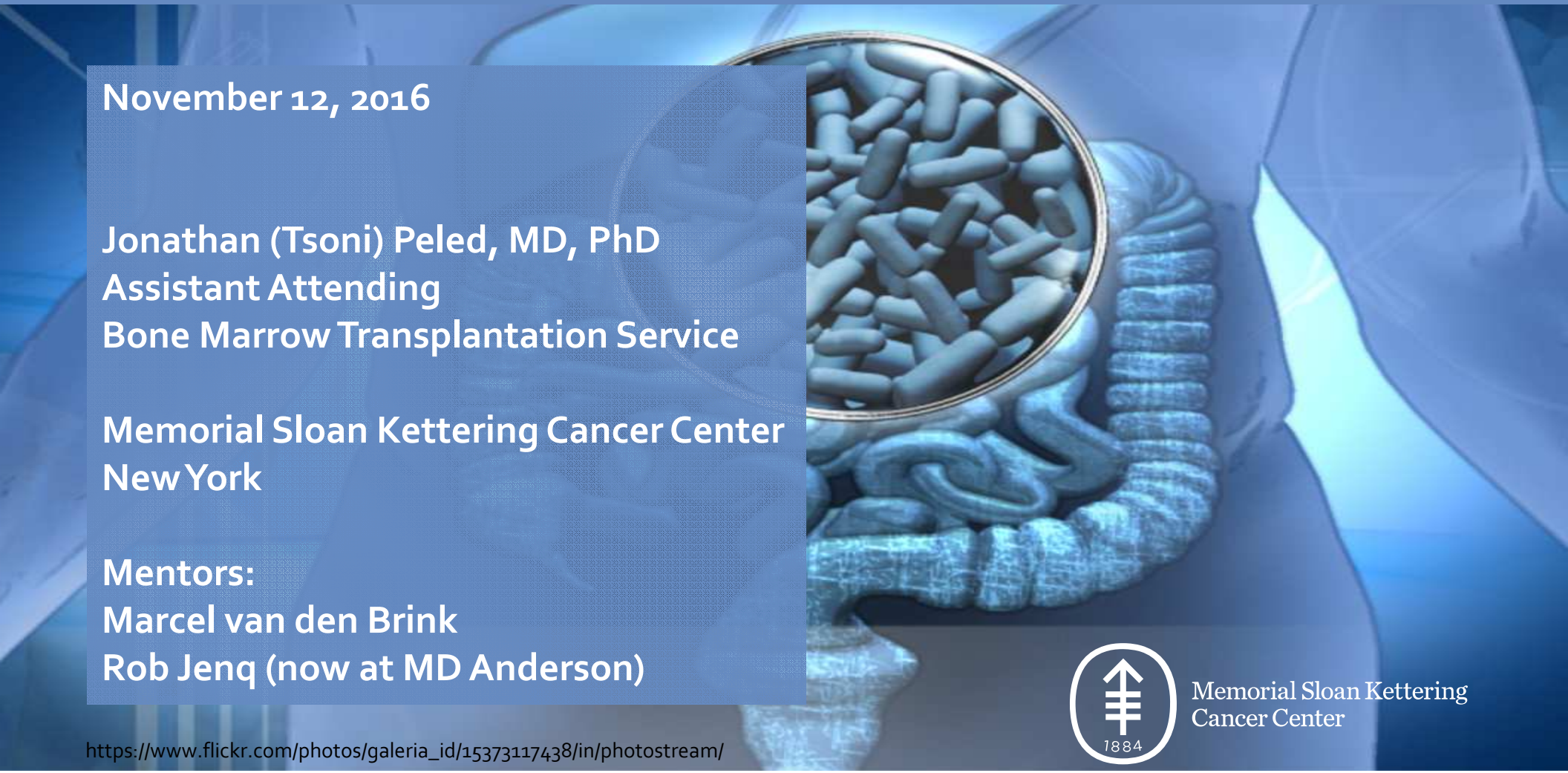
SITC 2016

NATIONAL HARBOR, MD
NOVEMBER 9-13, 2016

Society for Immunotherapy of Cancer

Microbiota biomarkers of relapse after allogeneic hematopoietic stem-cell transplantation



November 12, 2016

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New York

Mentors:
Marcel van den Brink
Rob Jenq (now at MD Anderson)

https://www.flickr.com/photos/galeria_id/15373117438/in/photostream/

Memorial Sloan Kettering Cancer Center

Presenter Disclosure Information

Jonathan Peled

The following relationships exist related to this presentation:

- Seres Therapeutics, Licensing Fees
- Merck/SITC, Grant Support

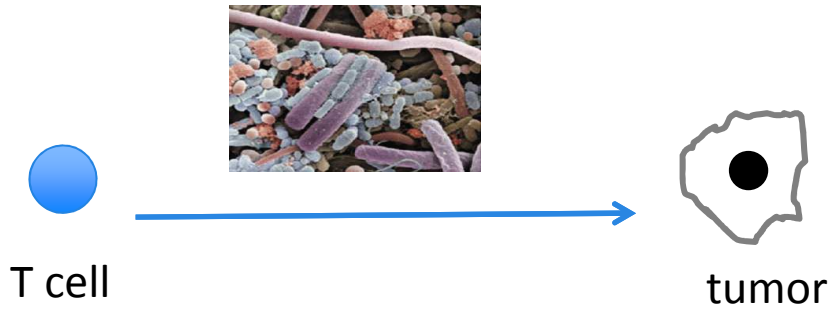
Allogeneic hematopoietic stem-cell transplantation is tumor immunotherapy

Lines of evidence for graft-vs-tumor (GVT) activity

1. Graft-vs-Host Disease (GVHD) associated with remission
2. animal models
3. regression after withdrawal of immune suppression
4. regression after donor lymphocyte infusion (DLI)
5. CRs can be seen after reduced-intensity conditioning

Thomas' Hematopoietic Cell Transplantation 2009

The intestinal microbiota modulates anti-tumor immunity in mouse models

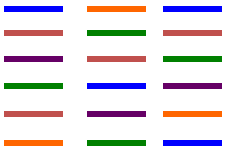


- Irradiation-induced T-cell antitumor activity is augmented by microbiota (Paulos *JCI* 2007)
- Chemotherapy-induced immunogenic cell death depends on microbiota (Iida *Science* 2013 & Viaud *Science* 2013)
- Anti-tumor checkpoint blockade is augmented by microbiota. (Vetizou *Science* 2015, Sivan *Science* 2015, Daillere *Immunity* 2016)
- Intestinal microbiota composition predicts immune-checkpoint-blockade-induced colitis (Dubin *Nature Communications* 2016)

16S sequencing allows culture-independent profiling of a microbial community

Extract DNA

Amplify 16S rRNA gene by PCR (V4-V5 region)



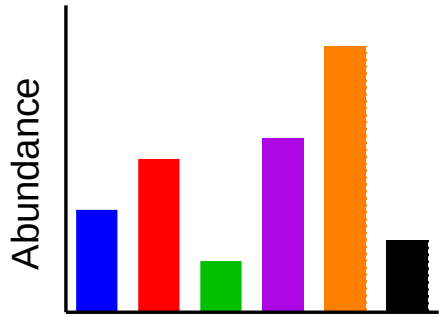
Sequence (Illumina MiSeq)

ATCGGTACCTATCGGATCCTAC
CATTAGGTACCATGCGGACCTA
CATTAGGTACGCGACCATACGA

Group sequences into OTUs (mothur software) and compared to NCBI 16S ribosomal RNA sequence database

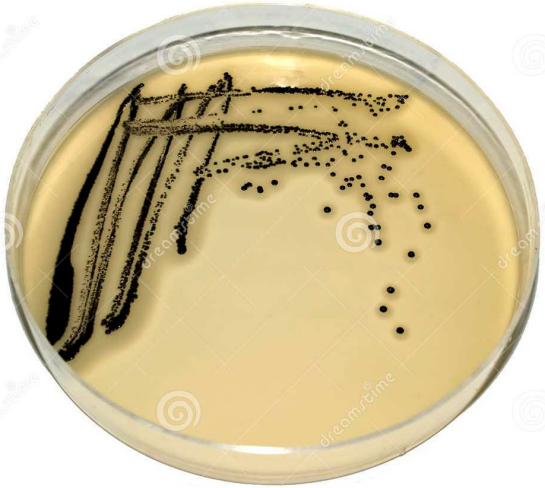
OTU 1 ATCGGTACCTATCGGATCCTAC
ATCGGTACCTATCGGATCCTAC
OTU 2 CATTAGGTACCATGCGGACCTA
CATTAGGTACCATGCGGACCTA
OTU 3 CATTAGGTACGCGACCATACGA
CATTAGGTACGCGACCATACGA

Identify OTUs in each sample and relative abundance (python & R scripts)

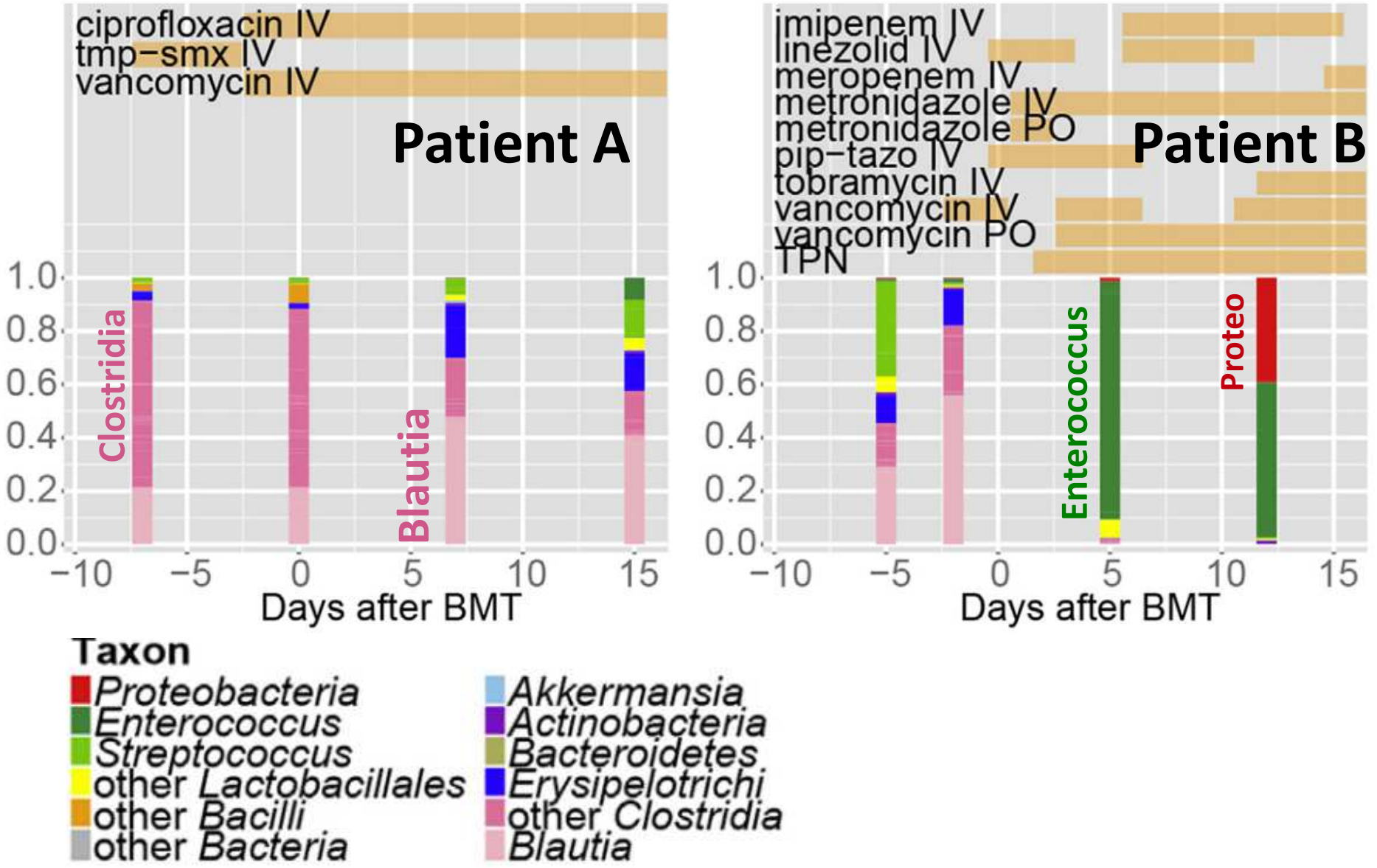


OTU
operational
taxonomic
unit

OTU vs. a cultured & cloned bacteria



Major shifts are observed in the microbiota during allo-HSCT admissions



Questions

Are there associations between the enteric flora and relapse after allogeneic transplantation?

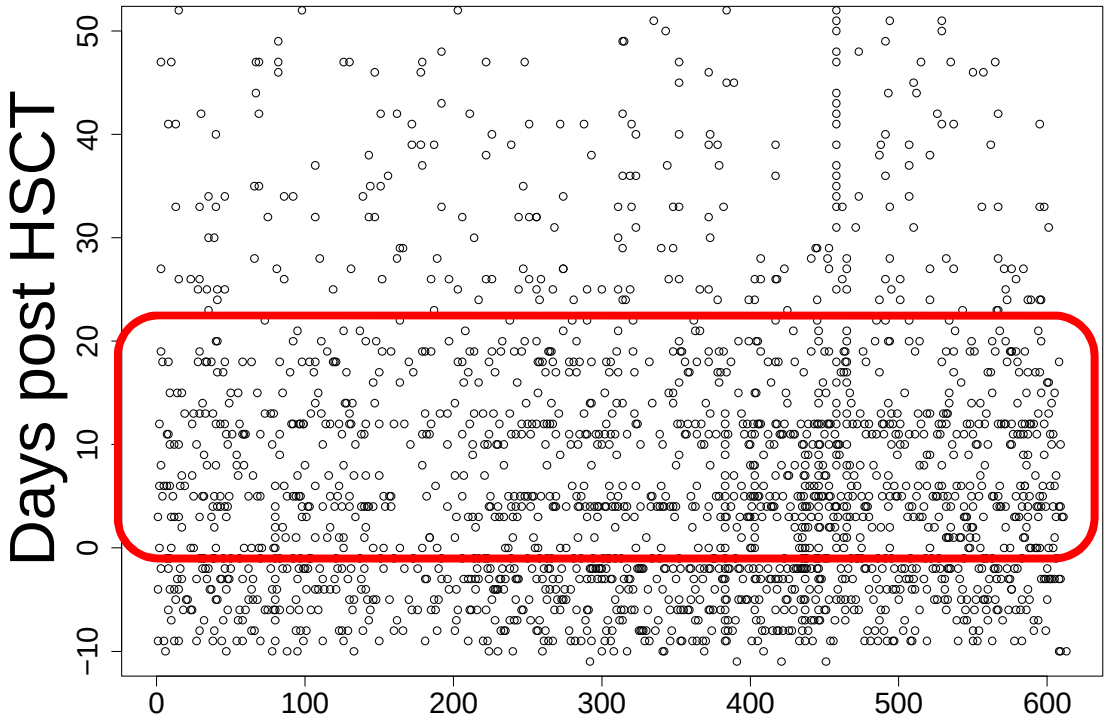
Can microbial biomarkers predictive of relapse be identified?

Approach

Retrospective observational single-institution study of patients undergoing transplantation whose stool samples have been prospectively banked.

