



ASSOCIAZIONE ITALIANA EMATOLOGIA
ONCOLOGIA PEDIATRICA

**LA SOMMINISTRAZIONE ORALE DI LATTOFERRINA
PREVIENE LA NEUTROPENIA FEBBRILE E LA SEPSI
E MANTIENE L'EUBIOSI INTESTINALE:
studio di fase II, randomizzato in doppio cieco in bambini
oncoematologici in trattamento chemioterapico di induzione**

Nunzia Decembrino

IRCCS Policlinico San Matteo Pavia OEPed

AOU Policlinico G. Rodolico Catania

XLVIII

CONGRESSO NAZIONALE

AIEOP

Bologna

2-4 Ottobre 2023

Il sottoscritto Nunzia Decembrino

ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

dichiara

- ☐ *che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario*
- ☐ *che negli ultimi due anni ha avuto rapporti diretti di finanziamento con i seguenti soggetti portatori di interessi commerciali in campo sanitario:*

Febrile Neutropenia

US: incidence 7.8 cases/1000 patients
Europe: rate ~8 cases/1000 patients
Among pediatric cancer, 10.1%-22.7% of hospitalizations
Hospitalization costs up to \$880 million (>\$2 billion for adults)

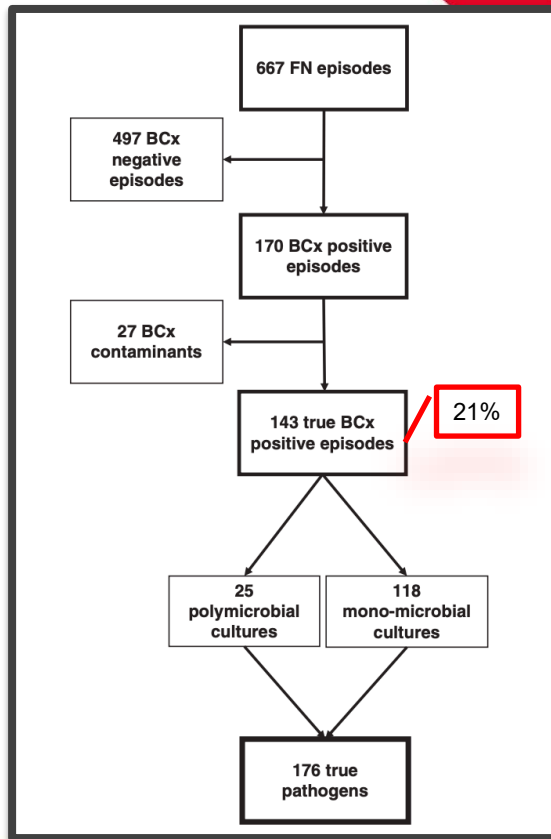
A multifactorial disease:

Infections

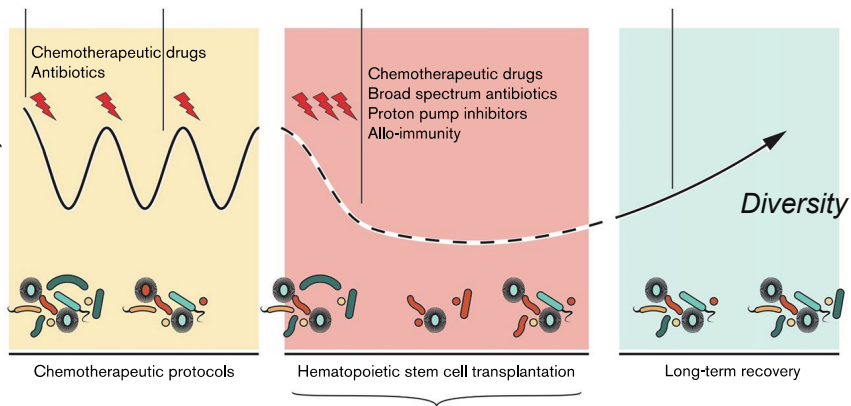
FUO

Cytokine storm

The Oncologist, 2022, 27, 625–636
J Pediatr Hematol Oncol, 2020, 42, 6,



Microbiome during chemotherapy



In HSCT patients, GM dysbiosis and *Enterococcus* overabundance have been linked with longer fever duration during neutropenia

Chemo-immunotherapy treatments can drastically impact GM, favoring the establishment of unbalanced low-diversity profiles, with potentially severe health implications even in the long-term.

