The role of the intestinal microbiome in GVHD

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Memorial Sloan Kettering Cancer Center

Acknowledgements

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Assistant Attending level 1
Infectious Disease Service

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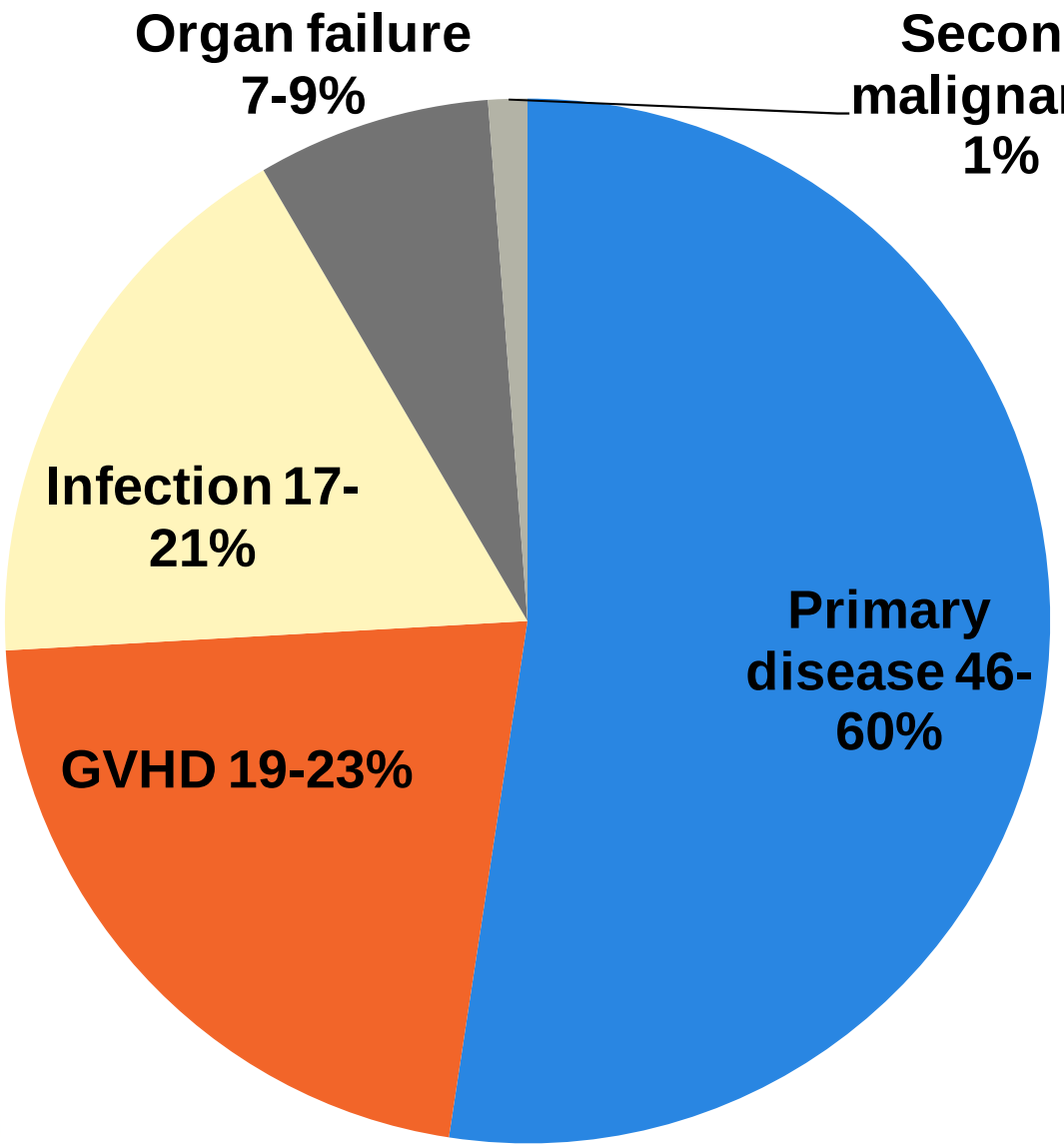
Bone Marrow Transplant in Numbers

- 66,000 global transplants[1] (2011-2012):
  - 36,000 autologous
  - 30,000 allogeneic
- 1 millionth patient transplanted in December 2012
- 22,222,377 donors[2] currently registered worldwide
- 1,400 transplant centers worldwide
- Mostly for patients with leukemia, lymphoma or myeloma

Allogeneic Hematopoietic Stem Cell Transplantation

- High dose therapy with hematopoietic stem cell rescue
- Only established stem cell therapy
- Immunotherapy of cancer (graft-versus-tumor)
- Personalized/precision medicine
- Adoptive cell therapy

Cause of death after allogeneic BMT (2010-2011)



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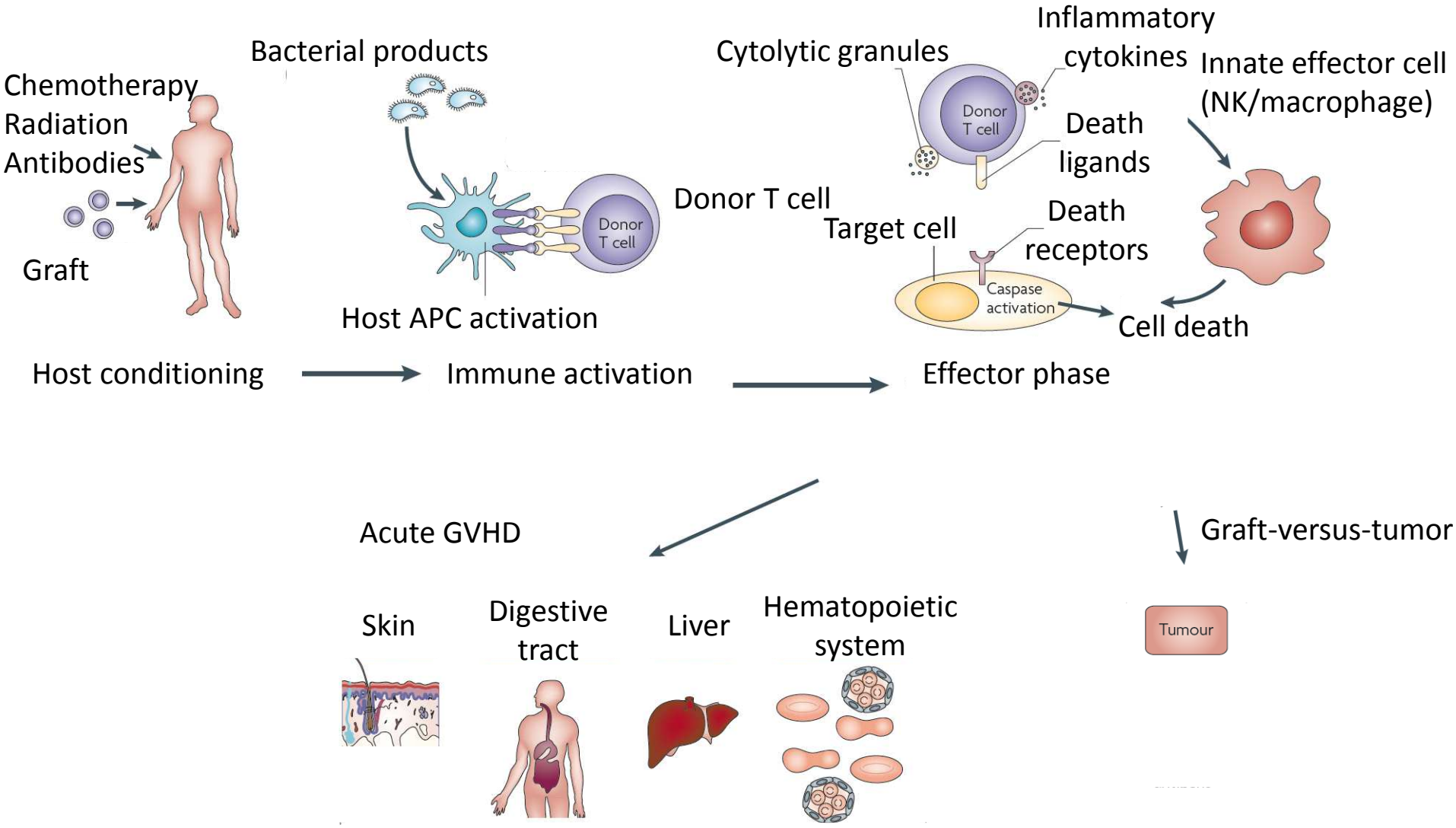
CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

Pathophysiology of Graft-versus-host disease



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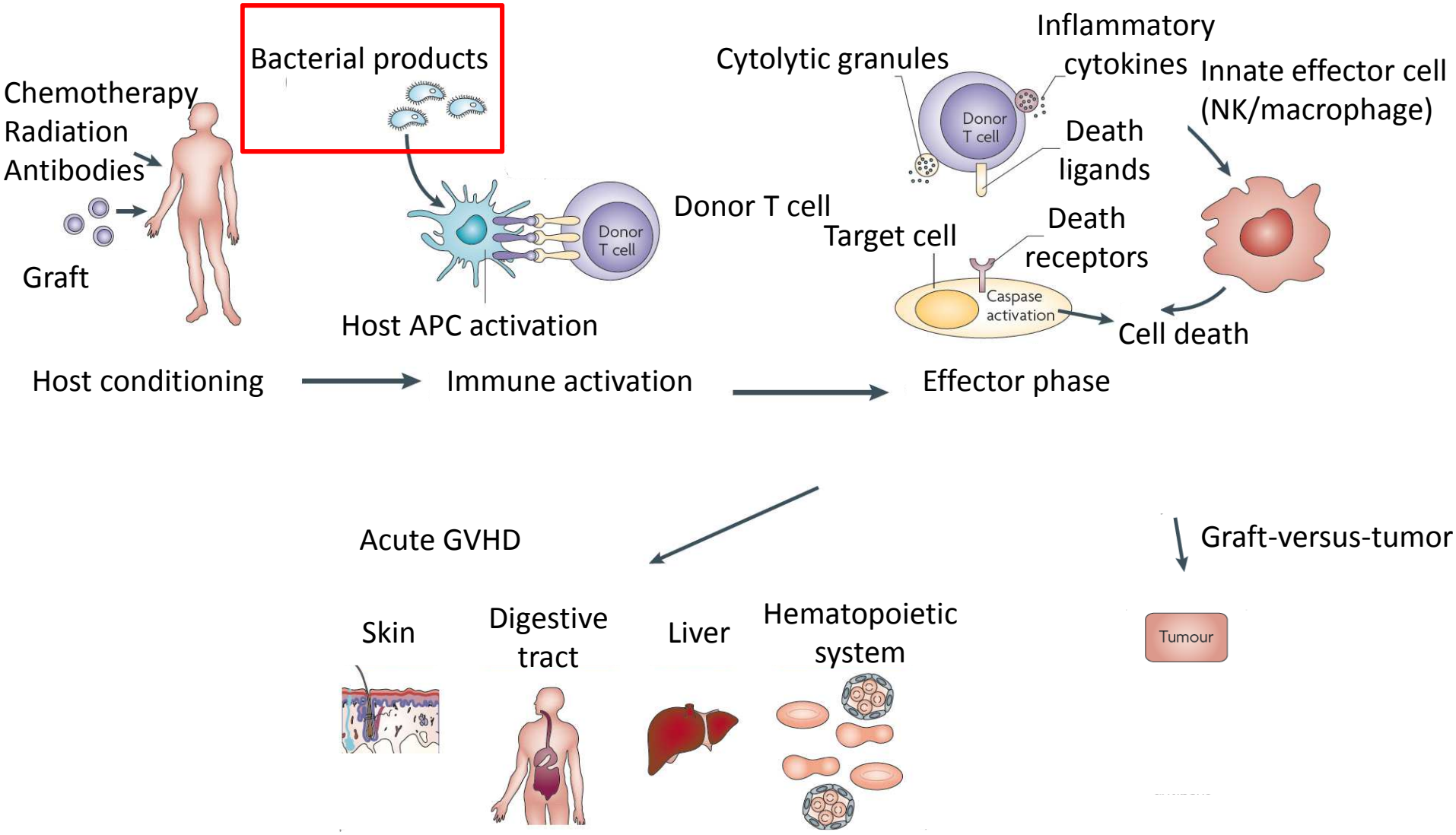

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An old question – can the flora impact on GVHD

Mortality and Gross Pathology of Secondary Disease in Germfree Mouse Radiation Chimeras[1]

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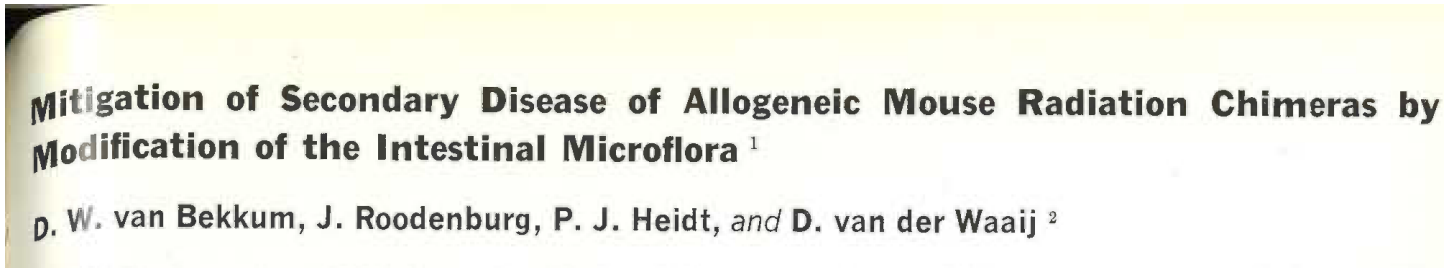

Mitigation of Secondary Disease of Allogeneic Mouse Radiation Chimeras by Modification of the Intestinal Microflora [1]

D. W. van Bekkum, J. Roodenburg, P. J. Heidt, *and* D. van der Waaij [2]

1974

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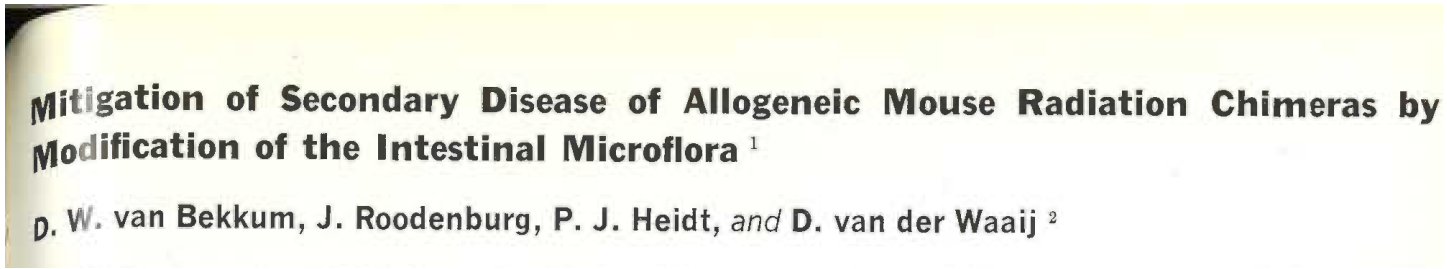

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GRAFT-VERSUS-HOST DISEASE AND SURVIVAL IN PATIENTS WITH APLASTIC ANEMIA TREATED BY MARROW GRAFTS FROM HLA-IDENTICAL SIBLINGS

Beneficial Effect of a Protective Environment

RAINER STORB, M.D., ROSS L. PRENTICE, PH.D., C. DEAN BUCKNER, M.D., R. A. CLIFT, F.I.M.L.S., FRED APPELBAUM, M.D., JOACHIM DEEG, M.D., KRISTINE DONEY, M.D., JOHN A. HANSEN, M.D., MARK MASON, JEAN E. SANDERS, M.D., JACK SINGER, M.D., KEITH M. SULLIVAN, M.D., ROBERT P. WITHERSPOON, M.D., AND E. DONNALL THOMAS, M.D.

1983

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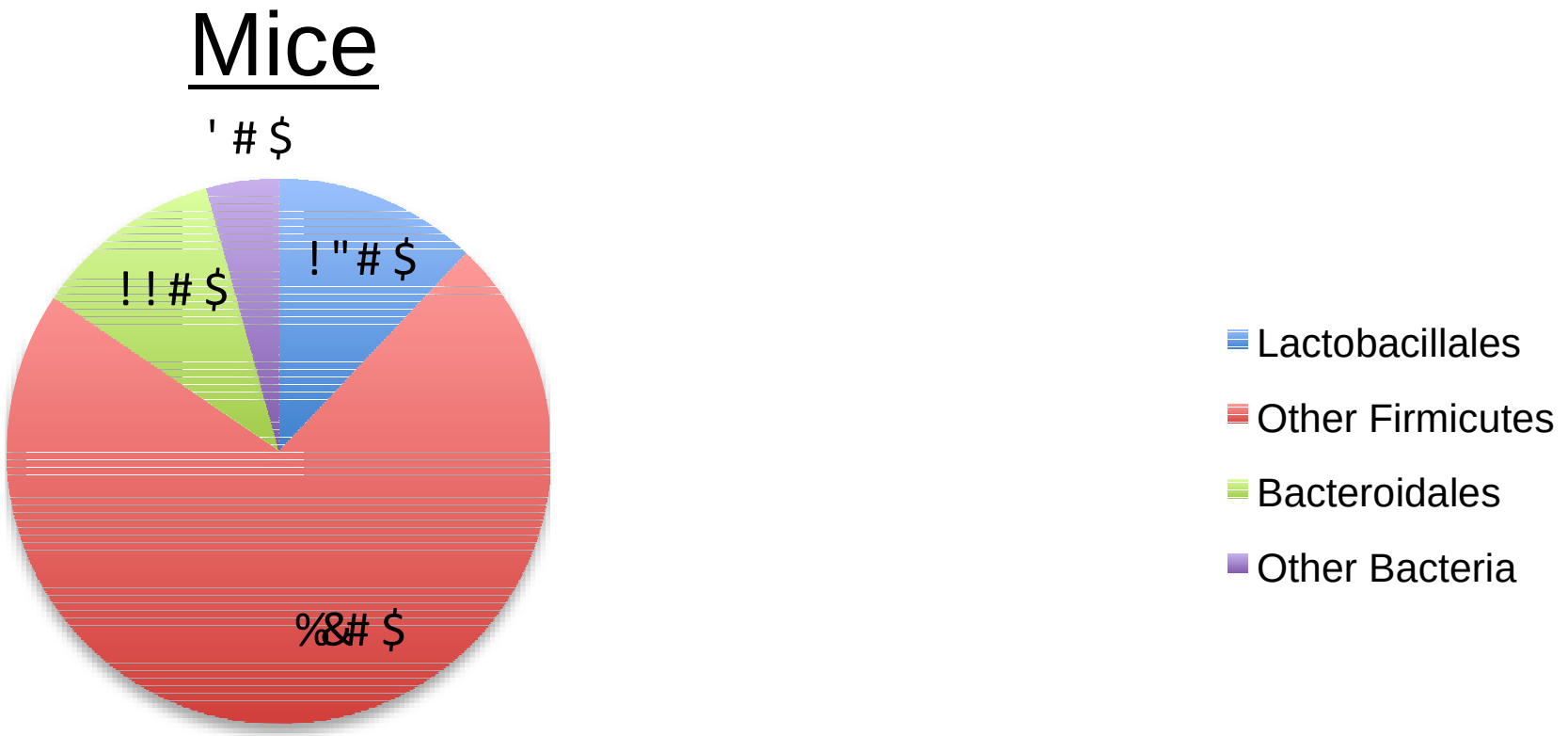
Bacterial Taxonomy Guide