Assembled a panel of 2,303 ~weekly stool amples from 541 adult allo-BMT patients

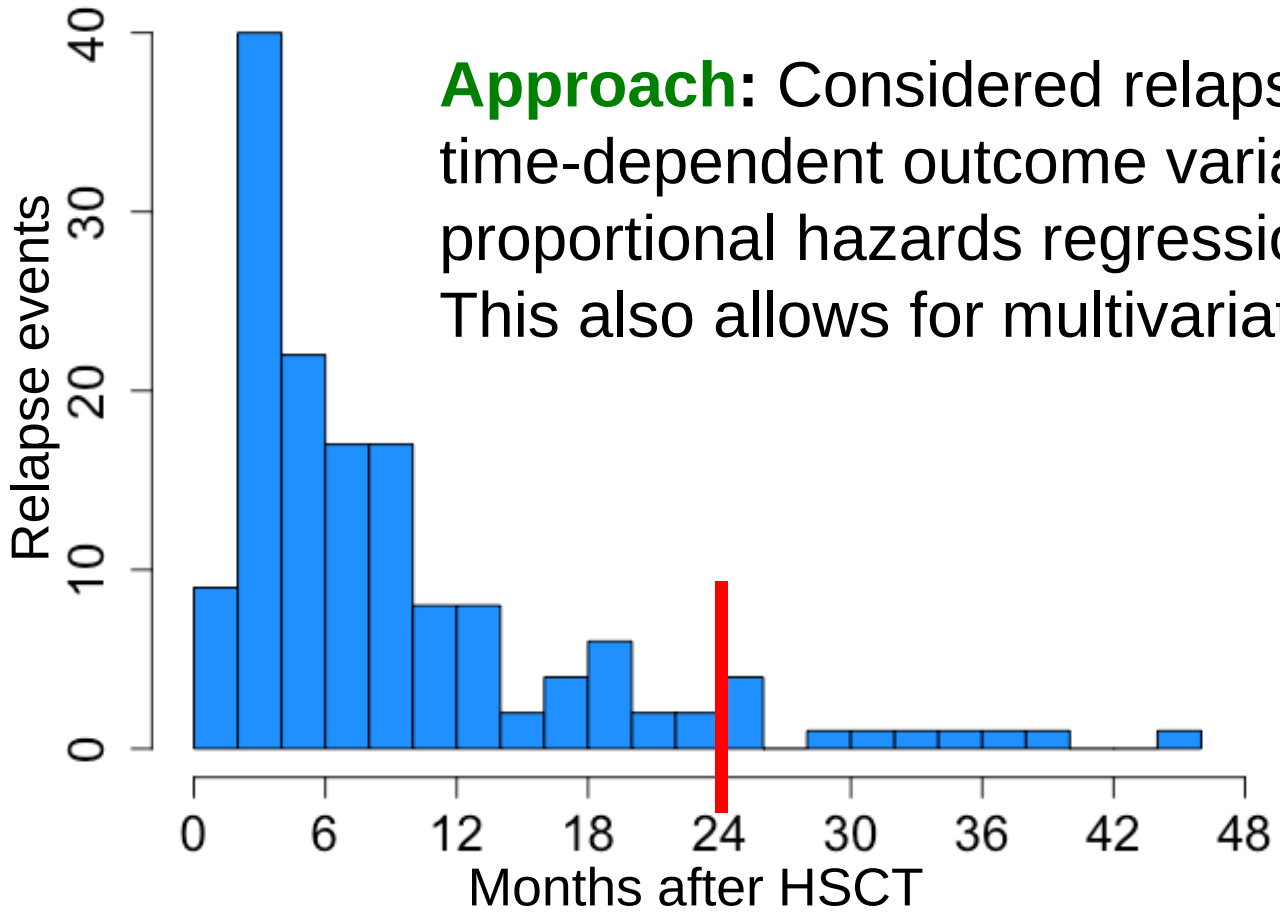
Distribution of collection times



As of Nov 2016
Stool bank contains
7,900 samples

Sample collection by BMT Service RNs
sequencing by MSKCC Castori Center for Microbes, Inflammation & Cancer
In collaboration with Eric Pamer and Ying Taur

Relapse was considered as a time-dependent variable



Approach: Considered relapse as a continuous time-dependent outcome variable in Cox proportional hazards regression models.
This also allows for multivariate adjustment.

Relapse was defined as

- Relapse/POD by disease-specific criteria
- MRD events that triggered an intervention

148 relapses in 541 patients (27% relapse rate)
After censoring at 2 years of follow-up, 138 events (25%)

Problem: Taxonomic classification splits and lumps OTUs imperfectly

> *Those who make many species are the 'splitters,' and those who make few are the 'lumpers.'*
>
> - Charles Darwin, letter to J.D. Hooker, 1857

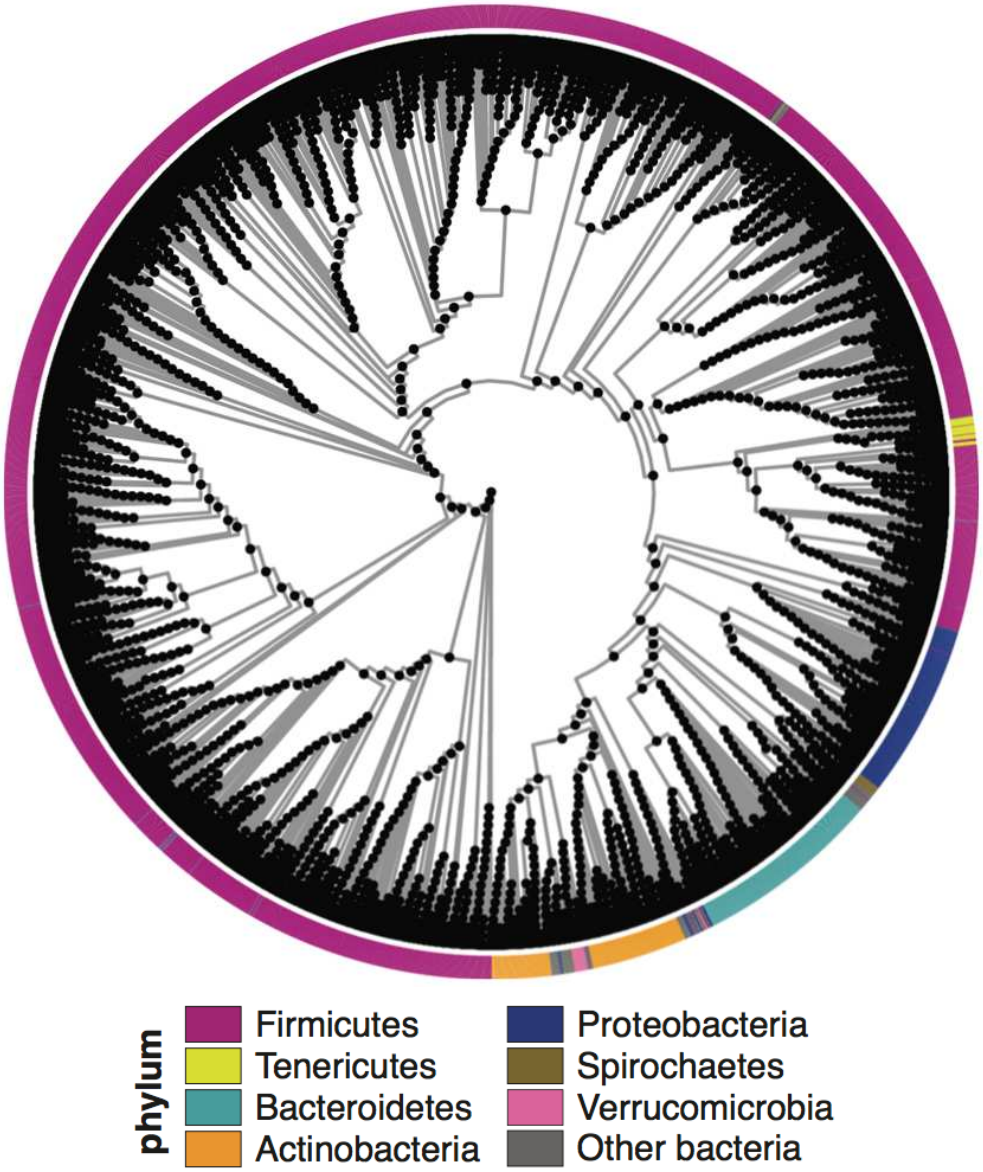
- association strength of a single species with clinical outcome can be distributed among multiple OTUs.

- at higher taxonomic levels (genus, family etc), potential associations may be lost when dozens or hundreds of OTUs are grouped together.

Approach:

Combine the abundances of evolutionarily related OTUs, as determined by 16S sequence similarity.

Empirically derived phylogentic tree largely recapitulates standard taxonomy and allows finer resolution of bacterial groups



4,100 OTUs

3,952 (96%) OTUs could be aligned

Assembled phylogenetic tree
FastTree algorithm in QIIME

Tree has $n - 1$ nodes

3,951 nodes (branchpoints) = "clusters of related OTUs" (crOTU)

Abundance of each crOTU is the sum of the abundance of subsidiary OTUs (tips)

Each crOTU becomes a feature to test for association with clinical outcome

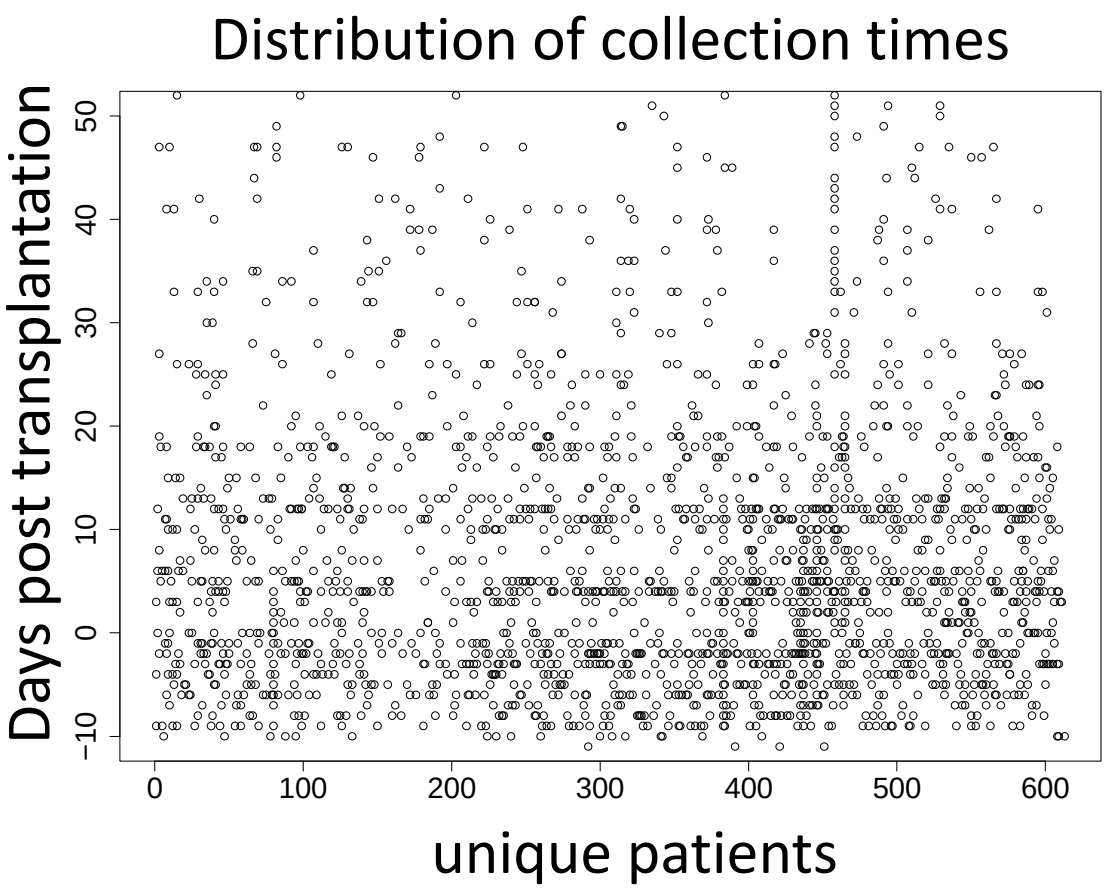
Problem: The “large *p*, small *n*” problem (multiple comparisons)

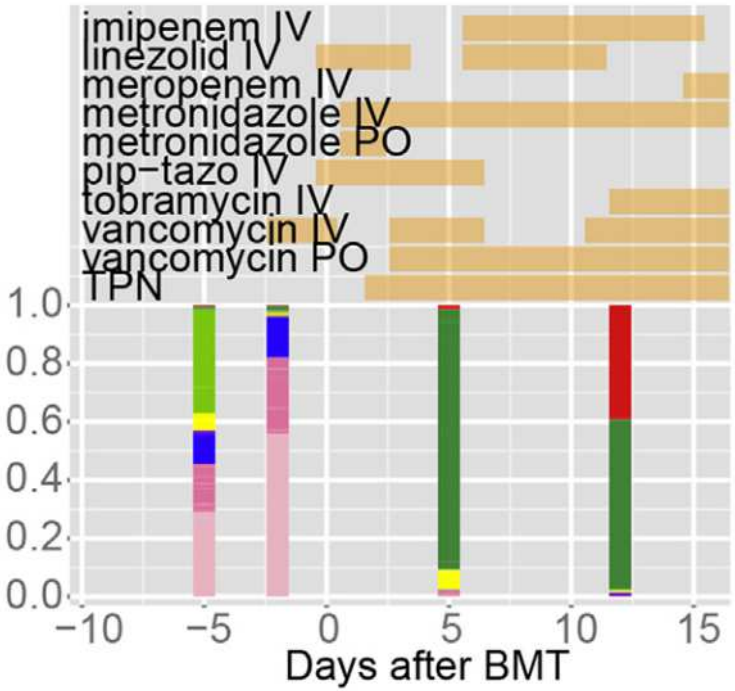
- 4,100 Operational Taxonomic Units (OTUs) were identified across all samples from 541 patients.

Approaches:

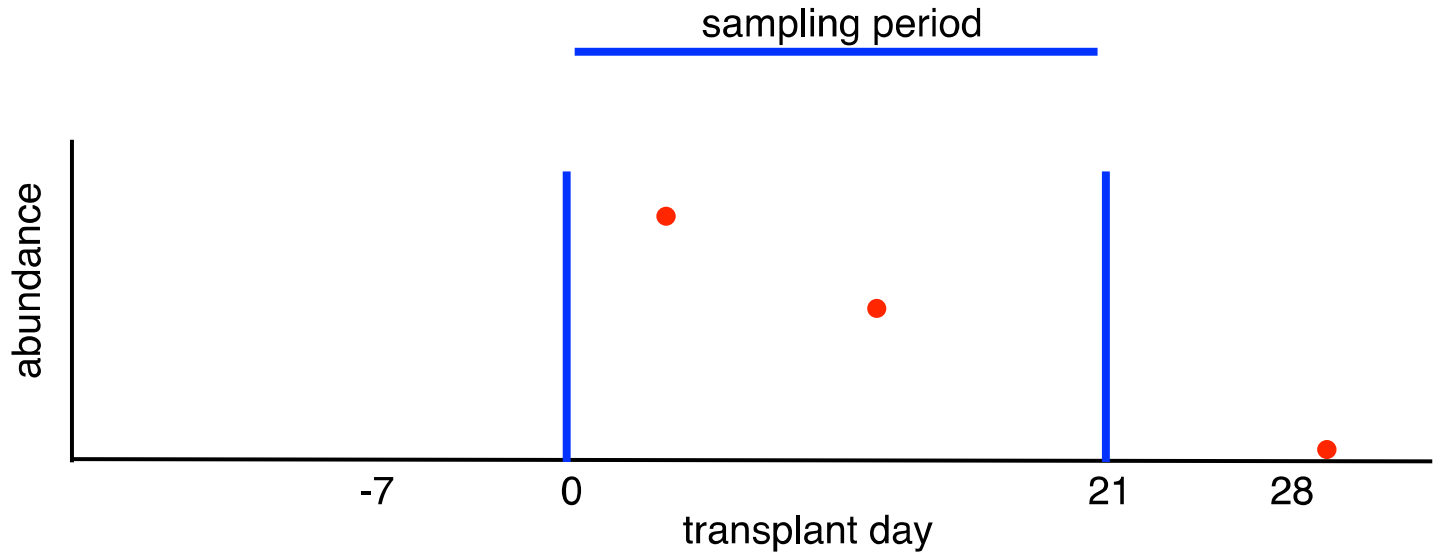
1. Exclude OTUs unlikely to be top hits based on abundance
   ≥ 0.01% abundance in ≥ 10% of the patients
2. Partition the patients into discovery and validation sets
   Test only a small number of hits in the validation set
3. Establishing collaborations to assemble multi-center external validation sets

Problem: how to deal with multiple samples per patient?