Masetti R, 2021, *Blood Advances*, 5, 22
Masetti R, 2022, *Cancers*, 14, 1932

Lactoferrin

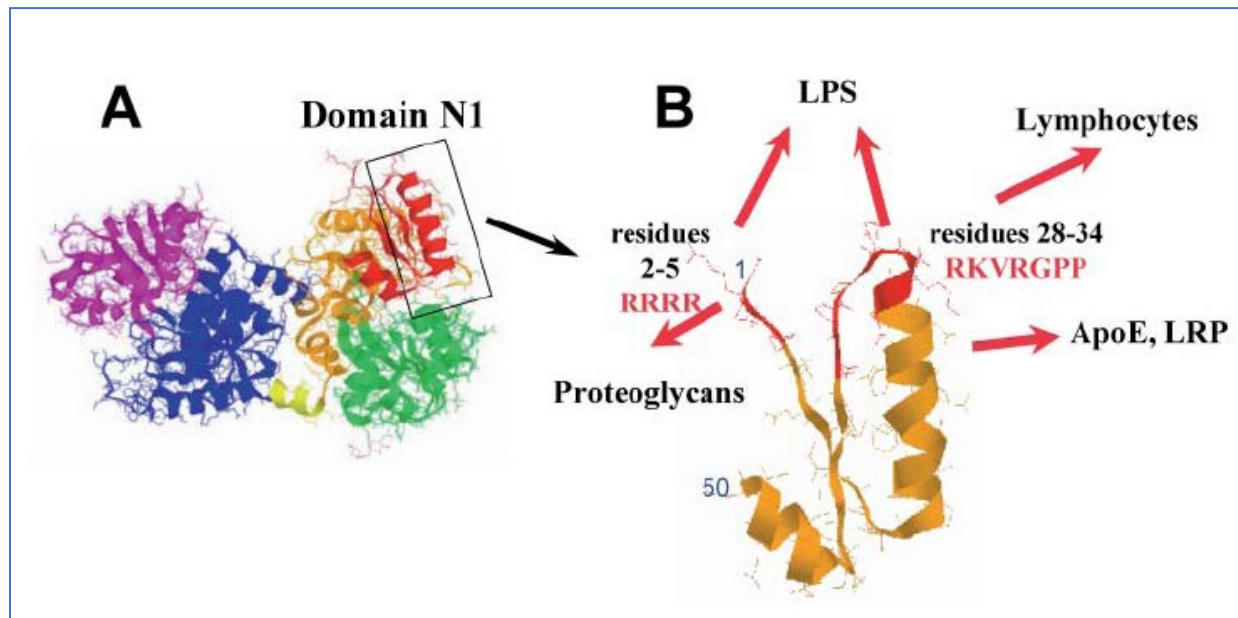
Fam. TRANSFERRINS

PRESENT IN:

Human milk
Tears
Saliva
Nasal secretions
Urogenital secretions
Respiratory secretions
Gastrointestinal fluids

RELEASED BY:

Epithelial cells
PMNs granules



Lactoferrin

BACTERIA

- Iron binding: bacteriostatic effect
- LPS-binding and Lipoteicoic ac.: bactericidal effect
- Preventing bacterial adhesion and entry to host cells
- Peptidoglycan degradation (synergic with lysozyme)

FUNGI

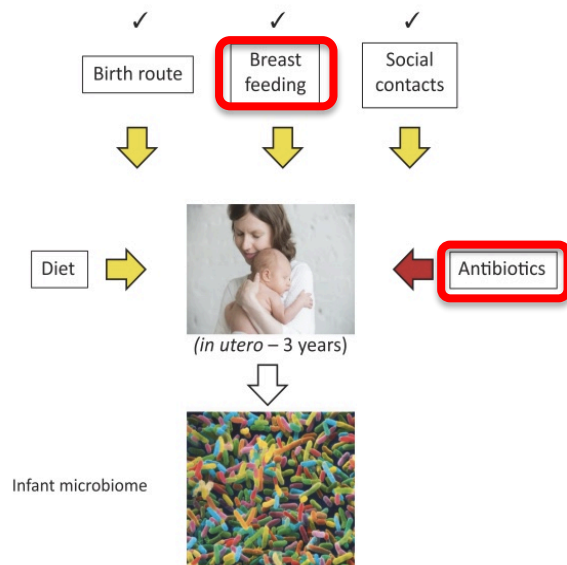
- Membrane disruption

VIRUSES

- Lf binding and blocking viral receptors (heparan sulfate proteoglycans)
- Reducing viral replication by inducing type I IFN production
- Increased phagocytic activity of macrophages enhanced NK cell activity

IMMUNE MODULATOR

- Antioxydant activity (via iron binding)
- Reduces SIRS response (LPS-binding and reduced IL-6, IL-10, IL-12 levels)
- Modulator, bridging Innate and Adaptive Immune response (increasing NK activity, enhancing neutrophils' phagocytic activity, activating macrophages, promoting maturation of T-cell precursors into competent T helper cells, promoting differentiation of immature B cells into efficient APCs)
- Augments the delayed type hypersensitivity (DTH) response to antigens