Phylum	Order	Genus	Gram stain	Facultative or obligate anaerobe
Firmicutes	Lactobacillales	Lactobacillus Enterococcus	+	facultative
	Clostridiales	Clostridium Blautia		obligate
Proteobacteria	Enterobacteriales	Escherichia Klebsiella	–	facultative
Bacteroidetes	Bacteroidales	Bacteroides Barnesiella		obligate

Comparison of baseline flora of mice and allo BMT patients



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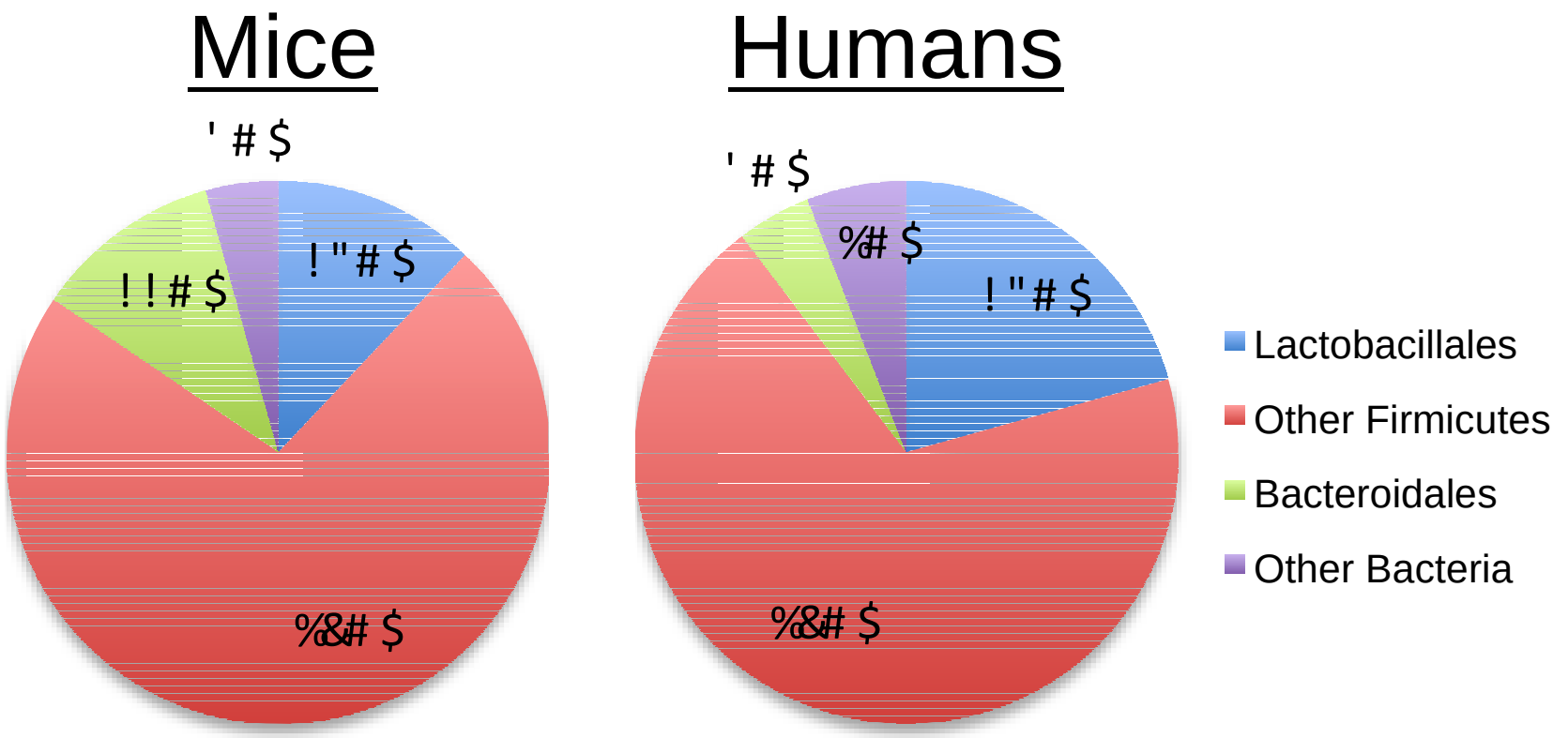

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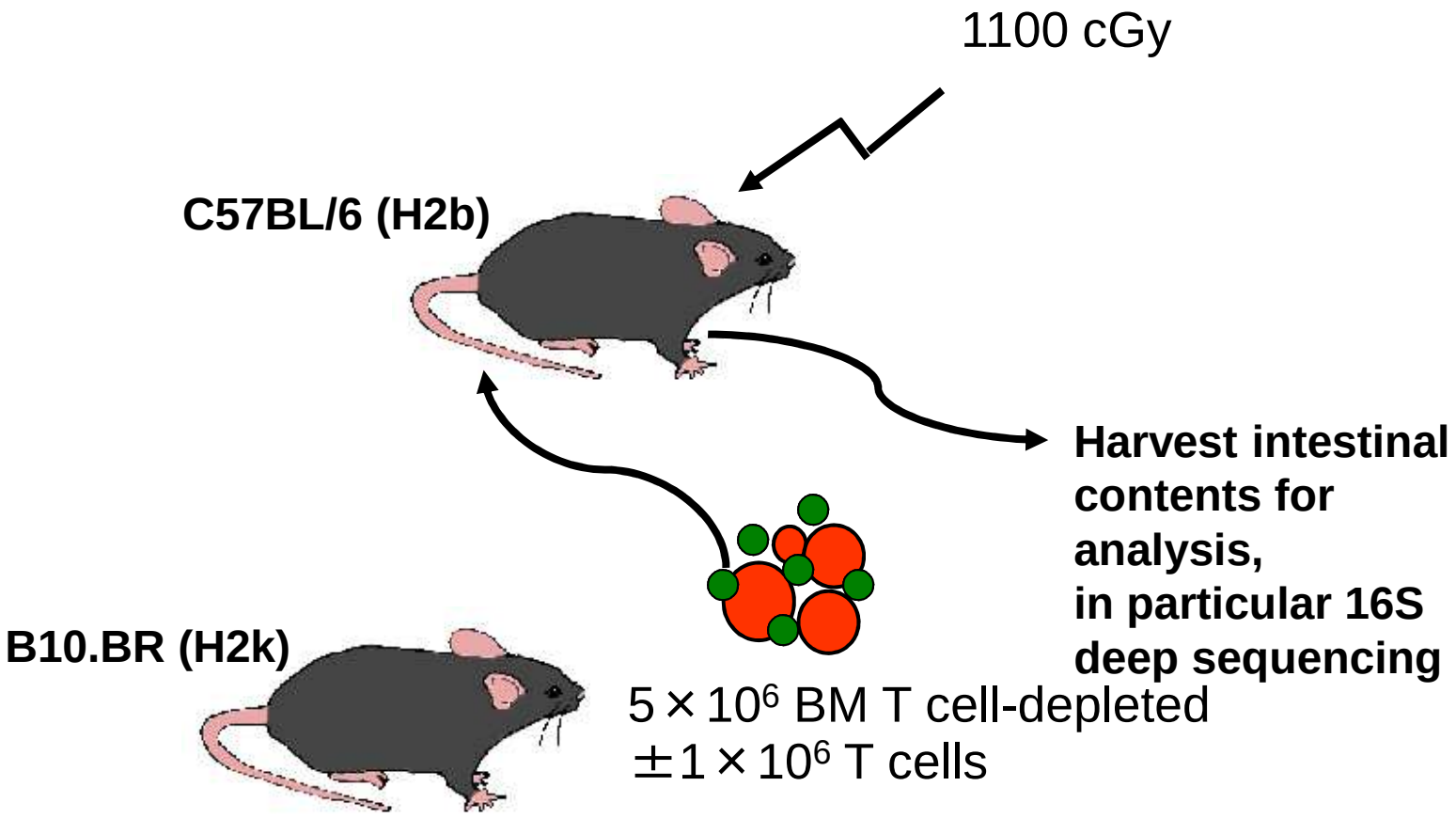
Studying effects of GVHD on the flora: MHC-mismatched model B10BR into B6



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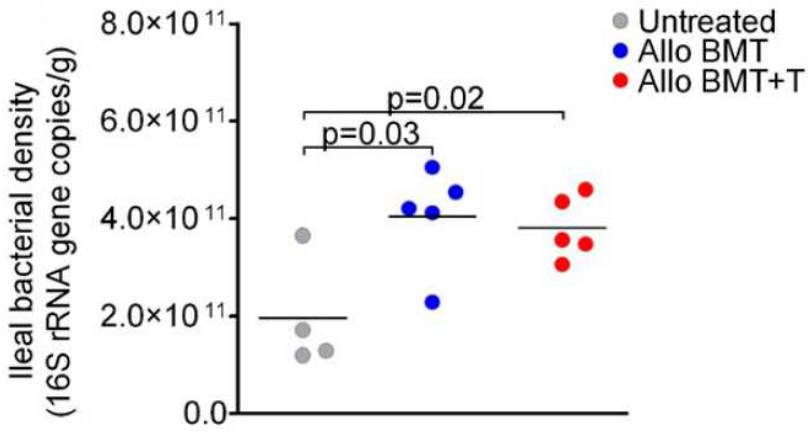
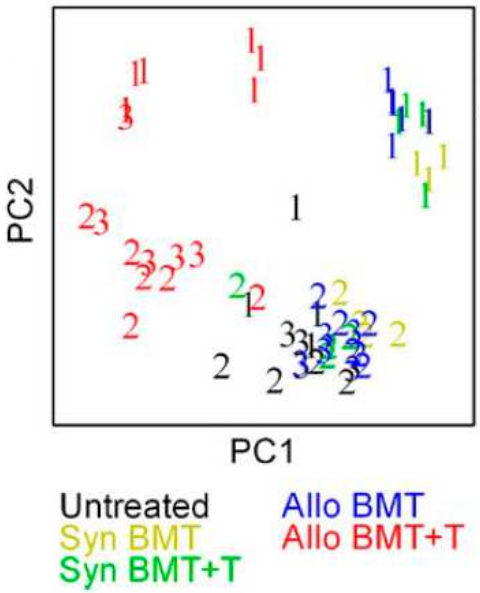
Effects of TBI/transplant and GVHD on the microbiota



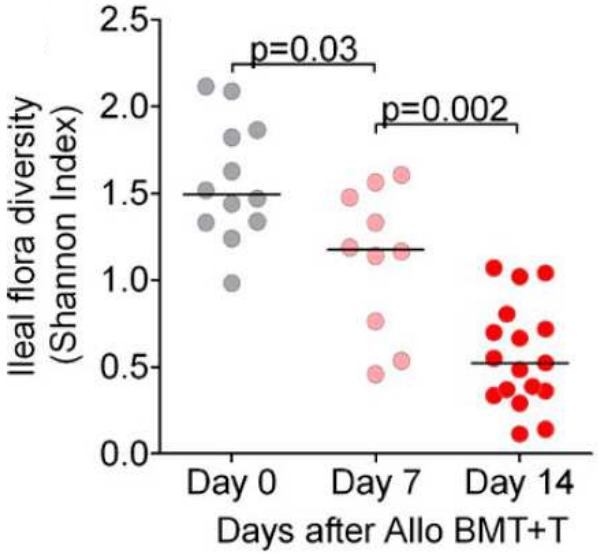
- Day 14 ileal flora bacteria density (16S qPCR)
- No difference was seen in the large bowel

- **TBI/transplant, without or with GVHD, results in a small increase in ileal flora bacterial density**



- 3 experiments indicated by number
- Day 14 ileal flora
- Unweighted UniFrac

- **TBI/transplant alone in absence of GVHD has only minor effects on the flora**



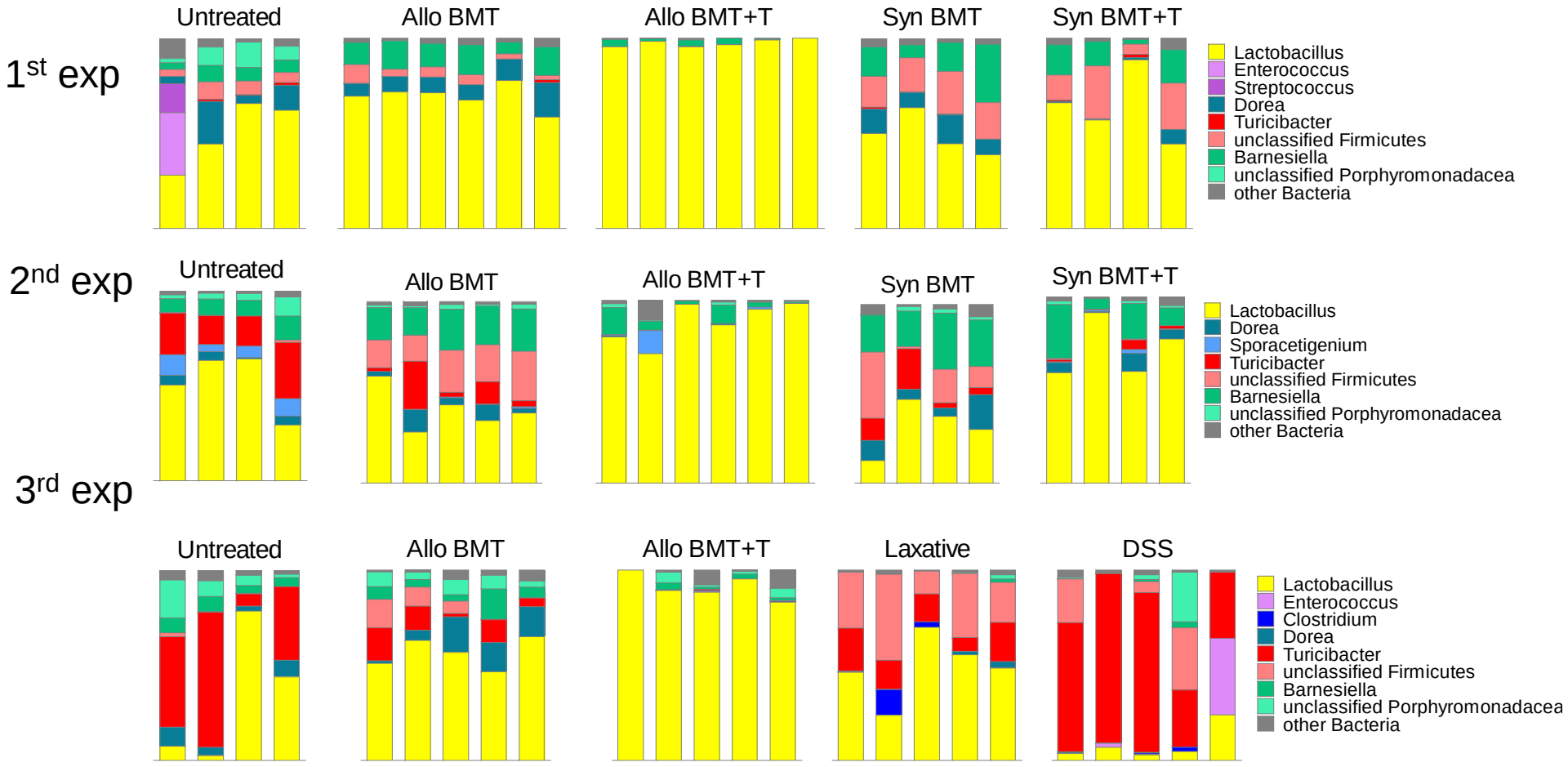
- Ileal flora diversity at indicated time points during GVHD

- **GVHD leads to reduced flora diversity**

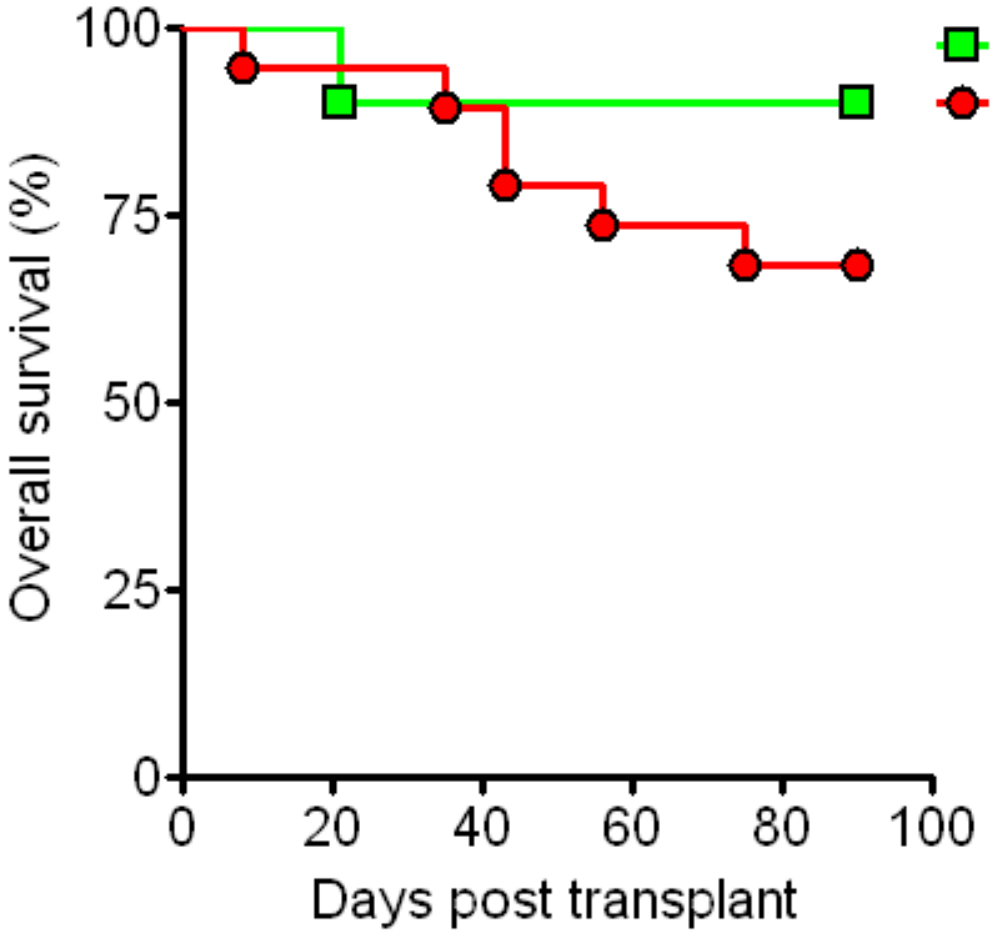
Despite variability in baseline flora, GVHD flora "signature" is highly reproducible and distinct: expansion of Lactobacillales

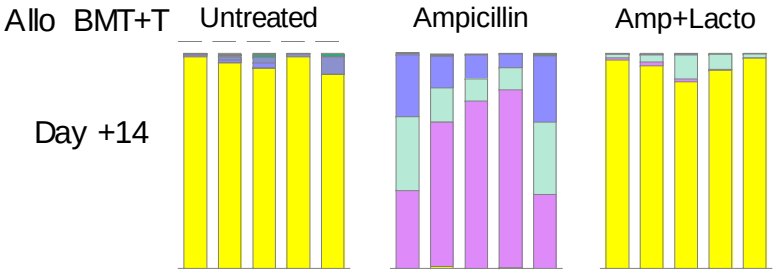
B10.BR into B6

Day 14



Effects of flora manipulation pre-BMT on GVHD





B10BR→B6
1100 cGy day 0
5×10^6 T cell depleted-BM day 0
2×10^6 $CD5^+$ splenic T cells day 0