Abundance-AUC approach to irregularly sampled time-series



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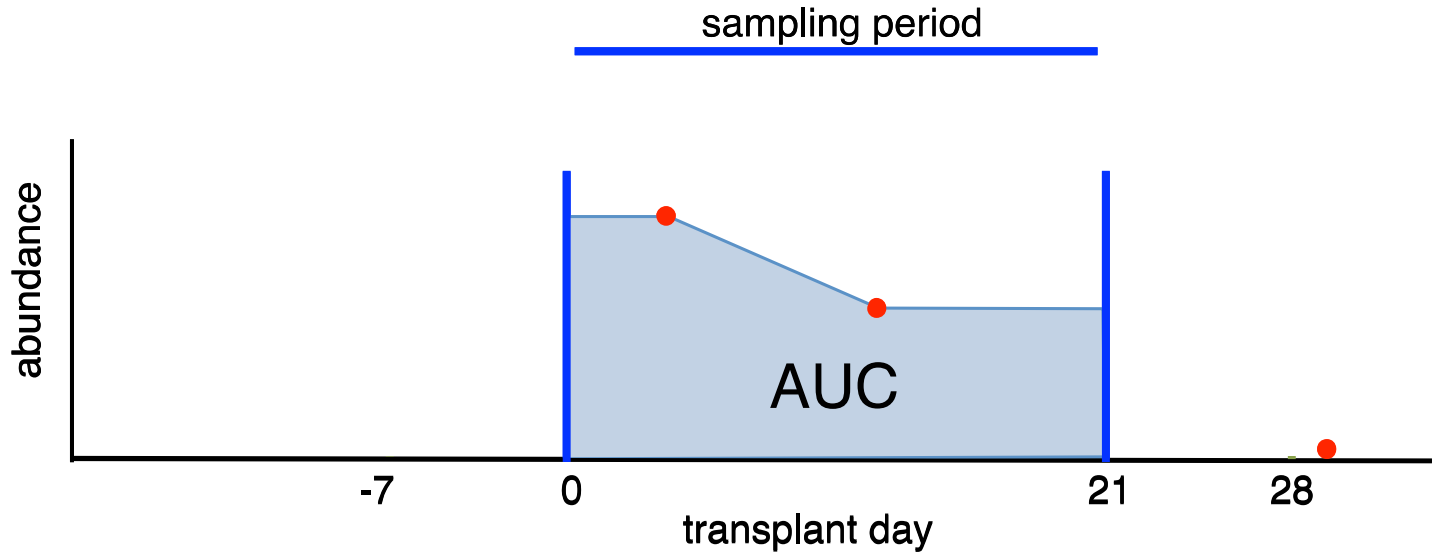


Potential Advantages of AUC-abundance approach

- Includes all samples
- Accommodates different # of samples and different timepoints per patient
- Takes time-dependent perturbations into account

Prior uses of AUC-biomarker concentration

CA-125, Mano *Gynecologic Oncology* 2005
Troponin, Chia *J Am Coll Cardiol* 2008
PSA, Oudard ASCO Abstract 2004

Patient Characteristics (n = 541)

Disease		
AML	195	36%
MDS	85	16%
NHL	68	13%
myeloma	61	11%
ALL	44	8%
T-NHL	24	4%
CLL/SLL	16	3%
Hodgkin's	9	3%
CML	12	2%
MPN	12	2%
Other	9	2%

Follow-up Duration
mean 21.5 months

Conditioning		
Ablative	317	59%
Reduced Intensity	162	30%
Nonablative	62	12%

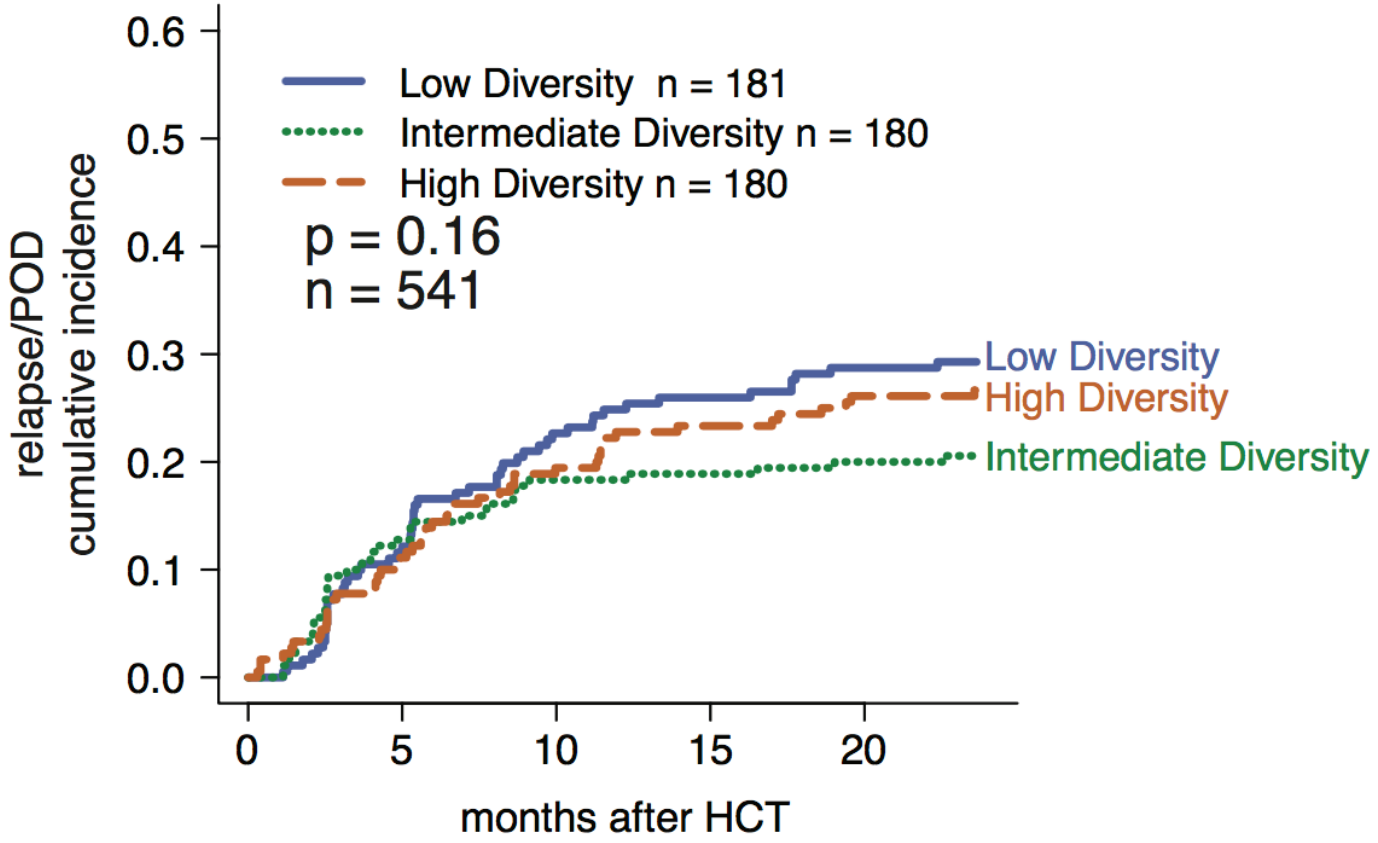
Graft		
T-cell Depleted	274	51%
Unmodified PBSC/BM	172	32%
Cord	95	18%

Age mean (range)	54 (19- 75)

Disease Risk Index		
Low	63	12%
Intermediate	353	65%
High	125	23%

Results

Diversity is not associated with relapse/Progression of Disease (POD)

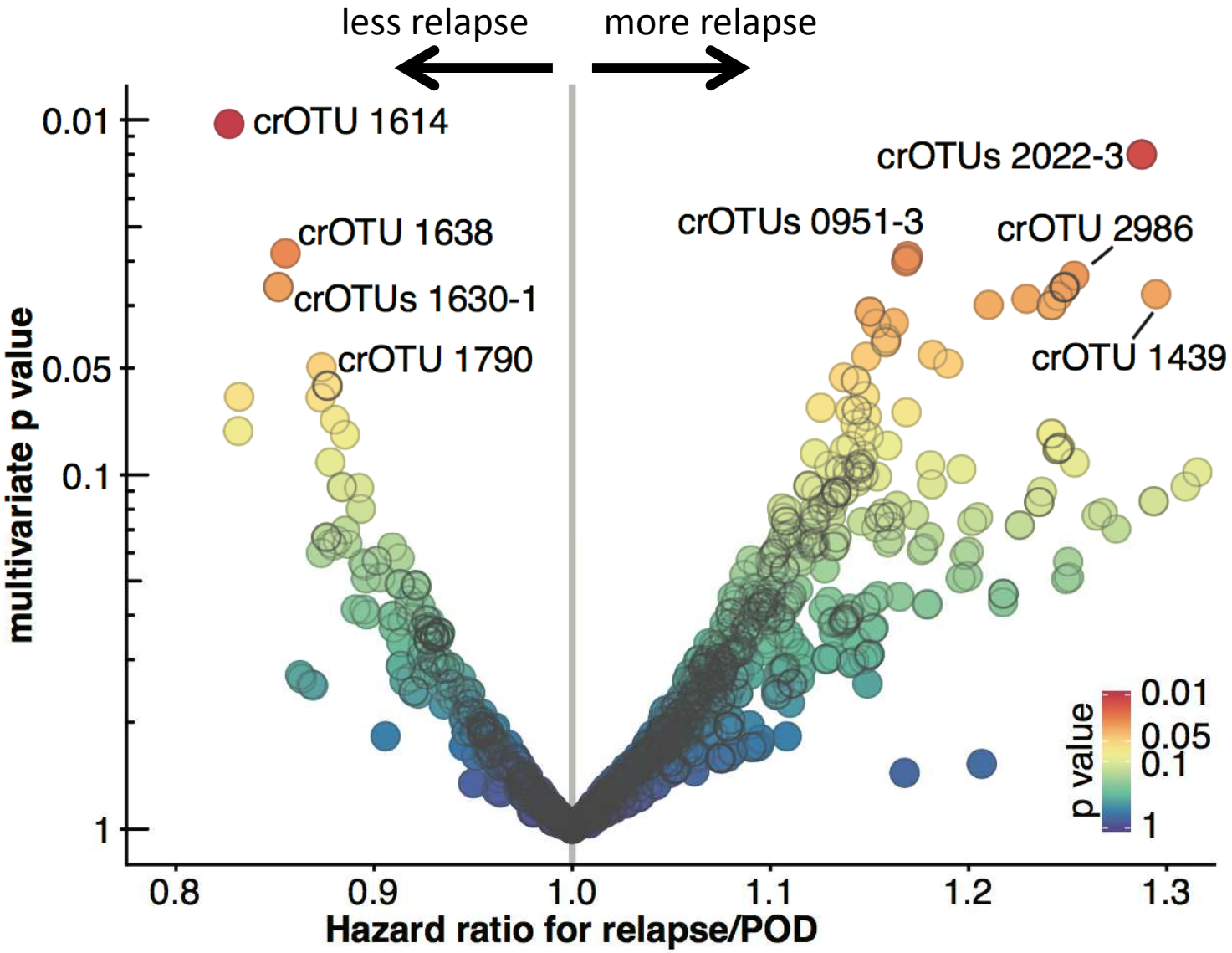


Inverse Simpson score categories

low	< 2.4
intermediate	≥ 2.4 and < 4.4
high	≥ 4.4.

submitted

Abundances of 208 OTUs & 1,343 crOTUs were tested for association in the Discovery set with relapse in a Cox Regression Models



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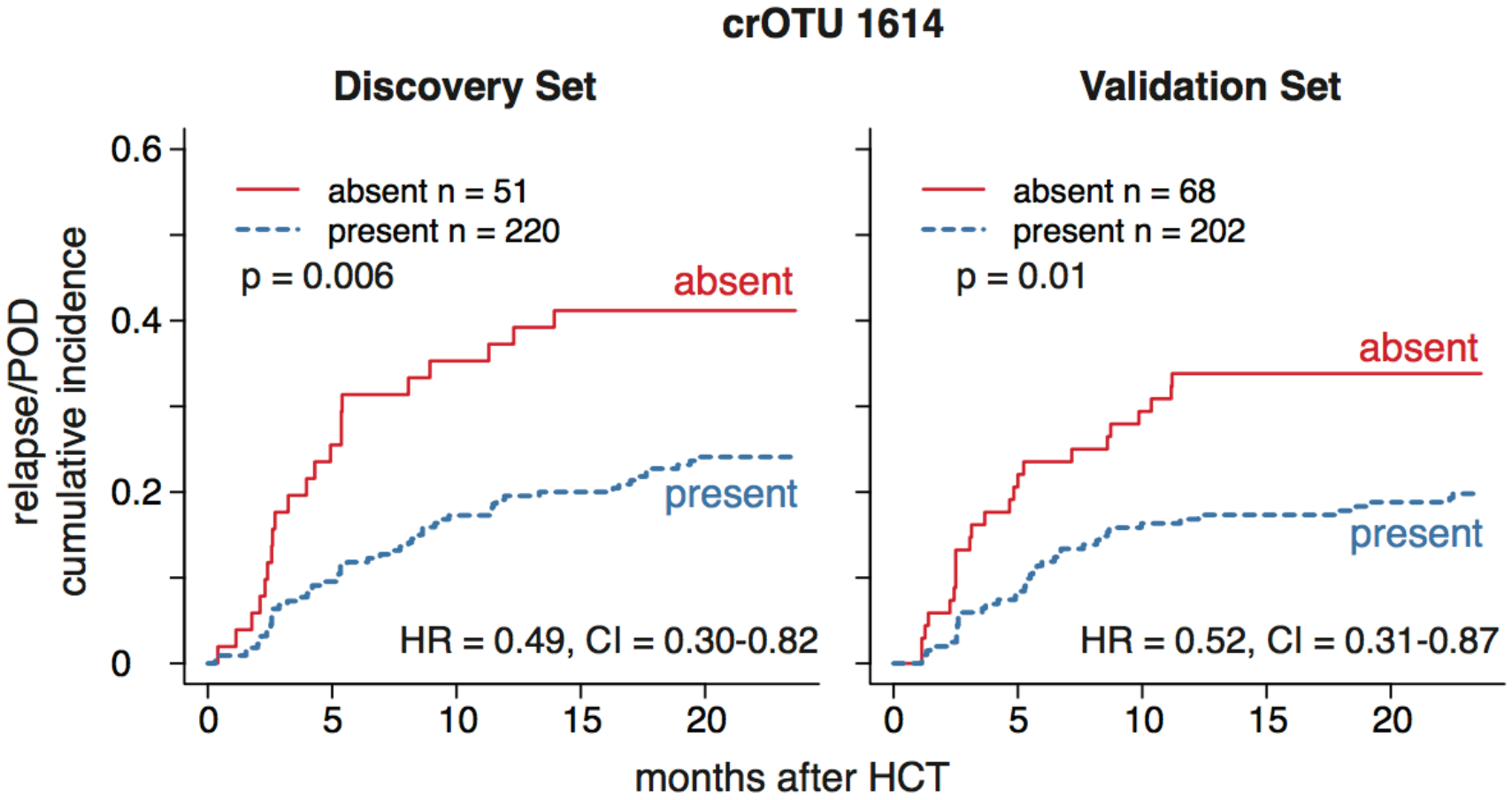
crOTU 1614 presence is associated with less relapse in both Discovery & Validation Sets



Relapse/POD was analyzed as cumulative incidence with death from other causes as competing risks

submitted

crOTU 1614 abundance is associated with less relapse after multivariate adjustment for relapse risk

Discovery Cohort	Univariate		Multivariate I crOTU log transformed	
n = 271	HR (95% CI)	P-value	HR (95% CI)	P-value
crOTU 1614, log transformed	0.84 (0.73-0.96)	0.01	0.83 (0.71-0.96)	0.01

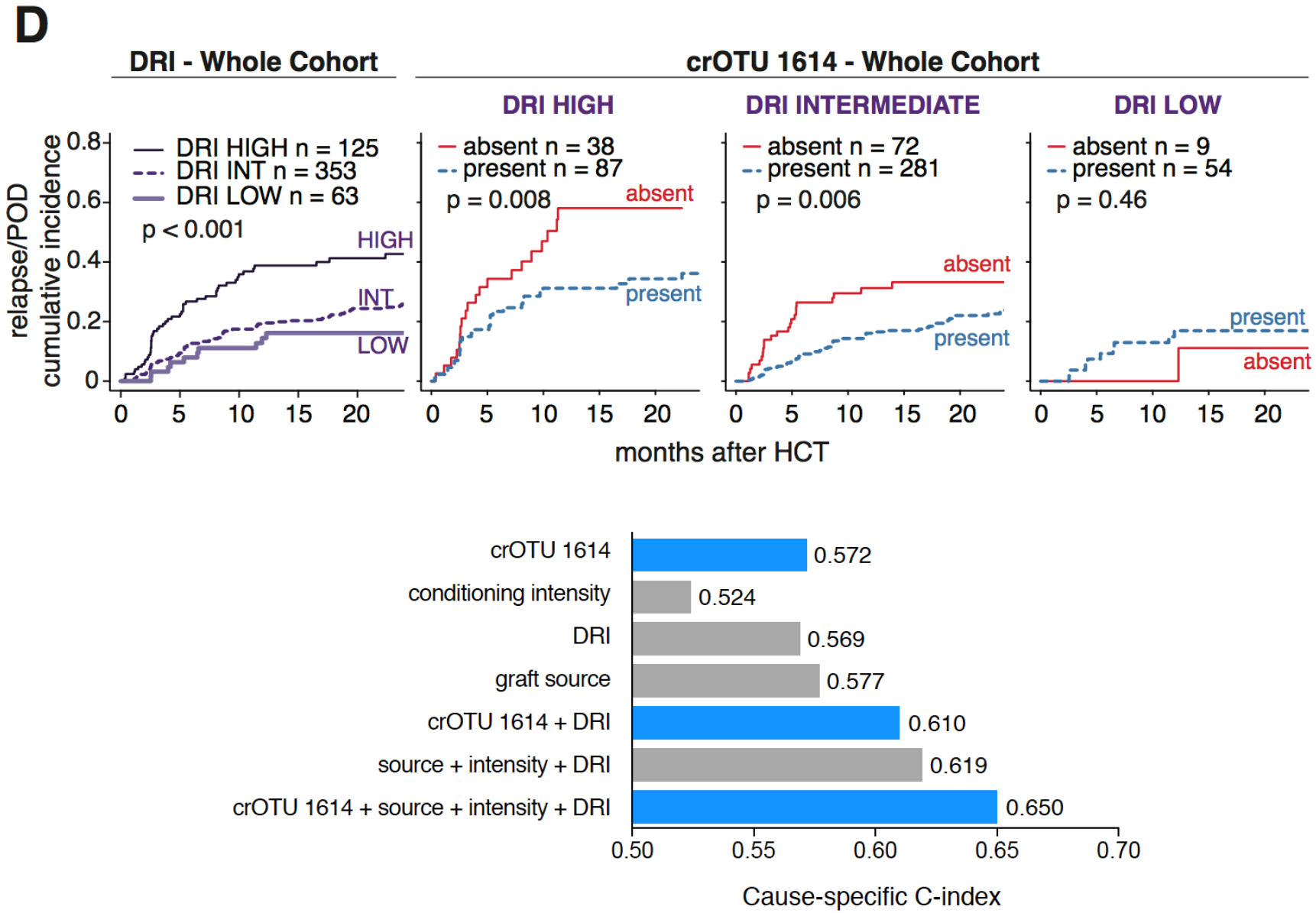
Validation Cohort	Univariate		Multivariate I crOTU log transformed	
n = 270	HR (95% CI)	P-value	HR (95% CI)	P-value
crOTU 1614, log transformed	0.82 (0.71-0.95)	0.009	0.82 (0.7-0.96)	0.01

