pro-inflammatory systemic component 2 compared to the SOC arm. In addition, colostrum led to higher levels of circulating GLP2 and angiopoietin, which are markers of increased epithelial turnover and repair[45]. Milk has extracellular vesicles which has shown to induce intestinal repair and improve intestinal permeability in mice[46]. It should be noted that amongst the extensive nutrition components of bovine colostrum[30], it is itself is a source of GLP2[47], but its bioavailability is poor[48]. Colostrum also led to significant reductions in circulating LPS, suggesting that improved epithelial repair may reduce translocation of LPS, although formal gut permeability testing, such as urinary excretion of lactulose:rhamnose, would be required to confirm this hypothesis.

The PLS path modelling examining the relationship between the enteropathy and inflammation components for each trial arm showed that, despite the intervention being aimed at reducing

enteropathy, changes occurred in both gut and systemic inflammatory markers, showing the interdependence between the gut and these systemic markers. This highlights the central role that malnutrition enteropathy may play in driving the inflammatory changes seen in SAM[13], and that utilising interventions targeting the gut may have downstream systemic effects. Few other trials have evaluated interventions targeting enteropathy in complicated SAM, although smaller trials of probiotics in Malawi[49], microbiota-directed foods in Bangladesh[50], pancreatic enzymes in Malawi[51], and a proof-of-concept trial of mesalazine in Kenya[52] have all shown benefits. Despite these promising initial studies, interventions have not yet progressed further to Phase III trials to assess effectiveness, and despite their recent 2023 update, the WHO guidance on the treatment of complicated SAM has not substantially changed in the last few decades, although it does now recognise the research need to 'study non-antibiotic pharmacologic and other medical interventions'[53].

Overall, most biomarkers significantly changed over a 2-week gut-targeted intervention: All markers of systemic inflammation and innate immune activation, except D-dimer, decreased, of which ten showed significant decreases. Markers of vascular inflammation and activation also declined (Supplementary Table 3). Collectively, this is likely to represent a partial natural resolution of the inflammatory processes acutely present in children with SAM as children recover, and may reflect the fact that many of these children present with infections. That some inflammatory biomarkers are even lower and some growth factors are higher in the treatment arms, compared to standard care, highlights the additional benefits arising from some of the treatments. Children with complicated SAM remain vulnerable and in need of potential therapeutic intervention long after their inpatient rehabilitation: a previous study showed that inflammatory markers in children with SAM remain higher than adequately-nourished community controls over the following year with current standard care[15]. In another paper, poorer outcomes over the following year were associated with higher PCA component scores representing systemic and vascular inflammation and lower component scores representing growth factors at baseline; increases in GLP-2 and I-FABP were both associated with improved outcome and each $\log_{10}$ rise of VEGF was independently associated with a 50% reduction in mortality or readmission[15]. A suggestion of this same interplay between inflammatory markers and growth factors was seen in the current study, in the PCA analysis by treatment group as well as negative correlations seen in simple correlation analyses (Supplementary Figure 3). Both colostrum and budesonide reduced the systemic component 2, primarily composed of pro-inflammatory biomarkers. In the case of colostrum, the reduction of LPS suggests the mechanism involved may be a reduction of intestinal translocation of bacterial products and

subsequent immune activation, and the effects of budesonide are likely mediated by its anti-inflammatory properties. N-acetyl glucosamine increased the level of systemic component 3, primarily composed of growth factors, and individually increased IGFBP-3, angiopoietin, and G-CSF, consistent with increased epithelial glycosaminoglycan synthesis, and likely reflecting increased repair and regeneration. This interplay between inflammation and growth/tissue accretion may well represent a therapeutic target in itself, suggesting that interventions that push the balance towards repair and growth could potentially be beneficial, given the respective concentrations of these biomarkers are associated with outcomes. The trial tested a two-week intervention period during hospitalization. Given the evidence that abnormalities in inflammatory markers remain for at least a year post-discharge, children might well benefit from prolonged therapy but further studies will be needed to assess any longer-term impact on outcomes in these children.

There are limitations to this analysis. This was a relatively small trial and in these exploratory analyses, which are tertiary trial outcomes, no correction was made for type 1 error, consistent with the view that this is not required for exploratory multi-arm multi-stage trials in Phase II[37]. However, particularly where there are larger dimensional data such as with our multiple biomarkers, this does limit the certainty of interpretation of individual biomarkers, as evidenced by the adjusted P-values in Supplementary Table 4, which have been included to highlight this limitation. Setting the α=0.1 is also consistent with an exploratory trial, but this means a larger adequately powered trial will be needed before any definitive conclusions regarding treatments can be reached. Additionally, there are some limitations in the applicability of the data to real-world situations, since adherence to these drugs was high in a clinical trial setting. Furthermore, as children were recruited following stabilisation, they are not reflective of all children who present with SAM, as emphasised by the low 28-day mortality rate of under 3%, compared with the higher mortality rates reported elsewhere[4]. Data on other factors, such as water, sanitation, and hygiene set-up at their homes, which have the potential to impact the severity of enteropathy seen in these children, was not collected, but there is no suggestion any such factors may be different between randomisation groups. It is possible individual sub-groups, such as those children with oedema or diarrhoea, were affected differently by these interventions, but this trial was not powered to pick up differences such as this.

In conclusion, we show that there is biological plausibility in extending the range of therapeutic interventions in the very high-risk population of children with complicated SAM. In this phase II randomised controlled trial, interventions designed to heal the gut led to reductions in systemic inflammation and increase in growth factors and biomarkers of tissue repair. Given that the

interplay between systemic inflammation and tissue accretion is central to recovery from SAM[15], the next step is to test these strategies in a larger phase III trial to evaluate whether laboratory improvements are accompanied by lower mortality and readmission to hospital, and better growth recovery.

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Conflicts of Interests

R.J.P. was previously an external consultant to Colostrum UK which provided the bovine colostrum used in these studies. RJP has also been an external consultant to Sterling Technology (USA) and an employee of Pantheryx Inc (USA) who produce and distribute bovine colostrum. There was no bovine colostrum company involvement in the production of this article or editing of its content. S.H. has had funding for teduglutide studies and lectured and participated in advisory boards on behalf of Takeda.

Data availability

The TAME dataset with demographics and primary/secondary outcomes is available here:

https://doi.org/10.6084/m9.figshare.24442699

The dataset for tertiary outcomes can be provided by the authors pending scientific review and a completed material transfer agreement. Requests for this dataset should be submitted to the corresponding author.

Supplementary Material

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	Colostrum	N-acetyl glucosamine	Teduglutide	Budesonide	Standard care
Preparation	Powder	Powder	Ampoule	Liquid	-
Route	Oral	Oral	Subcutaneous	Oral	-
N	25	25	25	25	25
Dose:					-
Days 1-7	1.5g tds	300mg tds	0.05mg/kg daily	1mg tds	
Days 8-11	1.5g tds	300mg tds	0.05mg/kg daily	1mg bd	
Days 12 – 14	1.5g tds	600mg tds	0.05mg/kg daily	0.5 mg bd	
Cost	£28 for 200g[1]	USD23.20 for 250g[2].	£7,307 for 28 1.25mg vials[3]	£41.19 for 20 1mg vials[3]	

Supplementary Table 1: Investigatory medical products and their preparations

[1]Costs taken from Colostrum UK; www.neovite.com. [2]Costs taken from Blackburn Distributions; www.blackburndistributions.com, [3]Costs are taken from https://bnf.nice.org.uk and are the UK nationally agreed prices, correct as of September 2022. Tds: four times a day; bd: twice a day.