PRETERM NEWBORNS

- Increased mucosal permeability
- Intestinal dysbiosis
- Immunodeficiency
- LOS important cause of mortality

LACTOFERRIN

Safe
Immuno-modulatory properties

Reduces sepsis and NEC
in preterm newborns

Wang et al 2016, J Ped
Manzoni 2016, J Ped

Lactoferrin in Pediatrics

Phase II randomized, double-blind, placebo-controlled trial to evaluate the efficacy of oral lactoferrin supplementation in reducing febrile neutropenia in children with hematologic malignancies during induction chemotherapy

Objective

To verify the efficacy of early administration of oral LF as prophylaxis of febrile neutropenia (FN) in pediatric patients undergoing chemotherapy treatment

Method

Multicentric phase II randomized, double-blind, placebo-controlled trial

Subjects

First line induction chemotherapy for:

ALL

AML

NHL

age ≥ 1 month ≤ 21 years

Ability to take oral therapy

Informed consent

Exclusion criteria

- gut colonization by multidrug-resistant bacteria before cancer diagnosis
- prior chemotherapy received,
- refusal or difficulty to take the product

Group A (LF): 200 mg/die LF (Mosiad[®]) for 60 days from the start of chemotherapy

Group B (placebo)

Every center provided supportive therapy according to local guidelines, including antibiotic prophylaxis

Primary endpoint

FN incidence in both groups

Secondary endpoints:

- **Sepsis** and severe sepsis incidence
- MDR infections
- *Clostridium difficile* infections
- VRE infections
- Fungal infections

Ancillary study

Gut microbiome composition
in a sub-group of patients of both groups

Sample size

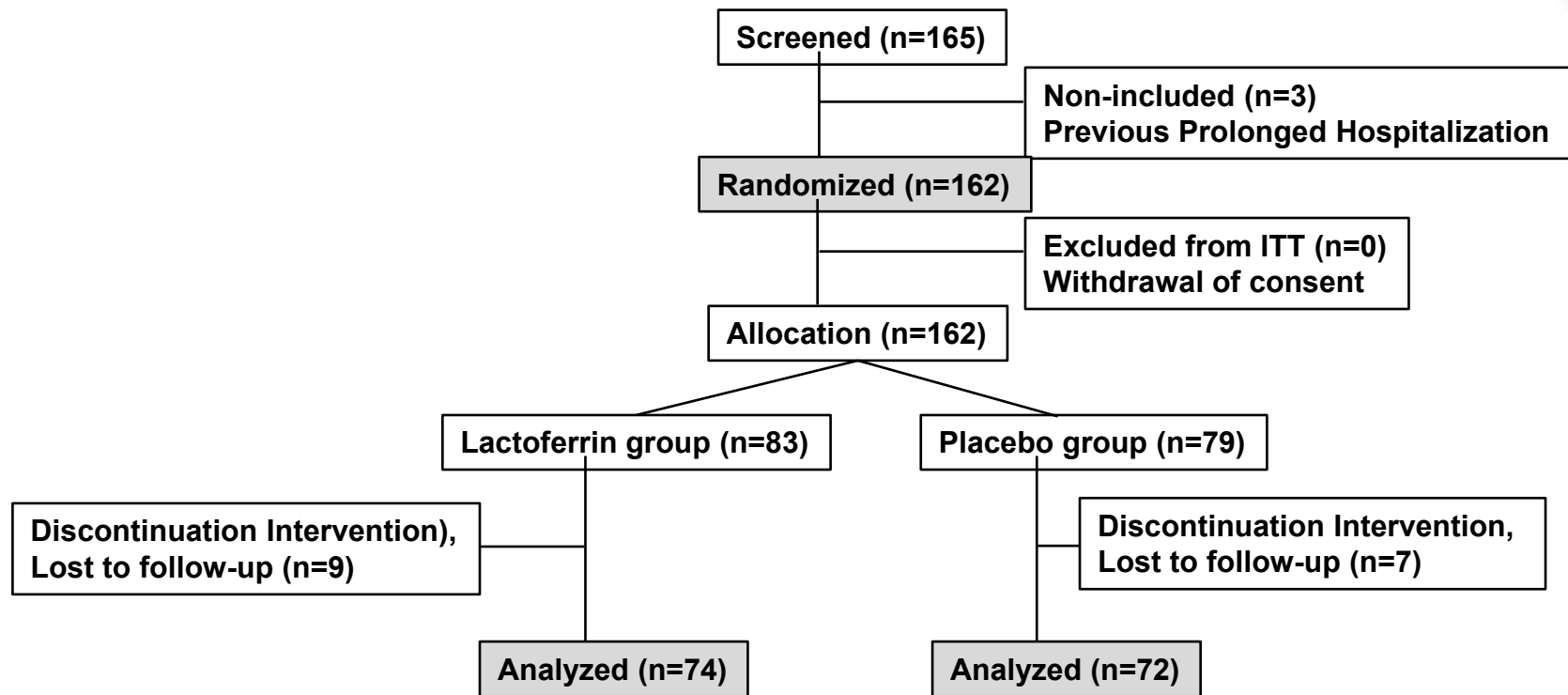
It was expected that in about a third of patients will experience febrile neutropenia. bLF reduced cases of infection in preterm infants by 2/3.