Multivariate adjustment for clinical variables predictive of relapse

1. Intensity of conditioning regimen
2. Stem-cell-graft source & ex vivo manipulation (T cell depletion)
3. Disease Risk Index

submitted

crOTU 1614 abundance further stratifies relapse risk beyond clinical risk variables



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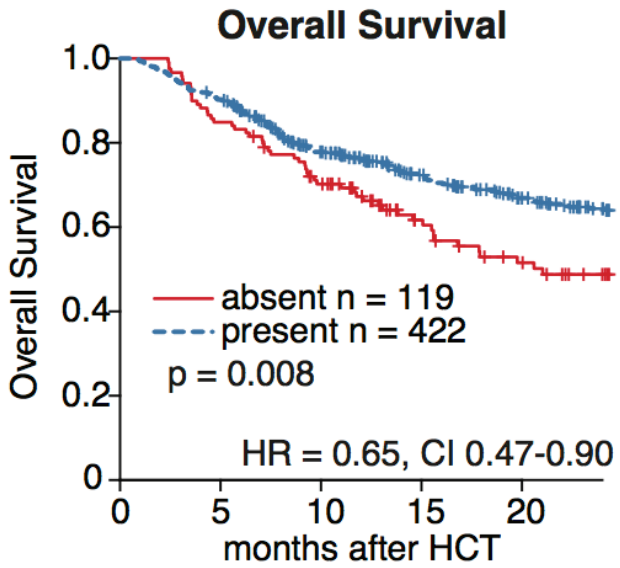
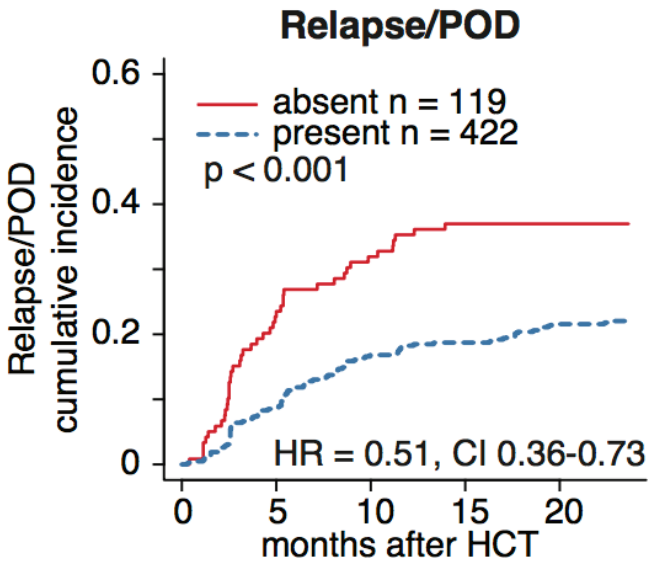
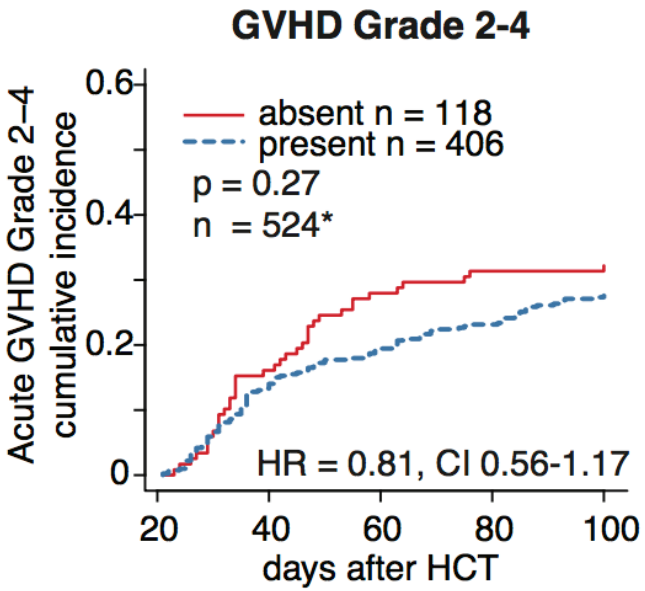
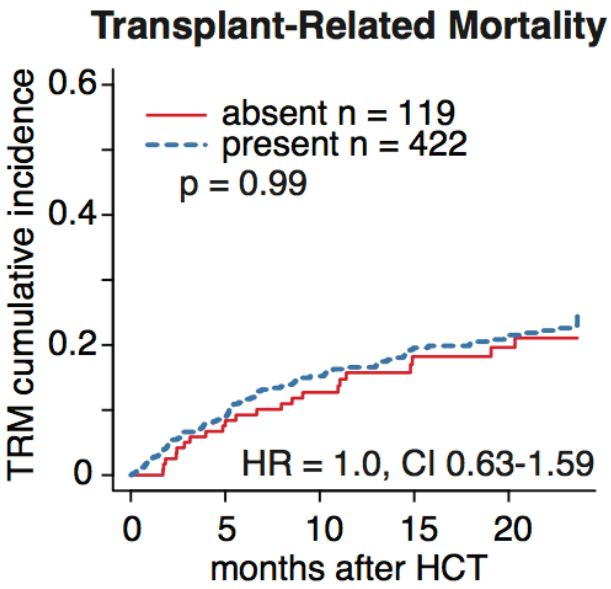
crOTU 1614 is associated with improved OS and no significant association with Day 100 Gr 2-4 GVHD nor TRM









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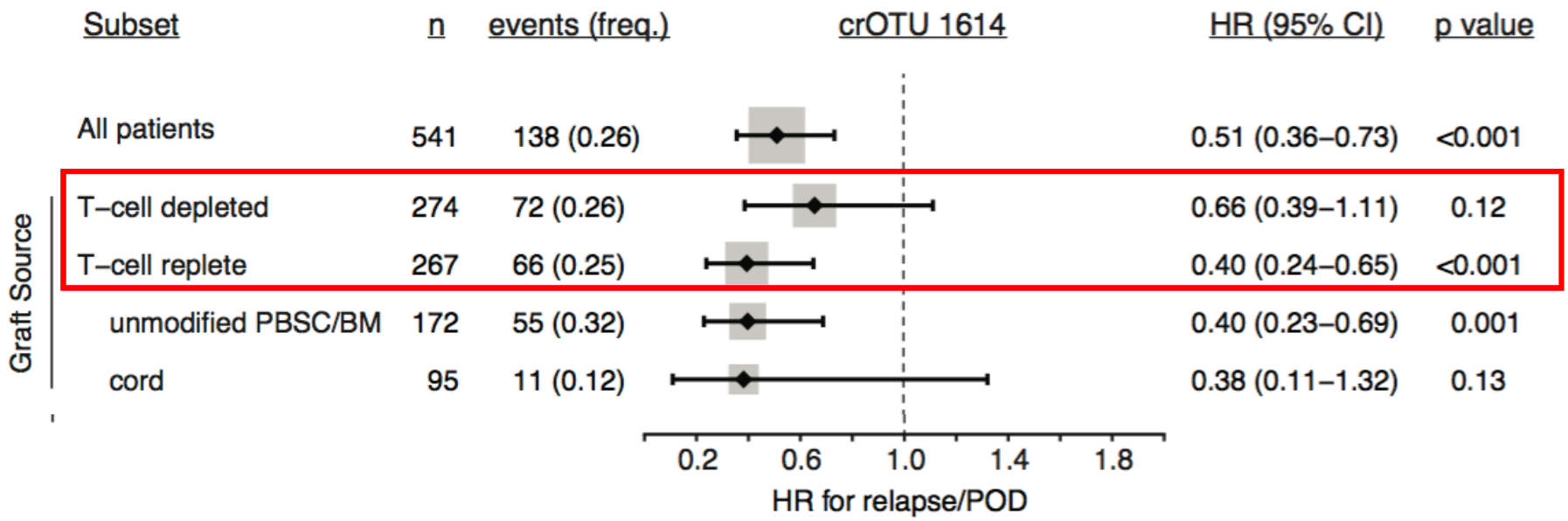
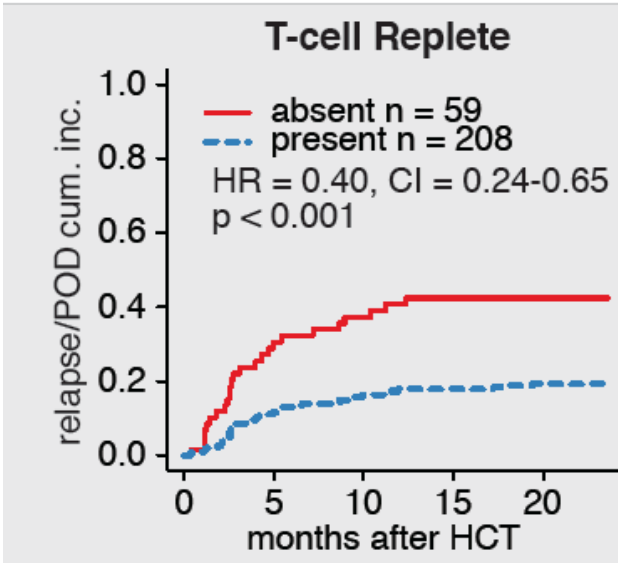
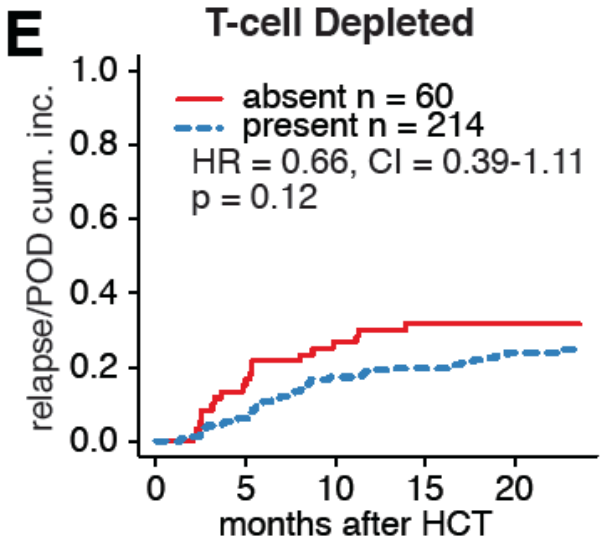
The association of Node 1614 with less relapse is driven primarily by T-cell replete, adult-donor transplants







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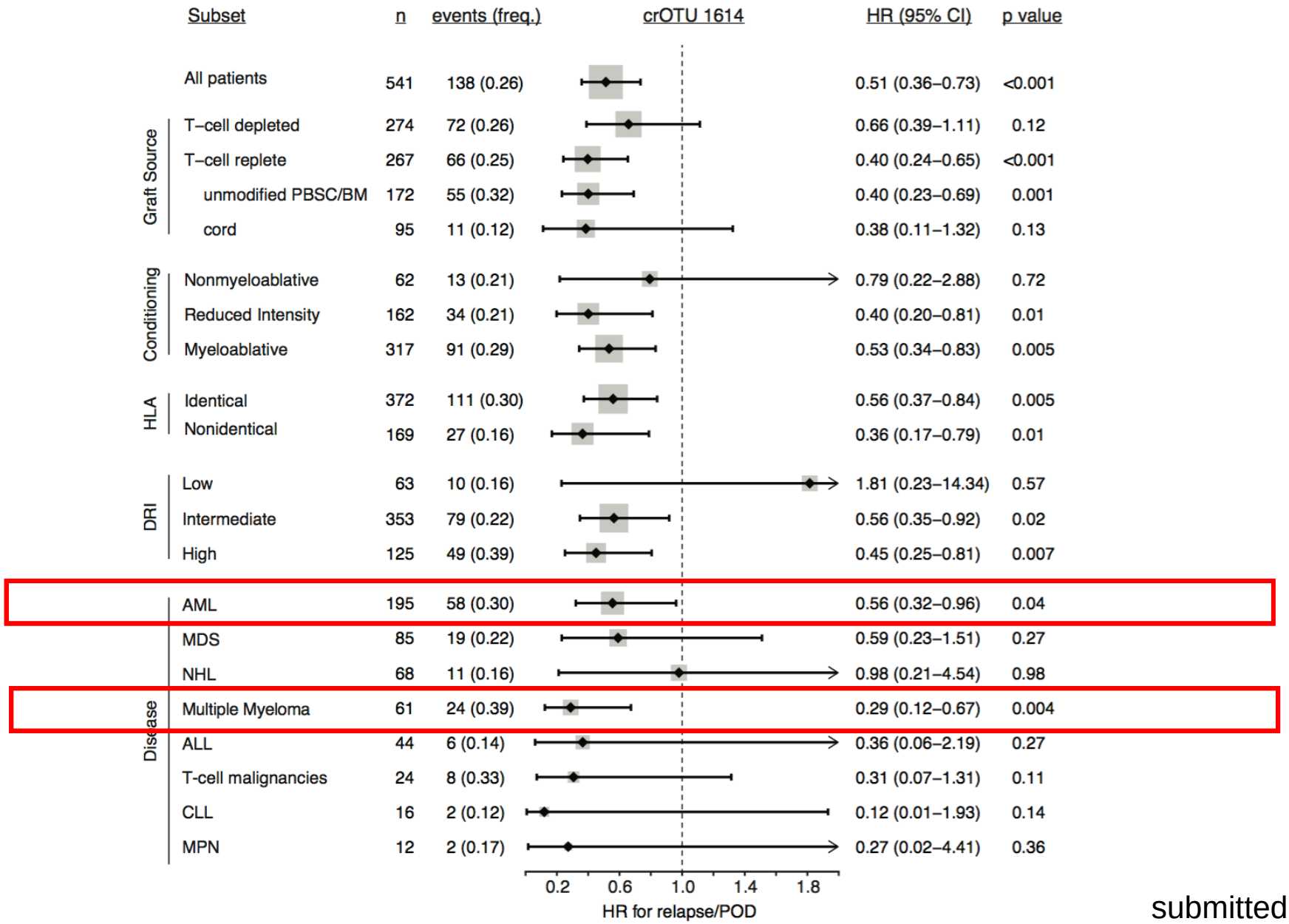
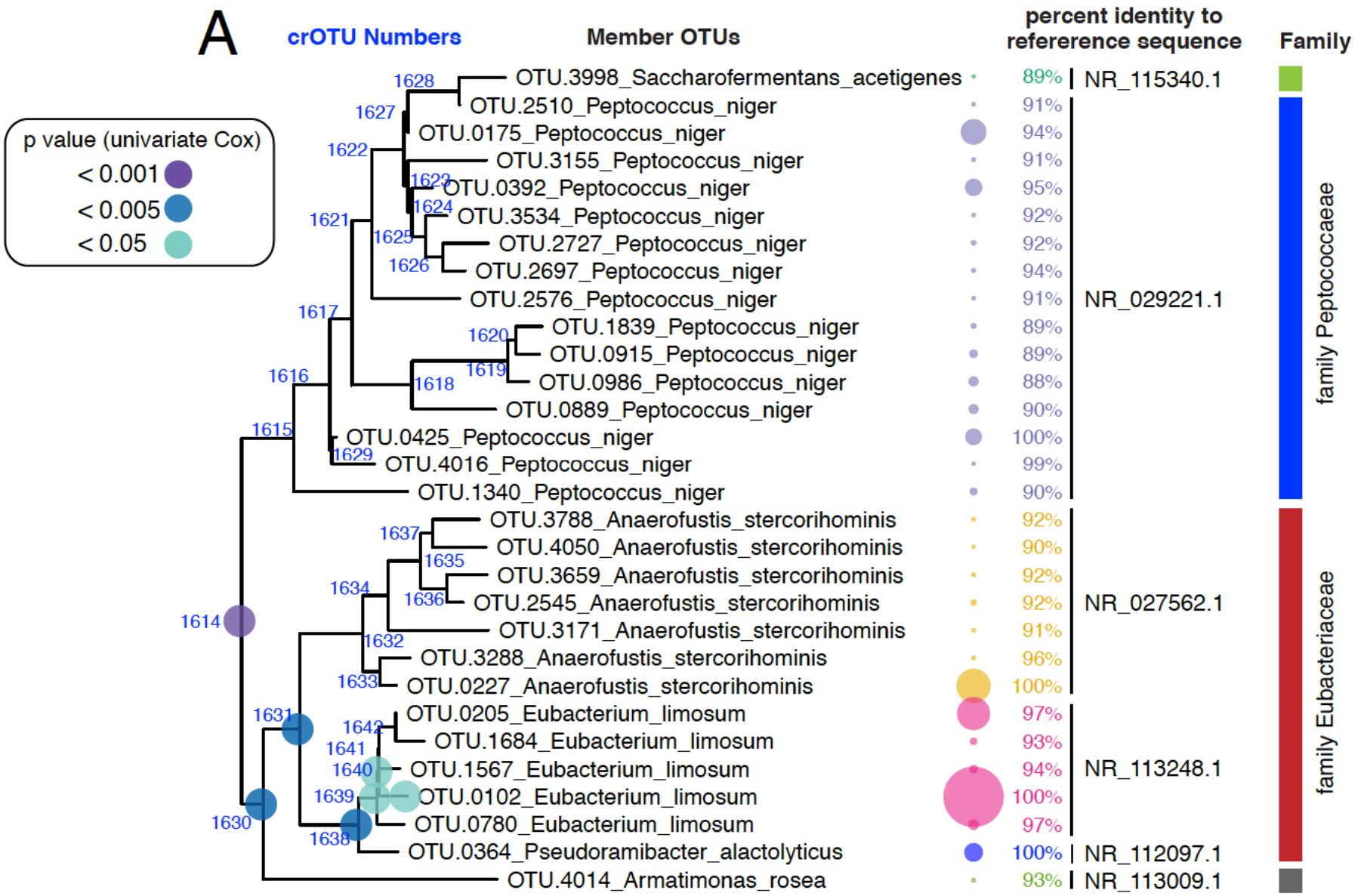


submitted

crOTU 1614 contains several members of family Eubacteriaceae, mostly *Eubacterium limosum*



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Eubacterium limosum is a commensal microbe that has been associated with health-related outcomes in other studies

E. limosum is an anaerobic, non-spore forming gram-positive rod

- Common human intestinal microbe
- markedly increased in the stools of centenarians
- ameliorates experimental colitis
- Produces SFCAs including butyrate, acetate, propionate, lactate
- Cell wall components utilized in animal models of arthritis
- Associated with susceptibility of *in vitro* PBSC production of IFNγ upon stimulation by *B. fragilis*.