	Protein ID	Full name	Method	Sample type	Limit of detection (LOD)
Inflammatory	**CRP**	C-reactive protein	ELISA	Plasma	0.004 mg/L
	sCD14	Soluble CD14	ELISA	Plasma	900000 pg/mL
	LBP	Lipopolysaccharide-binding-protein	ELISA	Plasma	200 ng/mL
	sCD163	Soluble CD163	ELISA	Plasma	250 pg/mL
	TNFα	Tumour necrosis factor-alpha	Luminex panel	Plasma	20 pg/mL
	IL-6	Interleukin-6	Luminex panel	Plasma	0.13pg/mL
	IL-33	Interleukin-33	Luminex panel	Plasma	47pg/mL
	IL-8	Interleukin-8	Luminex panel	Plasma	8.6pg/mL
	IL-10	Interleukin-10	Luminex panel	Plasma	0.72 pg/mL
	IL-2	Interleukin-2	Luminex panel	Plasma	13pg/mL
	IL-1β	Interleukin-1β	Luminex panel	Plasma	15pg/mL
	IFNγ	Interferon-gamma	Luminex panel	Plasma	71pg/mL
	IL-1ra	Interleukin -1 receptor antagonist	Luminex panel	Plasma	310 pg/mL
	CCL3	Chemokine (C-C motif) ligand 3	Luminex panel	Plasma	88pg/mL
	CCL4	Chemokine (C-C motif) ligand 4	Luminex panel	Plasma	650pg/mL
	D-dimer	D-dimer	Luminex panel	Plasma	235,000pg/mL
Endothelial	**P-selectin**	P-selectin	Luminex panel	Plasma	11900 pg/mL
	L-selectin	L-selectin	Luminex panel	Plasma	76000 pg/mL
	VCAM-1	Vascular cell adhesion molecule 1	Luminex panel	Plasma	210000 pg/mL
	ICAM-1	Intercellular adhesion molecule 1	Luminex panel	Plasma	3500 pg/mL
	TPO	Thrombopoietin	Luminex panel	Plasma	1600 pg/mL
	Eotaxin	Eotaxin-1 (CCL11)	Luminex panel	Plasma	29.5pg/mL
Growth	**GM-CSF**	Granulocyte-macrophage colony-stimulating factor	Luminex panel	Plasma	2.12pg/mL
	GCSF	Granulocyte colony-stimulating factor	Luminex panel	Plasma	55pg/mL
	EGF	Epidermal growth factor	Luminex panel	Plasma	2.98 pg/mL
	VEGF	Vascular endothelial growth factor	Luminex panel	Plasma	23 pg/mL
	Angiopoietin	Angiopoietin-1	Luminex panel	Plasma	30 pg/mL
	PlGF	Placental growth factor	Luminex panel	Plasma	1pg/mL
	IGFBP-3	IGF-binding protein 3	Luminex panel	Plasma	640 pg/mL
Enteropathy	**GLP-2**	Glucagon-like peptide 2	ELISA	Plasma	0.3 ng/mL
	IFABP	Intestinal fatty-acid binding protein	ELISA	Plasma	153 pg/mL
	MPO	Myeloperoxidase	ELISA	Stool	3.99 ng/mL
	Neopterin	Neopterin	ELISA	Stool	4.7 nmol/L
	α1-AT	Alpha-1-antitrypsin	ELISA	Stool	0.007 mg/mL
	LPS	Lipopolysaccharide (also called endotoxin)	LAL assay	Plasma	0.002EU/mL

Supplementary Table 2: List of biomarkers analysed

The Luminex panel was one single 25-plex panel (Assay code: UgRYKY2M; R&D Systems, Minneapolis, USA). ELISAs were run separately.

ELISA kits (R&D Systems, Minneapolis, USA) were used to analyse CRP (cat number:SCRP00) and sCD14 (cat number: DC140) at 1:200 dilution; and CD163 (cat number: DC1630) and LBP (cat number: DY870) at 1:40 dilution. GLP-2 (Merck, Darmstadt, Germany; cat number: EZGLP2) was analysed without dilution. IFABP (HycultBiotech, Uden , The Netherlands; Cat number: HK406) was analysed following 1:2 dilution. MPO (Immunodiagnostik, Bensheim, Germany; cat number: K6630) was analysed following 1:500 dilution. α1-AT (ImmunoChrom, Heppenheim, Germany; Cat number: IC6200) was analysed following 1:12500 dilution. Neopterin (Arigobio, Hsinchu, Taiwan; Cat number: ARG80878) was analysed following 1:50 dilution.

		Baseline [n=125]					Day 15 [n=122]				
				Included		Miss-ing			Included		Miss-ing
	Biomarker (pg/mL, unless stated)	**Median**	**[IQR]**	total	*of which, OOR*		**Median**	**[IQR]**	total	*of which, OOR*	
Inflammatory	CRP (mg/L)	1.823	[0.698-6.616]	n=123		2	0.910	[0.315-3.819]	n=121		1
	CD-163 (ng/mL)	1,191	[825-1,808]	n=123		2	1,104	[838-1,562]	n=121		1
	sCD14 (ug/mL)	2.2	[1.8-2.6]	n=123		2	2.0	[1.7-2.5]	n=121		1
	LBP (ng/mL)	5,982	[4,302-8,592]	n=123		2	5,840	[4,219-8,092]	n=121		1
	TNF-α	50.8	[38.5-65.1]	n=123		2	45.4	[34.3-61.6]	n=120		2
	IL-6	32.2	[11.9-40.9]	n=123		2	26.6	[7.7-35.8]	n=120	*1*	2
	IL-33	115.5	[93.9-135.1]	n=123		2	101.1	[86.3-127.6]	n=120		2
	IL-1β	54.1	[38.2-64.4]	n=123		2	46.8	[31.4-59.2]	n=120		2
	Interferon-γ	173	[143-211]	n=123		2	158	[125-206]	n=120		2
	IL-1ra	1,344	[864-2,232]	n=123		2	847	[647-1,337]	n=120		2
	CCL3	452	[241-525]	n=123		2	435	[220-499]	n=120		2
	CCL4	1,073	[920-1,222]	n=123		2	982	[860-1,147]	n=120		2
	IL-8	43.2	[33.3-59.3]	n=123		2	33.9	[26.6-45.7]	n=120		2
	IL-2	81.5	[43.8-98.9]	n=123		2	72.0	[34.4-92.6]	n=120		2
	IL-10	13.4	[8.9-17.2]	n=122		3	11.9	[6.2-15.9]	n=120		2
	D-dimer (ug/mL)	1.8	[1.1-2.4]	n=123		2	1.8	[1.3-2.7]	n=120		2
Enteropathy	α1-AT (/mg/mL)	0.114	[0.051-0.379]	n=121		4	0.332	[0.164-0.672]	n=117	*7*	5
	MPO (ng/mL)	1,361	[441-5,217]	n=121	*2*	4	809	[355-1,670]	n=117	*5*	5
	Neopterin (nmol/L)	372.4	[195.5-731.7]	n=120	*1*	5	576.3	[326.7-991.5]	n=118	*3*	4
	GLP-2 (ng/mL)	5.7	[3.7-8.0]	n=123	*1*	2	4.4	[3.0-5.9]	n=121	*1*	1
	IFABP	1,555	[795-2,680]	n=123		2	1,633	[1,027-2,893]	n=121		1
	LPS (EU/mL)	0.005	[0.002-0.015]	n=108	*47*	17	0.009	[0.004-0.034]	n=105	*34*	17
Endothelial	L-selectin (ng/mL)	777.1	[479.0-1072.9]	n=123		2	564.7	[383.3-915.4]	n=120		2
	P-selectin (ng/mL)	34	[27-40]	n=123		2	29	[24-34]	n=120		2
	Thrombopoietin	3,645	[2,976-4,175]	n=123		2	3,174	[2,678-4,065]	n=120		2
	VCAM-1 (ug/mL)	1.6	[1.2-2.1]	n=123		2	1.3	[1.0-1.8]	n=120		2
	ICAM-1	587	[370-897]	n=123		2	558	[361-790]	n=120		2
	Eotaxin	142.8	[104.6-182.1]	n=123	*5*	2	129.5	[90.4-167.4]	n=120	*5*	2
Growth	EGF	22.3	[16.8-33.4]	n=123		2	22.6	[17.4-33.8]	n=120		2
	VEGF	75.5	[57.6-104.8]	n=123		2	82.6	[63.3-120.4]	n=120		2
	PlGF	71.1	[8.1-88.2]	n=123		2	62.0	[7.0-78.5]	n=120		2
	Angiopoietin	5,583	[2,159-10,697]	n=123		2	4,420	[1,835-12,944]	n=120		2
	GCSF	197.8	[149.0-235.3]	n=123		2	184.0	[151.1-226.9]	n=120		2
	GM-CSF	24.9	[6.0-40.0]	n=123	*24*	2	20.4	[2.0-33.8]	n=120	*34*	2
	IGFBP-3 (ng/mL)	135	[68-200]	n=123		2	198	[92-342]	n=120		2