We expected bLF to reduce by no less than 5% the proportion of patients experiencing FN. This is a and preliminary efficacy study.

We wanted to exclude, with a **one sided 80% confidence interval**, that the difference in **incidence of febrile neutropenia in the treated group is less than 5%** compared to controls (i.e. that the incidence in the LF group is >25%).

We computed a sample size of 71 patients per group (Cocks et al), which was increased to 80 per group to account for dropouts

Randomization 1:1 was stratified by center, with blocks of different size
Nine centers (Pavia, Verona, Bologna, Monza, Perugia, Bari, S. Giovanni Rotondo, Cagliari, Trieste)



	Lactoferrin (83)	Placebo (79)
Age (years), SD	6 (4-12)	7 (4-13)
Female	32 (39%)	36 (46%)
Comorbidities	5 (6%)	3 (4%)
AML vs ALL	8 (10%)	9 (11%)
Haematological disease		
ALL	65 (78.3%)	58 (73.4%)
AML	8 (9.6%)	9 (12%)
NHL	10 (12%)	12 (15.2%)
Weight	31.08 (11-75)	30.2 (9.7-86)
Previous antibiotics	20 (24%)	13 (16%)
Previous prolonged hosp	4 (5%)	3 (4%)
WBC	3.07 (1.70-6.47)	3.32 (1.84-6.73)
PLT	82 (37-224)	124 (48-251)
Neutrophils	0.74 (0.25-1.90)	0.89 (0.32-2.10)
Lymphocytes	1.44 (0.70-2.46)	1.41 (0.80-2.21)

	Lactoferrin events (N°)	Lactoferrin rate per 100 person week (80%)	Placebo events (N°)	Placebo rate per 100 person week (95%)	HR (80%CI)	p-value
Primary endpoint						
Pts with FN	33	2.0 (1.4-2.8)	41	3.1 (2.3-4.3)	0.64 (0.45-0.89)	0,087
N° of episodes per patient	57	2.6 (1.9-3.4)	48	3.2 (2.4-4.1)	*0.82(0.53- 1.28)	0,571
Secondary endpoints						
Sepsis	13	1.0 (0.6-1.6)	24	1.3 (0.8-2.0)	0.46 (0.3-0.71)	0,349
Sepsis GRAM- MDR	2	0.1 (0.0-0.4)	0	0	NE	0.167#
Clostridium difficile	3	0.2 (0.1-0.5)	1	0.1 (0.0-0.4)	2.53 (0.73-8.83)	0,337
VRE	1	0.05 (0.0-0.4)	0	0	NE	0.327#
Safety						
Pts with AE	14 (17%)		17 (21%)			0.550°
Pts with SAE	8 (10%)		10 (13%)			0.621°

*IRR (80%CI); #log rank test; °Fisher exact test

Endpoints by treatment arm

variable	Placebo events N	Placebo rate per 100 person week (95%)	Lactoferrin events N	Lactoferrin rate per 100 person week (80%)	HR (80%CI)	p-value
Primary endpoint						
patients with febrile neutropenia	41	3.1 (2.3-4.3)	33	2.0 (1.4-2.8)	0.64 (0.45- 0.89)	0,087
number of episodes per patient	48	3.2 (2.4-4.1)	57	2.6 (1.9-3.4)	*0.82 (0.53- 1.28)	0,571
Secondary endpoints						
sepsis	19	1.3 (0.8-2.0)	16	1.0 (0.6-1.6)	0.73 (0.47- 1.12)	0,349
sepsis gram neg multiresistant	0	0	2	0.1 (0.0-0.4)	not evaluable	0.167#
clostridium difficile	1	0.1 (0.0-0.4)	3	0.2 (0.1-0.5)	2.53 (0.73- 8.83)	0,337
VRE	0	0	1	0.05 (0.0-0.4)	not evaluable	0.327#