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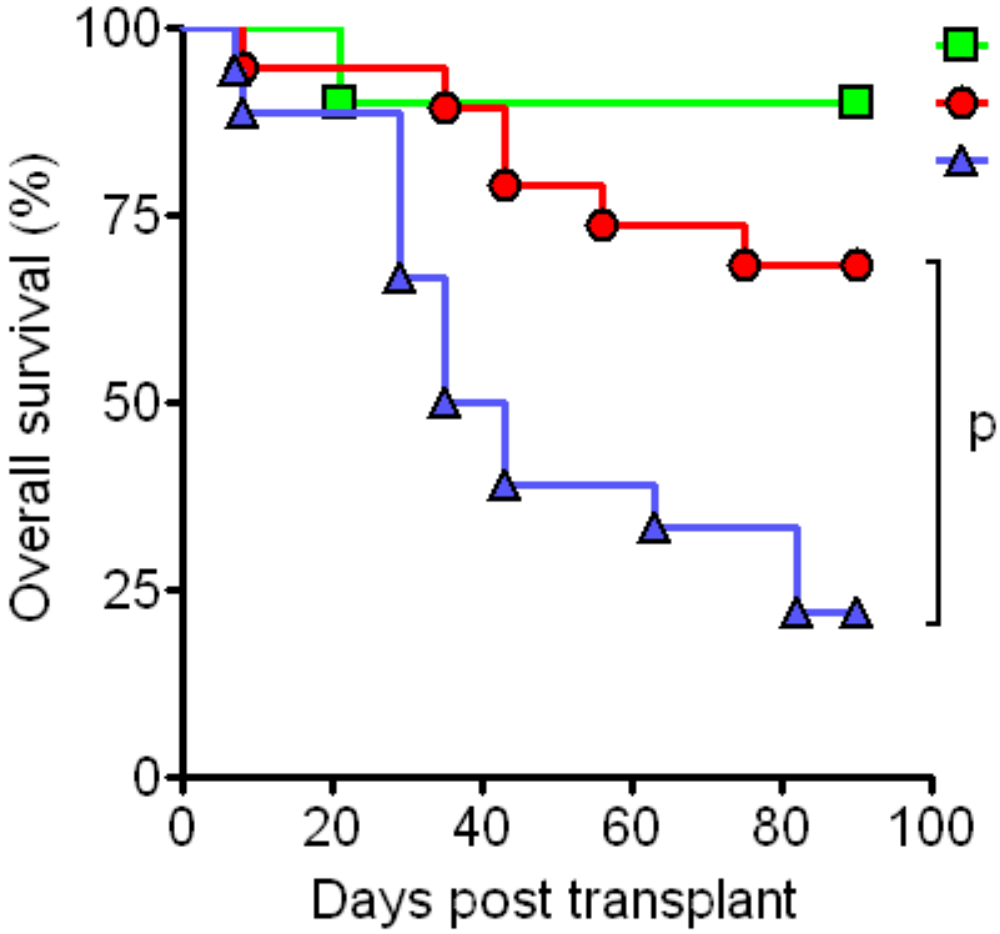
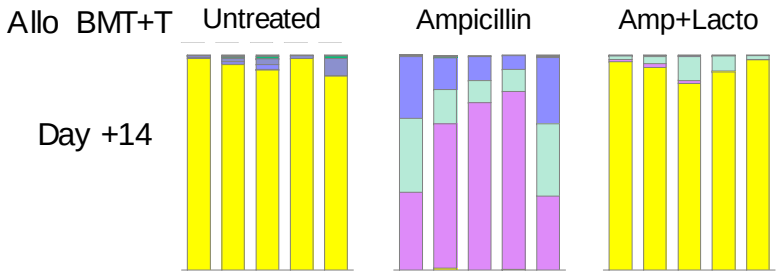
Effects of flora manipulation pre-BMT on GVHD





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Ampicillin treatment days -21 to -14

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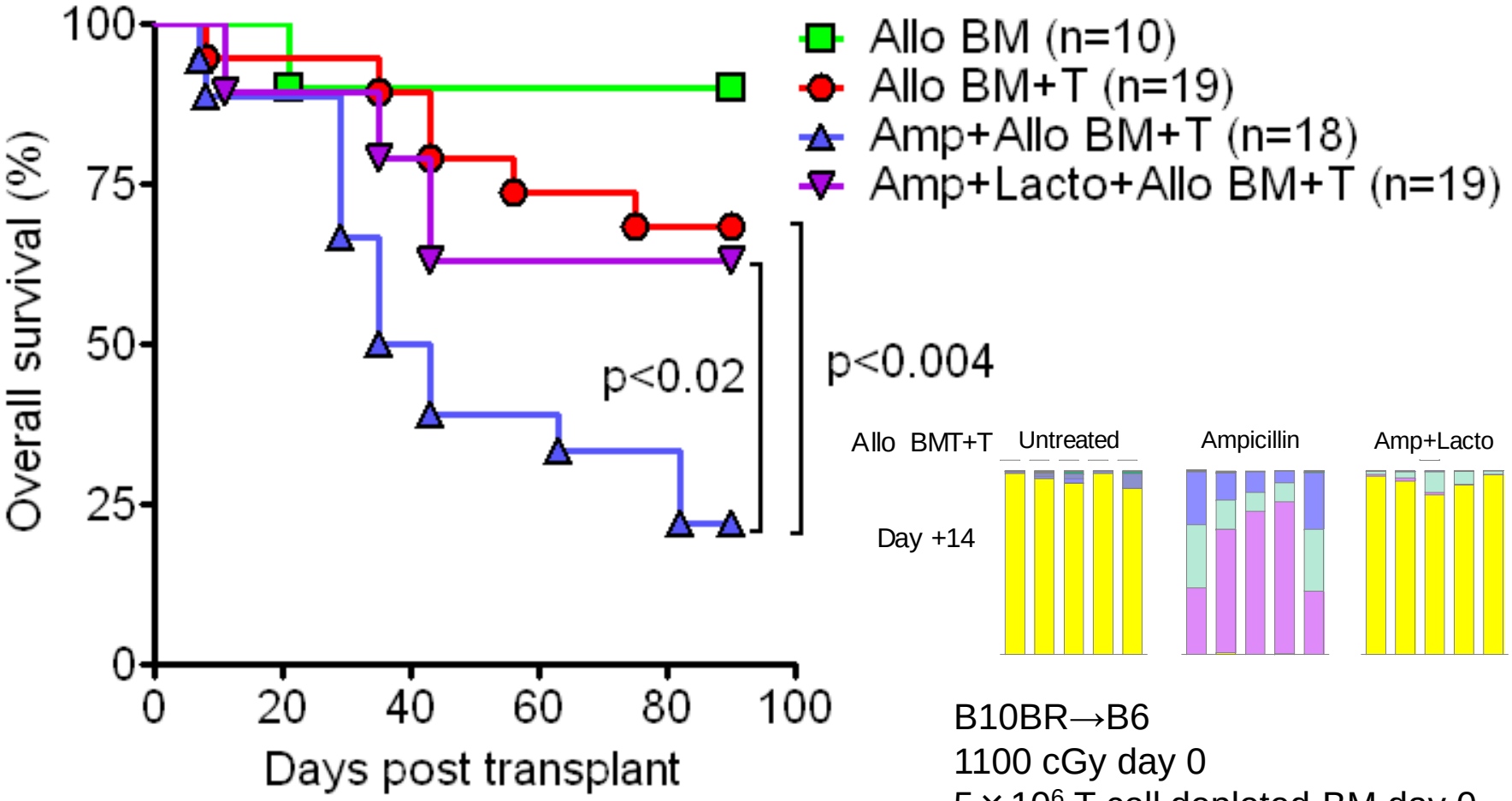

B10BR→B6
1100 cGy day 0
5×10^6 T cell depleted-BM day 0
2×10^6 $CD5^+$ splenic T cells day 0
Ampicillin treatment days -21 to -14
Lactobacillus treatment days -12 to -2

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Does GVHD produce changes in humans as well as in mice?

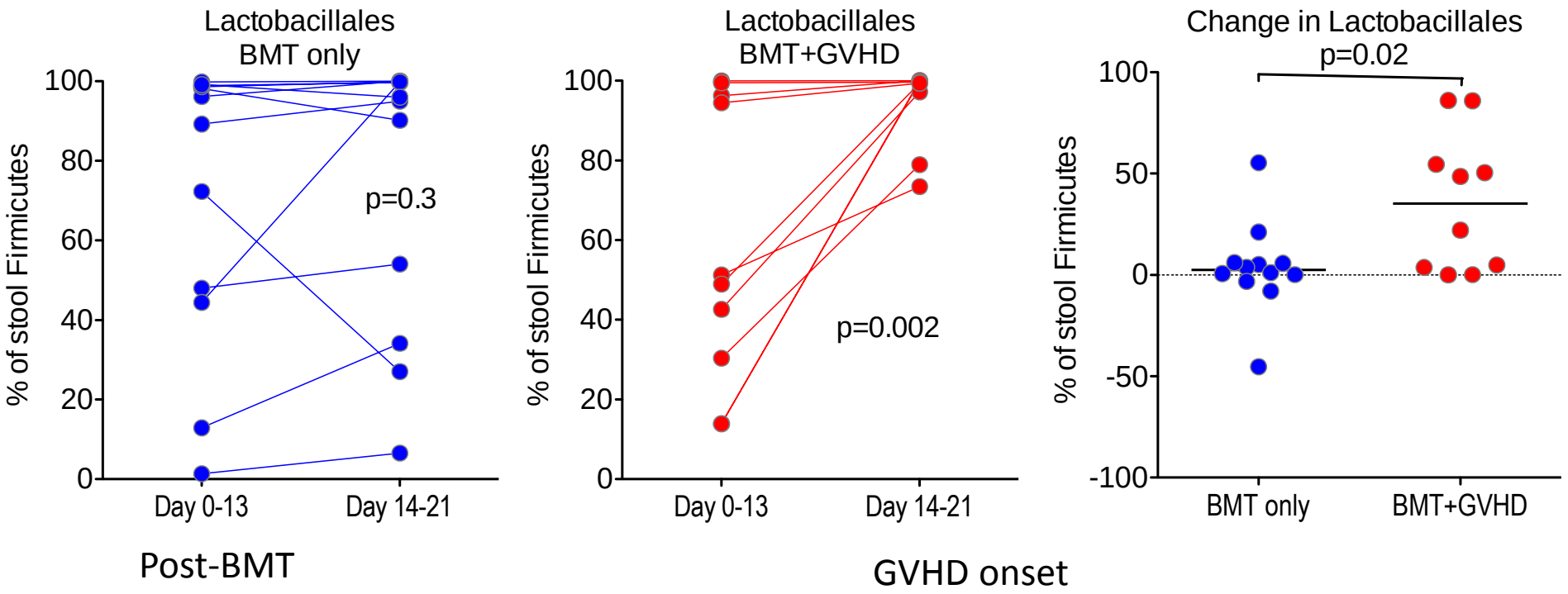
GVHD vs non-GVHD patient selection

- 8 patients developed gut GVHD during transplant hospitalization, between days +18 to +21
- 10 control patients had no evidence for GVHD at day +100

Patient characteristics

	non-GVHD	GVHD
Total patients	10	8
Male	4 (40%)	4 (50%)
Female	6 (60%)	4 (50%)
Median age (range)	58 (32-70)	52 (26-64)
Peripheral blood stem cell transplant	4 (40%)	2 (25%)
Cord blood stem cell transplant	6 (60%)	6 (75%)
Myeloablative conditioning	7 (70%)	7 (87.5%)
Non-myeloablative conditioning	3 (30%)	1 (12.5%)
NHL	2 (20%)	3 (37.5%)
Leukemia/MDS/MPD	8 (80%)	5 (62.5%)
Received therapy for febrile neutropenia	10 (100%)	8 (100%)
Received vancomycin	10 (100%)	8 (100%)
Received fluoroquinolone	5 (50%)	5 (62.5%)
Received metronidazole	2 (20%)	2 (25%)

Human GVHD increases Lactobacillales representation within stool Firmicutes

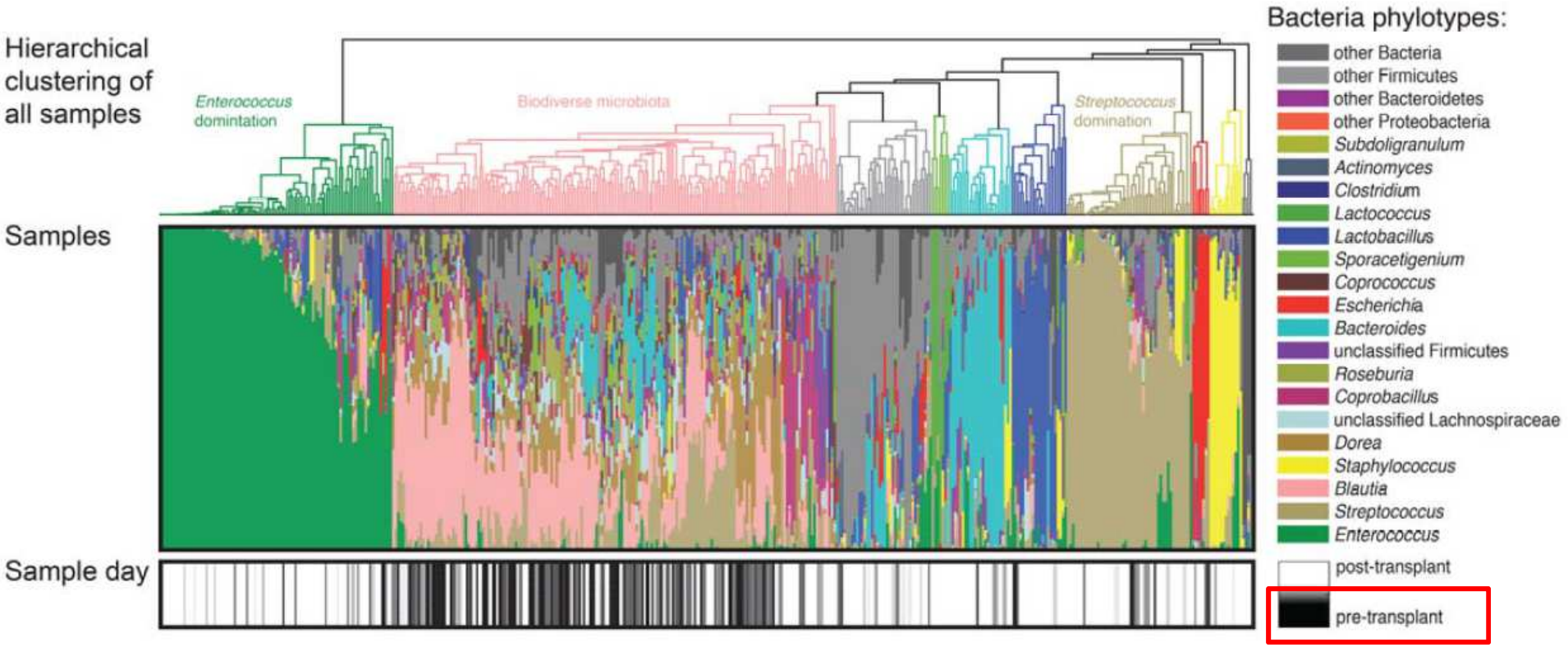


18 recipients of T-cell replete allo BMT
8 developed GVHD ~D21, and 10 did not
Analysis of stool flora composition before and after onset of GVHD symptoms

Jenq, et al., J Exp Med, 2012

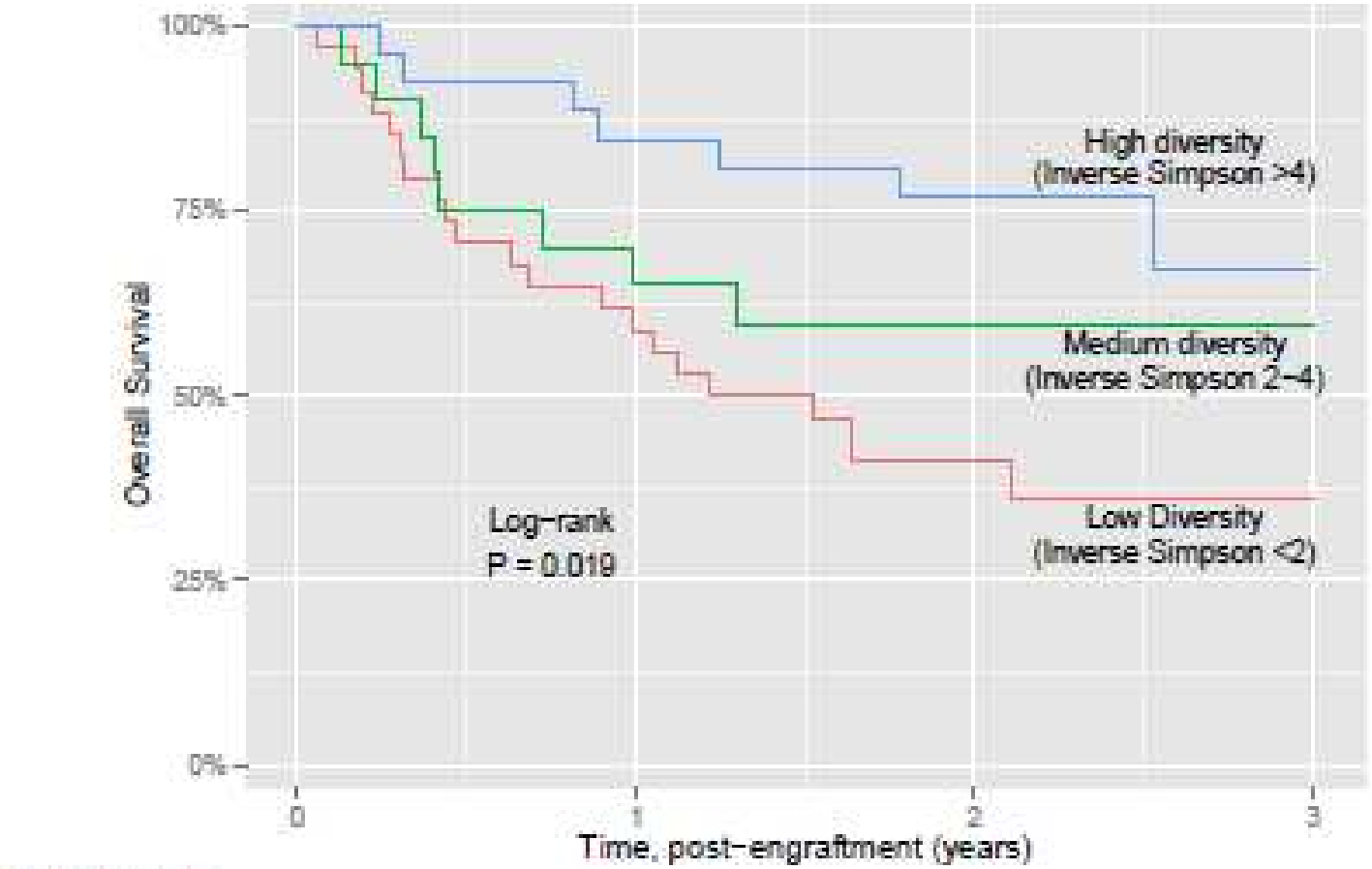
Are there changes in in the intestinal microflora that can predict the risk for GVHD?