Preliminary data: activates human TLR2, TLR4, and NOD2

Schwiertz, *Applied & Environmental Microbiology*, 2000
Kanauchi, *World J Gastroenterol* 2006
Biagi, *PLoS One*, 2010
Schirmer *Cell* 2016

Conclusions

- We have assembled a large single-institution bank of microbiota samples from patients undergoing allogeneic hematopoietic stem-cell transplantation.
- Abundance-AUC can be applied to time-course microbiota biomarker studies.
- The abundance of certain bacteria is associated with less relapse.
- This association is seen primarily in patients who received T-cell replete grafts.

Acknowledgements

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Seres Therapeutics
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MD Anderson Cancer Center
Robert Jenq

Intestinal bacteria

Phylum	Class	Order	Genus	Gram stain	Type of anaerobe
Firmicutes	Bacilli	Bacillales	Gemella Staphylococcus	+	Facultative
		Lactobacillales	Enterococcus Lactobacillus Streptococcus		
	Clostridia	Clostridiales	Blautia Clostridium Eubacterium Faecalibacterium Ruminococcus	+	Obligate
	Erysipelotrichia	Erysipelotrichiales	Erysipelatoclostridium Holdemania	+	Obligate
	Negativicutes	Selenomonadales	Acidaminococcus Megasphaera Phascolarctobacterium Veillonella	−	Obligate
Bacteroidetes	Bacteroidia	Bacteroidales	Bacteroides Prevotella	−	Obligate
Proteobacteria	Gamma-proteobacteria	Enterobacteriales	Escherichia Klebsiella	−	Facultative
Actinobacteria	Actinobacteria	Actinomycetales	Actinomyces	+	Facultative
		Bifidobacteriales	Bifidobacterium		
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Akkermansia	−	Obligate

Can we speculate that microbiota have an effect on GVT and not just an association?

A few hypotheses

1. PAMPs → host innate immune receptors → antigen presentation & donor T cell activation at distant sites

1. lymphocytes maintaining the low-intensity, large volume antigen load from flora are cytokine sinks

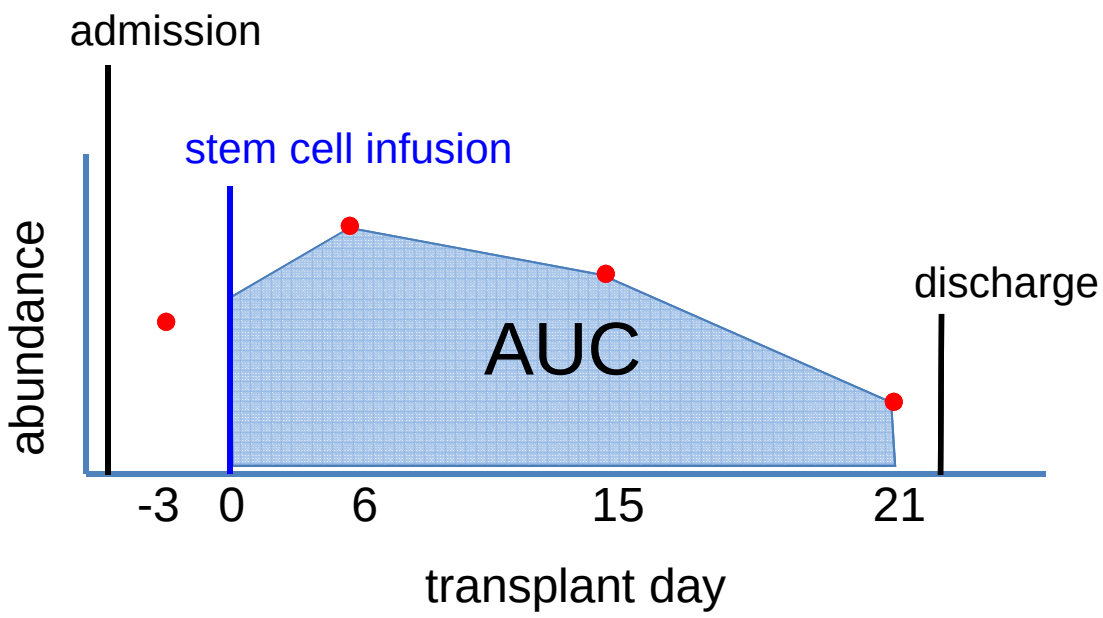
2. antigen mimicry

3. Direct effect of (genotoxic) microbial metabolite on tumor

1. Modulation of the cytotoxicity of chemotherapy

Solution: Area Under the Curve (AUC) of abundance as a measure of cumulative exposure.

Abundance of a taxon in four samples from a hypothetical patient



Prior uses of AUC-biomarker concentration

CA-125, Mano *Gynecologic Oncology* 2005
Troponin, Chia *J Am Coll Cardiol* 2008
PSA, Oudard ASCO Abstract 2004

Potential Advantages of AUC-abundance approach

- Includes all samples
- Accommodates different # of samples and different timepoints per patient
- Takes into account the dramatic changes that occur during transplant
- Applicable to serial immunophenotyping samples?

crOTU 1614 abundance is associated with less relapse in both Discovery & Validation Sets

Discovery Cohort	Univariate		Multivariate I crOTU log transformed		Multivariate II crOTU present/absent	
n = 271	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
crOTU 1614, log transformed	0.84 (0.73-0.96)	0.01	0.83 (0.71-0.96)	0.01		
OTU 1614, present	0.49 (0.3-0.82)	0.006			0.46 (0.27-0.78)	0.004

Validation Cohort	Univariate		Multivariate I crOTU log transformed		Multivariate II crOTU present/absent	
n = 270	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
crOTU 1614, log transformed	0.82 (0.71-0.95)	0.009	0.82 (0.7-0.96)	0.01		
OTU 1614, present	0.52 (0.31-0.87)	0.01			0.54 (0.31-0.92)	0.03

submitted

Acknowledgements

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Kristina Caban
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Ying Taur
Sergio Giralt
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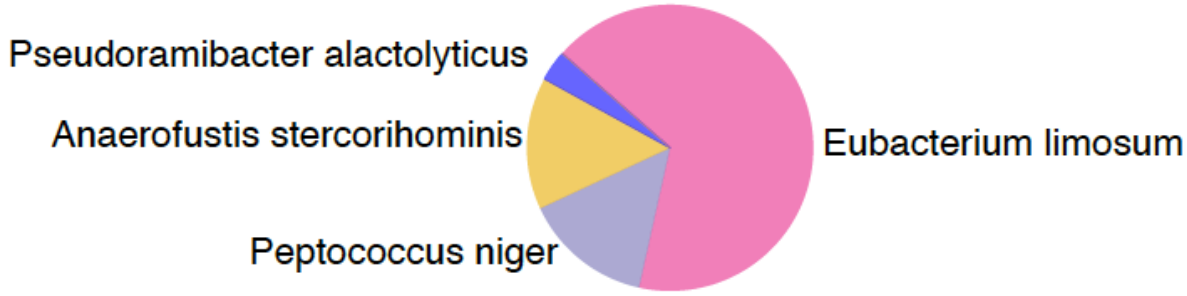
ASBMT Clinical Research Training Course

Seres Therapeutics

crOTU 1614 contains several members of family Eubacteriaceae, mostly *Eubacterium limosum*



Phylum	Class	Order	Family
Firmicutes	Clostridia	Clostridiales	Ruminococcaceae
Firmicutes	Clostridia	Clostridiales	Peptococcaceae
Firmicutes	Clostridia	Clostridiales	Eubacteriaceae
Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae

submitted

Ongoing work

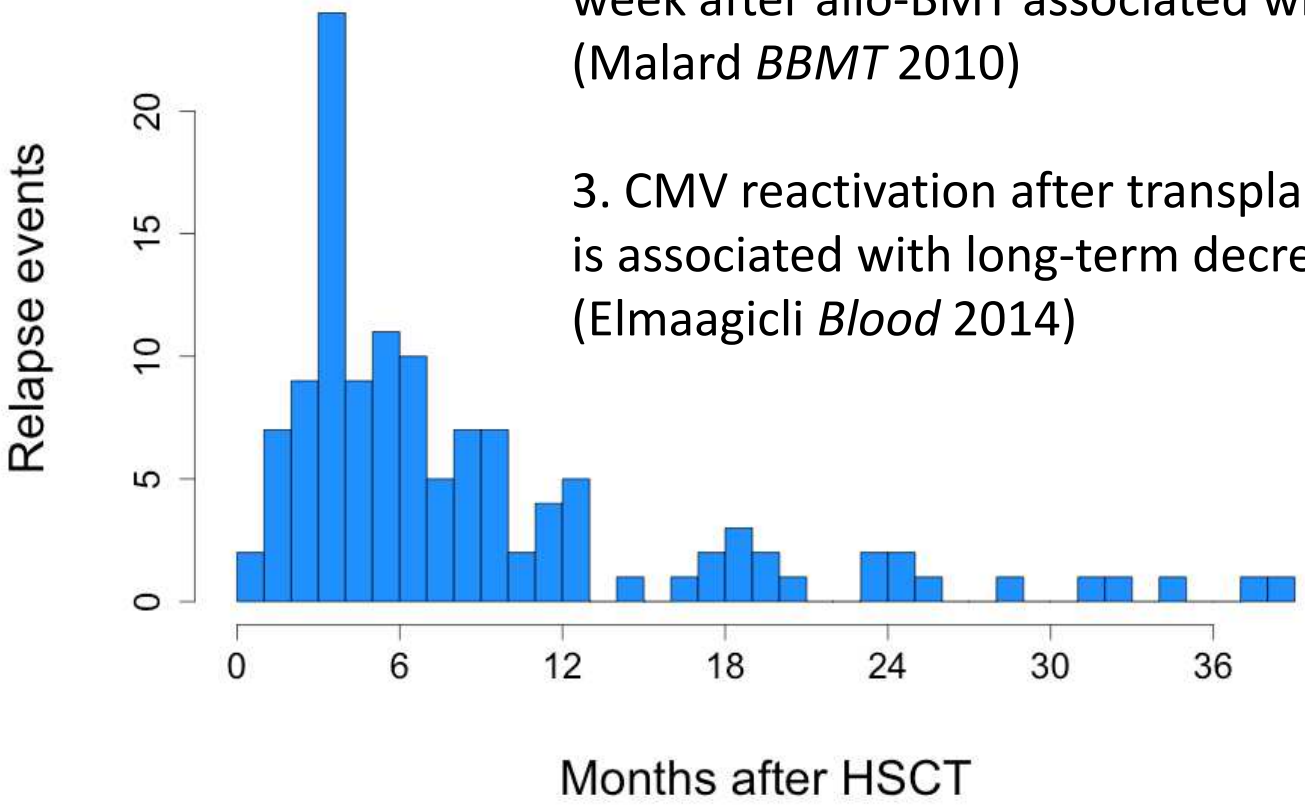
- Shotgun sequencing of selected samples
- External Validation Cohorts (Duke, Regensburg, MDACC, Rutgers, multicenter collaboration)
- Outpatient stool collection
- Genome comparisons between members of crOTU 1641 and neighbors
- Identifying signatures/consortia of bacteria
- Culturing *Eubacterium limosum* & its phylogenetic neighbors
  - effect on immune cells & tumor cells *in vitro*
  - mouse GVT models

Is a biological association between exposure during weeks 1-3 and an outcome at months-years later plausible?

1. Day 14-28 serum ST2 levels predict 6-month NRM & GVHD (van der Lugt *NEJM* 2013; Ponce *Blood* 2015)

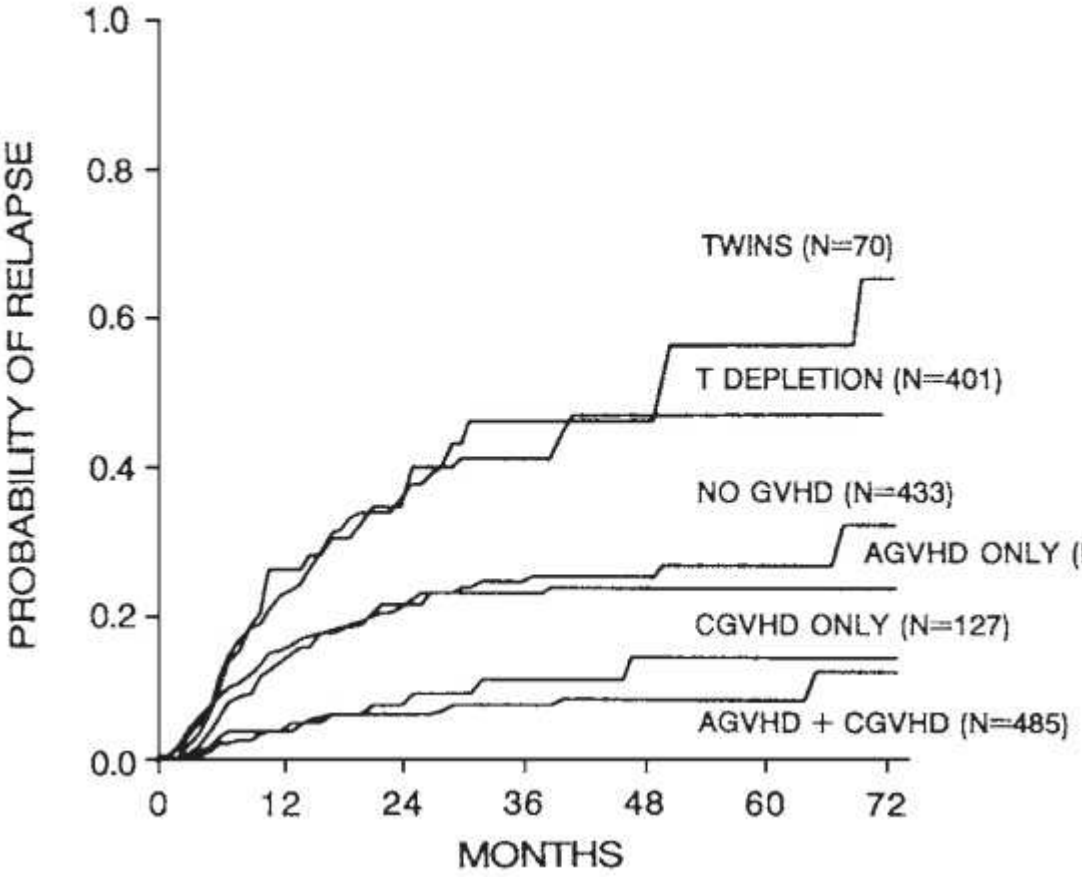
2. low serum cyclosporine concentrations in the first week after allo-BMT associated with GVHD after day 30 (Malard *BBMT* 2010)

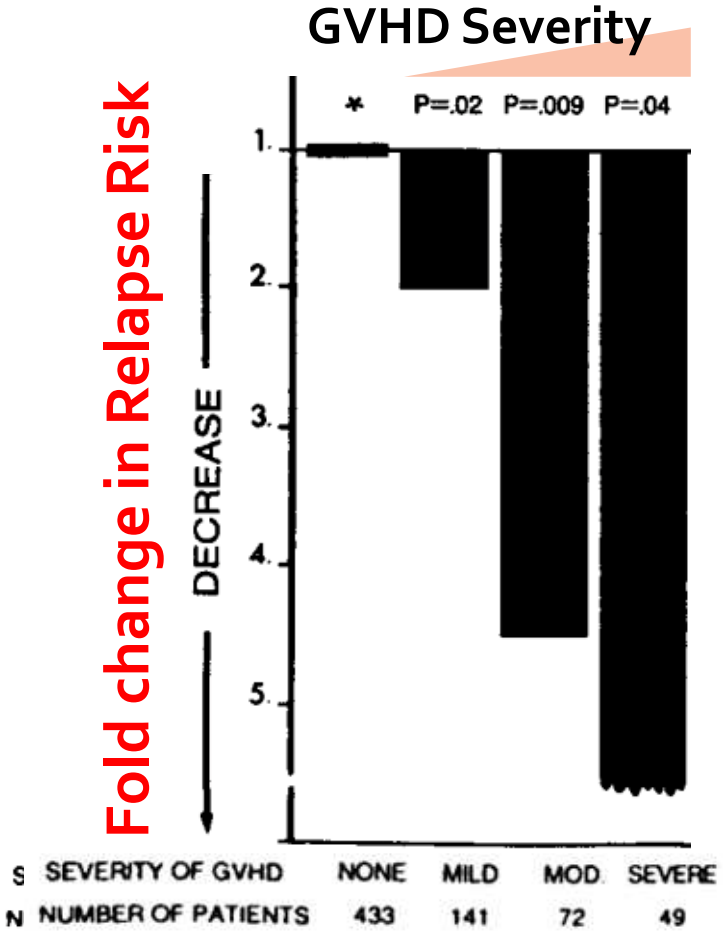
3. CMV reactivation after transplantation (median day 46) is associated with long-term decreased relapse risk (Elmaagicli *Blood* 2014)