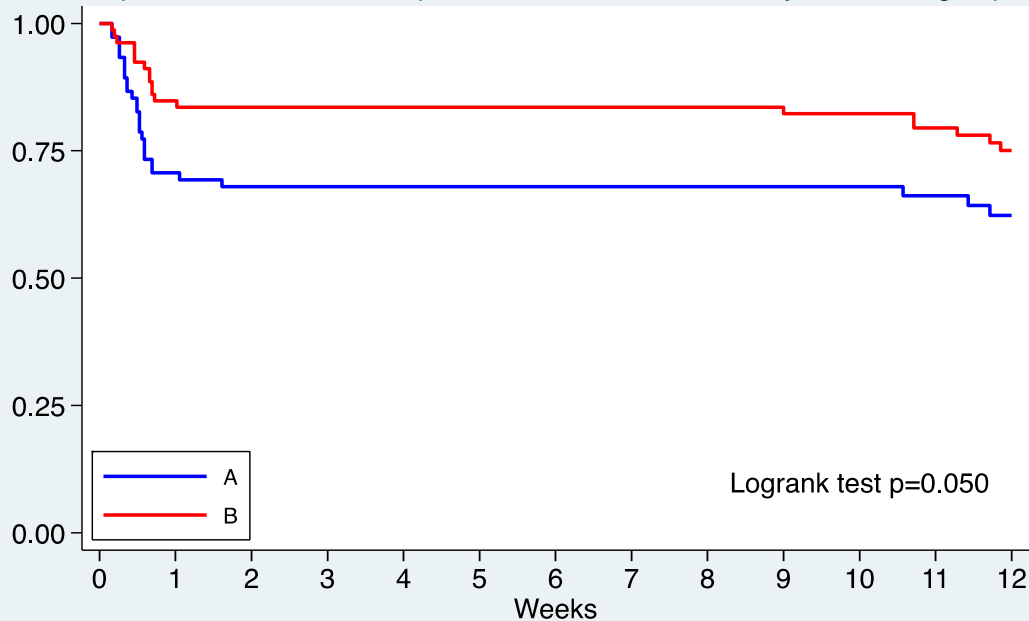
*IRR (80%CI); #log rank test; °Fisher exact test

Analysis at 8 weeks
(during LF administration)

variable	Placebo events N	Placebo rate per 100 person week (95%)	Lactoferrin events N	Lactoferrin rate per 100 person week (80%)	HR (80%CI)	p-value
patients with febrile neutropenia	24	6.0 (4.6-7.8)	13	2.4 (1.7-3.5)	0.46 (0.30-0.71)	0,023
sepsis	10	1.9 (1.3-2.9)	4	0.7 (0.3-1.3)	0.37 (0.22-0.62)	0,013

*IRR (80%CI); #log rank test; °Fisher exact test

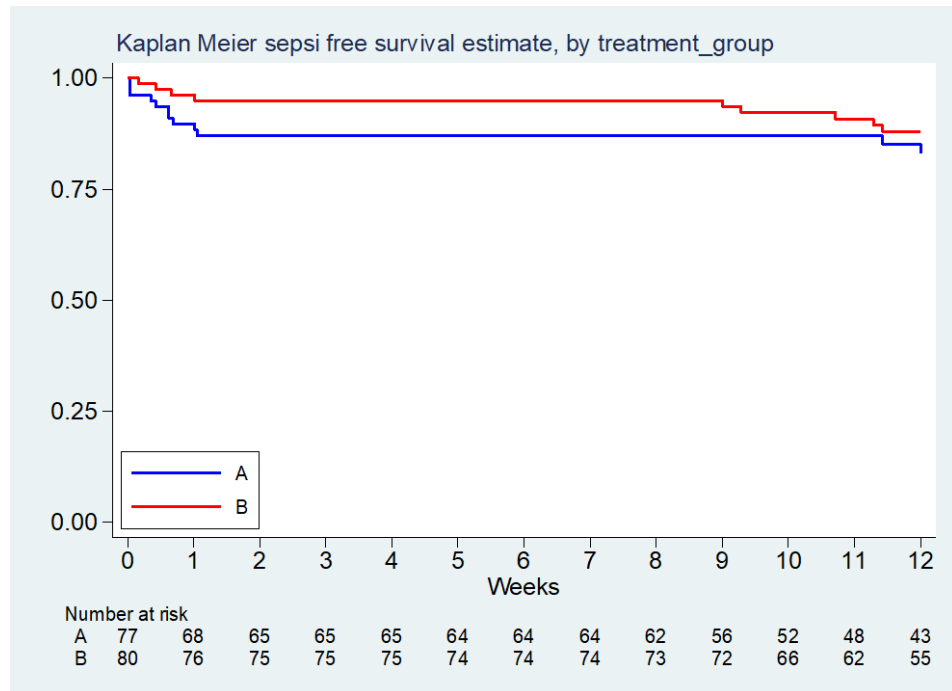
Kaplan Meier febrile neutropenia free survival estimate, by treatment_group