Hierarchical clustering of allo-BMT patient samples shows biodiverse flora pre-transplant, followed by marked changes following transplant



Taur, et al., CID, 2012

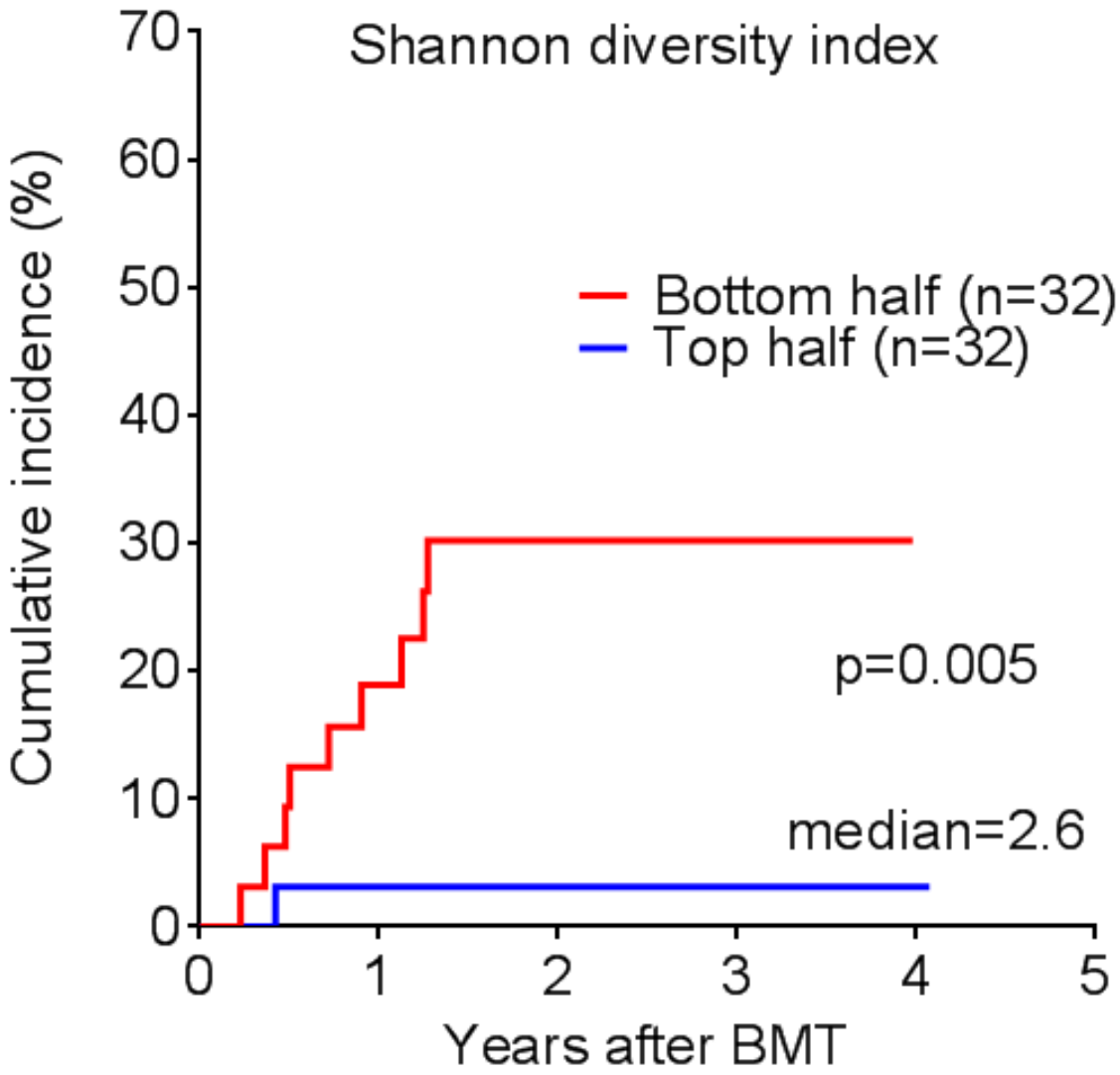
Decreased overall survival is associated with low diversity of intestinal microbial flora



Diversity is associated with protection from lethal GVHD

- 64 patients from MSKCC
- Underwent allo BMT 9/09 to 10/12
- Ex vivo or in vivo TCD excluded
- Analyzed day 12 (+/- 4 days) stool by V1-V3 (454)
- Stratified by Shannon diversity index

Intestinal flora diversity is associated with protection from lethal GVHD



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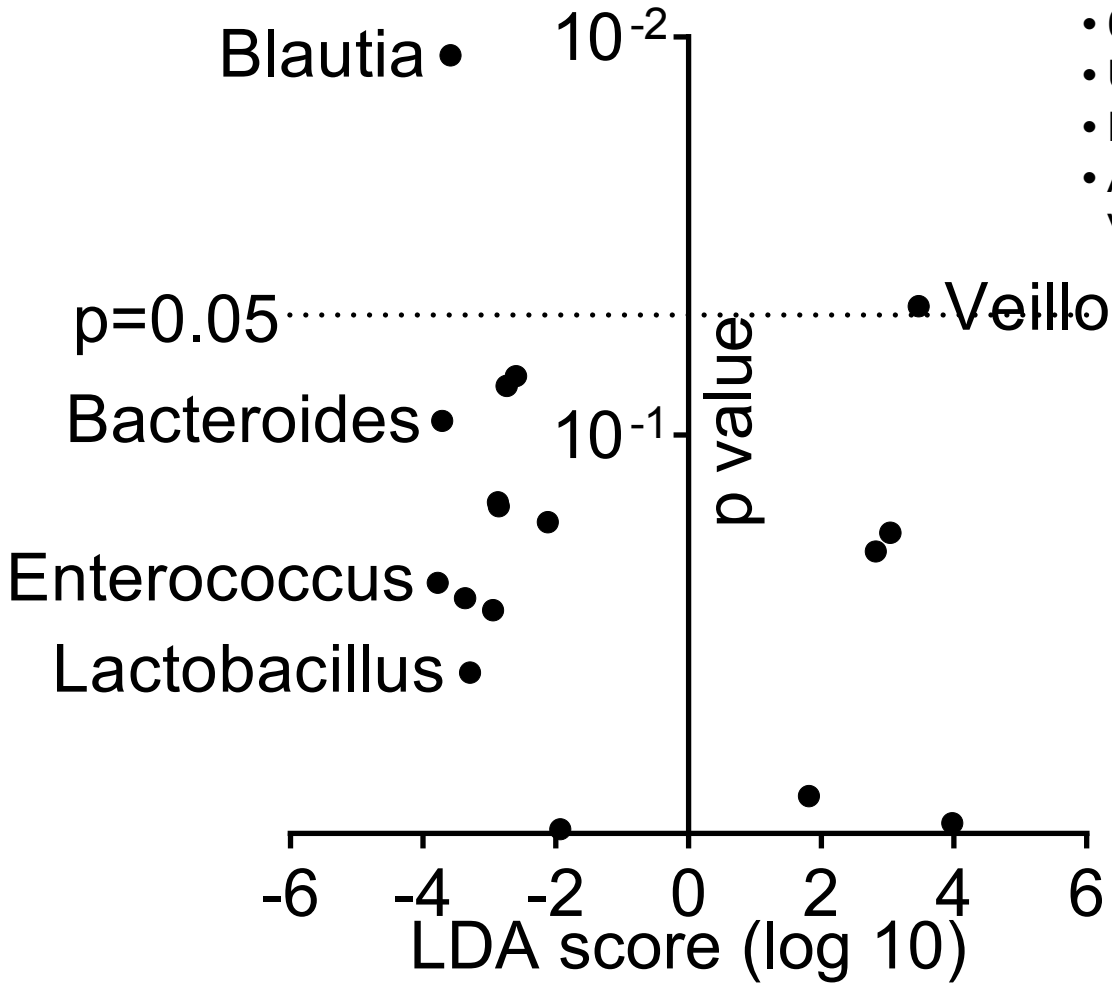
Bacterial genus associated with protection from lethal GVHD



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Favors no GVHD-related mortality

Favors GVHD-related mortality

Bacterial genus Blautia

- Named in 2008 in honor of Michael Blaut, a German microbiologist who studied human gut flora
- Group of species formerly classified as Clostridium or Ruminococcus
- Gram-positive, non-motile, coccoid or oval-shaped, obligately anaerobic
- Generally sensitive to vancomycin and metronidazole

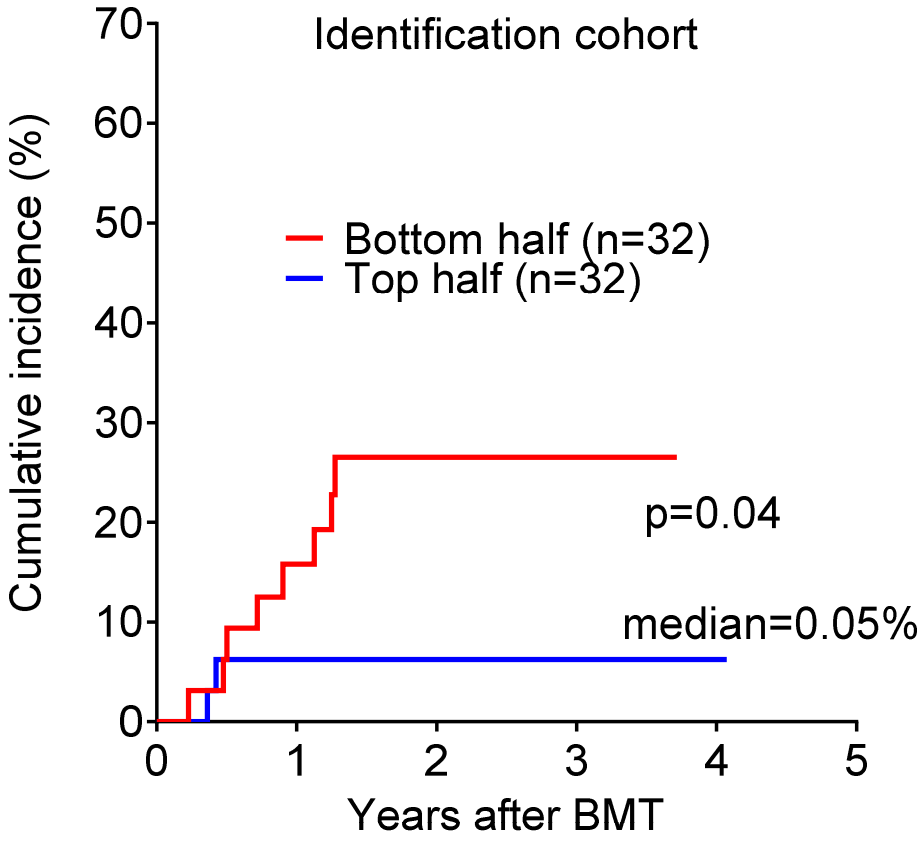
Unbiased approach: Do any bacterial subgroups impact on risk for gut GVHD?

- 64 adult patients transplanted from 9/09 to 10/12
- Evaluable for gut GVHD (survival until day 30, engrafted)
- 41% high-risk, 41% intermediate-risk, 17% low-risk
- 46% acute leukemia, 44% NHL
- 22% ablative, 42% reduced intensity, 36% nonablative
- 58% unmodified peripheral blood, 39% double cord blood
- Stool sample closest to BMT day 10, +/- 4 days
- Analyzed for abundance of bacterial subgroups, by 16S rRNA gene sequencing

Second cohort

- 51 adult patients transplanted from 8/11 to 8/13
- 34% high-risk, 30% intermediate-risk, 36% low-risk
- 56% acute leukemia, 34% NHL
- 18% ablative, 48% reduced intensity, 34% nonablative
- 60% unmodified peripheral blood, 36% double cord blood
- Evaluable for gut GVHD (survival until day 30, engrafted)
- Stool sample closest to BMT day 10, +/- 4 days
- Analyzed for abundance of bacterial subgroups, by 16S gene sequencing

Evaluating predictive power of Blautia abundance for lethal GVHD



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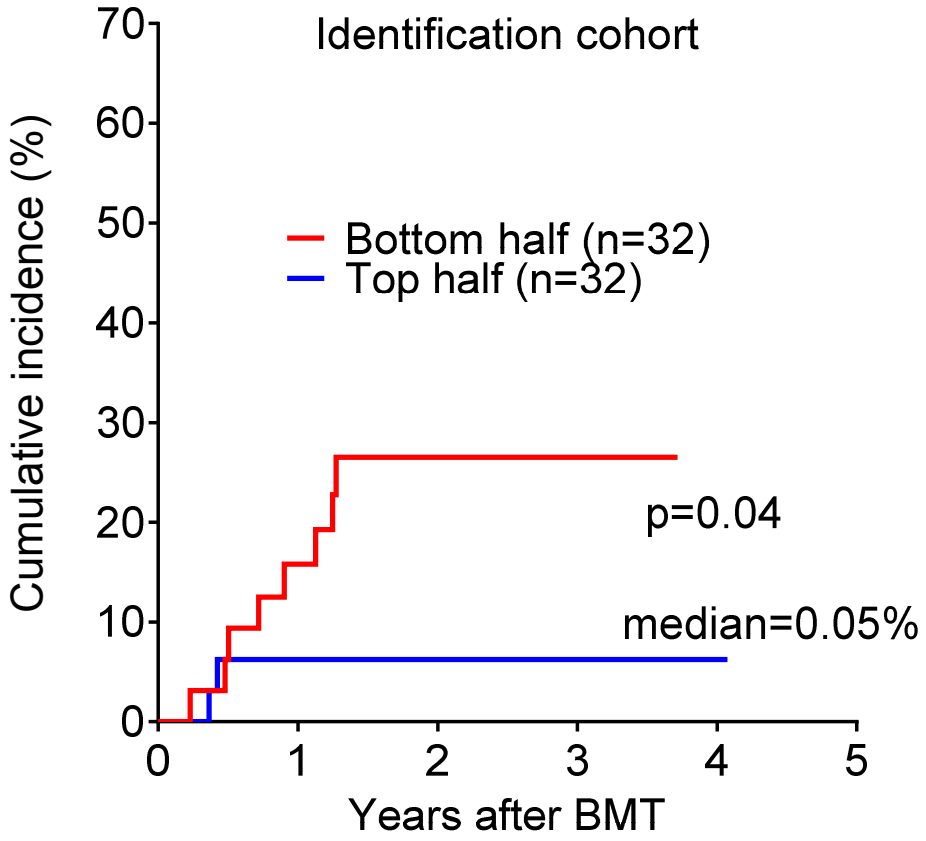
- Underwent allo BMT 9/09 to 10/12
- Analyzed by V1-V3 (454)

Evaluating predictive power of Blautia abundance for lethal GVHD



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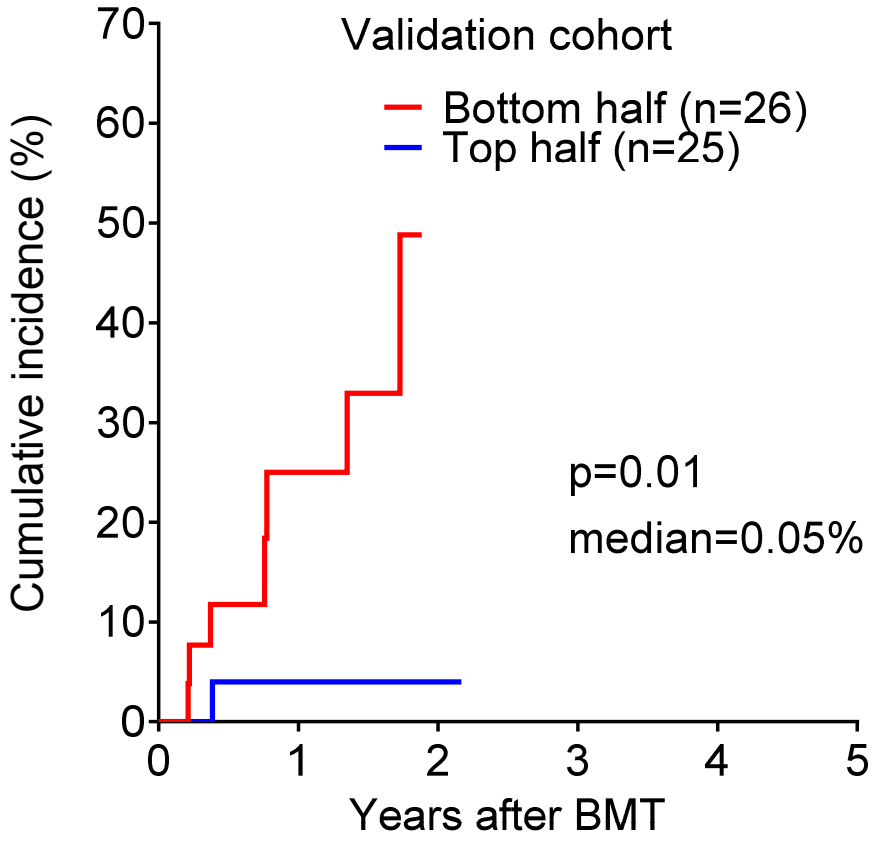
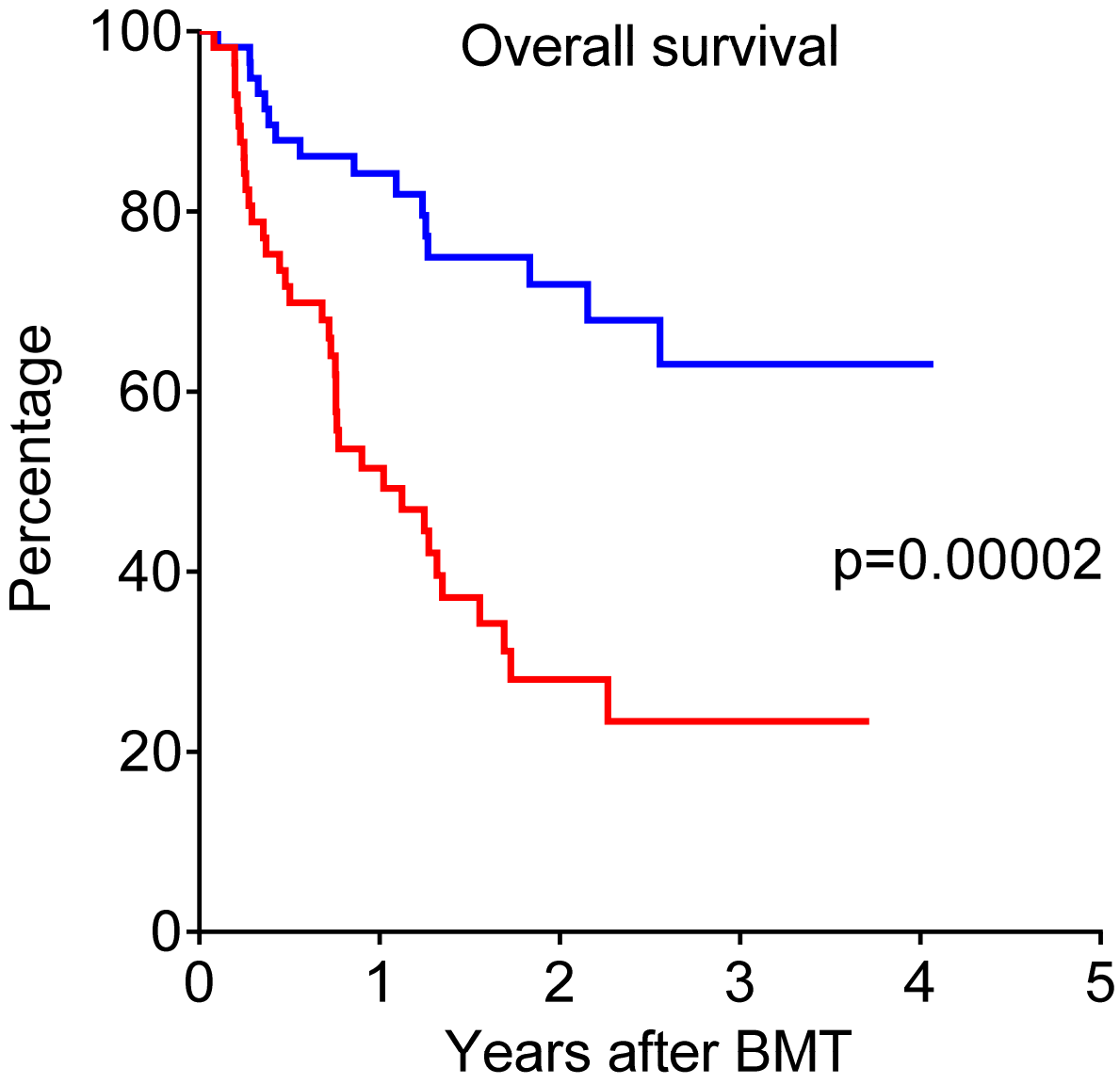
- Underwent allo BMT 9/09 to 10/12
- Analyzed by V1-V3