Supplementary Table 3: Biomarkers analysed, number of samples, and results shown at baseline, day 15 (D15) and the overall results

The total samples analysed is shown, and the number of this total which includes samples out of range (OOR), which were included at (limit of detection /√2) are shown. The number of samples missing, that being the number of children in the trial at that time without any result is shown. Missingness was due to insufficiency of samples.

	Biomarker (log_{10}) in pg/mL unless stated	Colostrum D15 level compared with SOC[1]	Colostrum 90% CI	Colostrum p-value	Colostrum *Adj p-value*[2]	NAG D15 level compared with SOC[1]	NAG 90% CI	NAG p-value	NAG *Adj p-value*[2]	Teduglutide D15 level compared with SOC[1]	Teduglutide 90% CI	Teduglutide p-value	Teduglutide *Adj p-value*[2]	Budesonide D15 level compared with SOC[1]	Budesonide 90% CI	Budesonide p-value	Budesonide *Adj p-value*[2]
Inflammatory	CRP (mg/L)	-0.20	(-0.52, 0.12)	0.30	*0.88*	-0.26	(-0.57, 0.06)	0.18	*0.67*	-0.17	(-0.48, 0.14)	0.37	*0.83*	**-0.39****	**(-0.71,-0.07)**	**0.048**	*0.56*
	CD-163 (ng/mL)	-0.04	(-0.13, 0.05)	0.46	*0.88*	-0.03	(-0.12, 0.05)	0.54	*0.78*	-0.07	(-0.16, 0.02)	0.18	*0.83*	**-0.11****	**(-0.20,-0.02)**	**0.046**	*0.56*
	sCD14	-0.02	(-0.09, 0.04)	0.57	*0.88*	0.04	(-0.02, 0.11)	0.25	*0.68*	0.03	(-0.03, 0.09)	0.42	*0.83*	0.00	(-0.06, 0.06)	1.00	*1.00*
	LBP (ng/mL)	-0.06	(-0.17, 0.06)	0.42	*0.88*	-0.01	(-0.13, 0.10)	0.84	*0.89*	-0.08	(-0.20, 0.03)	0.22	*0.83*	-0.08	(-0.20, 0.04)	0.27	*0.72*
	TNF-α	-0.02	(-0.08, 0.04)	0.55	*0.88*	0.01	(-0.05, 0.06)	0.84	*0.89*	0.02	(-0.04, 0.07)	0.61	*0.83*	-0.04	(-0.10, 0.01)	0.19	*0.70*
	IL-6	0.03	(-0.08, 0.15)	0.63	*0.88*	0.06	(-0.05, 0.17)	0.34	*0.75*	**0.12***	**(0.01, 0.23)**	**0.07**	*0.83*	0.01	(-0.10, 0.12)	0.90	*0.99*
	IL-33	0.01	(-0.04, 0.07)	0.68	*0.88*	0.02	(-0.03, 0.08)	0.45	*0.77*	0.03	(-0.03, 0.08)	0.42	*0.83*	-0.02	(-0.07, 0.04)	0.60	*0.95*
	IL-1β	-0.01	(-0.07, 0.04)	0.68	*0.88*	0.02	(-0.03, 0.08)	0.47	*0.77*	0.04	(-0.01, 0.10)	0.17	*0.83*	-0.01	(-0.07, 0.04)	0.70	*0.95*
	Interferon-γ	0.00	(-0.05, 0.06)	0.93	*1.00*	0.03	(-0.03, 0.08)	0.43	*0.77*	0.02	(-0.03, 0.08)	0.46	*0.83*	-0.01	(-0.06, 0.05)	0.77	*0.95*
	IL-1ra	-0.10	(-0.23, 0.03)	0.22	*0.88*	0.08	(-0.05, 0.21)	0.29	*0.68*	0.02	(-0.11, 0.15)	0.81	*0.90*	0.02	(-0.11, 0.16)	0.76	*0.95*
	CCL3	0.03	(-0.04, 0.09)	0.51	*0.88*	0.05	(-0.01, 0.11)	0.21	*0.67*	0.06	(-0.00, 0.12)	0.11	*0.83*	-0.01	(-0.07, 0.05)	0.79	*0.95*
	CCL4	0.02	(-0.03, 0.07)	0.46	*0.88*	0.03	(-0.01, 0.08)	0.23	*0.67*	0.03	(-0.01, 0.08)	0.20	*0.83*	-0.00	(-0.05, 0.05)	0.98	*1.00*
	IL-8	-0.00	(-0.09, 0.08)	0.96	*1.00*	0.04	(-0.05, 0.12)	0.46	*0.77*	-0.00	(-0.08, 0.08)	0.97	*0.97*	-0.02	(-0.11, 0.06)	0.65	*0.95*
	IL-2	0.01	(-0.06, 0.08)	0.77	*0.90*	0.01	(-0.06, 0.08)	0.81	*0.89*	0.02	(-0.05, 0.09)	0.61	*0.83*	-0.04	(-0.11, 0.03)	0.31	*0.77*
	IL-10	-0.00	(-0.12, 0.11)	0.99	*1.00*	-0.00	(-0.12, 0.11)	0.98	*0.98*	0.05	(-0.06, 0.16)	0.45	*0.83*	-0.01	(-0.13, 0.10)	0.84	*0.95*