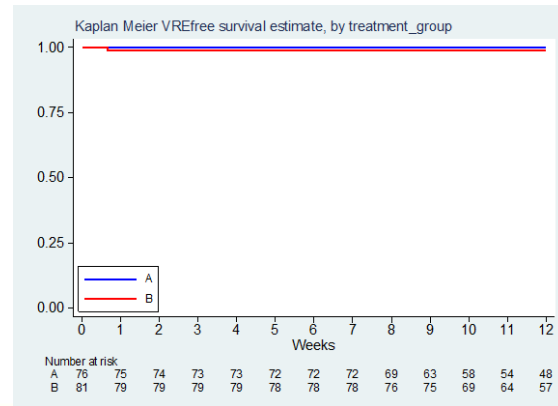
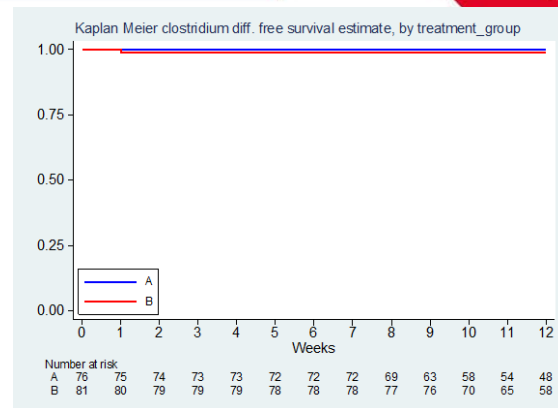
Number at risk

A	75	52	49	49	49	48	48	48	46	42	41	37	32
B	80	67	66	66	66	66	66	66	66	66	60	56	50

HR 0.46 (0.30-0.71)



HR 0.37 (0.22-0.62)












pharmaceutics



Article

Oral Lactoferrin Supplementation during Induction Chemotherapy Promotes Gut Microbiome Eubiosis in Pediatric Patients with Hematologic Malignancies

Federica D'Amico ^{1,†}, Nunzia Decembrino ^{2,3,†}, Edoardo Muratore ^{4,*}, Silvia Turrone ^{5,*}, Paola Muggeo ⁶,
Rosamaria Mura ⁷, Katia Perruccio ⁸, Virginia Vitale ⁹, Marco Zecca ³, Arcangelo Prete ⁴, Francesco Venturelli ^{4,10},
Davide Leardini ⁴, Patrizia Brigidi ¹¹, Riccardo Masetti ^{4,11}, Simone Cesaro ^{9,†} and Daniele Zama ^{11,12,†}

- 1) To evaluate gut microbiome dynamics in pediatric patients affected by acute leukemia/lymphoma and to compare it with healthy matched controls
- 2) To evaluate the impact of bovin Lactoferrin administration (77% homology with human LF) on GM, in children during induction chemotherapy for acute leukemia/lymphoma



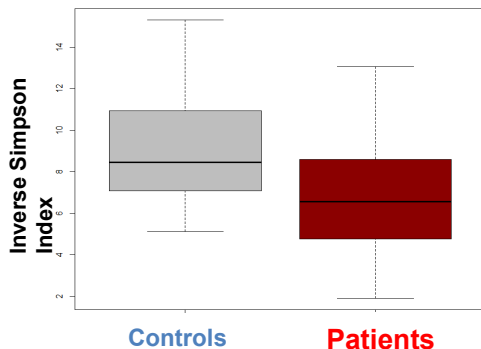
Table 1. Summary of patient characteristics in the two groups.

	Lactoferrin (<i>n</i> = 14)	Placebo (<i>n</i> = 20)	<i>p</i>
Age at diagnosis: years, median (range)	3.50 (1.76–18.35)	6.96 (1.49–16.34)	0.42
Weight at diagnosis: kg, median (range)	15.0 (9.7–33.8)	24.0 (12.0–67.5)	0.03
Diagnosis, <i>n</i> , (%):			
• ALL	13 (92.9)	17 (85.0)	0.47
• AML	1 (7.1)	1 (5.0)	
• Lymphoma	0 (0.0)	2 (10.0)	
Center, <i>n</i> , (%):			
• Bologna	4 (28.6)	6 (30.0)	0.78
• Pavia	0 (0.0)	1 (5.0)	
• Verona	1 (7.1)	1 (5.0)	
• Perugia	2 (14.3)	2 (5.0)	
• Bari	7 (50.0)	8 (40.0)	
• Cagliari	0 (0.0)	2 (10.0)	
Prior use of antibiotics, <i>n</i> , (%)	5 (38.5)	4 (20.0)	0.43
Neutropenic fever, <i>n</i> , (%)	8 (57.1)	18 (90.0)	0.04
Antibiotics during inductions (%)	10 (71.4)	14 (70.0)	1.00
BSI, <i>n</i> , (%)	6 (50.0)	7 (43.8)	0.74
Mucositis, <i>n</i> , (%)	5 (38.5)	6 (35.3)	0.90
Mucositis, grade:			
• I	4 (80.0)	3 (50.0)	0.50
• II	1 (20.0)	2 (33.3)	
• III	0 (0.0)	1 (16.7)	
• IV	0 (0.0)	0 (0.0)	