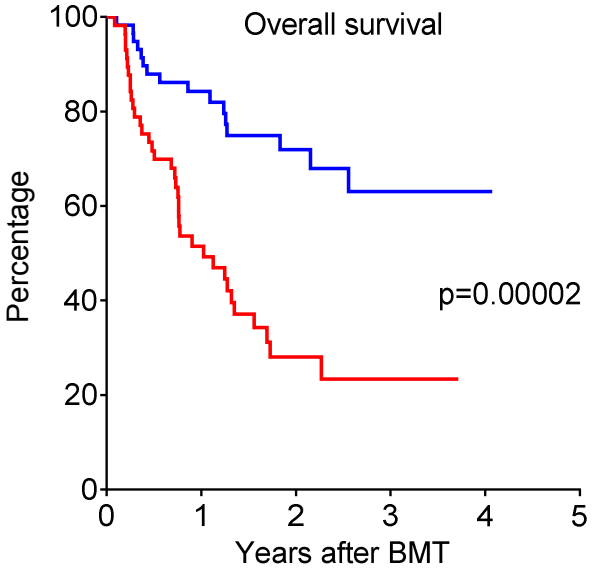
- 51 patients from MSKCC
- Underwent allo BMT 8/11 to 8/13
- Analyzed by V4-V5

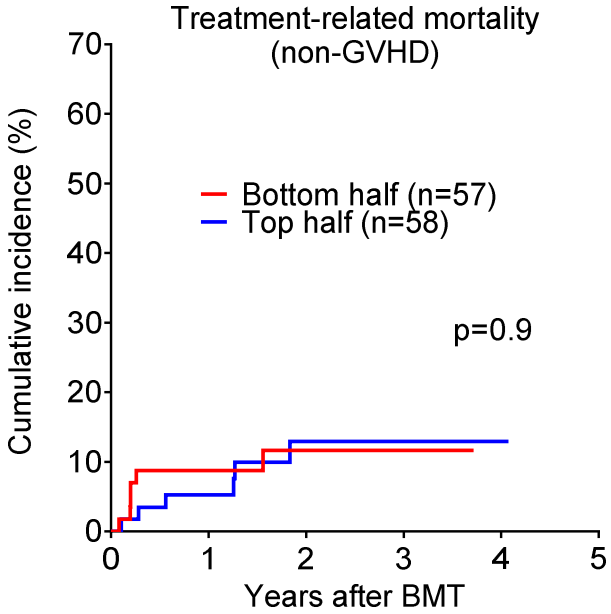
Blautia is associated with improved outcomes

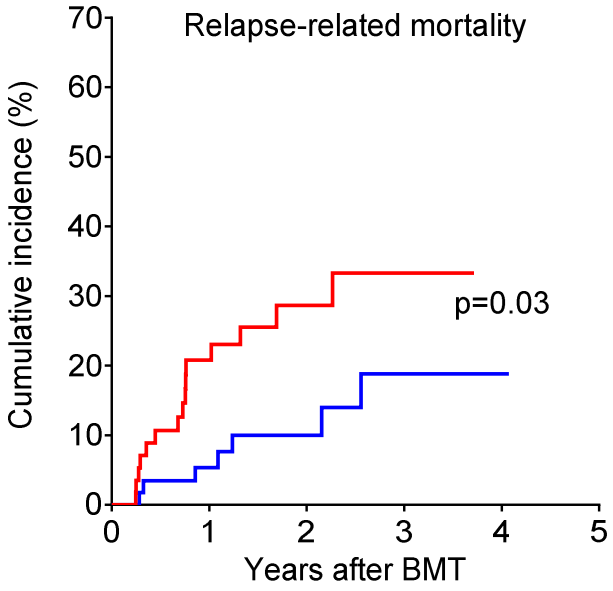


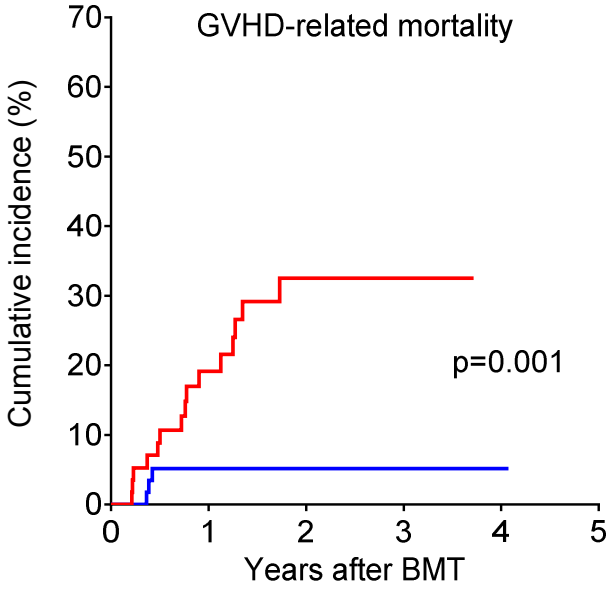
- Two cohorts combined

Blautia is associated with improved outcomes





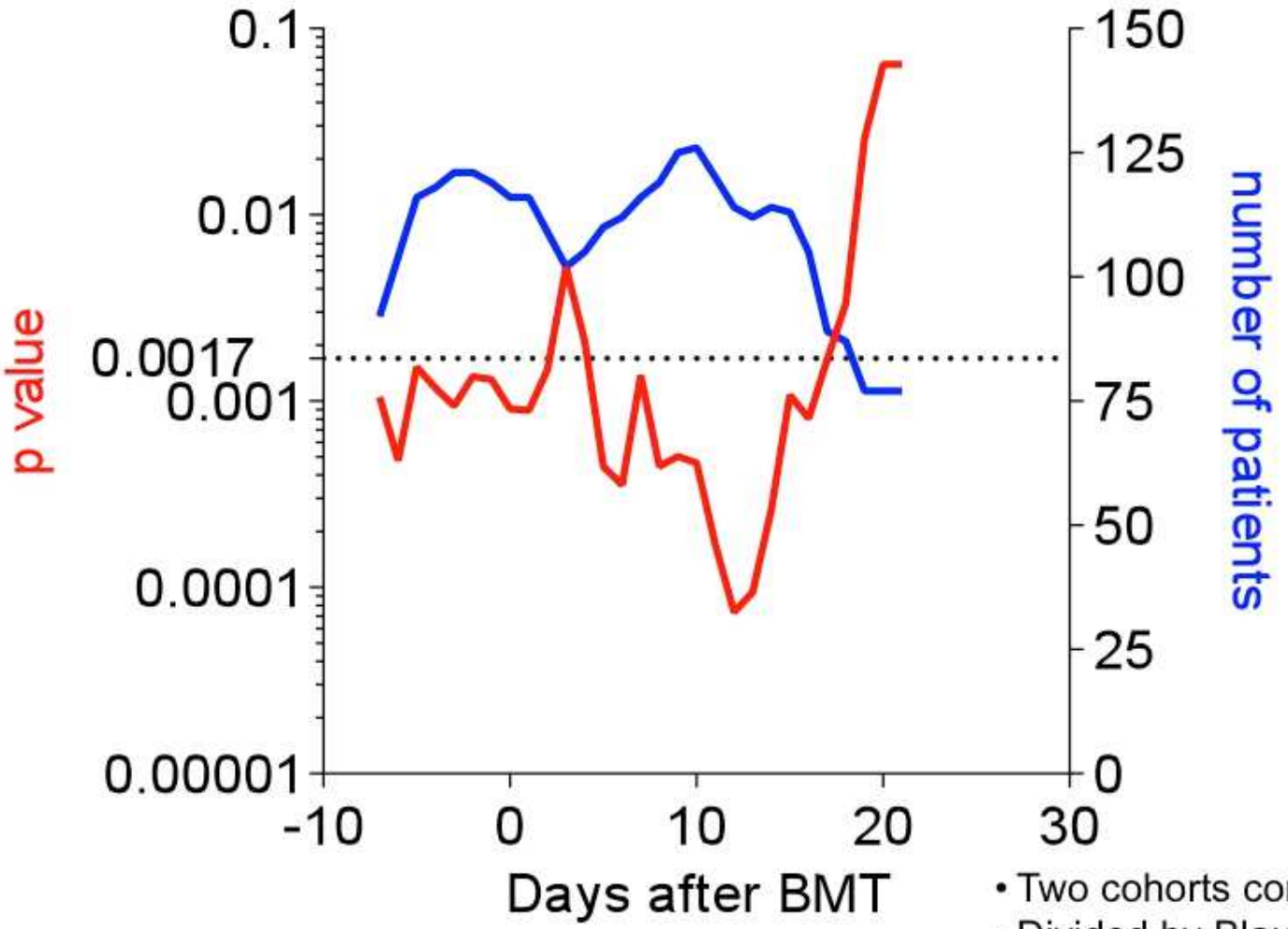




- Two cohorts combined

Blautia's ability to predict GVHD-related mortality – when to look?

Blautia is most predictive for reduced lethal GVHD shortly after day 10



- Two cohorts combined
- Divided by Blautia abundance cutoff of 0.0001 (bottom tertile)
- p value cutoff of 0.0017 using Bonferroni correction for multiple comparisons (30)

Do known GVHD risk factors impact on Blautia abundance?

- MUD vs MRD:
- Patient race: W/B vs Asian:
- CMV neg/neg vs any positive:
- Donor gender: M vs F:
- Donor/recipient gender: F/M vs non-F/M:
- Performance status: <90 vs 90-100: p=

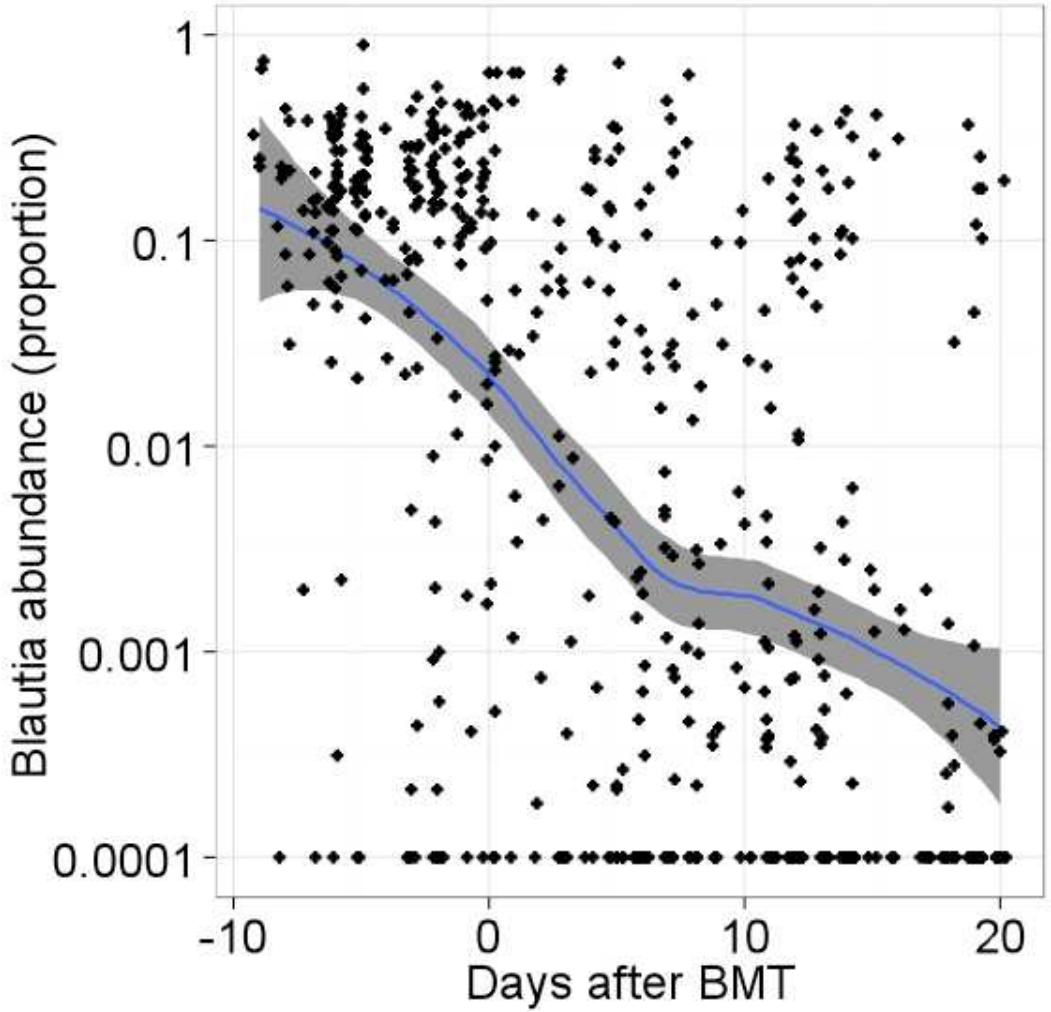
Acknowledgement: Sean Devlin

Do known GVHD risk factors impact on Blautia abundance?

- MUD vs MRD: p=0.62
- Patient race: W/B vs Asian: p=0.69
- CMV neg/neg vs any positive: p=0.93
- Donor gender: M vs F: p=0.24
- Donor/recipient gender: F/M vs non-F/M: p=0.85
- Performance status: <90 vs 90-100: p=0.62

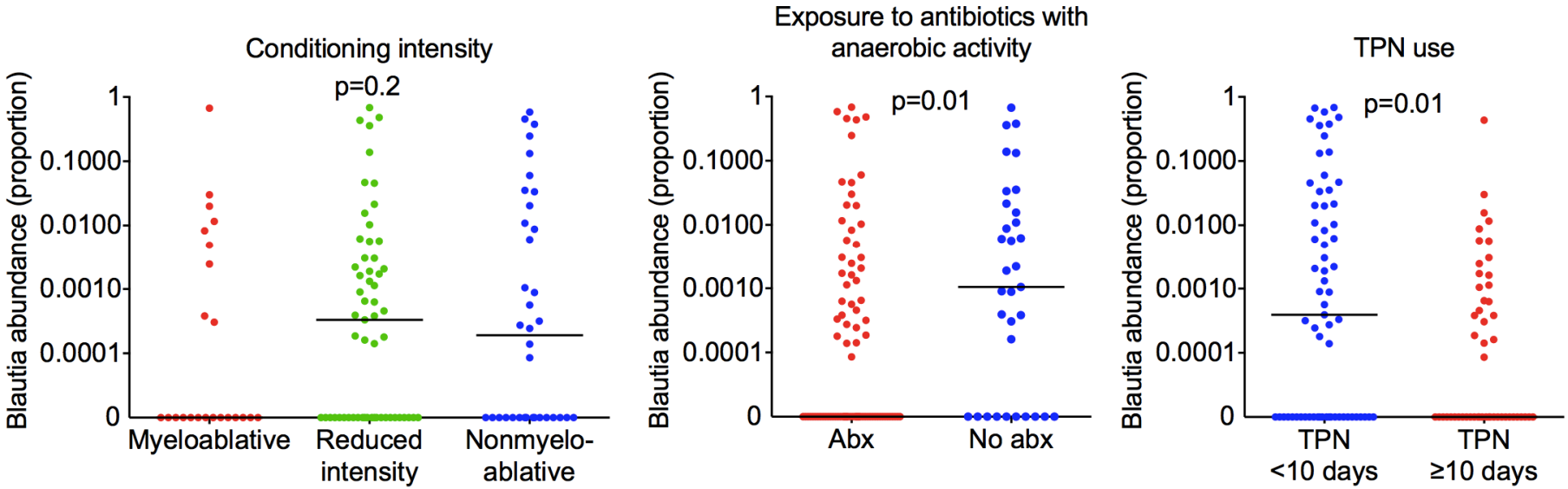
Acknowledgement: Sean Devlin

Blautia is abundant on admission but rapidly declines



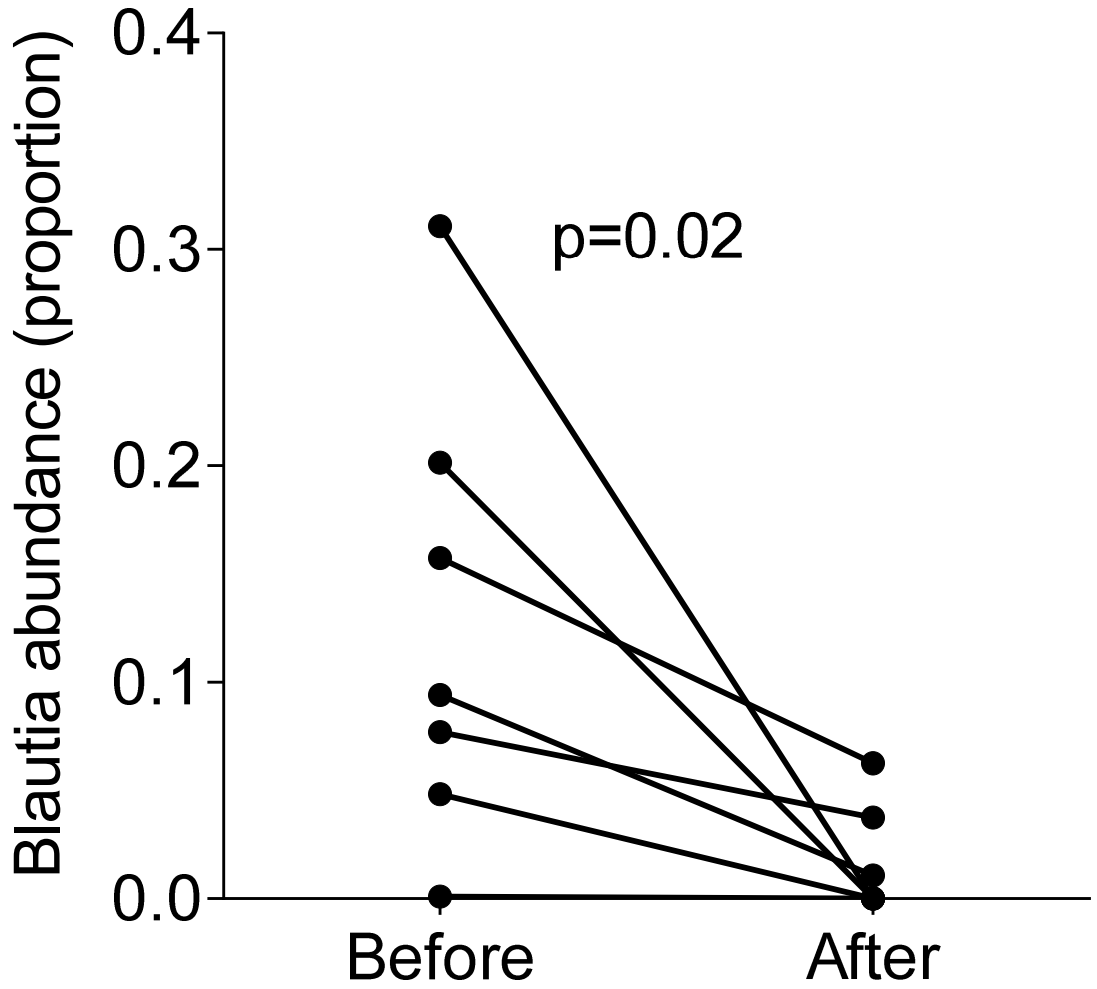
- Two cohorts combined
- Blautia abundance trend
- 95% confidence intervals shown in gray