	D-dimer	-0.03	(-0.13, 0.08)	0.67	*0.88*	0.02	(-0.08, 0.12)	0.69	*0.84*	-0.03	(-0.13, 0.06)	0.58	*0.83*	-0.09	(-0.19, 0.01)	0.16	*0.69*
Enteropathy	α1-AT (mg/mL)	-0.00	(-0.20, 0.20)	1.00	*1.00*	0.06	(-0.14, 0.26)	0.61	*0.82*	0.01	(-0.18, 0.20)	0.93	*0.96*	0.08	(-0.13, 0.28)	0.54	*0.95*
	MPO (ng/mL)	0.08	(-0.27, 0.43)	0.71	*0.88*	0.26	(-0.08, 0.61)	0.21	*0.67*	0.07	(-0.28, 0.41)	0.75	*0.87*	0.34	(-0.02, 0.70)	0.12	*0.68*
	Neopterin (nmol/L)	-0.05	(-0.31, 0.20)	0.73	*0.88*	0.22	(-0.03, 0.47)	0.15	*0.67*	**0.26***	**(0.02, 0.51)**	**0.08**	*0.83*	0.07	(-0.18, 0.33)	0.64	*0.95*
	GLP-2 (ng/mL)	**0.12***	**(0.01, 0.23)**	**0.08**	*0.88*	0.03	(-0.07, 0.14)	0.60	*0.82*	0.03	(-0.08, 0.13)	0.65	*0.83*	-0.02	(-0.13, 0.09)	0.81	*0.95*
	IFABP (/pg/mL)	0.11	(-0.06, 0.29)	0.29	*0.88*	0.17	(-0.00, 0.34)	0.10	*0.67*	0.15	(-0.02, 0.32)	0.14	*0.83*	0.10	(-0.08, 0.27)	0.37	*0.85*
	LPS (EU/mL)	**-0.49***	**(-0.97,-0.02)**	**0.09**	*0.88*	-0.10	(-0.53, 0.33)	0.70	*0.84*	-0.11	(-0.54, 0.31)	0.66	*0.83*	-0.43	(-0.85, 0.00)	0.10	*0.68*
Endothelial	L-selectin	0.02	(-0.07, 0.10)	0.73	*0.88*	**0.10***	**(0.01, 0.18)**	**0.05**	*0.63*	0.06	(-0.02, 0.14)	0.25	*0.83*	-0.02	(-0.10, 0.07)	0.74	*0.95*
	P-selectin	-0.03	(-0.08, 0.03)	0.46	*0.88*	0.01	(-0.05, 0.06)	0.80	*0.89*	0.01	(-0.04, 0.07)	0.67	*0.83*	-0.04	(-0.10, 0.01)	0.21	*0.70*
	Thrombopoietin	0.02	(-0.04, 0.08)	0.55	*0.88*	0.02	(-0.03, 0.08)	0.46	*0.77*	0.03	(-0.02, 0.09)	0.32	*0.83*	-0.01	(-0.07, 0.04)	0.75	*0.95*
	VCAM-1	-0.07	(-0.15, 0.02)	0.20	*0.88*	0.04	(-0.05, 0.12)	0.48	*0.77*	0.03	(-0.05, 0.12)	0.50	*0.83*	-0.02	(-0.11, 0.06)	0.64	*0.95*
	ICAM-1	-0.01	(-0.07, 0.05)	0.72	*0.88*	0.01	(-0.05, 0.07)	0.86	*0.89*	0.01	(-0.04, 0.07)	0.68	*0.83*	**-0.06***	**(-0.12,-0.00)**	**0.09**	*0.68*
	Eotaxin	-0.05	(-0.17, 0.08)	0.54	*0.88*	-0.05	(-0.17, 0.07)	0.52	*0.78*	-0.05	(-0.16, 0.07)	0.51	*0.83*	-0.06	(-0.19, 0.06)	0.39	*0.85*
Growth	EGF	0.11	(-0.03, 0.26)	0.20	*0.88*	0.11	(-0.03, 0.25)	0.21	*0.67*	0.06	(-0.08, 0.20)	0.46	*0.83*	-0.02	(-0.16, 0.12)	0.83	*0.95*
	VEGF	-0.00	(-0.13, 0.13)	0.99	*1.00*	0.10	(-0.03, 0.23)	0.20	*0.67*	0.01	(-0.11, 0.13)	0.90	*0.96*	-0.00	(-0.13, 0.12)	0.95	*1.00*
	PlGF	-0.02	(-0.11, 0.07)	0.68	*0.88*	-0.02	(-0.11, 0.06)	0.64	*0.83*	0.04	(-0.05, 0.12)	0.48	*0.83*	-0.08	(-0.17, 0.01)	0.15	*0.69*
	Angiopoietin	**0.26***	**(0.01, 0.51)**	**0.09**	*0.88*	**0.35****	**(0.10, 0.59)**	**0.02**	*0.50*	0.22	(-0.02, 0.46)	0.13	*0.83*	0.18	(-0.07, 0.42)	0.24	*0.71*
	GCSF	0.06	(-0.01, 0.14)	0.17	*0.88*	**0.10****	**(0.03, 0.17)**	**0.03**	*0.50*	0.02	(-0.05, 0.09)	0.69	*0.83*	0.01	(-0.06, 0.09)	0.80	*0.95*
	GM-CSF	-0.08	(-0.22, 0.07)	0.39	*0.88*	-0.09	(-0.23, 0.05)	0.28	*0.68*	-0.02	(-0.16, 0.12)	0.82	*0.90*	**-0.24*****	**(-0.38,-0.10)**	**0.007**	*0.23*
	IGFBP-3	0.07	(-0.12, 0.26)	0.55	*0.88*	**0.20***	**(0.01, 0.39)**	**0.08**	*0.67*	0.09	(-0.10, 0.27)	0.44	*0.83*	0.14	(-0.05, 0.34)	0.22	*0.70*