Graft vs. host disease is accompanied by **graft vs. tumor (GVT) activity**

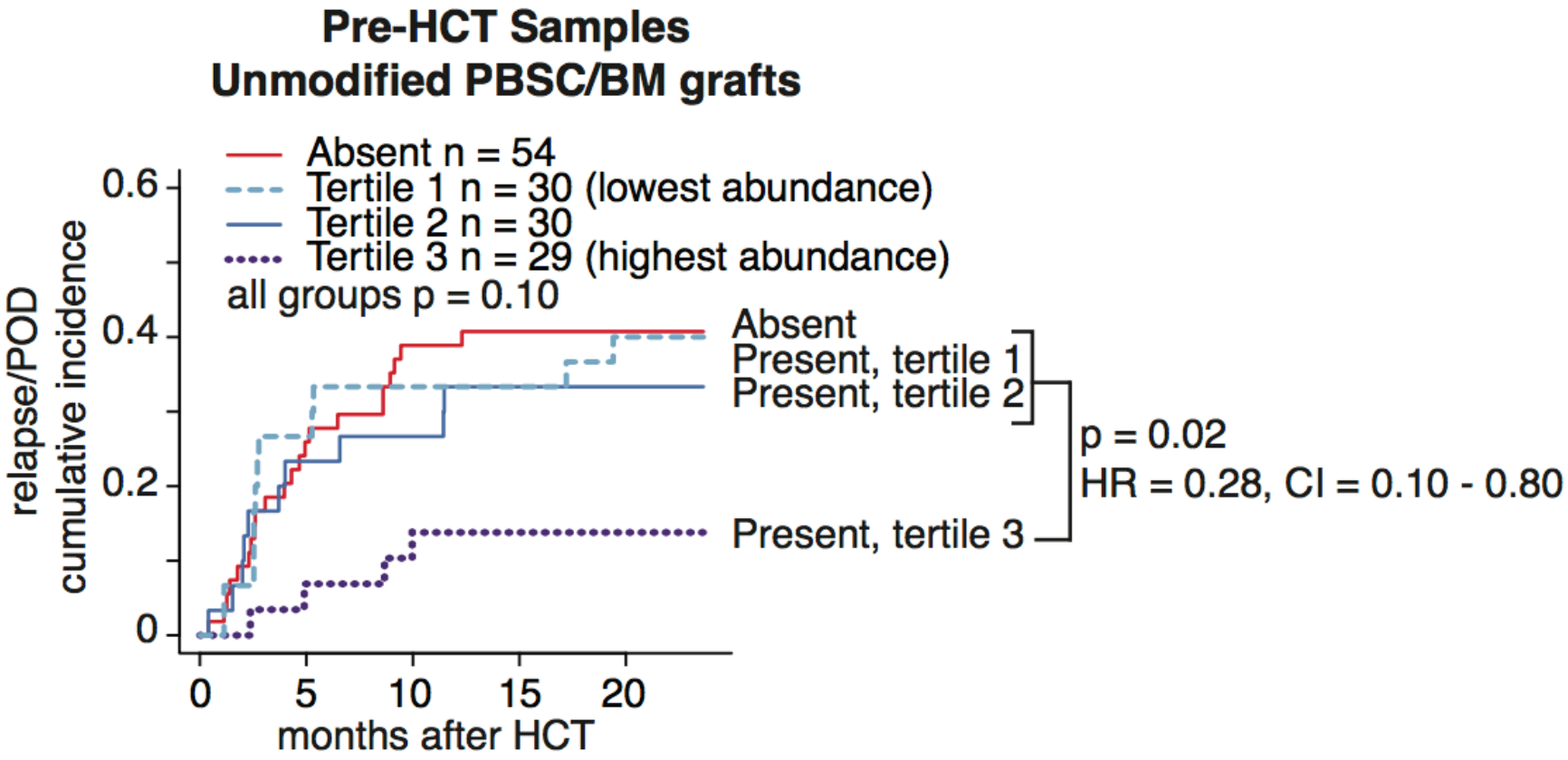
2,254 matched related transplants for CML, ALL, AML 1978-1988





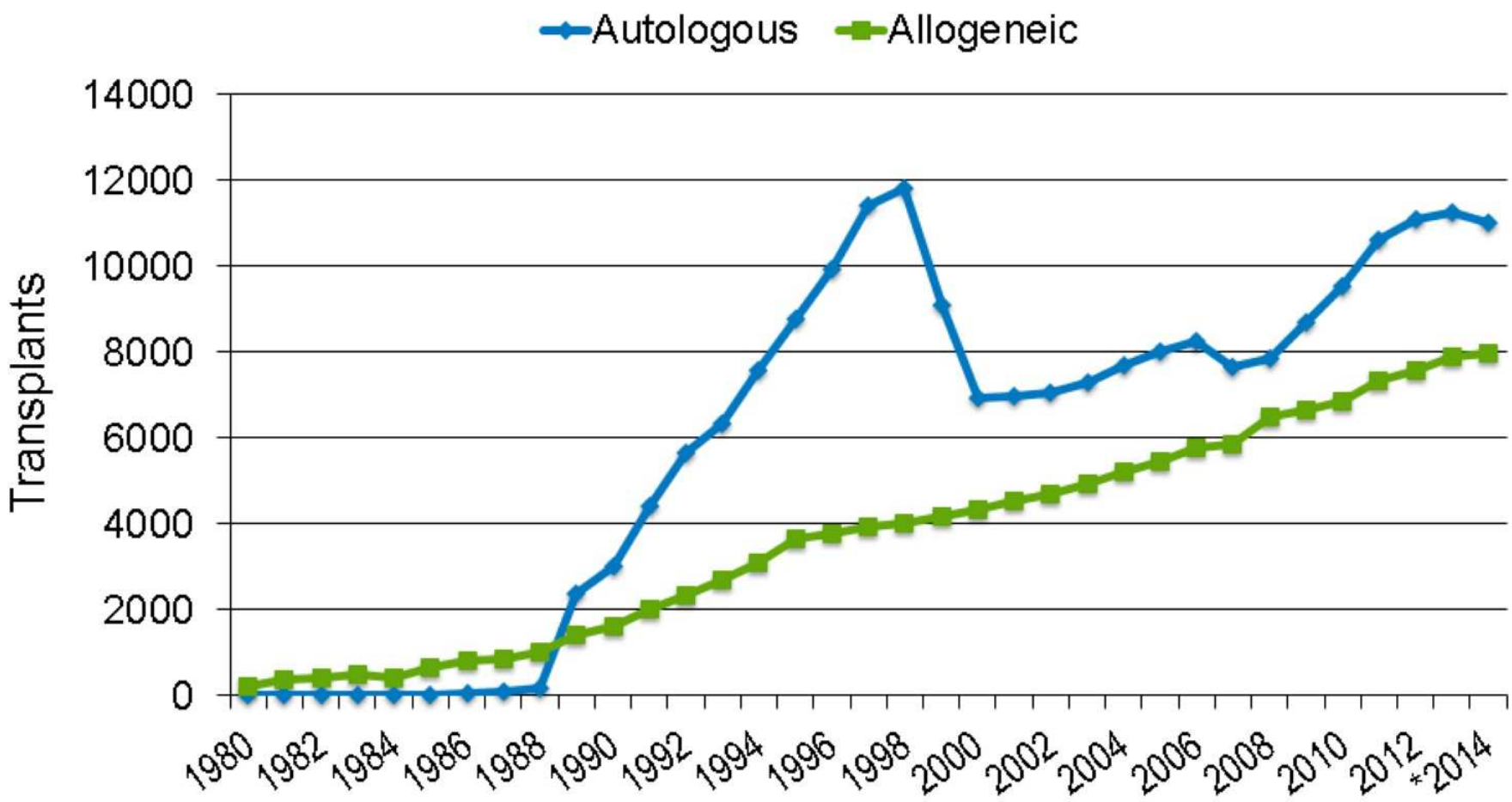
Horowitz *Blood* 1990

crOTU 1614 abundance also predicts relapse/POD when measured in a single pre-transplant samples from recipients of PBSC/BM recipients



submitted

Annual Number of Transplant Recipients in the US by Transplant Type



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*2014 Data incomplete

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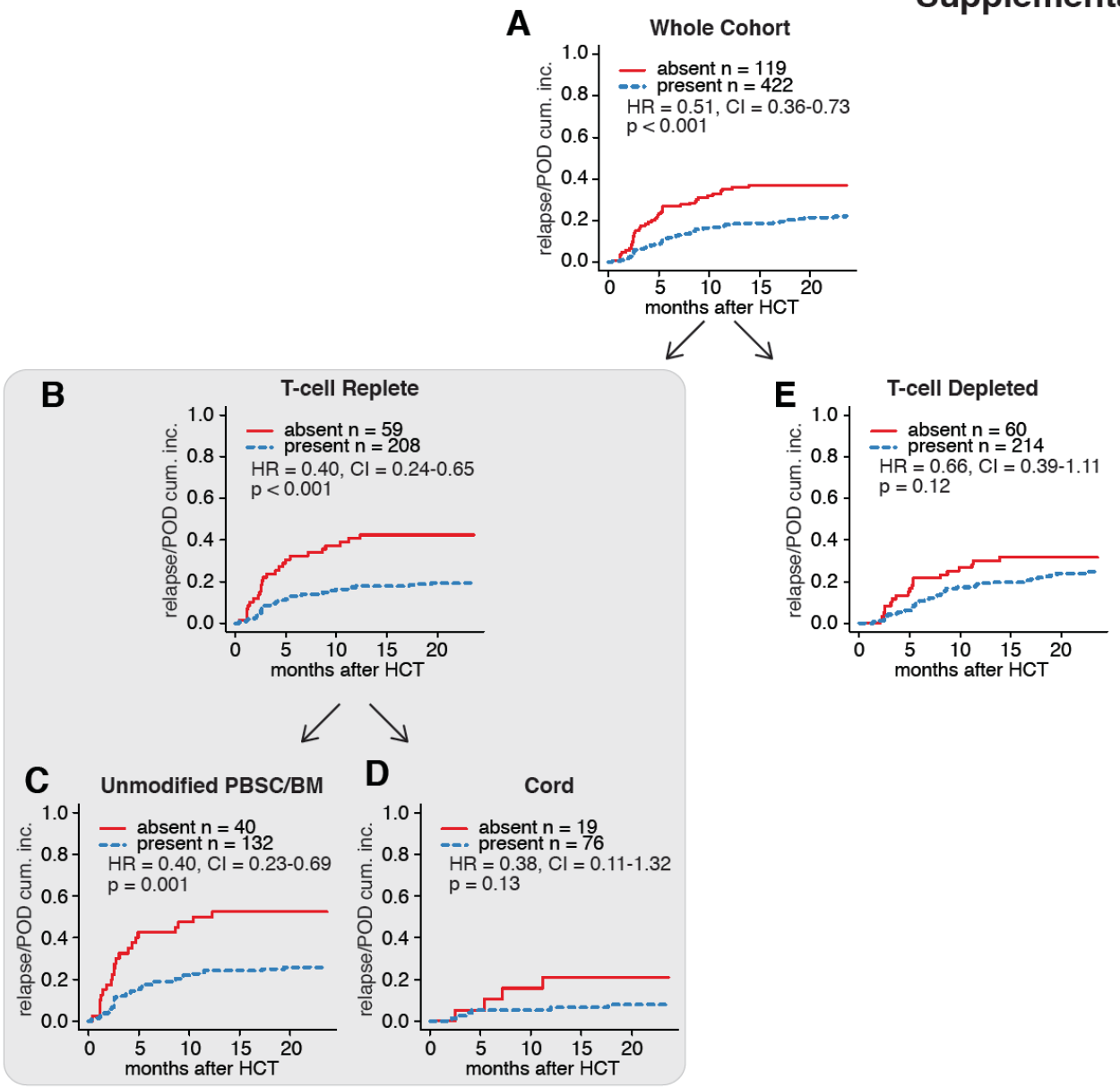
There are no major differences in baseline characteristics among patients with and without crOTU 1614

crOTU 1614	**Absent** N = 119	**Present** N = 422	**P**
Disease - no. (%)			0.3
AML	47 (39.5)	148 (35.1)	
MDS	23 (19.3)	62 (14.7)	
NHL	12 (10.1)	56 (13.3)	
other	37 (31.1)	156 (37.0)	
Conditioning Intensity - no. (%)			0.7
Myeloablative	74 (62.2)	243 (57.6)	
Reduced Intensity	33 (27.7)	129 (30.6)	
Nonmyeloablative	12 (10.1)	50 (11.8)	
Graft Source - no. (%)			0.8
Unmodified PBSC/BM	40 (33.6)	132 (31.3)	
Cord	19 (16.0)	76 (18.0)	
T-cell depleted	60 (50.4)	214 (50.7)	
DRI - no. (%)			0.02
Low	9 (7.6)	54 (12.8)	
Intermediate	72 (60.5)	281 (66.6)	
High	38 (31.9)	87 (20.6)	
mean age (SD) - yr	51.6 (13.1)	54.4 (11.8)	0.04

submitted

The association of Node 1614 with less relapse is driven primarily by T-cell replete, adult-donor transplants