Determinants of Blautia abundance: exposure to anaerobic antibiotics and TPN use



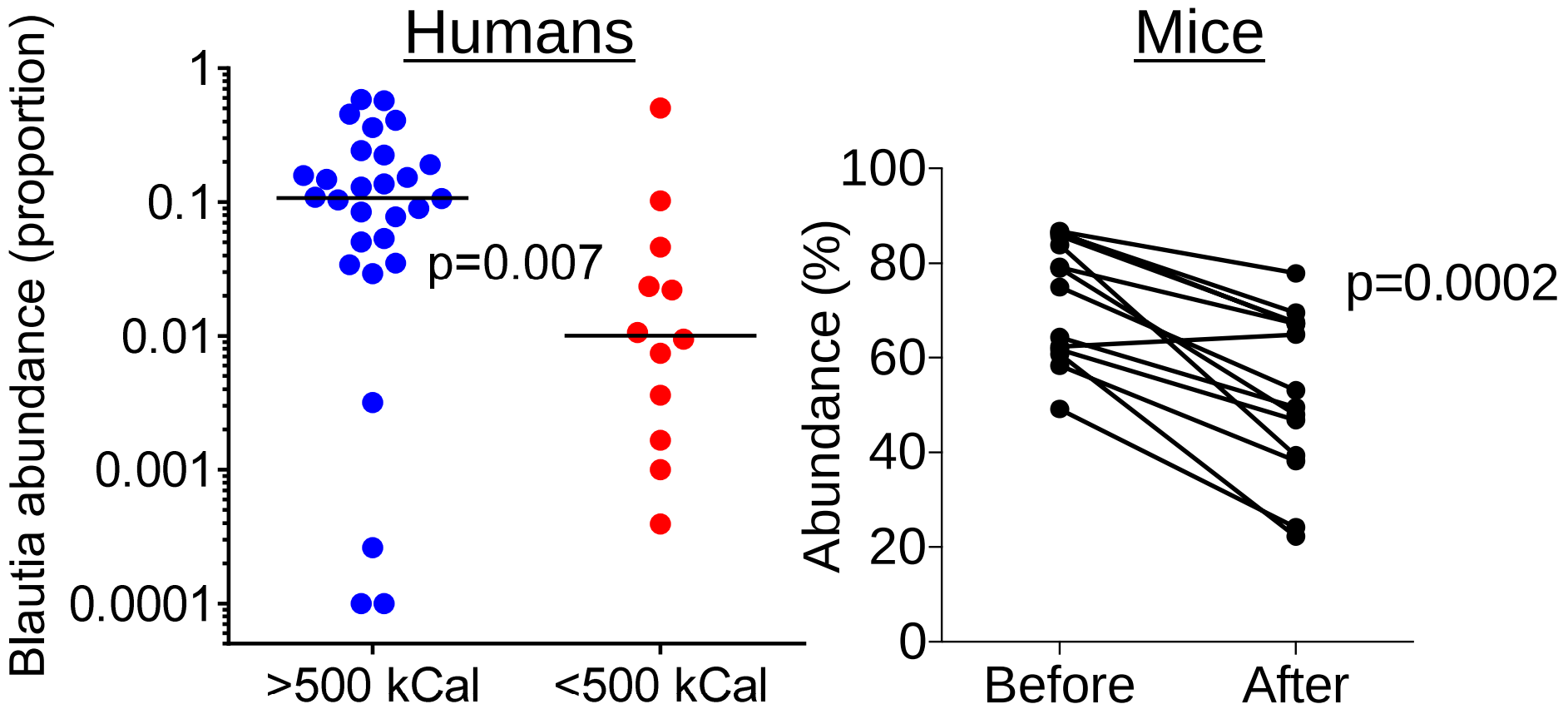
•Two cohorts combined

Blautia is reduced upon exposure to anaerobic antibiotics



- 7 patients with frequent stool monitoring
- 1-2 days prior to treatment and 2-3 days following treatment

Reduced caloric intake leads to loss of Blautia and Clostridiales



Acknowledgements: Tatanisha Peets and Melissa Lumish

Effects of GVHD on Blautia and Clostridiales

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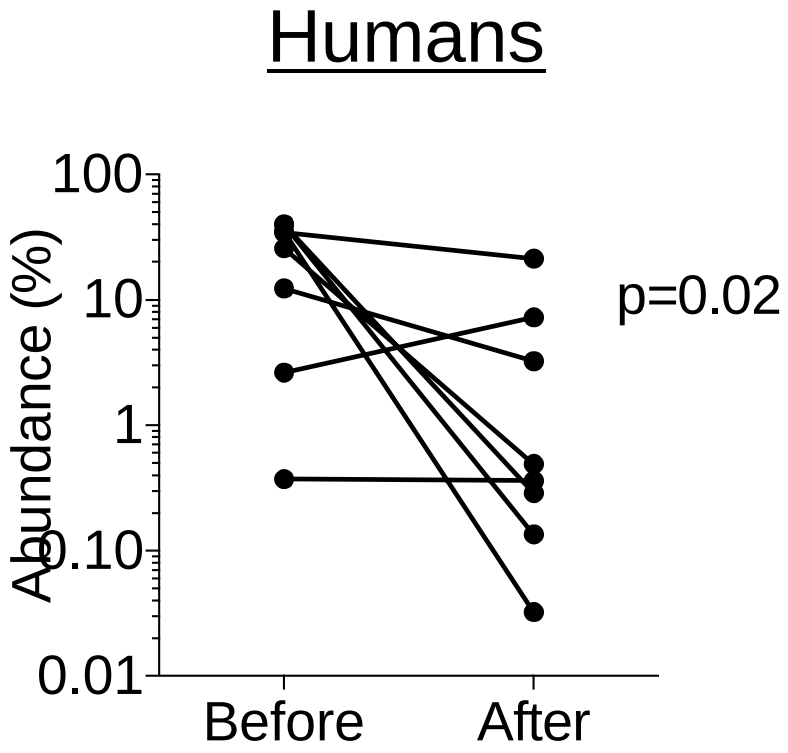
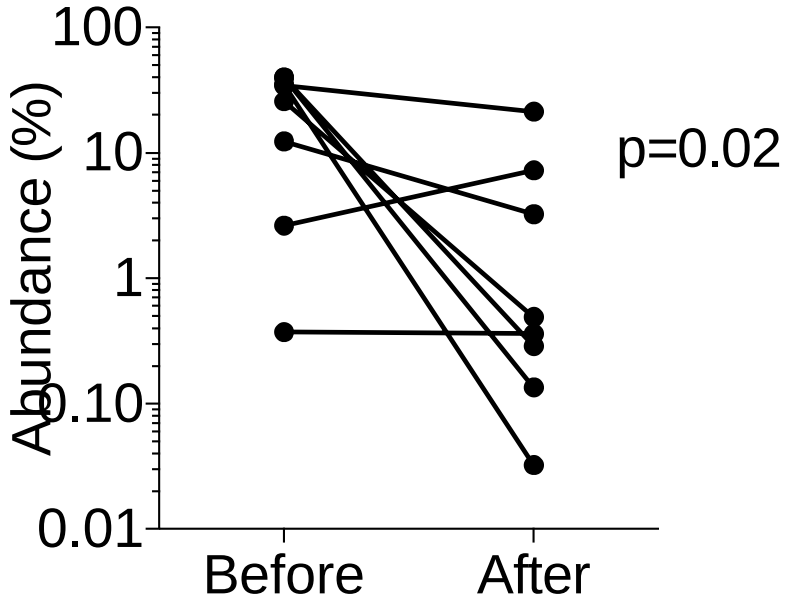
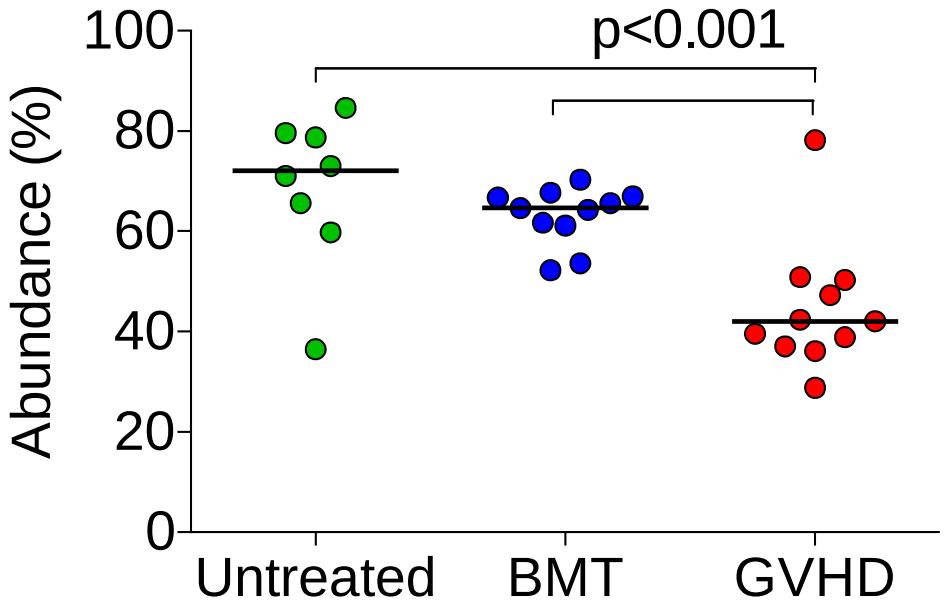


Effects of GVHD on Blautia and Clostridiales

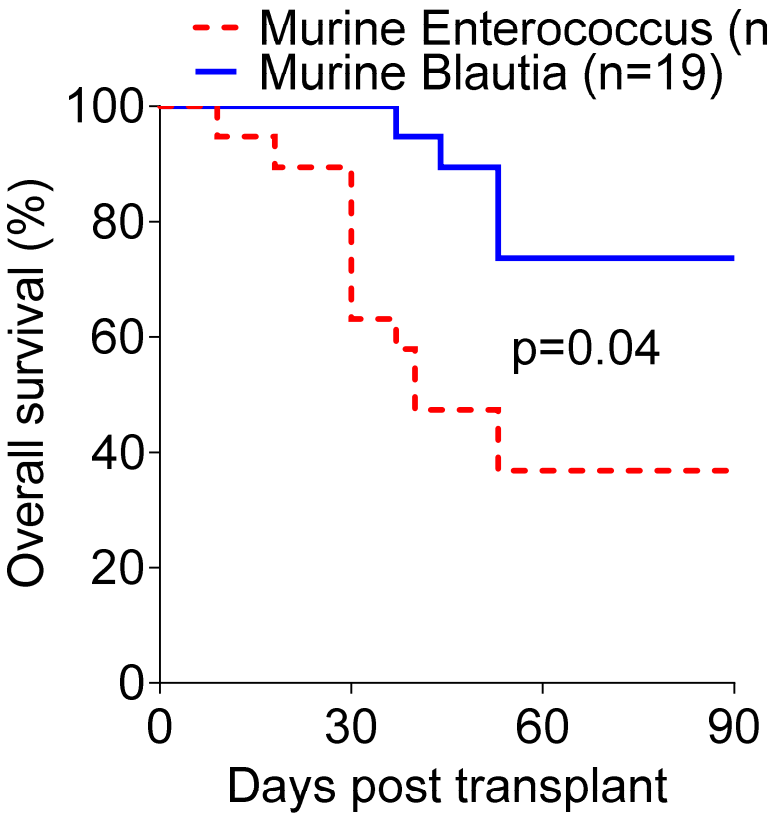
Humans



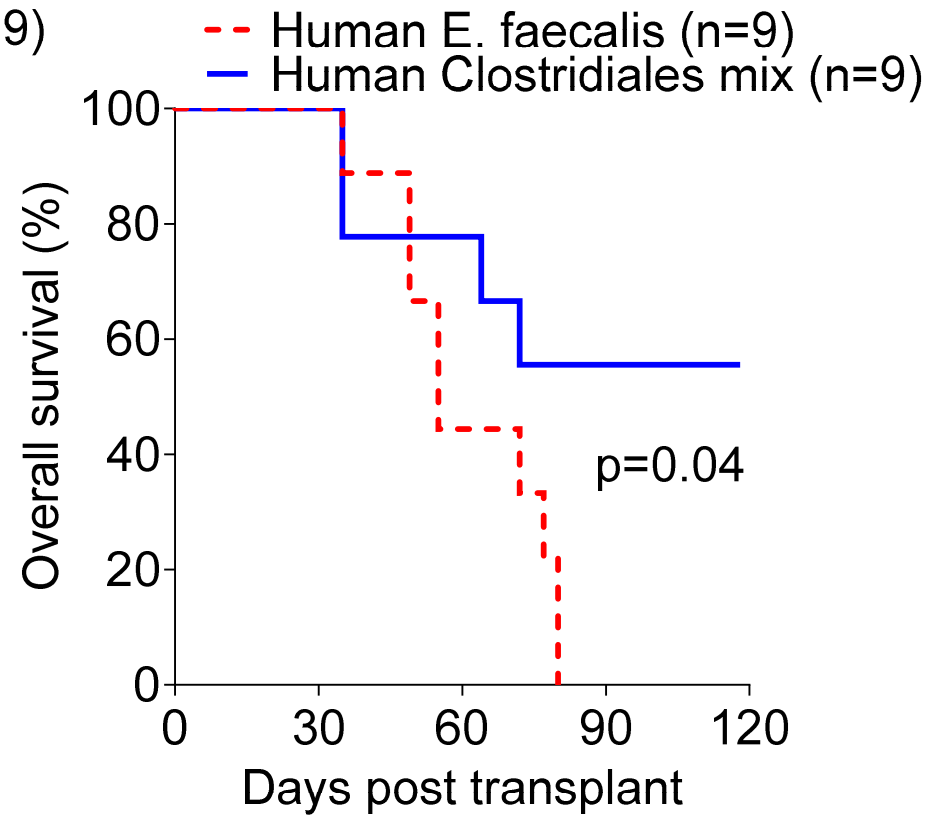
Mice



Bacterial re-introduction after microbiota injury reduces experimental GVHD



- Two experiments
- B10.BR into B6, BM + T
- Mice treated pre-BMT with cocktail of abx and indicated bacteria by gavage twice
- Acknowledgement: Silvia Caballero



- Single experiment
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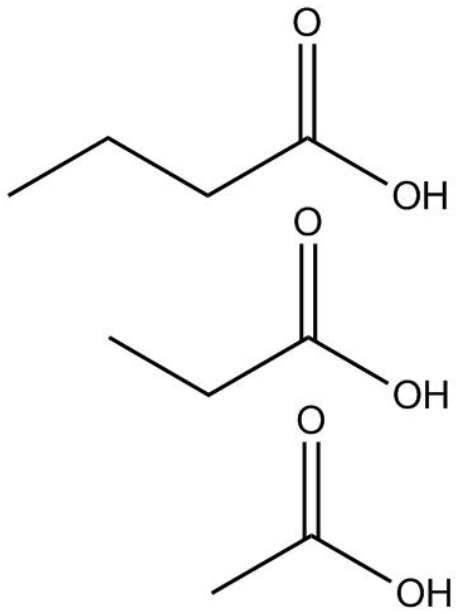
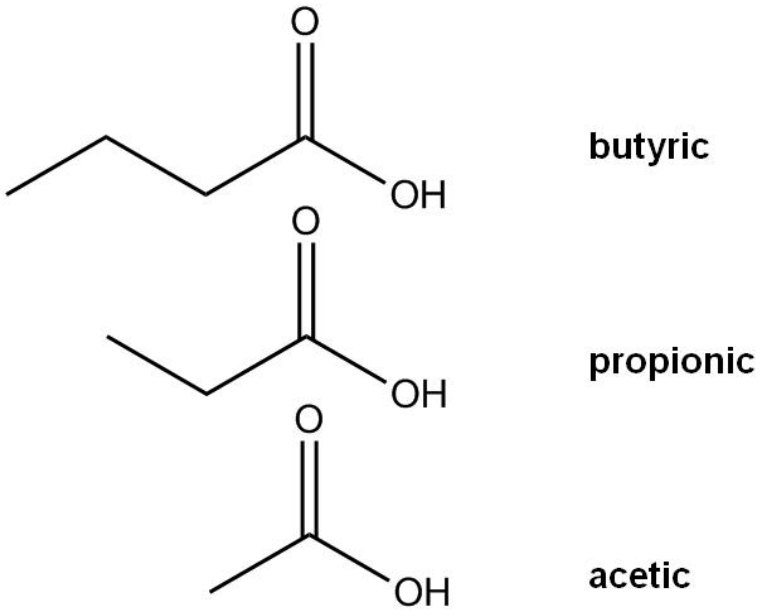
- Mice treated pre-BMT with cocktail of abx and indicated bacteria by gavage twice
- Acknowledgement: Kenya Honda