*** p-value<0.01, ** p-value<0.05, * p-value<0.10

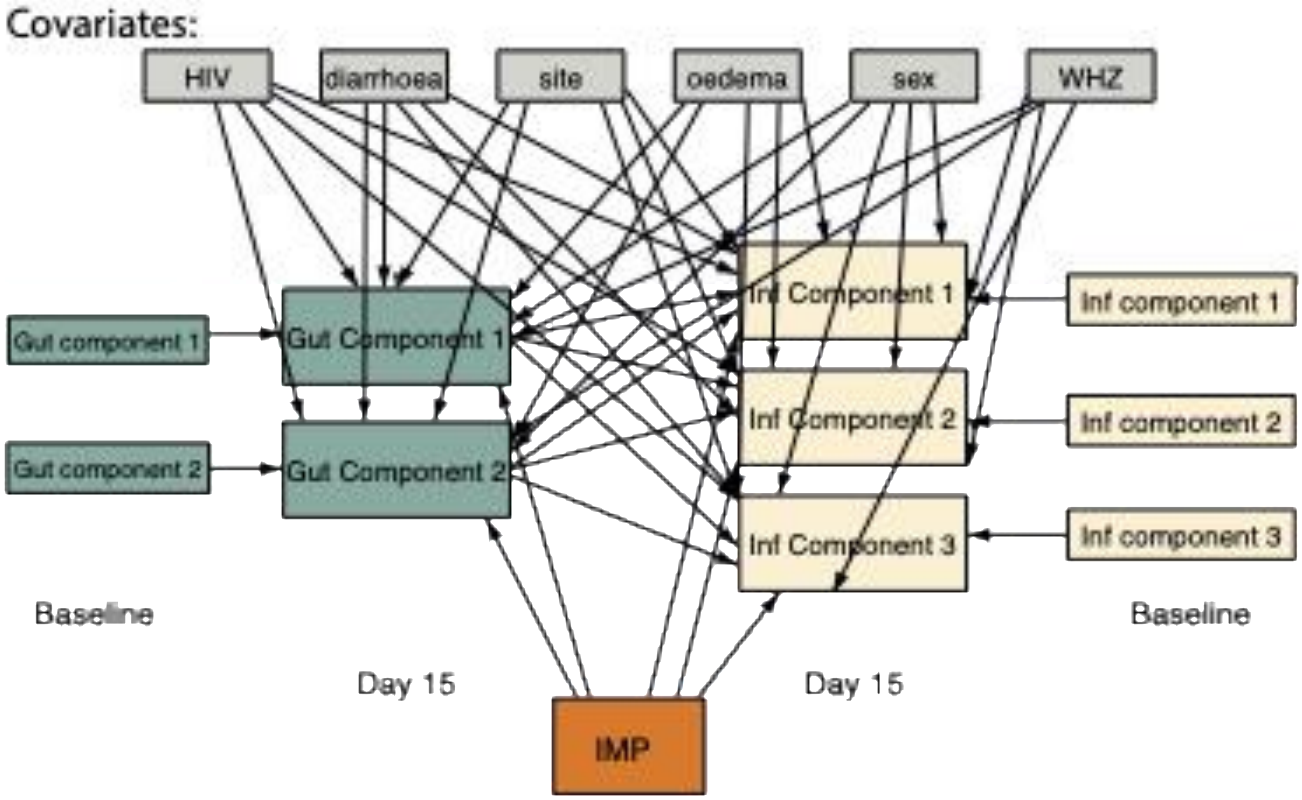
Supplementary Table 4: Changes of the D15 biomarker concentration attributable to randomized intervention, over the standard of care (SOC) group

[1]Results show the change in D15 biomarker concentration associated with the intervention, adjusted for the baseline biomarker level, HIV, sex, site, diarrhoea, WHZ, and oedema. [2]The Benjamini-Hochberg adjusted P-value is included for reference only and has not been used to adjust any significance. P-values were generated using Ancova as described in the methods.

	Budesonide					Teduglutide					N-acetylglucosamine					Colostrum				
Variable	**Inf1**	**Inf2**	**Inf3**	**Ent1**	**Ent2**	**Inf1**	**Inf2**	**Inf3**	**Ent1**	**Ent2**	**Inf1**	**Inf2**	**Inf3**	**Ent1**	**Ent2**	**Inf1**	**Inf2**	**Inf3**	**Ent1**	**Ent2**
Hiv	0.01	-0.21	0.04	-0.33	-0.19	-0.03	0.03	0.05	0.05	0.07	-0.04	0.01	0.05	-0.03	-0.09	0.00	0.00	0.11	-0.11	-0.02
	(0.96)	(0.06)	(0.79)	(0.01)	(0.17)	(0.77)	(0.79)	(0.68)	(0.66)	(0.64)	(0.66)	(0.90)	(0.69)	(0.80)	(0.53)	(0.97)	(0.97)	(0.37)	(0.41)	(0.85)
Site	0.42	-0.15	-0.19	0.02	0.06	0.50	-0.17	-0.03	-0.13	0.07	0.35	-0.08	-0.22	0.04	0.18	0.54	0.02	-0.23	0.12	0.04
	(0.003)	(0.15)	(0.22)	(0.90)	(0.70)	-	(0.14)	(0.82)	(0.31)	(0.63)	(0.01)	(0.44)	(0.13)	(0.76)	(0.27)	-	(0.86)	(0.09)	(0.40)	(0.78)
Oedema	0.21	0.01	0.17	0.05	0.06	0.13	-0.03	0.23	-0.16	0.13	0.05	0.02	0.04	0.02	-0.08	0.02	0.09	0.12	0.02	-0.18
	(0.05)	(0.95)	(0.19)	(0.69)	(0.69)	(0.17)	(0.77)	(0.10)	(0.24)	(0.41)	(0.59)	(0.88)	(0.77)	(0.88)	(0.62)	(0.81)	(0.40)	(0.34)	(0.91)	(0.19)
Diarrhoea	-0.03	0.06	0.05	0.20	0.01	-0.06	-0.05	-0.08	0.29	-0.12	-0.06	-0.26	-0.14	0.15	-0.18	0.05	-0.10	-0.18	0.17	-0.26
	(0.76)	(0.59)	(0.72)	(0.14)	(0.94)	(0.50)	(0.65)	(0.54)	(0.02)	(0.39)	(0.51)	(0.02)	(0.25)	(0.26)	(0.24)	(0.59)	(0.38)	(0.12)	(0.18)	(0.05)
Whz	-0.25	0.06	-0.02	0.36	0.02	-0.27	-0.18	-0.14	0.49	-0.23	-0.22	-0.28	-0.11	0.40	0.17	-0.22	-0.27	-0.06	0.39	0.07
	(0.03)	(0.57)	(0.87)	(0.01)	(0.90)	(0.02)	(0.16)	(0.38)	(0.00)	(0.14)	(0.03)	(0.02)	(0.45)	(0.01)	(0.29)	(0.03)	(0.03)	(0.65)	(0.01)	(0.64)
Random	0.00	-0.13	0.09	-0.04	0.27	0.09	-0.08	0.07	0.01	0.13	0.11	-0.05	0.20	0.02	0.18	0.04	-0.27	0.09	0.20	0.18
	(0.99)	(0.21)	(0.50)	(0.78)	(0.05)	(0.30)	(0.42)	(0.58)	(0.91)	(0.36)	(0.21)	(0.59)	(0.09)	(0.85)	(0.23)	(0.62)	(0.02)	(0.45)	(0.12)	(0.17)
Inf1(base)	0.42					0.40					0.54					0.31				
	(0.002)					(0.001)					-					(0.01)				
Inf2(base)		0.74					0.61					0.74					0.67			
		-					-					-					-			
Inf3(base)			0.49					0.51					0.43					0.57		
			(0.00)					(0.001)					(0.002)					-		
Ent1(base)				0.32					0.44					0.43					0.30	
				(0.01)					-					(0.003)					(0.04)	
Ent2(base)					0.45					0.46					0.28					0.52
					(0.00)					(0.003)					(0.10)					-
Ent1	0.18	-0.09	0.25			0.27	0.21	0.26			0.35	0.19	0.39			0.13	0.15	0.22		
	(0.10)	(0.42)	(0.10)			(0.01)	(0.08)	(0.07)			(0.001)	(0.08)	(0.01)			(0.20)	(0.23)	(0.10)		
Ent2	-0.13	0.09	-0.04			-0.24	0.39	-0.19			-0.15	0.09	-0.06			-0.31	0.17	0.00		
	(0.23)	(0.39)	(0.75)			(0.01)	(0.001)	(0.17)			(0.12)	(0.40)	(0.67)			(0.00)	(0.13)	(0.99)		