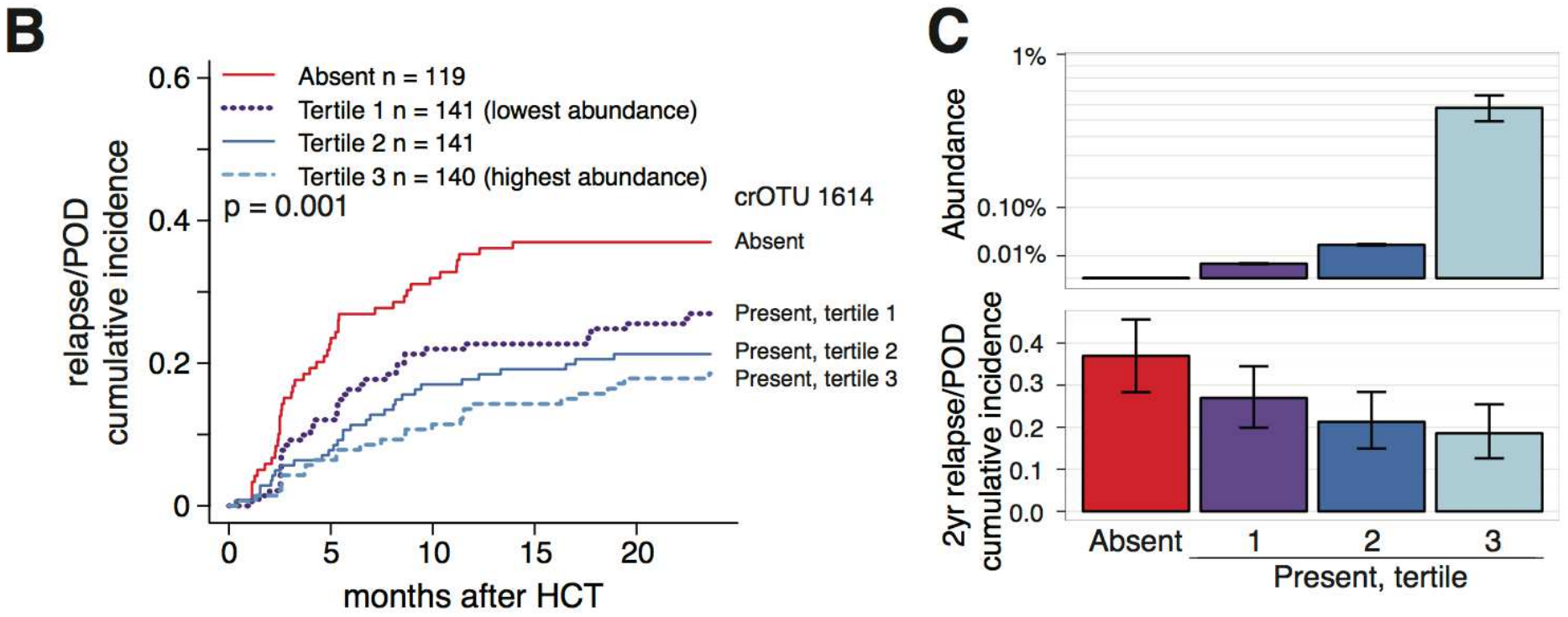
Supplemental Figure S8



submitted

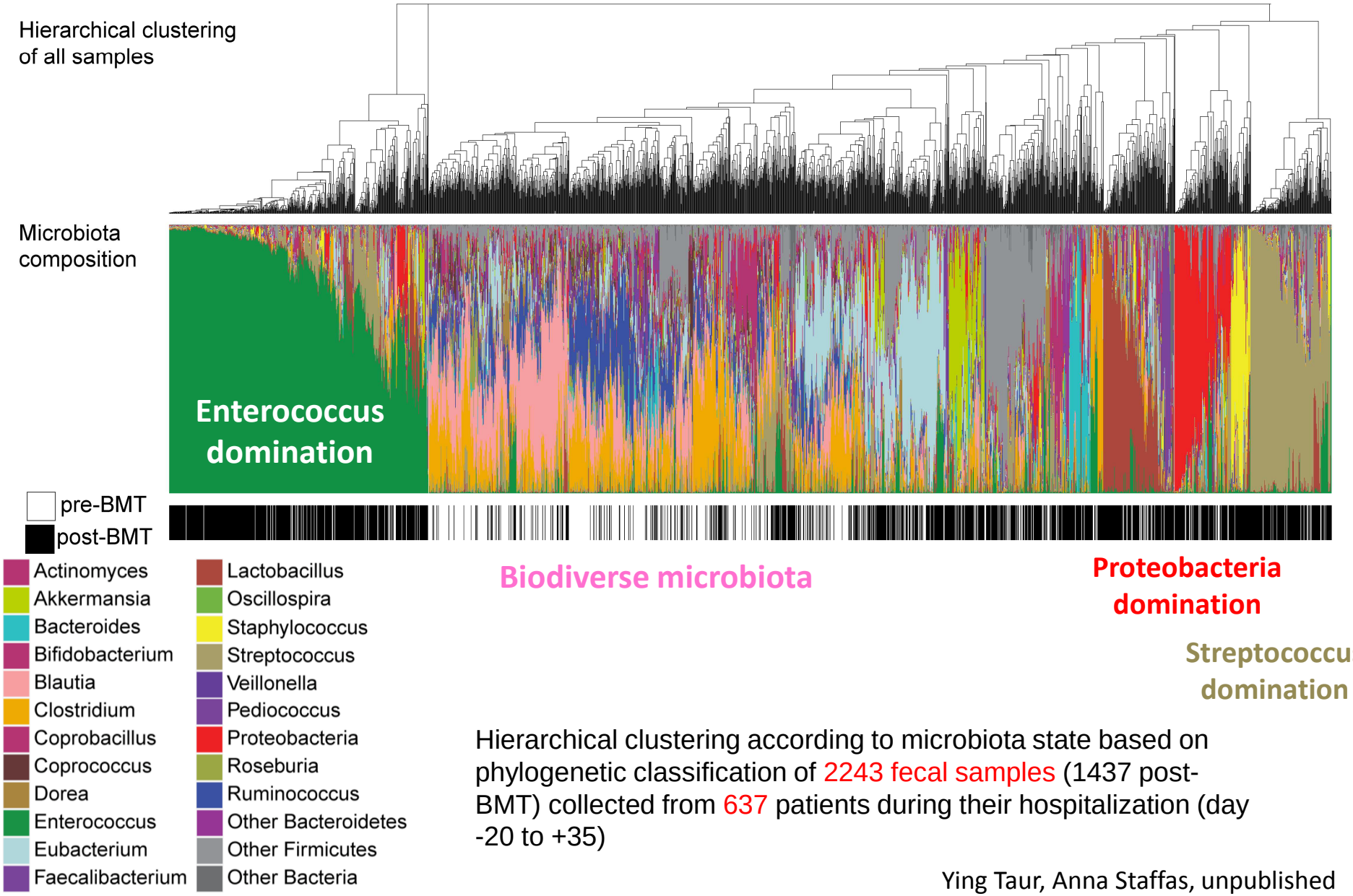
The association of crOTU 1614 abundance with less relapse exhibits a dose dependent relationship

Combined cohorts (n = 541)



submitted

Hierarchical clustering of allo-BMT patient samples shows loss of diversity and increased bacterial domination after transplant

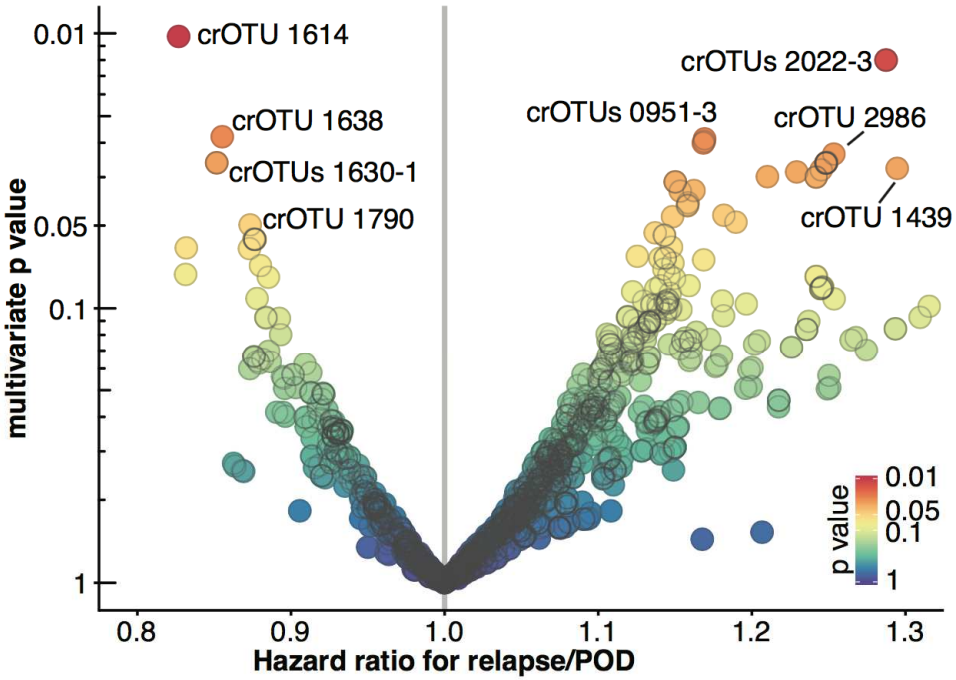


Hierarchical clustering according to microbiota state based on phylogenetic classification of 2243 fecal samples (1437 post-BMT) collected from 637 patients during their hospitalization (day -20 to +35)

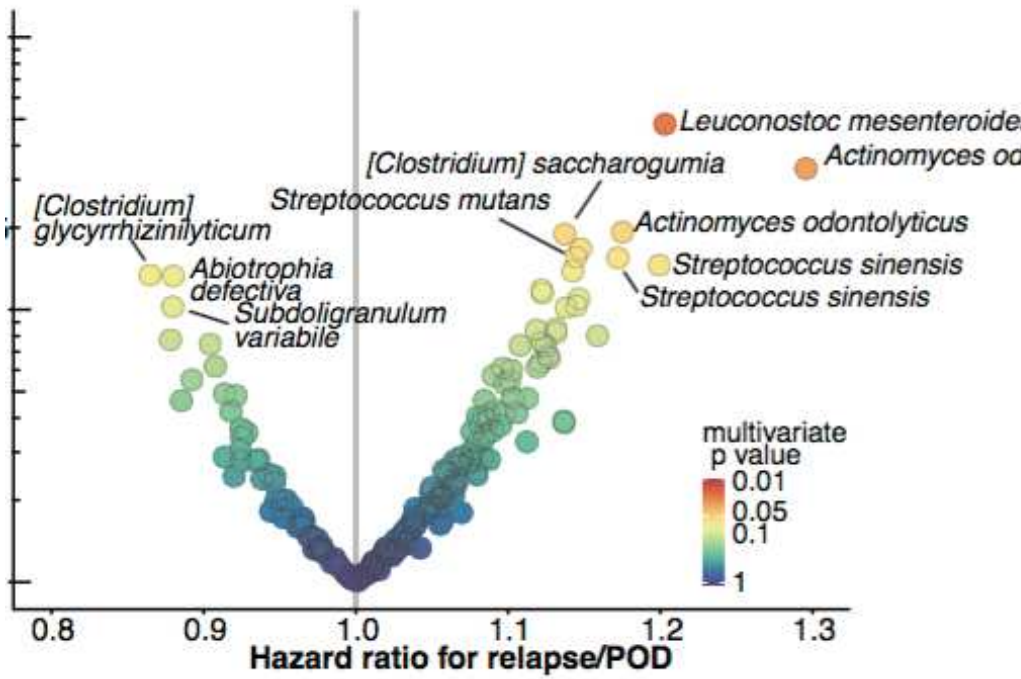
Ying Taur, Anna Staffas, unpublished

crOTUs seemed to yield better p values than OTUs

crOTUs

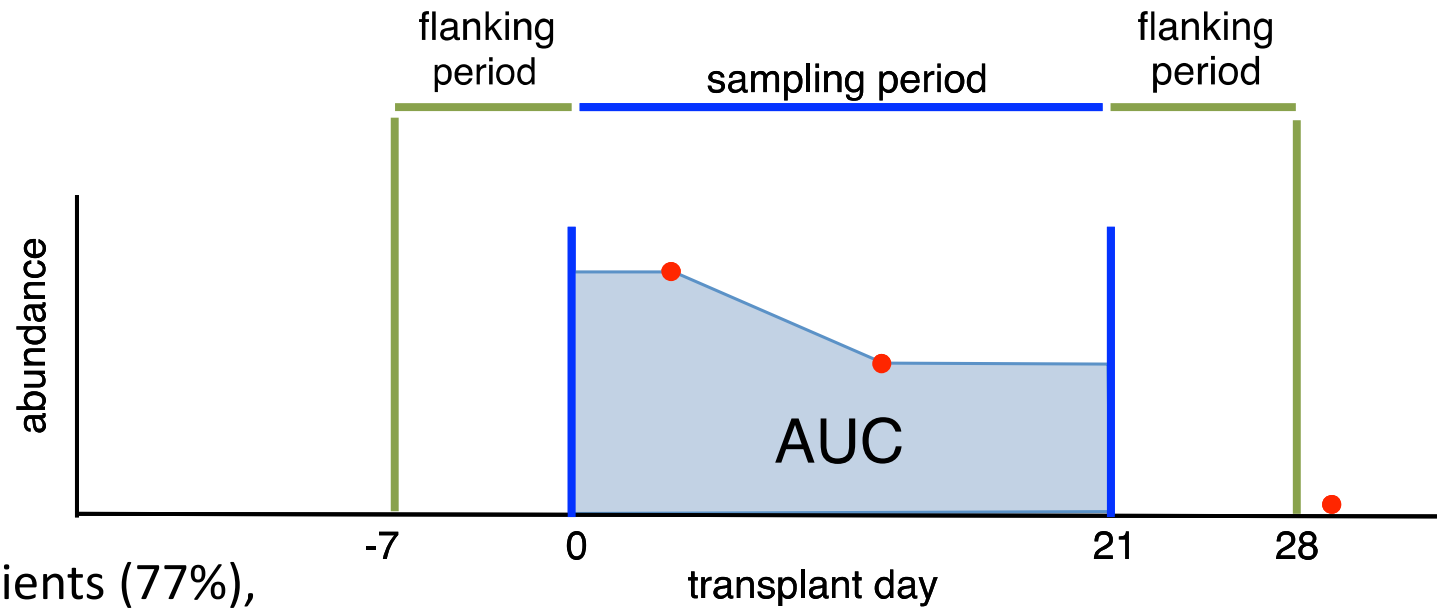


OTUs

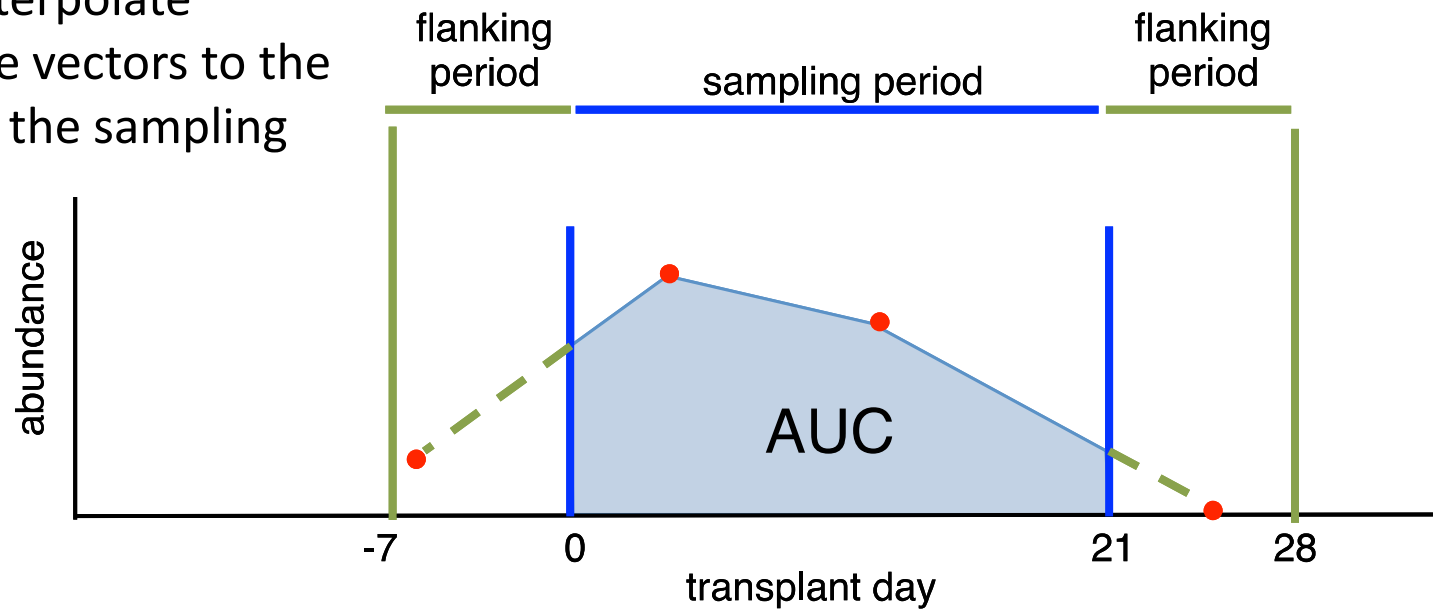


submitted

Abundance-AUC approach to irregularly sampled time-series



In 420 patients (77%), flanking samples were used to interpolate abundance vectors to the bounds of the sampling period



The Intestinal Microbiota and allo-HSCT

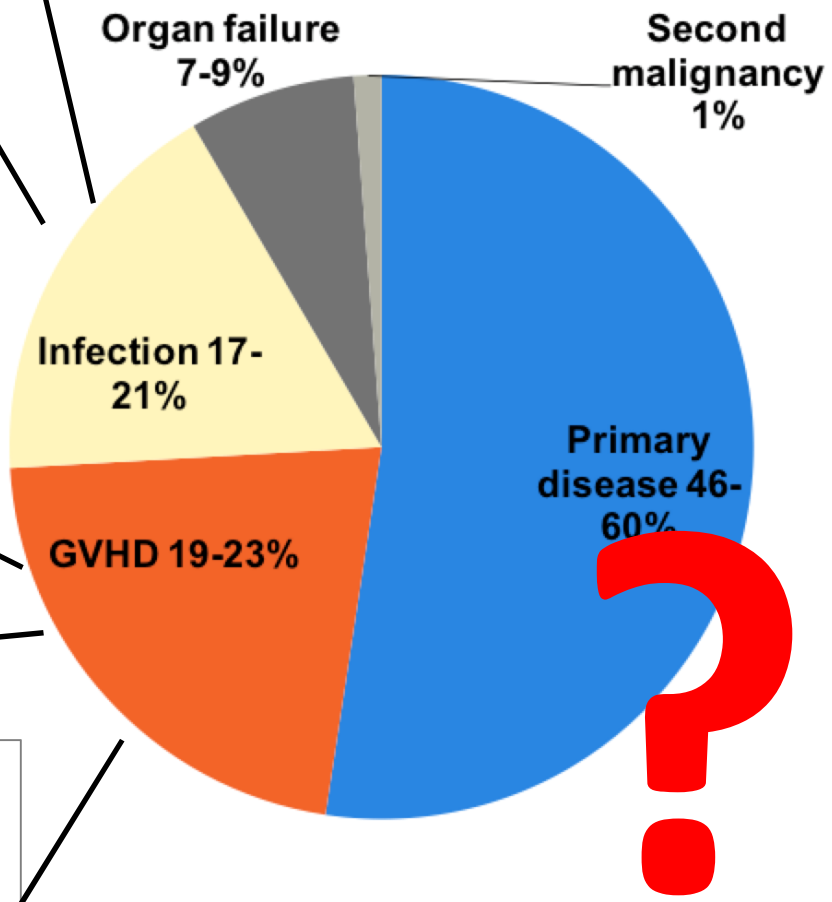
Microbial diversity predicts overall survival
Taur *Blood* 2014

Flora monodomination precedes bacteremia and predicts pulmonary complications
Taur *CID* 2012, Harris *AJRCCM* 2016