Possible anti-inflammatory mechanism of Blautia

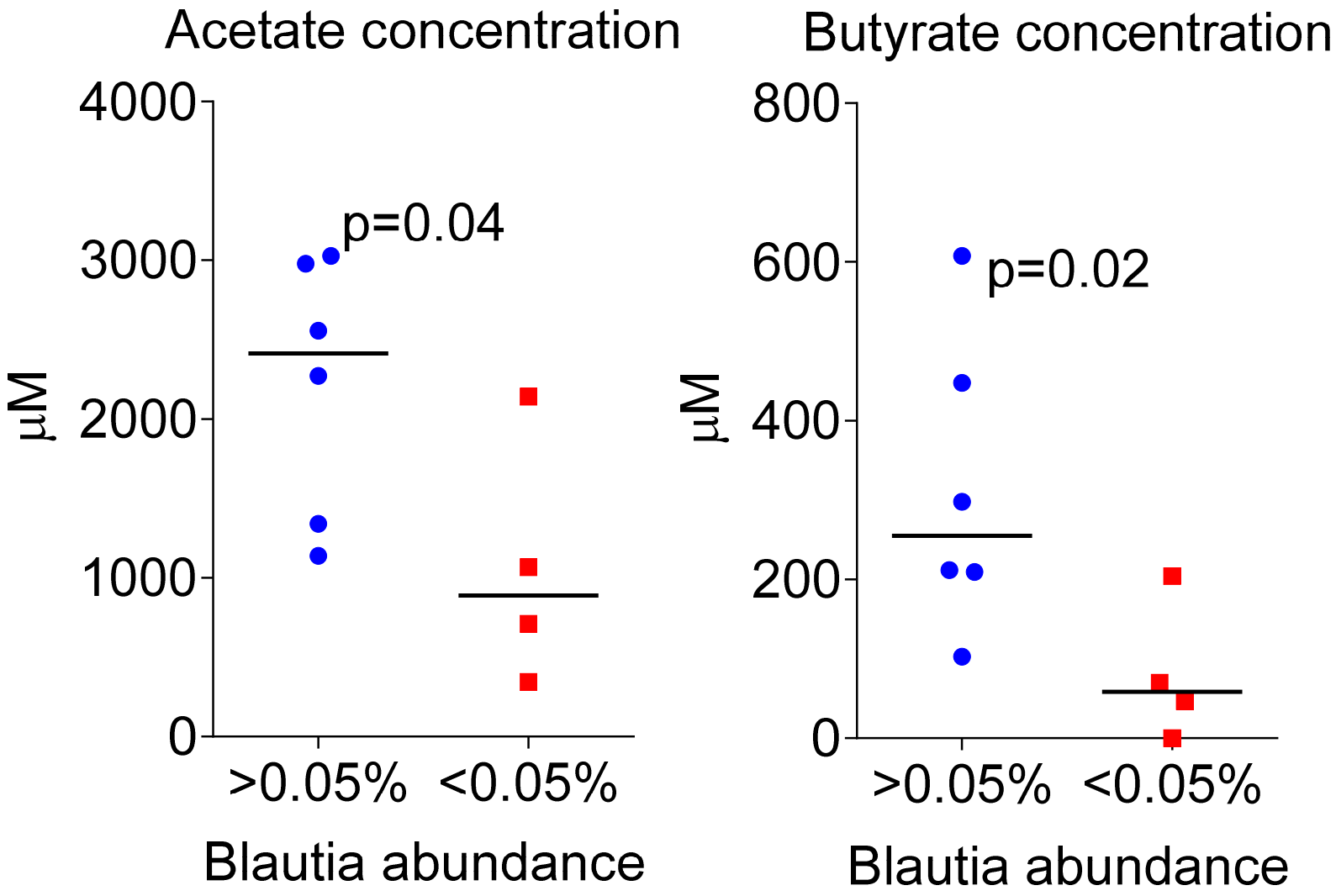


Possible anti-inflammatory mechanism of Blautia

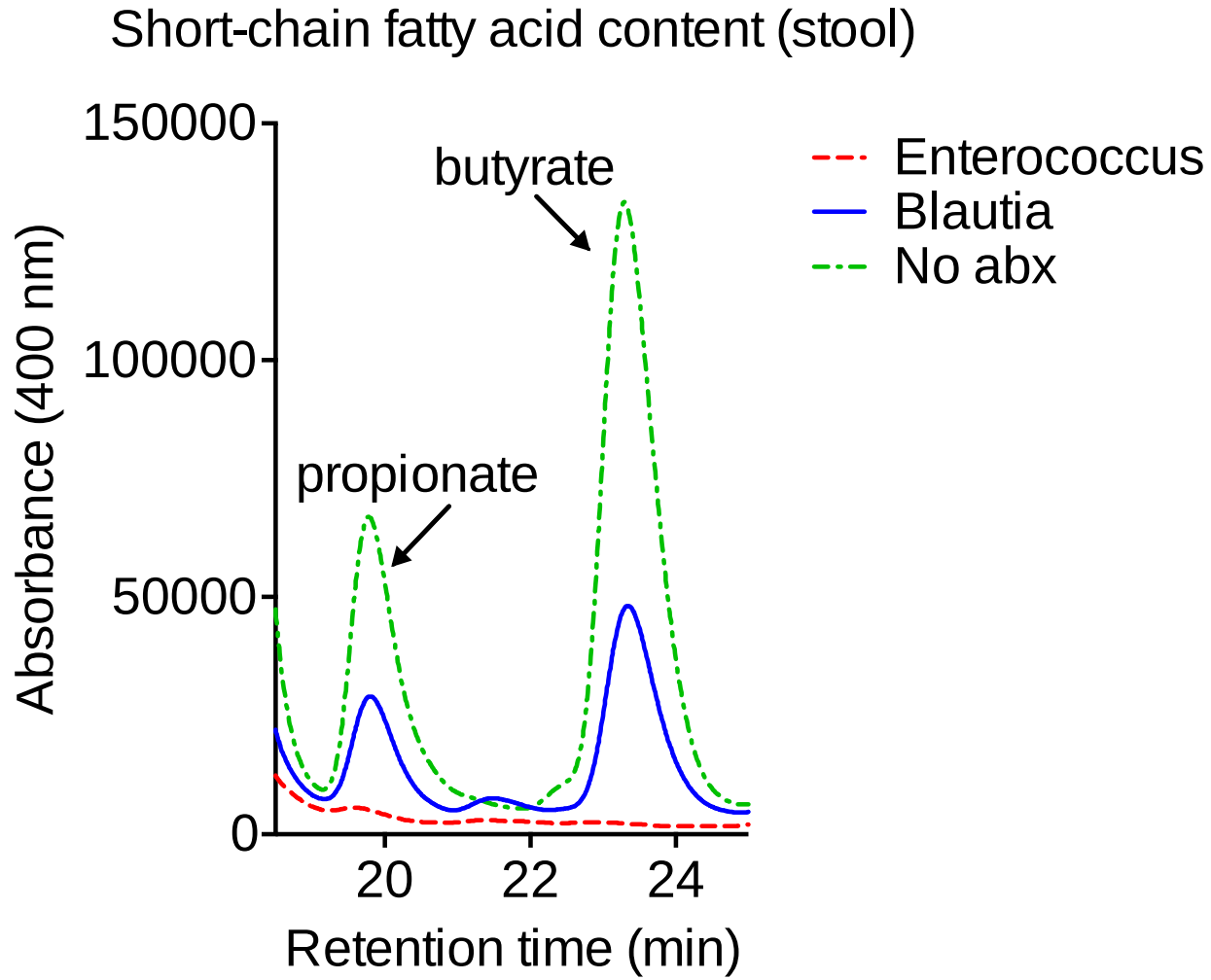
- Clostridial strains support colonic Tregs and produce short-chain fatty acids (Honda, 2011, 2013)
- Administration of acetate, propionate or butyrate increase colonic Tregs (Garrett et al, 2013)
- Administration of butyrate induces Foxp3 via HDAC inihibitor effects on CD4T cells and dendritic cells (Rudensky et al, 2013, Ohno et al, 2013)
- HDAC inhibition reduces GVHD in mice and humans (Reddy, Ferrara et al, 2004, 2008, 2014)



Association of Blautia abundance with short-chain fatty acid concentrations



Blautia introduction after antibiotics restores short-chain fatty acid levels



Acknowledgements: Nick Arpaia and Paul DeRoos

Prebiotics

- Encouraging eating
- Gastric nutritional supplementation
- Flora-targeted nutritional supplements

Probiotics

- Re-introducing endogenous flora (autologous fecal microbiota transplant)
- Re-introducing selected bacteria with beneficial potential

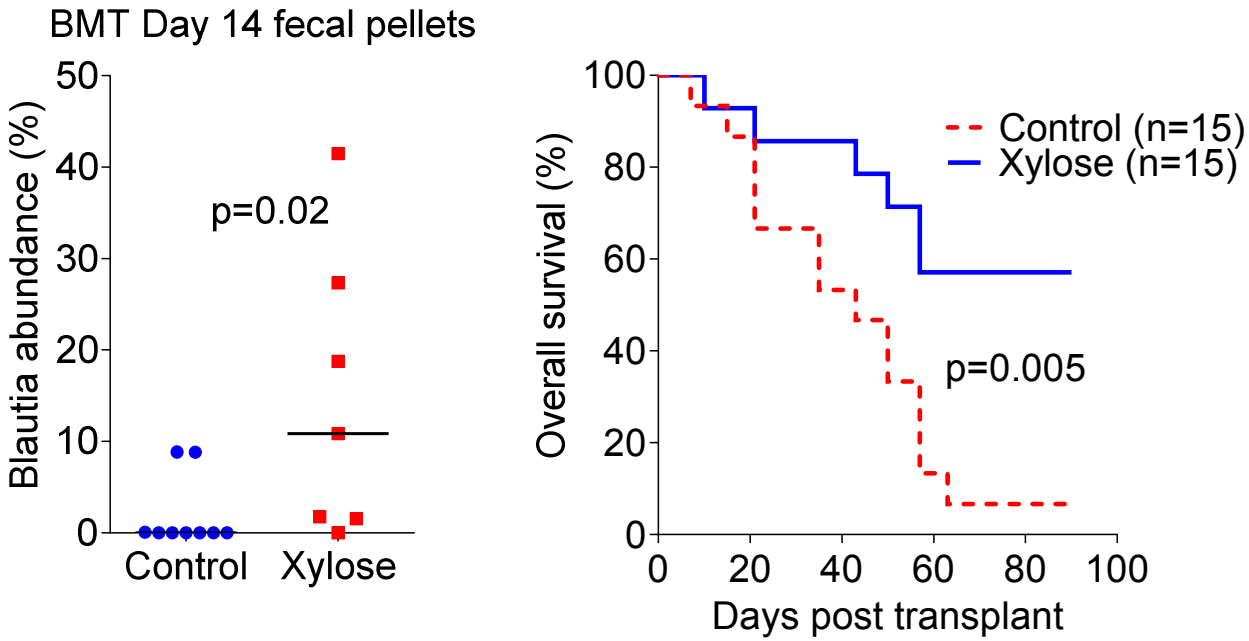
Antibiotics

- Selecting antibiotics that spare bacteria with beneficial potential

Postbiotics

- Identifying and introducing bacterial metabolites that mediate the anti-inflammatory effects

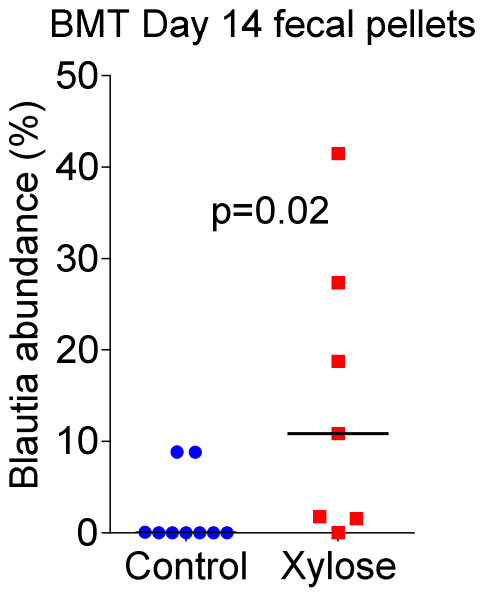
Strategies to prevent GVHD: prebiotics and postbiotics

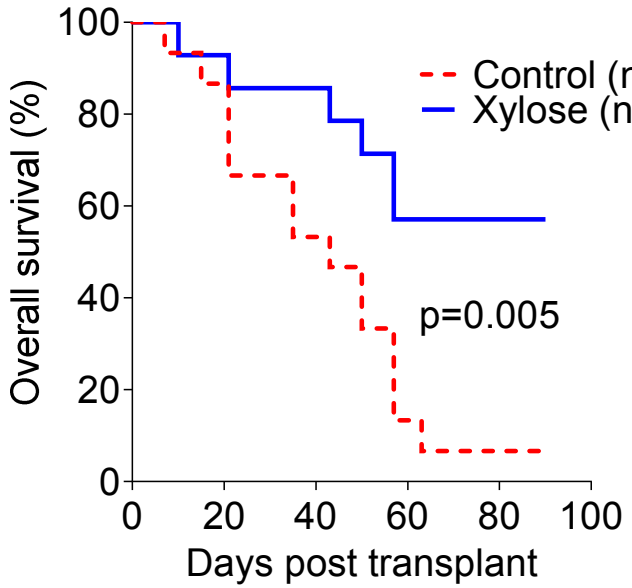


- Two experiments
- B10.BR into B6, BM + T
- Mice treated with xylose drinking water beginning 1 week pre-BMT

- Three experiments
- B6 into 129S1
- Mice treated with sodium acetate drinking water beginning 1 week pre-BMT

Strategies to prevent GVHD: prebiotics and postbiotics





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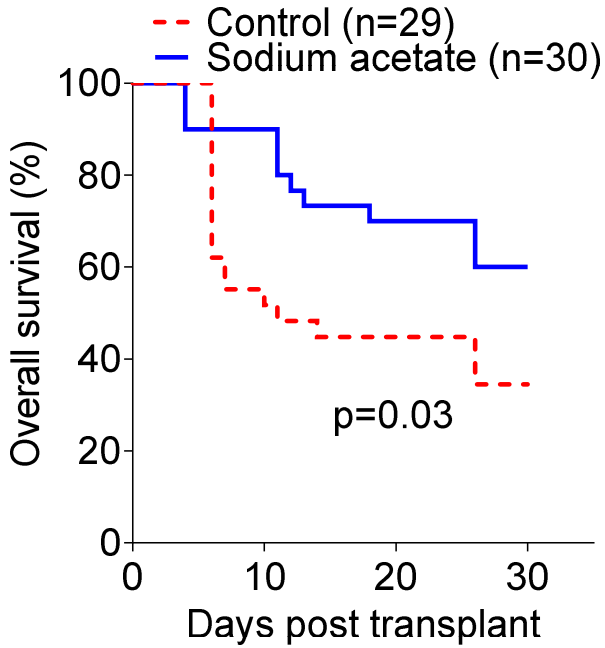

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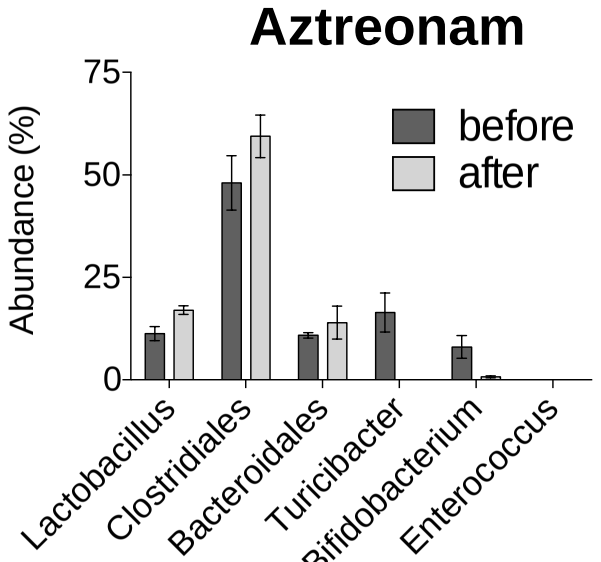
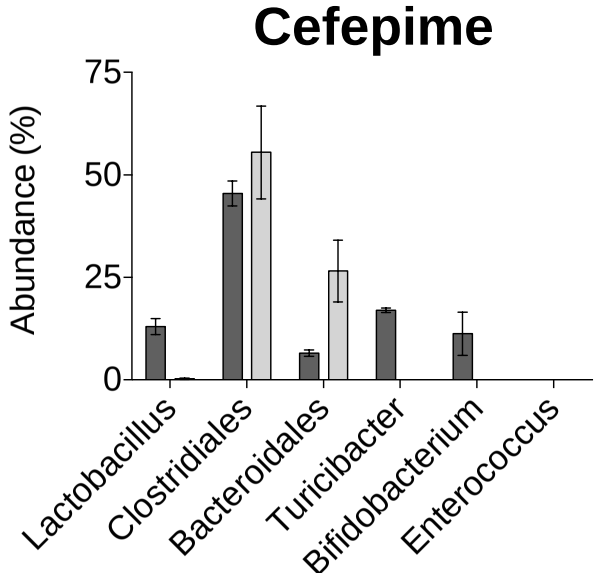
Antibiotic strategies to prevent GVHD: selecting those that spare Clostridiales





Antibiotic Treatment; BID SC x2 daily, 3 days at 100 mg/kg

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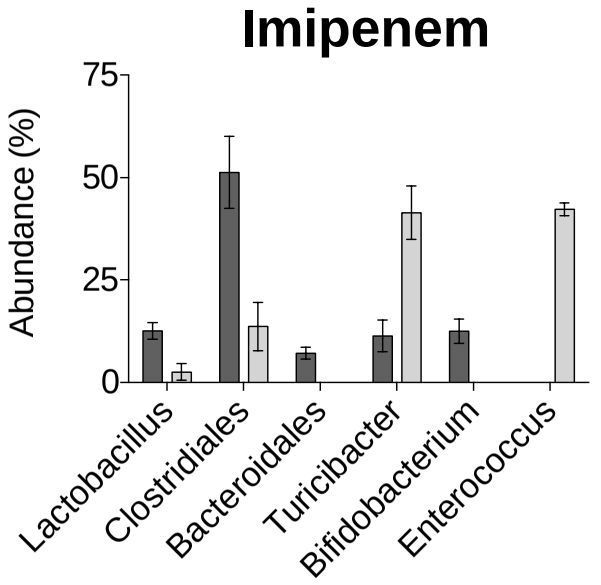
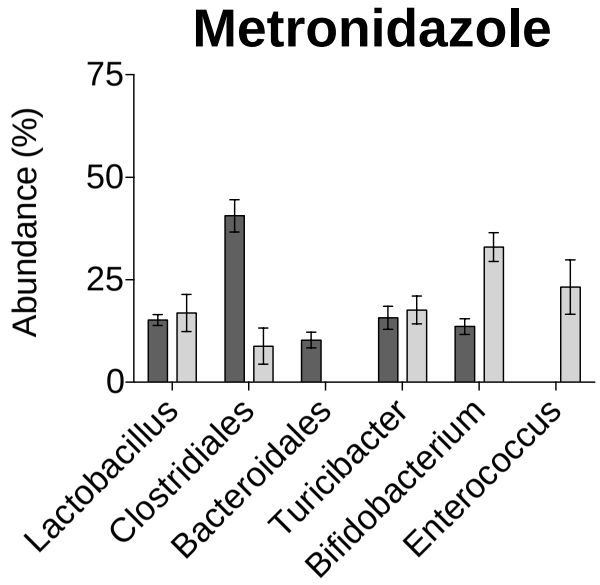
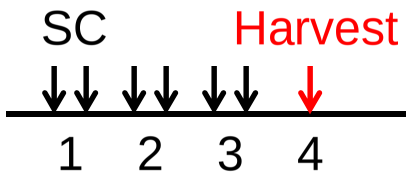
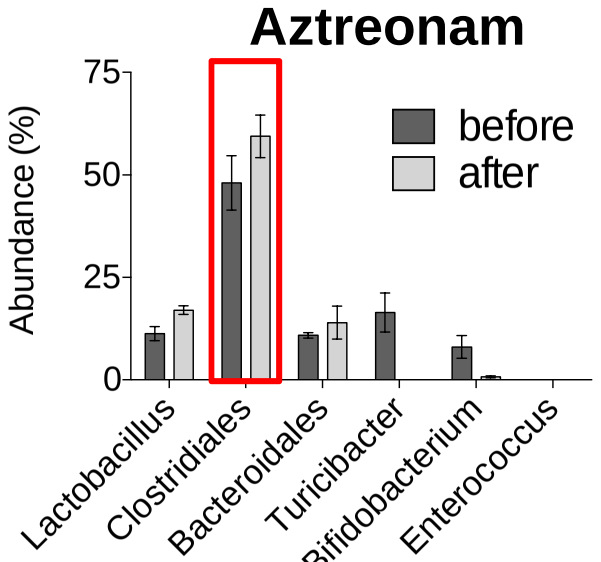
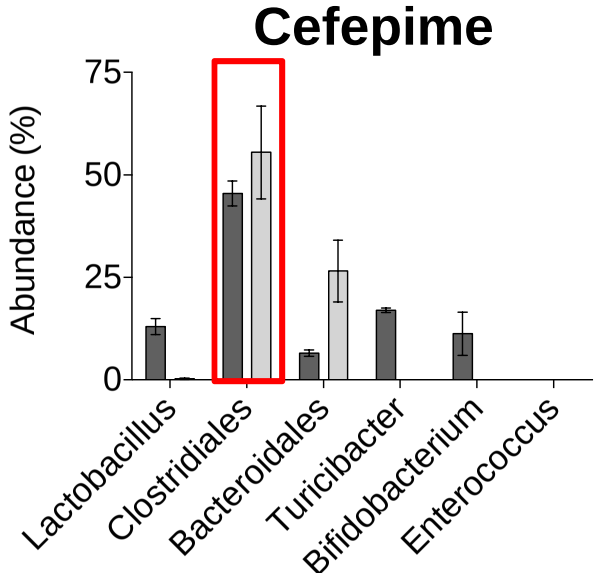