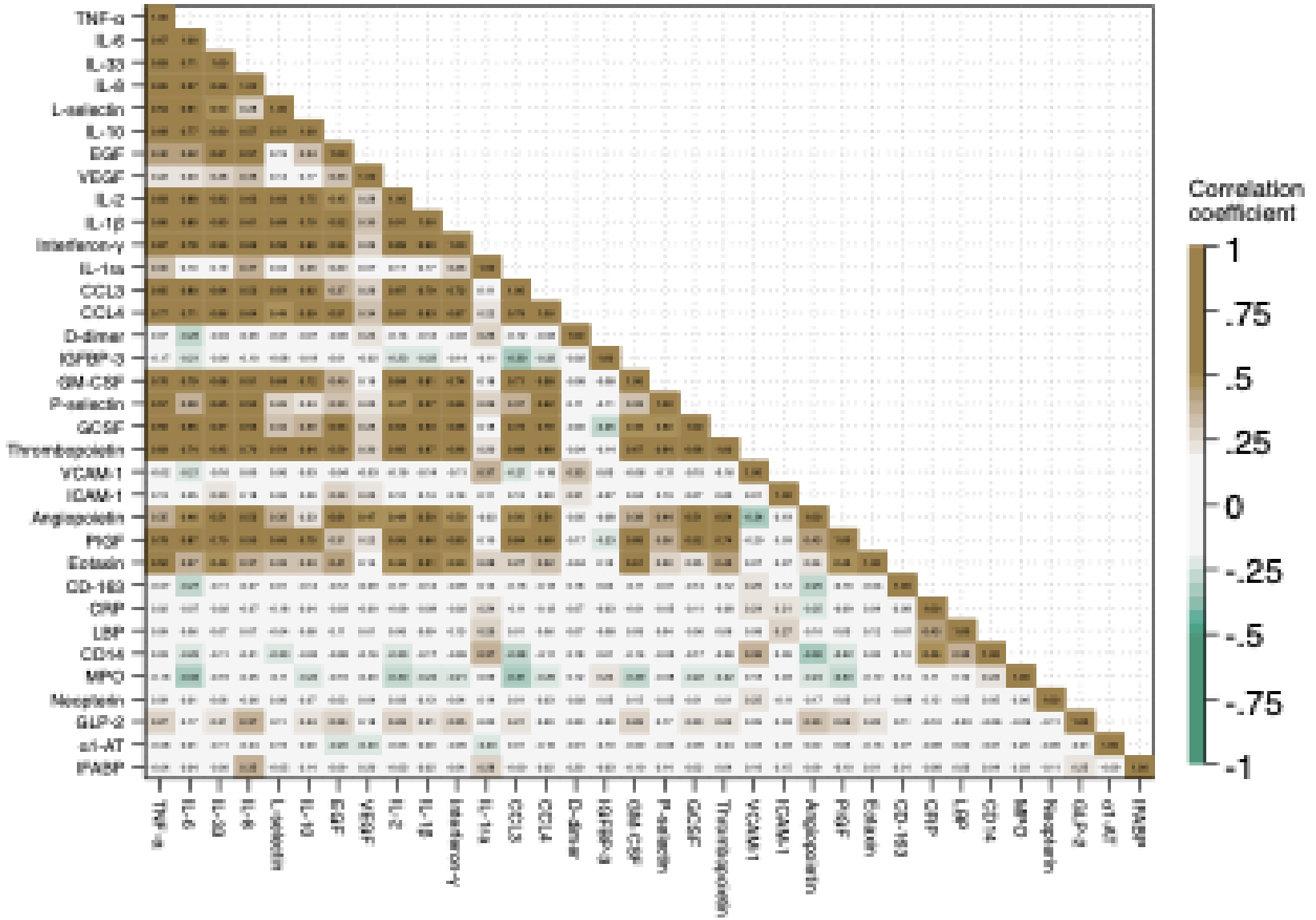
Supplementary Table 5: Standardized path coefficients from PLS-Path modelling by each randomization group

Standardized path coefficients with the appropriate p-value from the PLS modelling displayed beneath in brackets.



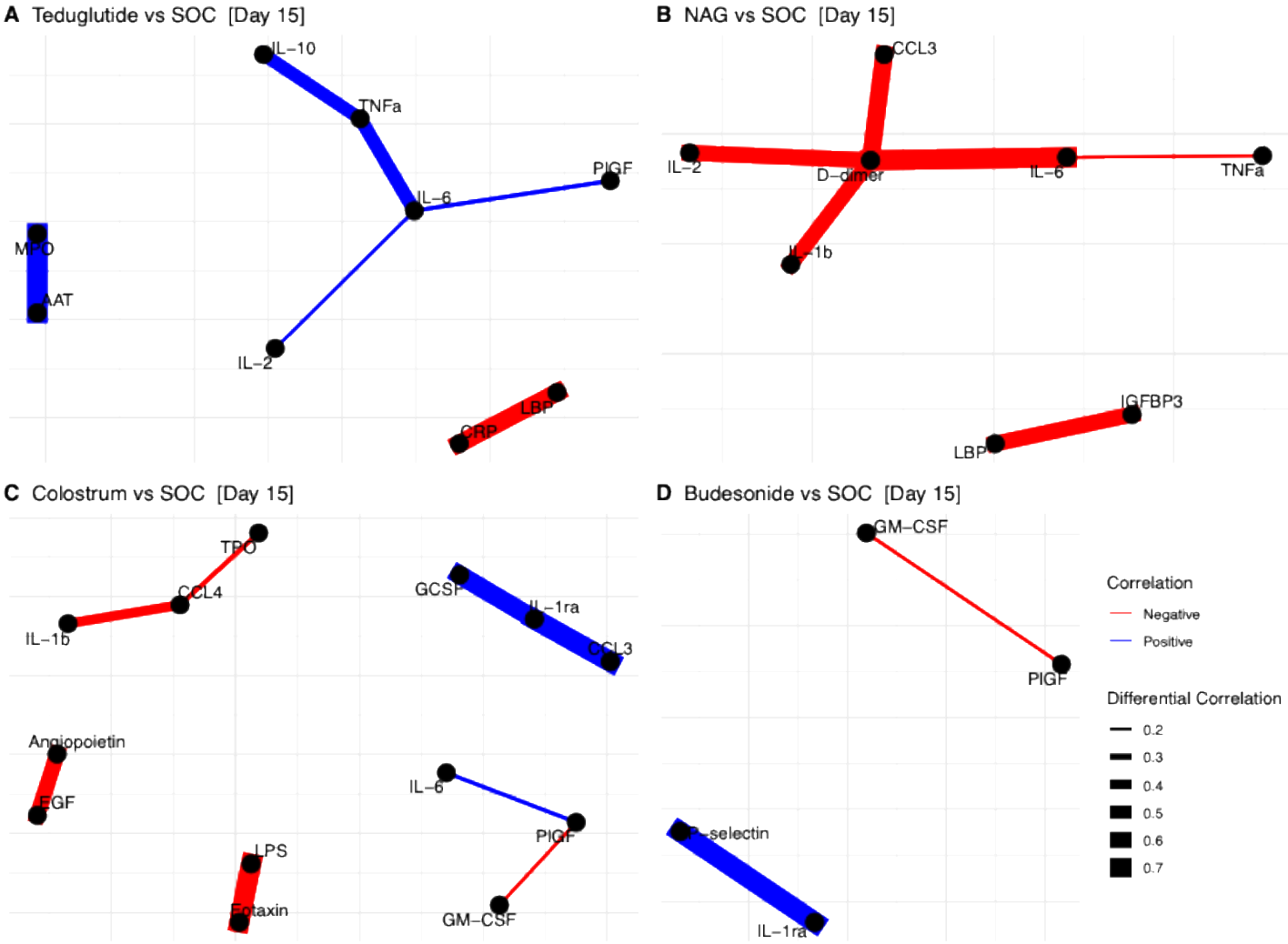
Supplementary Figure 1: Base model tested in structural path modelling

This shows all the base model tested by PLS-path modelling, with the results displayed in Figure 4, and full results shown in Supplementary Table 5. Connections were only shown in Figure 4 if they were significant. All were included in the model regardless of whether they were significant or not. IMP: investigatory medical product, which is the randomization group involved. The groups were tested separately against the standard of care arm.