210 x 297 mm

ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; BSI: Blood Stream Infection.

Diversity

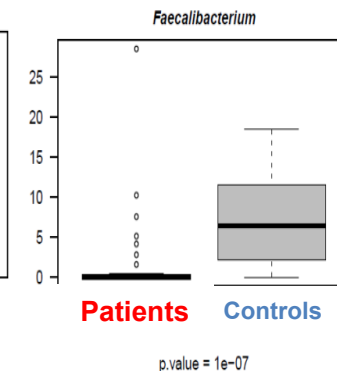
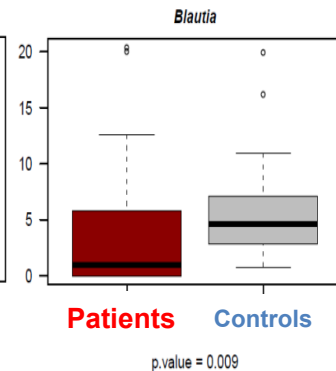
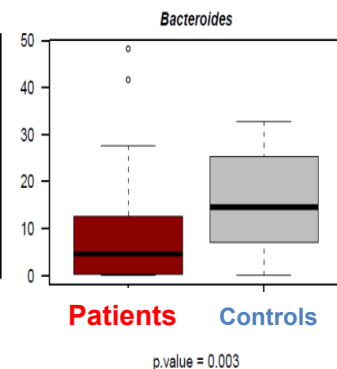
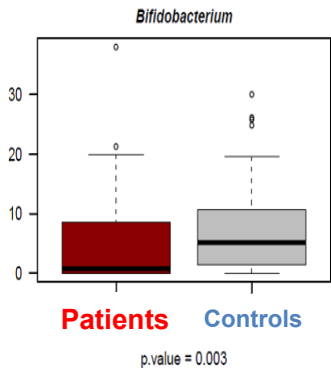


GM: patients vs healthy controls

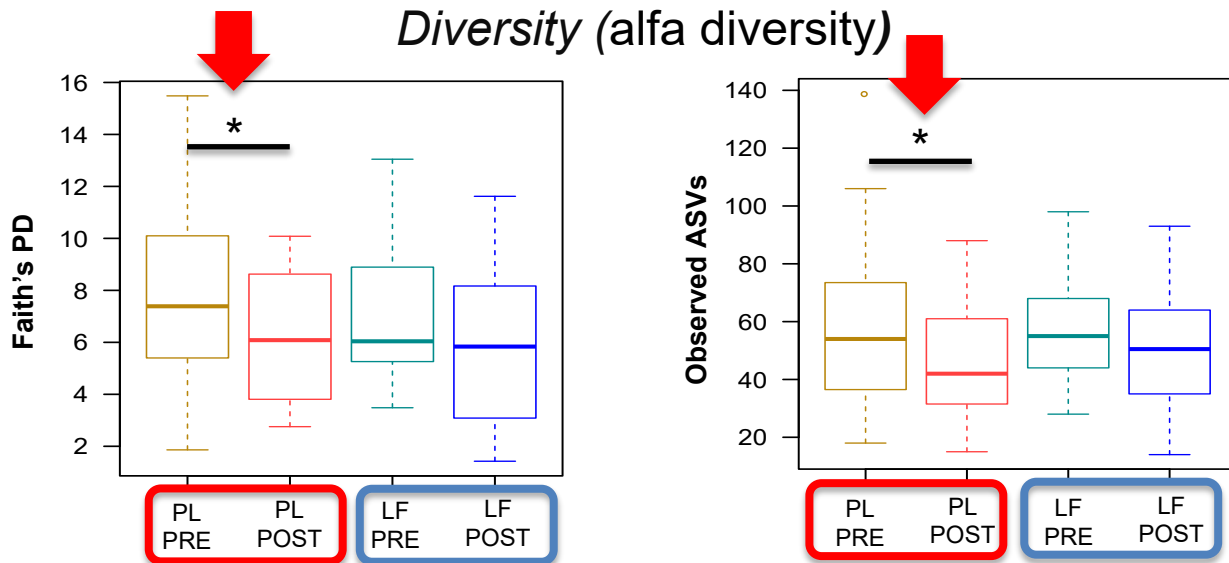
Significative difference:

- Alfa diversity and beta diversity
- Taxonomic composition of GM in patients at disease onset vs healthy controls

Composition



LF vs Placebo during induction chemotherapy



alpha diversity reflects the richness (number) or distribution (evenness) of a microbial sample

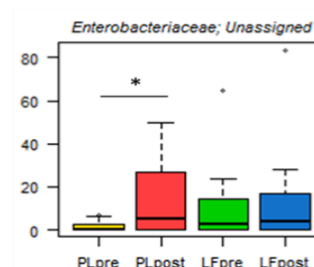
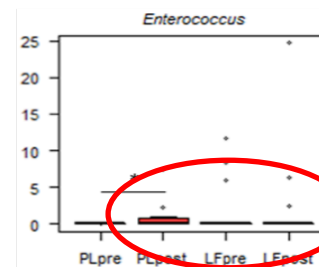
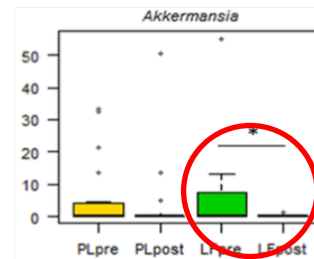
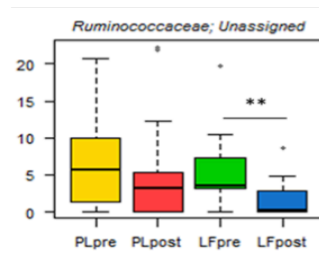
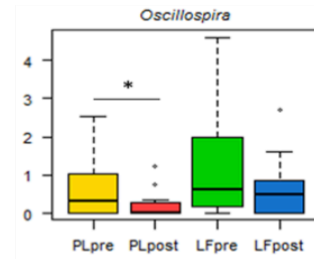
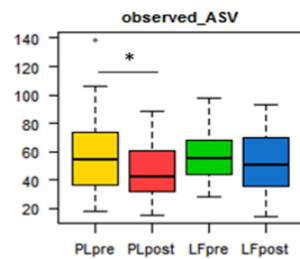
- **PLACEBO**: significant reduction
- **LATTOFERRINA**: stable

Composition

PLACEBO: significant reduction in health-associated taxon (*Oscillospira*) compared to LF treated group.

LACTOFERRIN: lower abundance of the mucin-degrading bacterium *Akkermansia*, which has been associated with neutropenic fever

LACTOFERRIN: reduced growth of *Enterococcus*, a pathogen associated with bacteremia and febrile neutropenia



Conclusions

LACTOFERRIN:

- **Reduces the incidence of febrile neutropenia**
- **Reduces the incidence of sepsis**
- **Promote the maintenance of diversity** (indirect sign of eubiosis)
- **GM composition in LF treated patients:**
 - ✓ **lower abundance of pathobionts** (es. Enterobacteriaceae, Enterococcus), which are correlated to the risk of bacteremia
 - ✓ **Lower abundance of Akkermansia** -> associated to the erosion of the mucus barrier, which favors metabolite absorption (es. gamma-glutamyl associated to oxidative stress) and bacterial translocation
 - ✓ No effect of age, weight, prior or concomitant antibiotic use
 - ✓ **No adverse effects reported**
 - ✓ **Good compliance**, even for the smallest children



**New approach, never tested before, to modulate
GM administering oral lactoferrin to cancer
patients**



Open questions

- Further studies are needed to evaluate the better dosage of LF (body weight-tailored? BMI, age or...?) and length of treatment
- To explore efficacy of LF in HSCT and colonized patients with MDR bacteria
- LF alone or combined with other strategies? (es. Prebiotics, postbiotic, diet, antibiotic sparing strategies...)
- To explore the immunomodulatory effects of LF



D. Zama- Bologna



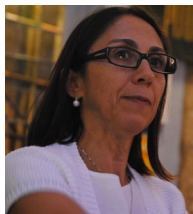
P. Muggeo- Bari



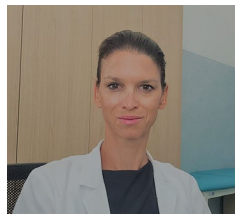
K. Perruccio-Perugia



R. De Santis- S. Giovanni Rotondo



R. Mura- Cagliari



N. Giurici-Trieste



A. Colombini- Monza



AIEOP GdS Terapia di Supporto e Infezioni

Grazie!