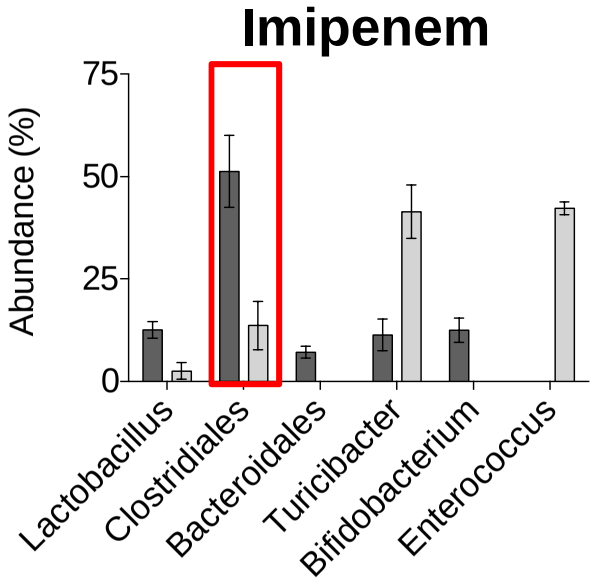


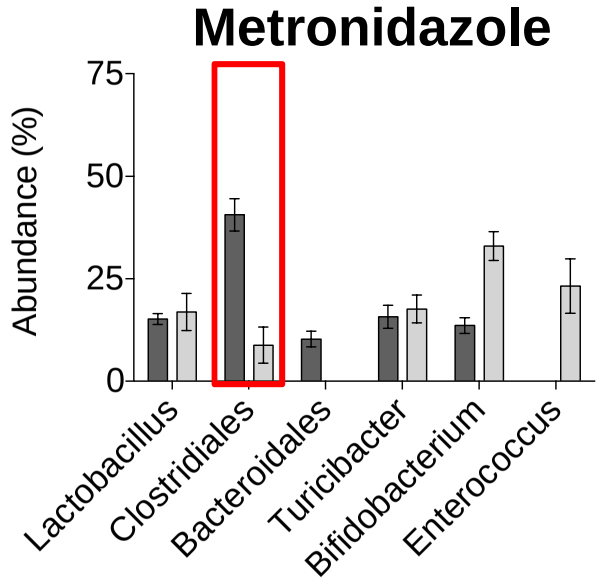


Antibiotic strategies to prevent GVHD: selecting those that spare Clostridiales









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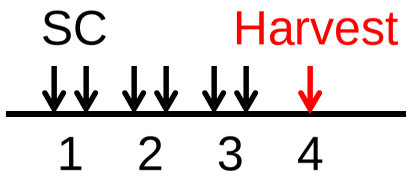

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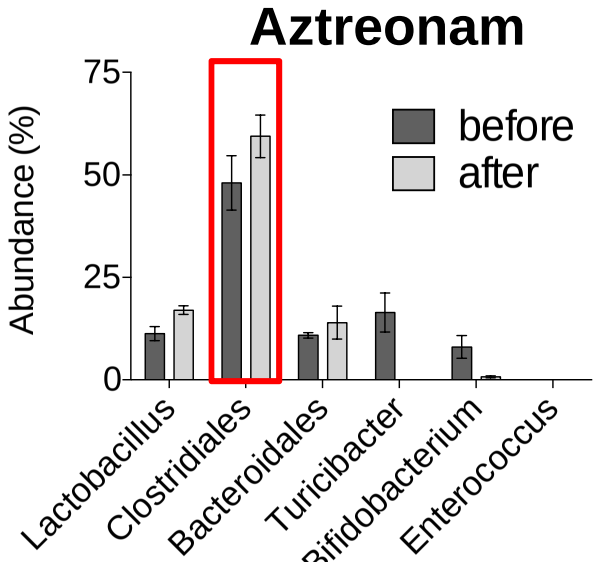

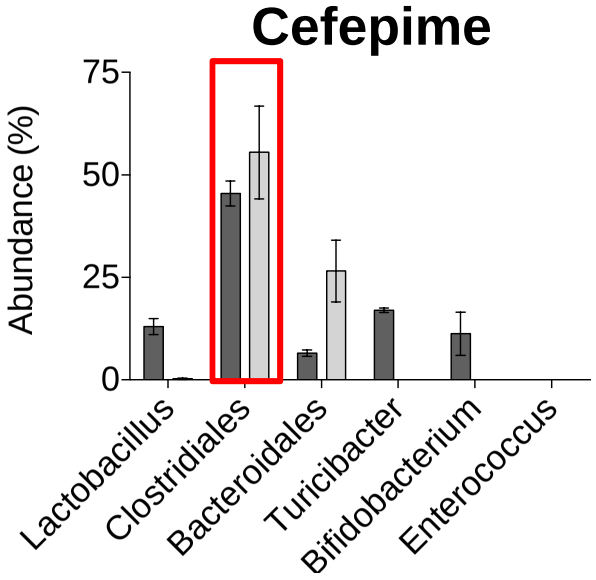

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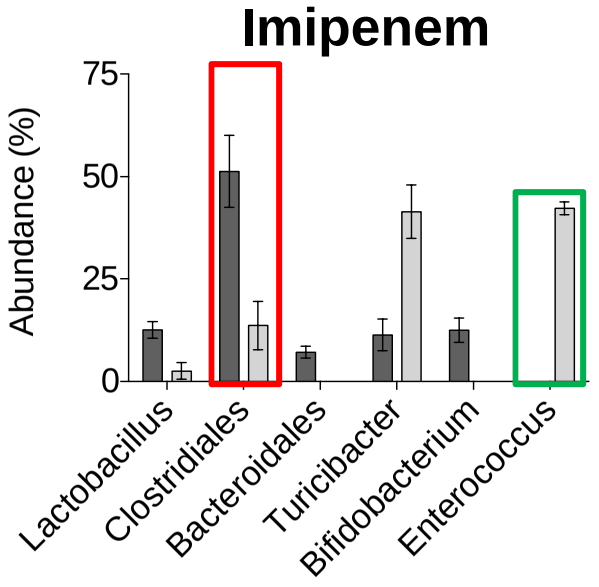
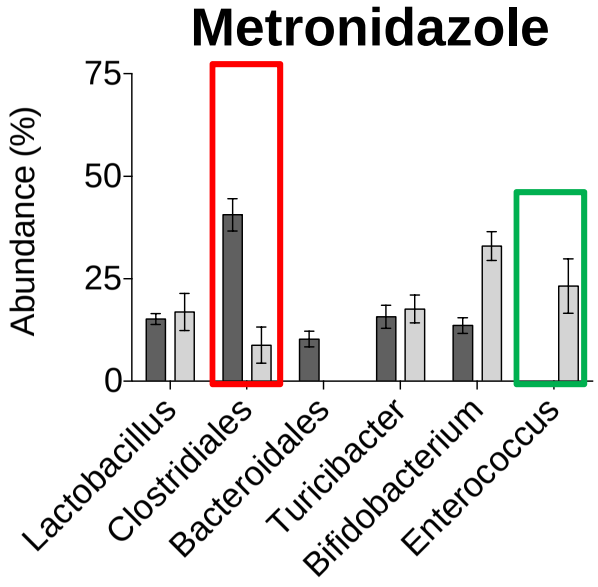
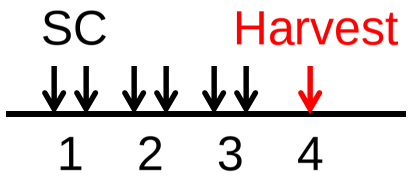
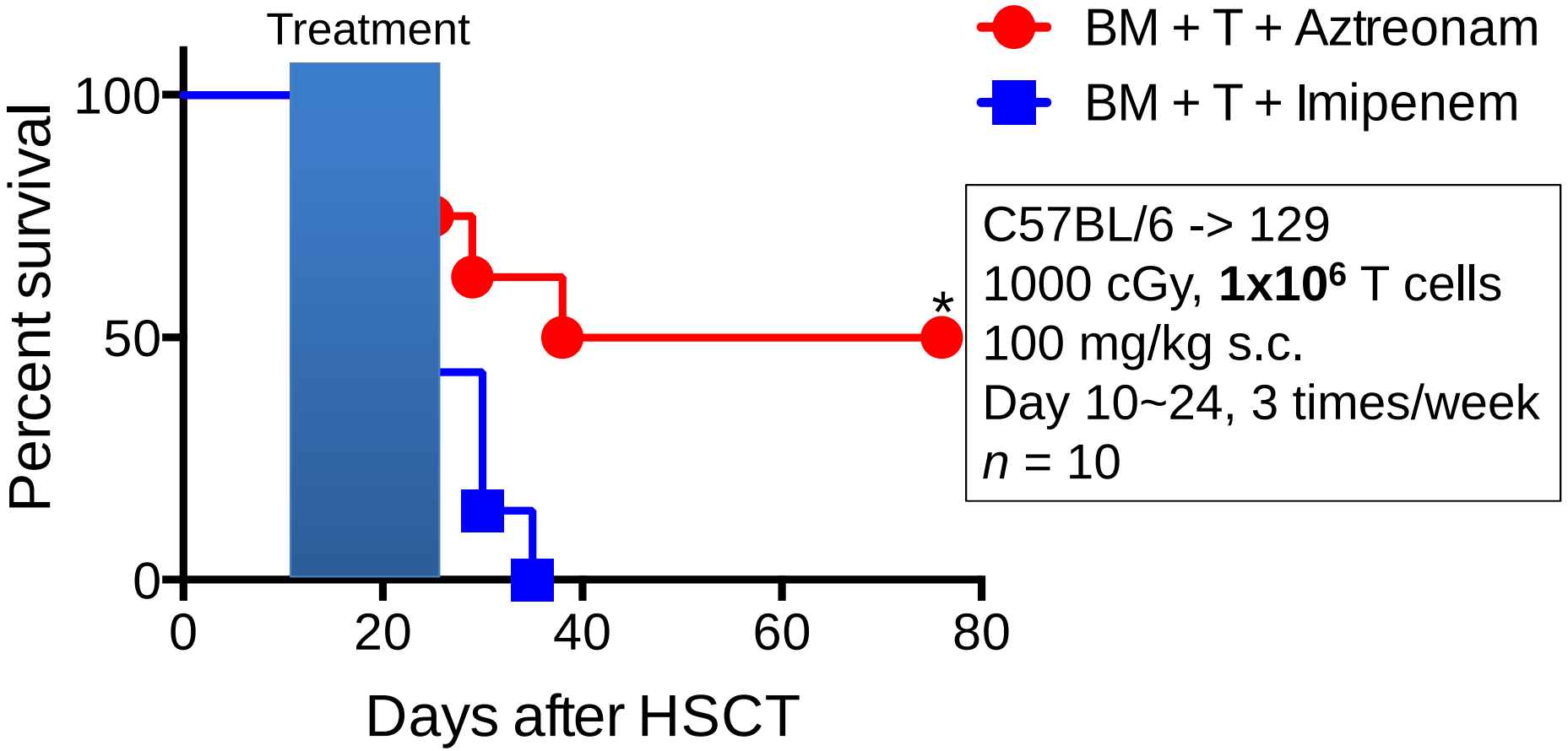






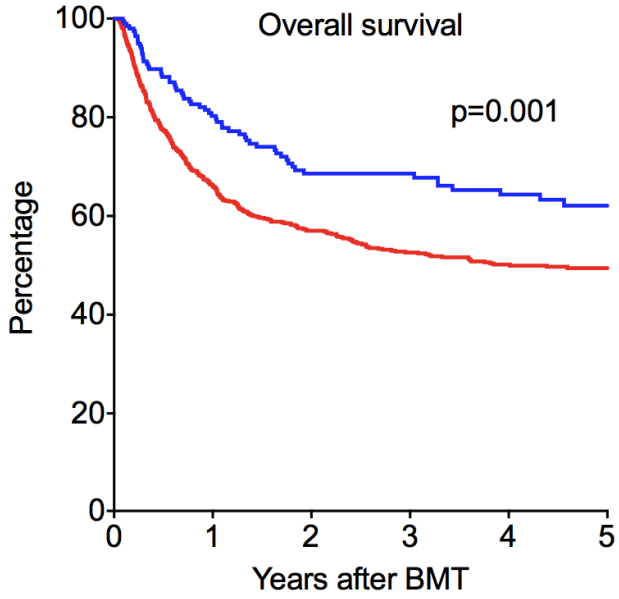
Clostridiales eliminating antibiotic Imipenem worsens GVHD survival

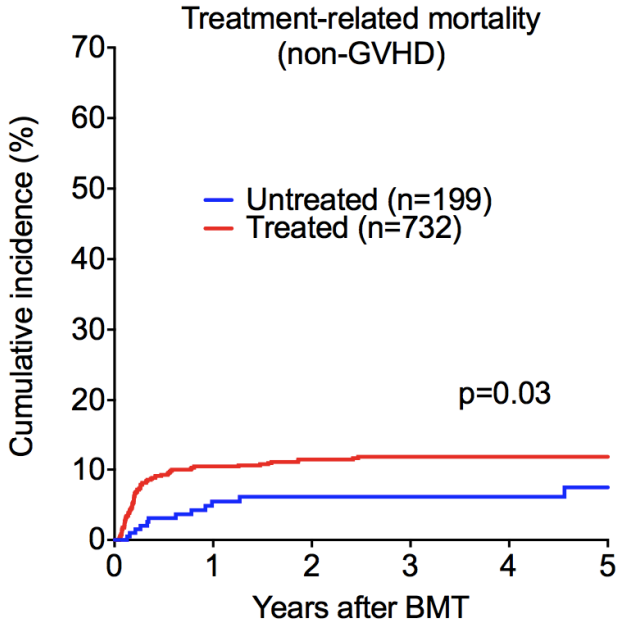


Lethal GVHD and anaerobic antibiotics

- 931 patients from MSKCC
- Underwent allo BMT 1991 to 2013
- Ex vivo or in vivo TCD excluded
- No oral antibiotic prophylaxis
- Stratified by exposure to antibiotics with anaerobic coverage during transplant hospitalization

Lethal GVHD and anaerobic antibiotics





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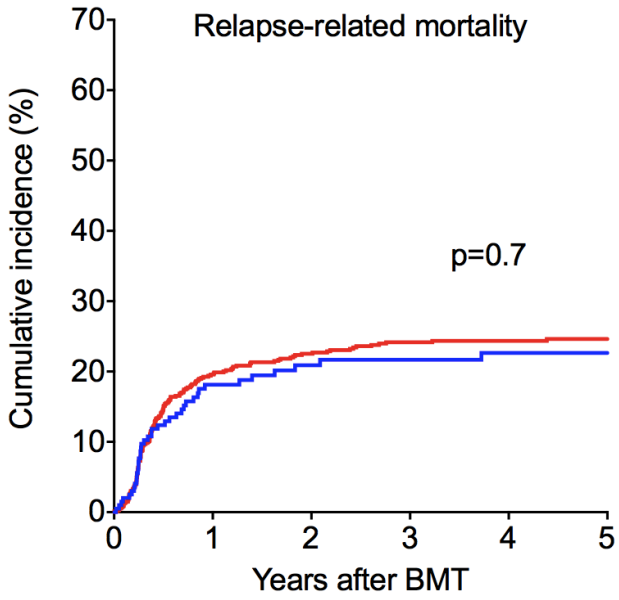

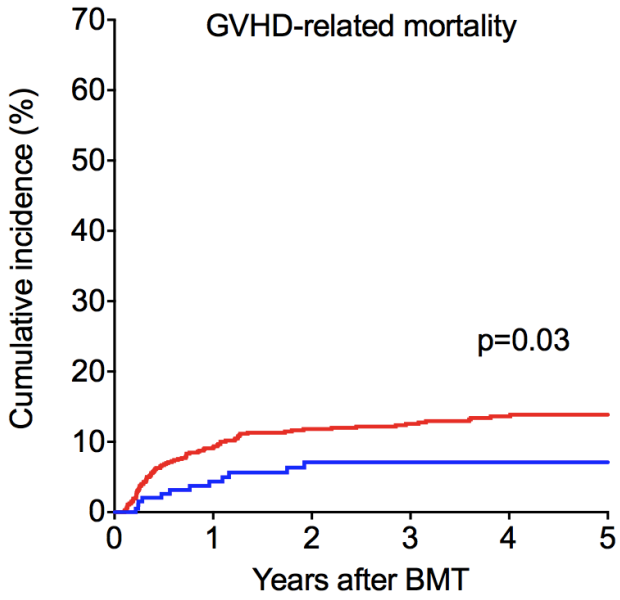

Lethal GVHD and anaerobic antibiotics

- To address a “sick bias”, specifically looked at subset of patients, all treated for neutropenic fever
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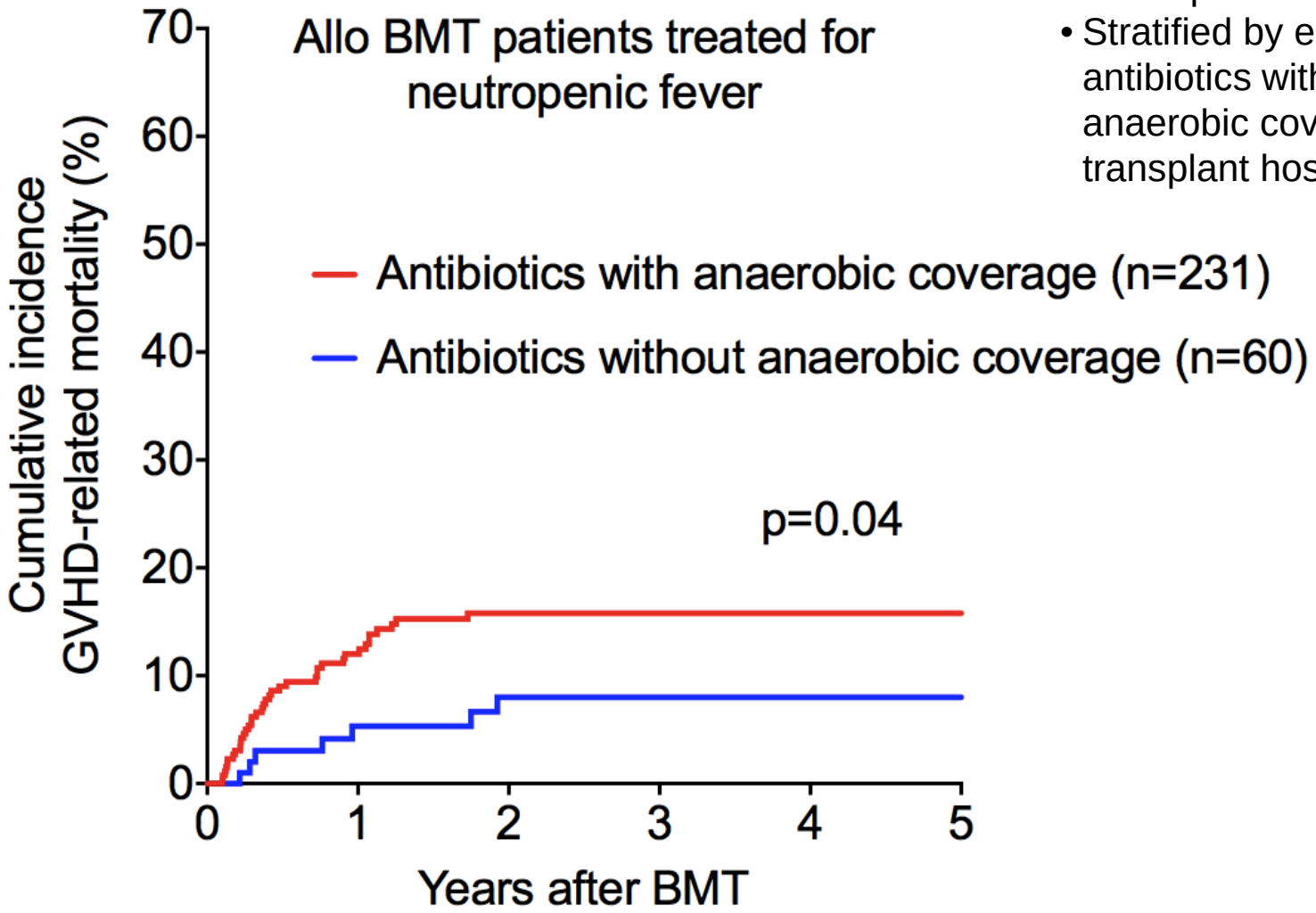

Summary

- GVHD is associated with:
  - Increase in Lactobacillales in gut flora
  - Loss of microbial diversity
  - Loss of Blautia
- Potential therapies to decrease gut GVHD
  - Prebiotics that support Clostridiales
  - Probiotic therapy with Blautia or pre-BMT flora
  - Short chain fatty acids (“postbiotics”)
  - Antibiotics which spare Clostridiales

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