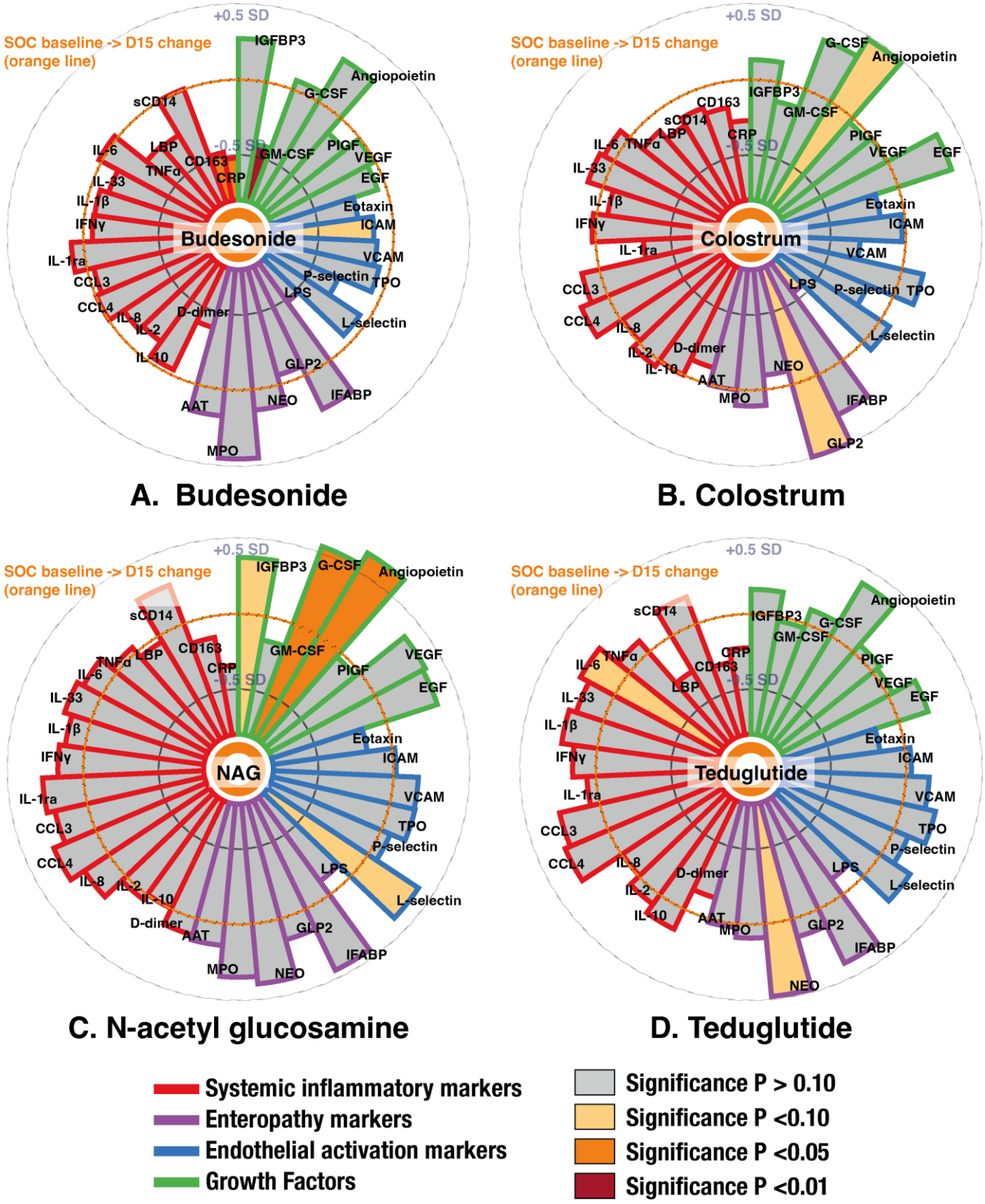
Supplementary Figure 2: Correlation coefficients between the biomarker levels at Day 15

Correlation coefficients between each of the 35 biomarkers concentrations, following normalization by $\log_{10}$ transformation.



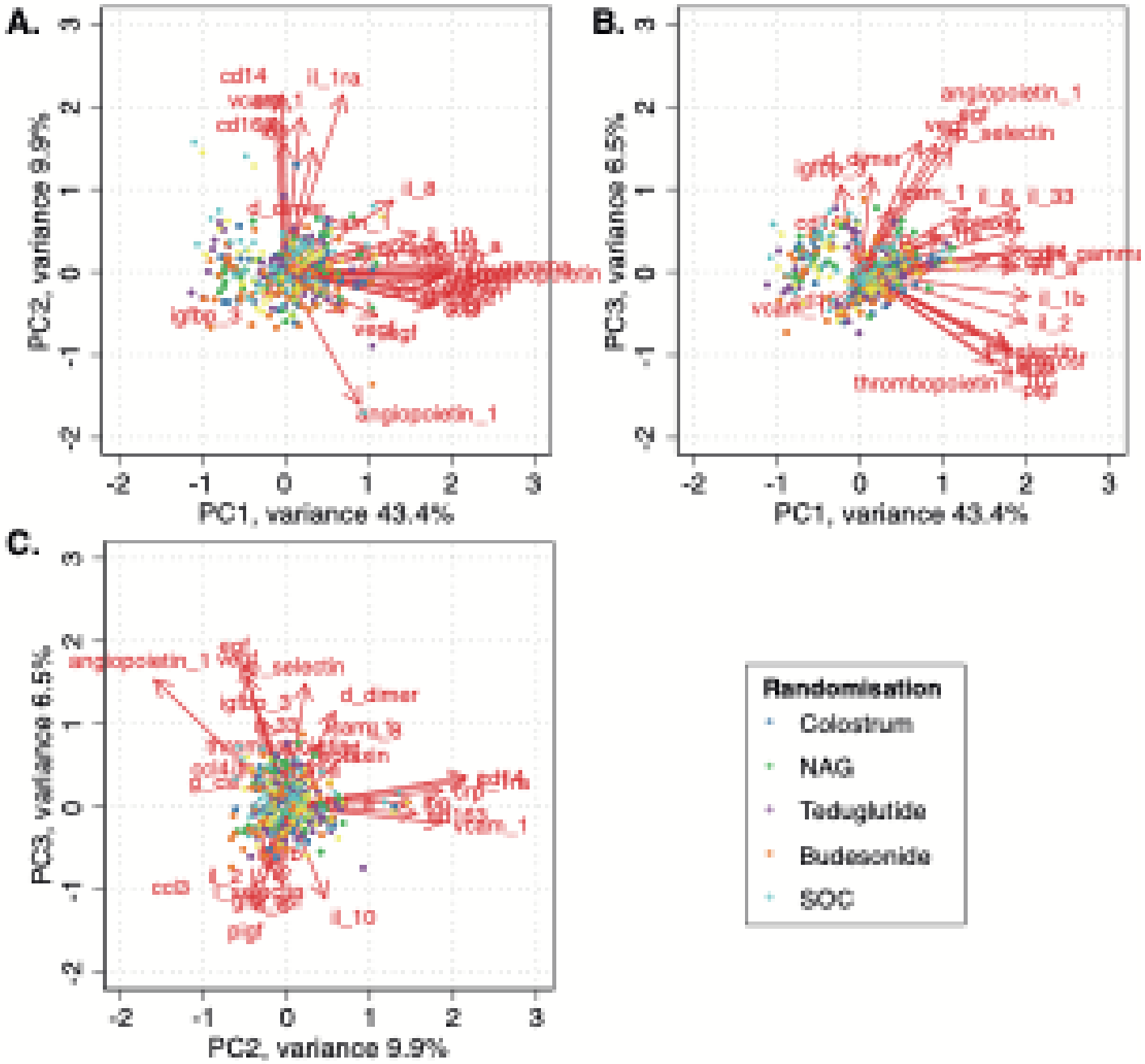
Supplementary Figure 3: Differential correlation network analysis between groups