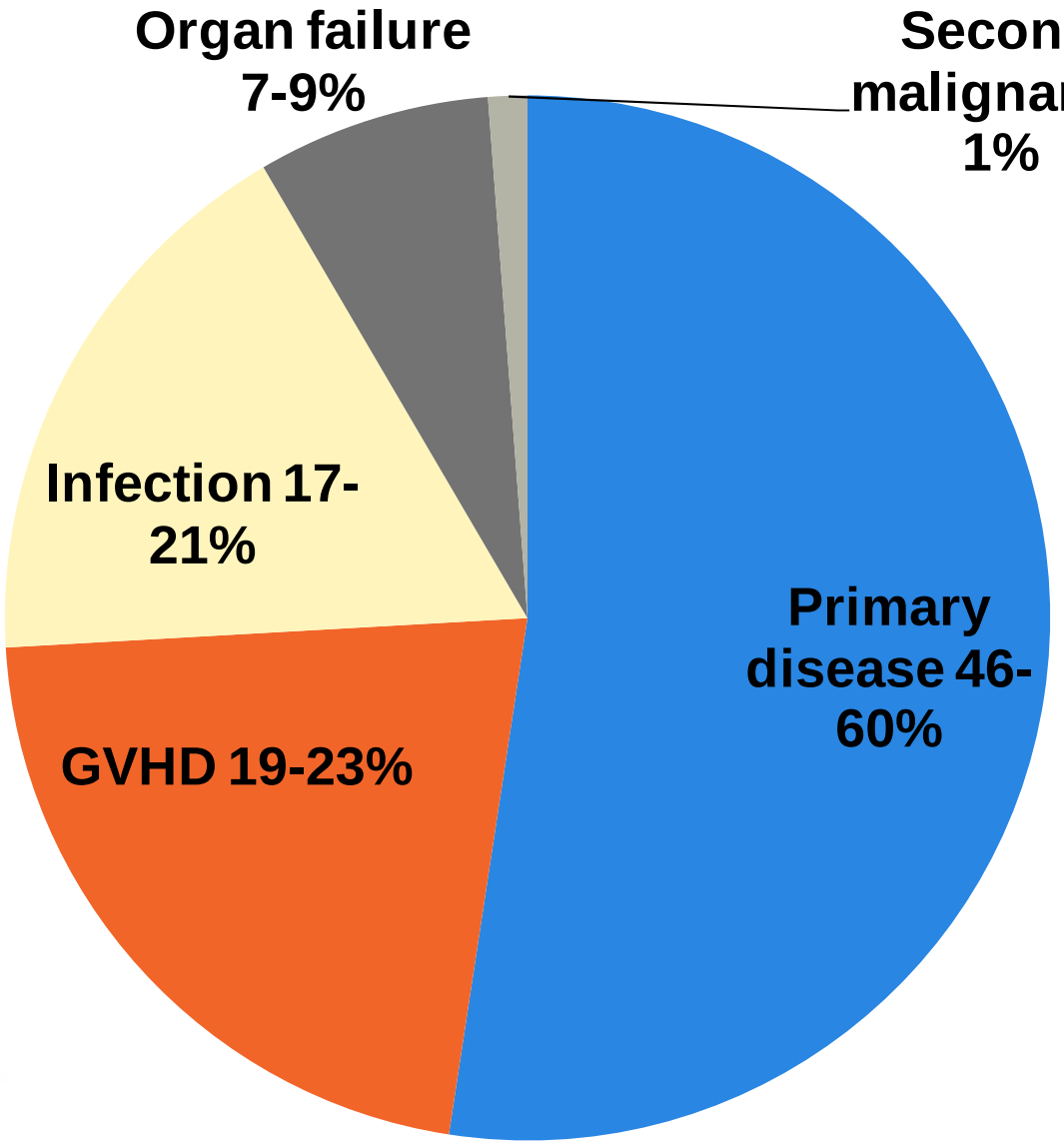
Microbiota affects GVHD
van Bekkum *JNCI* 1974
Jones *Rad Res* 1971
Jenq *JEM* 2012

Blautia predicts less GVHD mortality
Anaerobic-targeting abx → GVHD
Jenq *BBMT* 2015, Shono *SciTrans Med* 2016

Human studies of flora manipulation & GVHD
Storb *NEJM* 1983
Beelen *Blood* 1992 & 1999
Vossen *Eur J Clin Micr Inf* 1990
Vossen *PLoS One* 2014
Holler *BBMT* 2014



Cause of death after allogeneic BMT (2010-2011)



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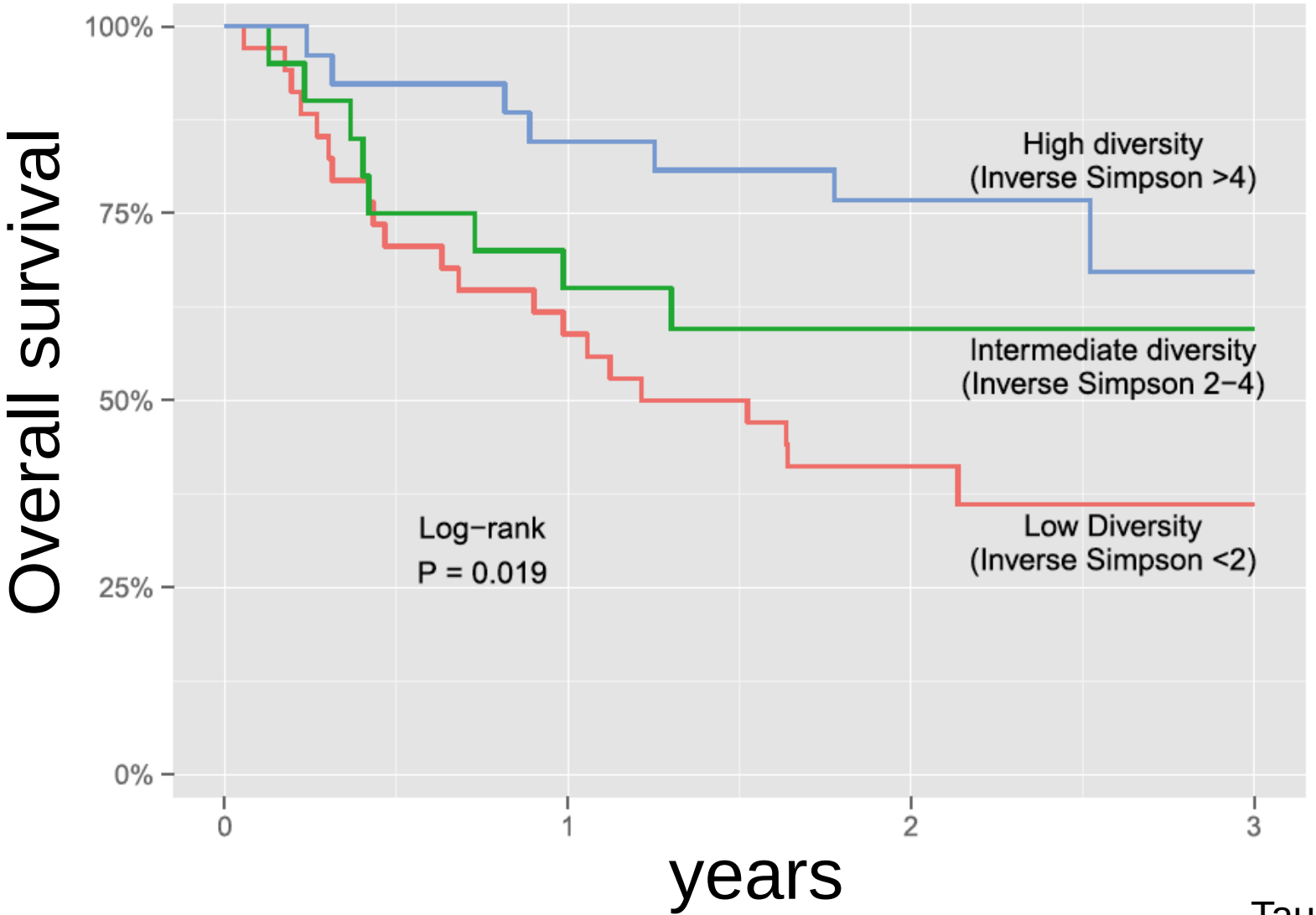
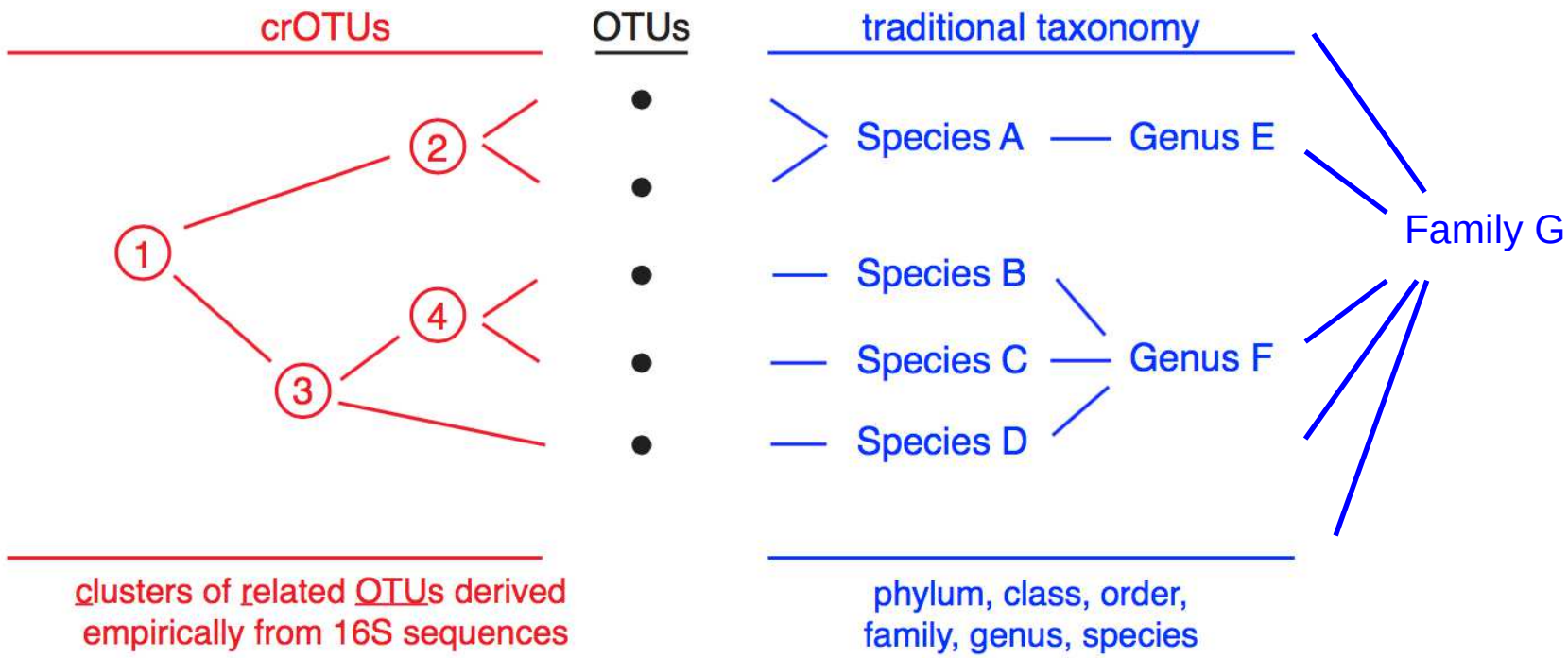
Intestinal microbiota α-diversity predicts overall survival after allo-HSCT



Taur *Blood* 2014

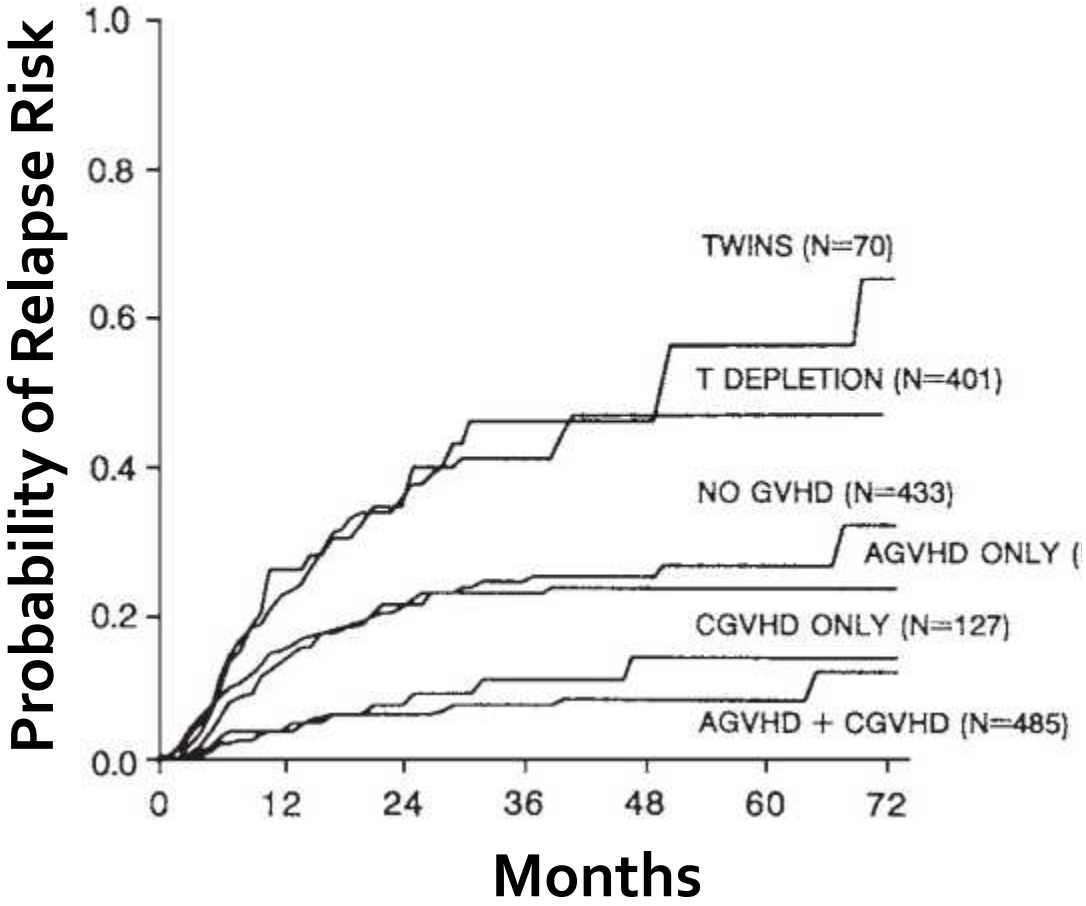
Empirically derived phylogentic tree largely recapitulates standard taxonomy and allows finer resolution of bacterial groups

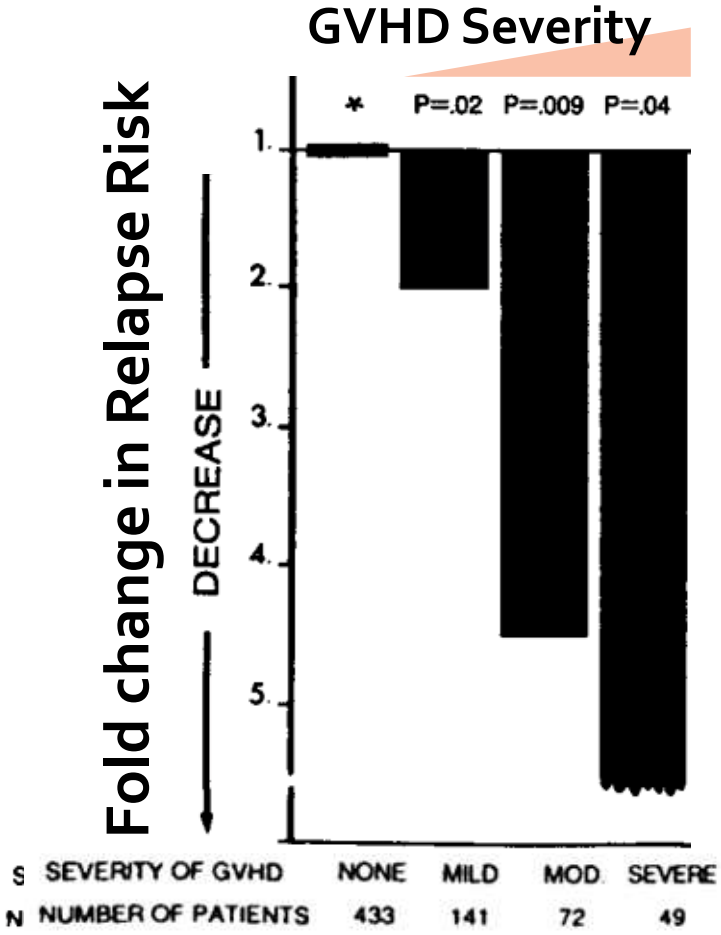


- Each **node** or **crOTU** can be evaluated for association with clinical outcome
- Empirically derived phylogenetic relationships from the **OTU** sequences
- Removes biases of historical nomenclature and 7 levels of taxonomy

Graft vs. host disease (GVHD) is accompanied by **graft vs. tumor (GVT) activity**

2,254 matched related transplants for CML, ALL, AML 1978-1988





Horowitz *Blood* 1990