The correlations between normalized $\log_{10}$ biomarker values at day 15, post-intervention, were compared between A) Teduglutide B) N-acetylglucosamine (NAG), C) colostrum, and D) Budesonide groups and the standard of care (SOC) using the Fisher's Z transformation of the Pearson correlation coefficient in each group, with significance determined by Fisher's Z test. The threshold of significance was P<0.10, with false discovery rate correction.



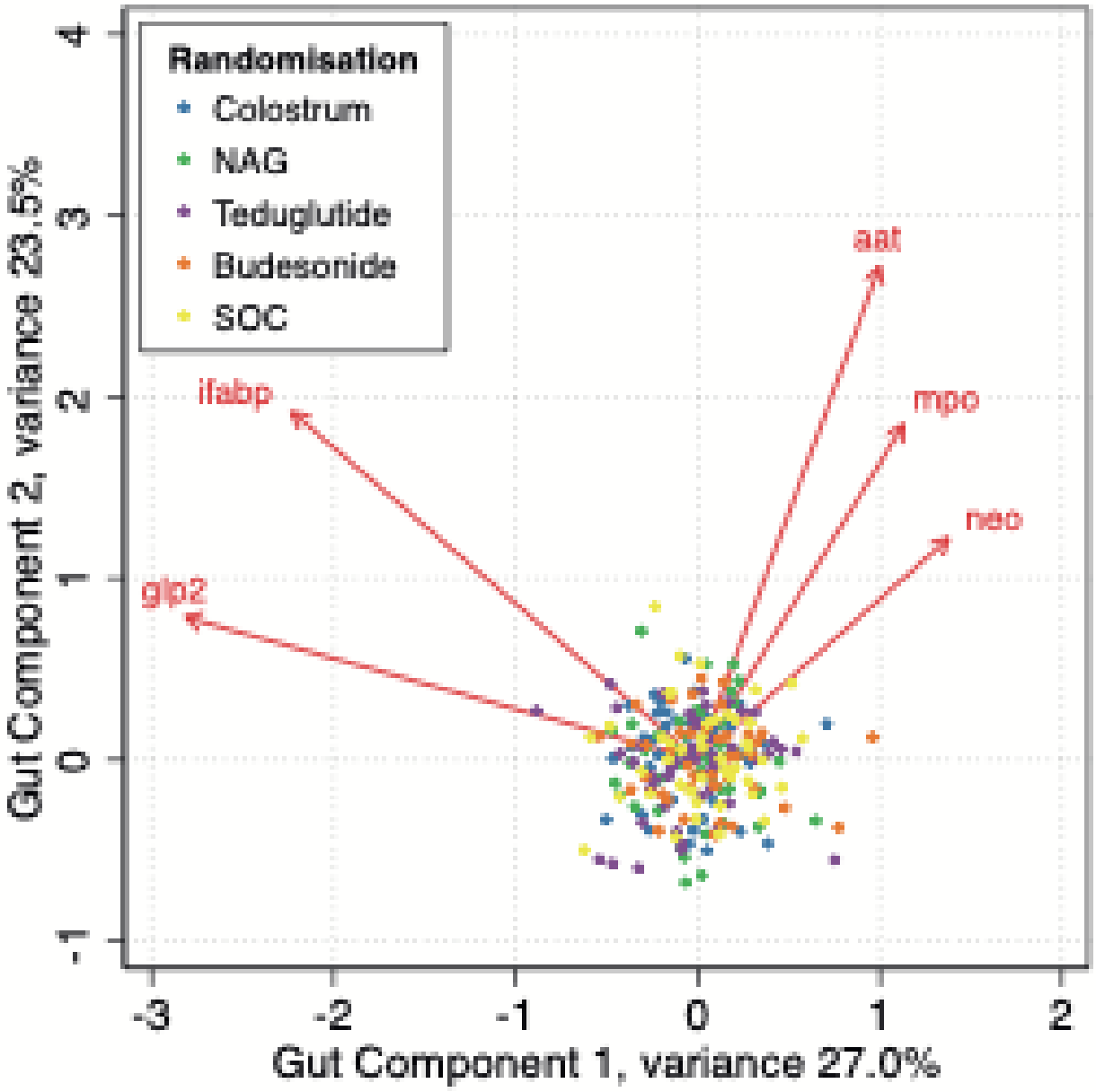
Supplementary Figure 4: Changes of the adjusted log10 D15 biomarker value attributable to randomized intervention, over the standard of care (SOC) group

Differences are shown in A) budesonide B) colostrum C) N-acetylglucosamine (NAG) and D) teduglutide groups. Results were adjusted for sex, oedema, HIV, diarrhoea, WHZ, site, and the baseline biomarker value. Each biomarker has its own assigned ray, and the length of the ray shows how the result differs in comparison to the SOC group, which is shown as the level of the labelled orange circle. If the ray is outside the orange circle, the biomarker value in this treatment group is higher than in the SOC group; if the ray is inside the orange circle the biomarker level in this treatment group is lower than in the SOC group. The ray is coloured according to the p value from the multivariable model. A p value threshold <0.10 from ancova modelling was pre-specified as statistically significant since this is a phase II trial.The full numerical results are shown in Supplementary Table 4.



Supplementary Figure 5: Principal component analysis plots of the all-timepoint results showing the individual participants split by randomization

This shows A) PC2 against PC1; B) PC3 against PC1; C) PC3 against PC2; the colours of the dots represent the randomization arms.



Supplementary Figure 6: PCA plots for the gut components at all timepoints

This shows A) Gut Component 2 against Gut Component 1; and B) the screeplot for the PCA analysis of gut biomarkers